

1 **Youth and long-term dietary calcium intake with risk of impaired glucose metabolism**
2 **and type 2 diabetes in adulthood**

3 Feitong Wu, PhD¹, Markus Juonala, MD, PhD^{2,3,4}, Katja Pahkala, PhD^{5,6}, Marie-Jeanne
4 Buscot, PhD¹, Matthew A. Sabin, MD, PhD⁷, Niina Pitkänen, PhD⁵, Tapani Rönkä, MD,
5 PhD², Antti Jula, MD, PhD⁸, Terho Lehtimäki, MD, PhD⁹, Nina Hutri-Kähönen, MD, PhD¹⁰,
6 Mika Kähönen, MD, PhD¹¹, Tomi Laitinen, MD, PhD¹², Jorma S.A. Viikari, MD, PhD², Olli
7 T. Raitakari, MD, PhD^{5,13*}, Costan G. Magnussen, PhD^{1,5*}

8 ¹ Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia.

9 ² Department of Medicine, University of Turku, Turku, Finland.

10 ³ Division of Medicine, Turku University Hospital, Turku, Finland.

11 ⁴ Murdoch Children's Research Institute, Parkville, Victoria, Australia.

12 ⁵ Research Centre of Applied and Preventive Cardiovascular Medicine; University of Turku,
13 Turku, Finland.

14 ⁶ Paavo Nurmi Centre, Sports & Exercise Medicine Unit, Department of Physical Activity and
15 Health, University of Turku, Turku, Finland.

16 ⁷ Murdoch Children's Research Institute, Royal Children's Hospital, and Department of
17 Paediatrics, University of Melbourne, Melbourne, VIC, Australia.

18 ⁸ National Institute for Health and Welfare, Turku, Finland.

19 ⁹ Department of Clinical Chemistry, Fimlab Laboratories and Faculty of Medicine and Health
20 Technology, Finnish Cardiovascular Research Center - Tampere, Tampere University,
21 Tampere, Finland.

22 ¹⁰ Department of Pediatrics, University of Tampere and Tampere University Hospital,
23 Tampere, Finland.

24 ¹¹ Department of Clinical Physiology, Tampere University Hospital and University of
25 Tampere, Tampere, Finland.

26 ¹² Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital and
27 University of Eastern Finland, Kuopio, Finland.

28 ¹³ Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital,
29 Turku, Finland.

30 ***These authors contributed equally to this work.**

31 **Abbreviated title: Youth calcium with adult glucose metabolism**

32 **Key terms:** pediatric, dietary calcium intake, glucose metabolism, type 2 diabetes, cohort

33 **Word count:** 2497

34 **Number of tables and figures:** 4 (2 supplemental tables and 1 supplemental figure)

35 Corresponding author and person to whom reprint requests should be addressed:

36 Costan G. Magnussen, PhD

37 Menzies Institute for Medical Research

38 University of Tasmania

39 Private Bag 23, Hobart, 7000 Tasmania, Australia.

40 E-mail: cmagnuss@utas.edu.au; Fax: +61(0)3 62267704

41 **Funding:** The Young Finns Study has been financially supported by the Academy of Finland:

42 grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and

43 41071 (Skidi); the Social Insurance Institution of Finland; Competitive State Research

44 Financing of the Expert Responsibility area of Kuopio, Tampere and Turku University

45 Hospitals (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish
46 Foundation for Cardiovascular Research ; Finnish Cultural Foundation; The Sigrid Juselius
47 Foundation; Tampere Tuberculosis Foundation; Emil Aaltonen Foundation; Yrjö Jahnsson
48 Foundation; Signe and Ane Gyllenberg Foundation; Diabetes Research Foundation of Finnish
49 Diabetes Association; and EU Horizon 2020 (grant 755320 for TAXINOMISIS); and
50 European Research Council (grant 742927 for MULTIEPIGEN project); Tampere University
51 Hospital Supporting Foundation. This study was supported by a grant from the National
52 Health and Medical Research Council Project Grant (APP1098369). CGM was supported by
53 a National Heart Foundation of Australia Future Leader Fellowship (100849). F.W. is
54 supported by a NHMRC Early Career Fellowship (APP1158661). They did not have any role
55 in the study concept, design, data analysis, writing of the manuscript, or submission of the
56 manuscript for publication. The researchers are totally independent of the funders.
57 **Disclosure Statement:** The authors have nothing to disclose.

