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Youth and long-term dietary calcium intake with risk of impaired glucose metabolism and type 2 diabetes in adulthood: The Cardiovascular Risk in Young Finns Study (YFS) and the Special Turku coronary Riskfactor Intervention Project (STRIP)

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Abstract

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

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

Design, Setting, and Participants The Cardiovascular Risk in Young Finns Study (YFS) is a 31-year prospective cohort study (n=1134, aged 3-18 years at baseline). The Special Turku coronary Risk factor Intervention Project (STRIP) is a 20-year prospective, randomized, infancy-onset intervention trial; data from age 2 to 20 years were used for observational analysis (n=420).

Exposures Youth and long-term (mean between youth and adulthood) dietary calcium intake. In the YFS, dietary calcium intake was assessed at baseline (1980) and adult follow-ups (2001, 2007 and 2011). In the STRIP, dietary calcium intake was assessed annually from age 2 to 20 years.

Main outcome measures Adult impaired fasting glucose (IFG), high HOMA-IR, high insulin levels, and T2D.

Results We found no evidence for non-linear associations between calcium intake with IFG or T2D among females and males (all p for non-linearity>0.05) in neither the YFS or STRIP. Higher youth and long-term dietary calcium intake was not associated with the risk of IFG, high HOMA-IR, high insulin levels or T2D among females or males after adjustment for confounders including youth and adult BMI in neither studies.

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

Introduction

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

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

Methods

Participants from the two studies were analysed separately.

The prospective Cardiovascular Risk in Young Finns Study (YFS)

Participants

The YFS began in 1980 and was followed up in 2001, 2007 and 2011. At baseline, 3596 participants aged 3-18 years were randomly selected from the national register of the study areas. A 50% random sample of the participants