58 **Abstract**

59 **Context** No previous studies have examined the role of youth calcium intake in the
60 development of impaired glucose metabolism, particularly those with long-term high calcium
61 intake.

62 **Objectives** To examine whether youth and long-term (between youth and adulthood) dietary
63 calcium intake is associated with adult impaired glucose metabolism and T2D.

64 **Design, Setting, and Participants** The Cardiovascular Risk in Young Finns Study (YFS) is a
65 31-year prospective cohort study (n=1134, aged 3-18 years at baseline).

66 **Exposures** Dietary calcium intake was assessed at baseline (1980) and adult follow-ups
67 (2001, 2007 and 2011). Long-term (mean between youth and adulthood) dietary calcium
68 intake was calculated.

69 **Main outcome measures** Adult impaired fasting glucose (IFG) and T2D.

70 **Results** We found no evidence for non-linear associations between calcium intake with IFG
71 or T2D among females and males (all p for non-linearity>0.05). Higher youth and long-term
72 dietary calcium intake was not associated with the risk of IFG or T2D among females or
73 males after adjustment for confounders including youth and adult BMI.

74 **Conclusions** Youth or long-term dietary calcium intake is not associated with adult risk of
75 developing impaired glucose metabolism or T2D.

76 **Introduction**

77 Due primarily to the rise in obesity over recent decades, the incidence of type 2 diabetes
78 (T2D) has dramatically increased among children and adolescents (herein termed youth)(1).
79 As a result, it is important that the prevention of T2D begins at an early stage. However, only
80 few modifiable risk factors in youth have been examined for their associations with the
81 development of adult T2D(2).

82 Recent data have raised concern that calcium intakes higher than the recommended levels are
83 associated with increased risk for cardiovascular diseases(3) and mortality(4). For T2D,
84 studies among adults have demonstrated conflicting results on the association of calcium
85 intake with T2D(5-7). Moreover, no studies have examined the relationship between calcium
86 intake in youth and the risk of developing impaired fasting glucose or T2D in adulthood. This
87 is important as calcium requirements vary by age with past studies in adults generally focused
88 on populations with low or moderate average calcium intake(5-8). In particular, people in
89 Northern European countries (e.g., Finland and Iceland) have globally high calcium intake
90 (9). Therefore, we aimed to describe the association between calcium intake in youth and
91 from youth to adulthood with the risk of developing adult impaired fasting glucose (IFG) and
92 T2D in a study among Finns with a generally high calcium intake.

93 **Methods**

94 *Participants*

95 Participants were from the prospective Cardiovascular Risk in Young Finns Study (YFS),
96 which began in 1980 and was followed up in 2001, 2007 and 2011. At baseline, 3596
97 participants aged 3-18 years were randomly selected from the national register of the study
98 areas. A 50% random sample of the participants was selected to participate in the dietary
99 recall interview (n=1768). Participants who had Type 1 diabetes or were pregnant at each
100 follow-up were excluded from all analyses. The current analyses used data from 1134

101 participants who had dietary and risk factor data from baseline, and adult T2D data. All
102 participants gave written informed consent, and local ethics committees approved the study.

103 ***T2D and IFG***

104 Participants were classified as having T2D if they met one of the following: fasting plasma
105 glucose ≥ 7 mmol/L (126 mg/dl); T2D diagnosed by a physician(10); HbA1c $\geq 6.5\%$ (48
106 mmol/mol) at the 2011 follow-up; use of glucose-lowering medication at 2007 or 2011
107 follow-ups; or being confirmed by National Social Insurance Institution Drug Reimbursement
108 Registry. IFG was defined as having a fasting plasma glucose ≥ 5.6 but ≤ 6.9 mmol/L using the
109 latest available measurement(11).

110 ***Dietary intake/Diet***

111 Diet was assessed by trained dietitians using a 48-hour dietary recall method in 1980 and
112 2001, and food frequency questionnaire in 2007 and 2011. We recorded the type and amount
113 of food eaten by the participant during the two days prior to the interview(12). Special
114 computer software was used to calculate dietary calcium intake(12). Long-term calcium
115 intake was calculated as the mean value of calcium intake in youth (1980) and adulthood
116 (mean of 2001, 2007, and 2011).

117 ***Other factors***

118 Height and weight were measured in 1980, 2001, 2007 and 2011 and body mass index (BMI)
119 calculated as weight/(height²) (kg/m²). The latest available measures were used as adulthood
120 BMI. Baseline serum 25-hydroxyvitamin D (25OHD) levels were measured as previously
121 described(2). Information on smoking habits was collected during a medical examination in a
122 solitary room. Youth smoking for participants aged <12 years in 1980 was defined on a daily
123 basis between ages 12-18. For those aged 12-18 years in 1980, youth smoking was defined as
124 regular cigarette smoking on a weekly basis (or more often). A physical activity index was
125 calculated as previously described(13). Briefly, we asked and summed up different variables
126 about exercise/physical activity habits, including intensity and frequency of exercise, athletic

127 club attendance (frequency of participating in training at an athletic club), athletic
128 competitions (whether participated in club, district or national level competitions), leisure
129 time (usual activities during spare time: indoors, mostly indoors and mostly outdoors) and
130 sports participation. A parent-completed questionnaire was used for participants aged 3 and 6
131 years, while self-completed questionnaires were used for children aged 9 to 18 years. This
132 physical activity measure has been shown to be reliable and valid(14). Physical activity
133 indices were standardised by age. Questionnaires were used to obtain information on parental
134 history of T2D and years of education.

135 **Statistical analysis**

136 Mean (standard deviation) and number (%) were used, as appropriate, to describe variables.
137 We compared baseline characteristics between participants who participated the baseline
138 dietary recall interview and those who did not, and between participants with complete data
139 and those lost to follow-up (or with incomplete baseline characteristics). Univariable and
140 multivariable modified Poisson regression models (using a robust error variance)(15) were
141 used to estimate the relative risk (RR) and 95% confidence intervals (CI) for youth and long-
142 term dietary calcium intake and the risk of adult IFG and T2D. All analyses were stratified by
143 sex. We selected potential confounders based on the biological plausibility of an association
144 of a factor with both the outcome and the exposure of interest, including age, BMI, serum
145 25OHD levels, parental history of diabetes, fruit and vegetable consumption, physical
146 activity, smoking, socio-economic status (parent's years of education) at baseline and adult
147 BMI. The association of tertiles of long-term dietary calcium intake with the risk of adult IFG
148 and T2D was further examined using above mentioned method. We used restricted cubic
149 splines to examine the potential non-linear associations between calcium intake and
150 outcomes(16). Non-linearity was tested by comparing the log-likelihood of the new model
151 with that of the linear model. A cut-off of 800 mg/d (the median of recommended intake for
152 youth aged 6-17 years in Finland) was used to estimate the RR (95% CIs) of developing IFG
153 and T2D at different calcium intakes. We created 10 imputations using linear regression for

154 missing data for adulthood BMI (n=13 (1%); predictors including sex and childhood BMI and
155 age) and long-term calcium intake (n=198 (17%); predictors including sex, childhood calcium
156 intake and BMI and adulthood BMI). We assumed all values were missing at random. We
157 also performed sensitivity analysis for the association of long-term calcium intake with IFG
158 and T2D by using available data for long-term dietary calcium intake. All analyses were
159 performed in Stata version 15.1 (Stata Corporation, Texas, USA). A two-tailed p value <0.05
160 was considered statistically significant.

161 **Results**

162 Of the 1134 participants (51% female) in the YFS, 50 developed T2D and 240 developed
163 IFG. **Table 1** shows the comparison of participants' characteristics between females and
164 males in youth and adulthood. At baseline, the mean (SD) intake of dietary calcium was 1019
165 (366) mg/d in females and 1270 (514) mg/d in males; only five participants were taking
166 calcium supplements (<0.5 %). The long-term mean (SD) intake was 1181 (340) mg/d for
167 females and 1398 (424) mg/d for males. There were no differences in baseline characteristics
168 between those who participated in the dietary interview and those who did not (data not
169 shown), or between participants who were followed up and those who were lost to follow-up
170 (**Table S1**(17)). A flowchart of participation is given in **Figure S1**(17).

171 We found no evidence of non-linear associations between youth or long-term calcium intake
172 and IFG or T2D in females or males (p for non-linearity>0.05 for all, **Figure 1** and **2**). In
173 unadjusted models, higher youth and long-term (youth to adulthood) dietary calcium intake
174 was associated with increased risk of IFG and T2D among males but these associations were
175 attenuated and no longer statistically significant after adjustment for confounders including
176 youth and adult BMI (**Table 2**). Youth or long-term dietary calcium intake was not associated
177 with IFG or T2D among females (**Table 2** and **Table S2**(17)). Results remained largely
178 similar in sensitivity analysis using available data for long-term dietary calcium intake (data
179 not shown).

180 **Discussion**

181 Using data from a cohort with on average high calcium intake, we found that neither youth
182 nor long-term (child to adult) dietary calcium intake was associated with increased risk of
183 developing IFG or T2D in adulthood. Our findings are novel as this is the first study to
184 describe the association of youth and long-term dietary calcium intake with these outcomes in
185 adulthood in cohorts with a high average intake of calcium. These findings suggest that higher
186 dietary calcium intake might not confer an increased risk of developing impaired glucose
187 metabolism or T2D in a population with calcium intake much higher than the recommended
188 level (but lower than the tolerable upper intake level).

189 **Important findings and possible explanations**

190 Findings for the association between calcium intake and risk of T2D in adults have been
191 contradictory(5-8). Overall, participants in previous studies had a low to moderate average
192 intake of calcium with the authors of these works concluding that increased calcium intake
193 was not, or was inversely, associated with T2D. For example, Lorenzo et al. found that an
194 increased serum calcium level but not dietary calcium intake was associated with increased
195 risk of T2D in adults during a mean follow-up of 5.2 years (mean calcium intake=942 mg/d;
196 aged 40-69 years)(5). In contrast, the Nurses' Health Study showed that women (aged 30-55
197 years, mean calcium intake =731 mg/d) in the highest category of calcium intake (>1200
198 mg/d) from all sources had 21% lower risk of developing T2D compared with those in the
199 lowest category (\leq 600 mg/d)(6). However, the association of dietary calcium intake with T2D
200 is similar to our findings in females in the fully adjusted model. Importantly, the analyses in
201 the Nurses' Health Study were stratified by pre-specified cut-offs, which risk missing
202 important associations. For example, it is unclear whether the association is linear and if not,
203 where and how the association changes particularly in those with high calcium intake. In the
204 Shanghai Women's Health Study, similar findings were observed (high calcium intake
205 associated with lower risk of T2D) when data were analysed by fifths of calcium intake(7).
206 However, the average intake of calcium was low (median=466 mg/d). The median calcium

207 intake of the highest fifth in the study was only 650 mg/d; much lower than the recommended
208 level for adults. Therefore, these previous findings might not apply to populations with higher
209 average dietary calcium intake.

210 Although the exact mechanisms for the association between calcium and T2D remain unclear,
211 those supporting a favourable role of calcium suggest an adverse effect of low serum calcium
212 concentration on insulin secretion and other insulin actions(8). In contrast, increased serum
213 calcium levels were associated with decreased insulin sensitivity but not insulin secretion in
214 elderly men, even in participants with normal glucose and normal levels of serum
215 calcium(18). In line, recent epidemiological studies have found a positive association between
216 increased serum calcium levels and the risk of T2D in adults(5,19-22). The conflicting
217 evidence may be due to the differences in serum calcium levels of the studied population as
218 the association between serum calcium concentration and the risk of T2D may differ by
219 calcium levels(5). In addition, a higher serum calcium level may not reflect high calcium
220 intake but rather an indicator of hyperparathyroidism, which might be attributed to long-term
221 insulin insufficiency or insulin resistance, leading to increased risk of T2D(23). Future studies
222 should consider the potential threshold effect of calcium intake or serum calcium levels on
223 T2D and related outcomes considering the impact of serum parathyroid hormone levels.

224 Only a few randomised controlled trials (RCT) have examined the effect of calcium
225 supplementation on T2D in adults and the results were also conflicting(24,25). In 20
226 nondiabetic patients with essential hypertension, calcium supplementation of 1,500 mg/d vs.
227 placebo for 8 weeks improved insulin sensitivity but did not affect fasting glycemia(25).
228 However, a 2-by-2 factorial-design RCT of 92 adults found no effect of twice-daily 400 mg
229 calcium supplementation (calcium + vitamin D or vitamin D placebo) vs. no calcium (calcium
230 placebo + vitamin D or vitamin D placebo) for 16 weeks on pancreatic β cell function, acute
231 insulin response, insulin sensitivity, or measures of glycemia(24). Of note, participants in the
232 control group of the smaller RCT were maintained on a low calcium intake (\approx 500 mg/d)
233 while participants in the larger study had a moderate calcium intake at baseline (mean= 976

234 mg/d). These data suggest calcium supplementation might only be effective at reducing the
235 risk of T2D among those with low calcium intake. Importantly, it is suggested that calcium
236 supplementation but not high intake of dietary calcium increases the risk of cardiovascular
237 diseases(3,26). However, our ability of examining calcium supplement is limited due to the
238 low rate of supplement (<0.5% in youth and 8% in adulthood in the YFS) and this should be
239 examined in future research in people with high rate of calcium supplementation. Moreover, a
240 6-month small RCT (n=95) showed that daily supplementation of calcium (1,200 mg calcium
241 carbonate) in combination with vitamin D (2,000-6,000 IU/d cholecalciferol) improved
242 insulin sensitivity in middle-aged adults with prediabetes and low vitamin D status(27).
243 However, future research is needed to clarify whether this benefit is due to calcium or vitamin
244 D.

245 **Methodological considerations and limitations**

246 The strength of this study is the analysis using data from a cohort with long-term follow-up in
247 a population-based sample, enabling the examination of childhood factors with adult health
248 outcomes. However, this study has limitations. Youth dietary calcium intake was measured by
249 the 48-hour recall method, which captures limited intra-individual variability. However, the
250 long-term calcium intake was based on four time points (two time points using food
251 frequency questionnaire), partly overcoming this limitation. Moreover, we had a small
252 number of T2D patients and participants with very low calcium intake (only 5% <800 mg/d
253 for the long-term intake). Therefore, we could not rule out the possible association between
254 calcium intake and T2D in those with very low calcium intake. Our total sample size is
255 relatively small. While the statistical power for IFG appears to be sufficient, studies of similar
256 settings but larger sample size are needed to confirm our findings about T2D before any
257 potential risk of high calcium intake could be ruled out. Although no T2D patients were
258 reported at baseline, we could not determine baseline status of IFG because fasting glucose
259 levels were not measured. Nevertheless, the rate of IFG at baseline is likely very low because

260 of the younger age (mean=10.6 years) and very low rate of obesity (1%) in our childhood
261 sample. Indeed, only 3.2% participants aged 18 had IFG (measured in 2008) in the STRIP
262 study among Finns, which had an obesity rate of 3.6% (unpublished data). We had
263 participants lost to follow-up but we have previously shown that these samples are
264 representative of the original cohorts(28,29), which was again confirmed in the current study.
265 Moreover, results remained largely similar when complete case analysis was conducted (i.e.,
266 no imputation for long-term calcium intake), suggesting minor influence of missing data on
267 our findings.

268 **Conclusions and policy implications**

269 In conclusion, dietary calcium intake in youth and between youth and adulthood is not
270 associated with the risk of IFG or T2D in adulthood in a population with calcium intake much
271 higher than the recommended level (but lower than the tolerable upper intake levels). This
272 finding should be considered in assessing the balance of risks and benefits of taking high
273 calcium intake to improve calcium associated health outcomes.

274 **Acknowledgement:** We thank Noora Kartiosuo for compiling data from the YFS for this
275 study. We thank all the volunteers and participants involved in the present study.

276 **Authors' roles:** F.W., C.G.M and M.J. were involved in study design. M.J., N.P., A.J., T.L.,
277 K.P., M.K., T.L., J.S.A.V., and O.T. R. were responsible for data collection and management.
278 F.W. performed data analysis, in consultation with C.G.M. and M.J.. F.W. drafted the
279 manuscript. All authors revised manuscript content and approved the final manuscript and had
280 access to the data. J.S.A.V. contributed to the initial design of Young Finns. O.T.R. leads
281 Young Finns and contributed to obtaining funding and to the study design. C.G.M. and
282 O.T.R. are the guarantors of the study and accept full responsibility for the finished article,
283 had access to any data, and controlled the decision to publish.

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383

384 **Figure Legend**

385

386 **Figure 1** Restricted cubic splines for the non-linear associations between youth dietary
387 calcium intake, IFG and T2D in females (A and B) and males (C and D) in the YFS. A
388 calcium intake of 800 mg/d was used as the reference to estimate the relative risk of
389 developing IFG and T2D at different calcium intakes. Solid and dashed lines denote relative
390 risks and corresponding 95% confidence intervals.

391

392 **Figure 2** Restricted cubic splines for the non-linear associations between long-term dietary
393 calcium intake, IFG and T2D in females (A and B) and males (C and D) in the YFS. A
394 calcium intake of 800 mg/d was used as the reference to estimate the relative risk of
395 developing IFG and T2D at different calcium intakes. Solid and dashed lines denote relative
396 risks and corresponding 95% confidence intervals.

397 **Table 1** Participant characteristics in youth (1980) and adulthood in the YFS

	Females (n=578)	Males (n=556)
Youth		
Age (year)	10.6 (4.9)	10.5 (5.0)
BMI (kg/m ²)	17.9 (3.1)	18.0 (3.1)
25OHD (nmol/L)	50.3 (15.6)	53.4 (14.7)
Dietary calcium intake (mg/d)	1019 (366)	1270 (514)
Physical activity index (z score)	-0.25 (0.90)	0.22 (1.03)
Parental history of diabetes, n (%)	13 (2)	7 (1)
Fruit consumption (>6 times/week), n (%)	485 (84)	429 (77)
Vegetable consumption (>6 times/week), n (%)	199 (34)	196 (35)
Smokers, n (%)	125 (22)	180 (32)
Parental years of education	10.1 (3.4)	10.0 (3.3)
Adulthood^b		
Age (year)	41.6 (4.9)	41.5 (5.0)
BMI (kg/m ²)	25.7 (5.1)	27.0 (4.1)
Smokers, n (%)	94 (16)	119 (22)
Education status, n (%)		
Grammar school	76 (15)	79 (16)
College or vocational school	232 (44)	242 (48)
University degree	212 (41)	184 (36)
Fasting glucose (mmol/L)	5.19 (0.73)	5.54 (0.92)
Glucose categories, n (%)		
NFG	483 (84)	361 (65)
IFG	76 (13)	164 (29)
T2D	19 (3)	31 (6)
Fruit consumption (g/day)	216 (209)	172 (213)
Vegetable consumption (g/day)	294 (194)	244 (172)

398 Data are mean (standard deviation) unless otherwise stated.

399 Abbreviations: NFG, normal fasting glucose; IFG, impaired fasting glucose; T2D, type 2
400 diabetes mellitus; BMI, body mass index; 25OHD, 25-hydroxyvitamin D.

401 ^a IFG cut-off is 5.6 mmol/L.

402 ^b all variables used data from the latest available values in adulthood (from 2001, 2007 and
403 2011).

404 For adult variables, number of participants were 1121 for BMI, 1128 for fasting glucose, 936
405 for fruit and vegetable consumption, 1118 for smoking and 1025 for education.

406 Bold denotes significant difference between females and males, p<0.05.

Table 2 Associations of youth and long-term dietary calcium intake with IFG and T2D in adult females and males in the YFS

		Females		Males		
		n	RR (95% CI) ^a	n	RR (95% CI) ^a	
Youth calcium	Model 1	NFG	483	1.00 (Ref)	361	1.00 (Ref)
		IFG	76	0.90 (0.72, 1.13)	164	1.17 (1.05, 1.30)
		T2D	19	1.08 (0.73, 1.61)	31	1.55 (1.20, 2.01)
	Model 2	NFG	483	1.00 (Ref)	361	1.00 (Ref)
		IFG	76	0.93 (0.74, 1.17)	164	1.11 (0.99, 1.24)
		T2D	19	1.12 (0.71, 1.79)	31	1.31 (0.98, 1.75)
	Model 3	NFG	483	1.00 (Ref)	361	1.00 (Ref)
		IFG	76	0.93 (0.74, 1.17)	164	1.11 (0.99, 1.24)
		T2D	19	1.11 (0.68, 1.80)	31	1.17 (0.83, 1.64)
Long-term calcium	Model 1	NFG	483	1.00 (Ref)	361	1.00 (Ref)
		IFG	76	1.04 (0.84, 1.29)	164	1.14 (1.02, 1.28)
		T2D	19	1.37 (0.94, 2.00)	31	1.41 (1.01, 1.98)
	Model 2	NFG	483	1.00 (Ref)	361	1.0 (Ref)
		IFG	76	1.11 (0.91, 1.36)	164	1.08 (0.97, 1.21)
		T2D	19	1.38 (0.98, 1.94)	31	1.05 (0.71, 1.53)
	Model 3	NFG	483	1.0 (Ref)	361	1.0 (Ref)
		IFG	76	1.11 (0.90, 1.36)	164	1.09 (0.97, 1.22)
		T2D	19	1.39 (0.93, 2.06)	31	1.10 (0.72, 1.69)

Abbreviations: RR, relative risk; CI, confidence interval; NFG, normal fasting glucose; IFG, impaired fasting glucose (cut-off 5.6 mmol/L); T2D, type 2 diabetes mellitus.

^a relative risk for every standard deviation (youth: 366 mg/d for females and 514 mg/d for males; long-term: 302 mg/d for females and 387 mg/d for males) higher dietary calcium intake.

Bold denotes statistical significance, $p < 0.05$.

Model 1, unadjusted; Model 2, adjusted for age and childhood and adulthood body mass index; Model 3, model 2 + baseline serum 25OHD levels, parental history of diabetes, fruit and vegetable consumption, physical activity, smoking, and socioeconomic status (parental education years).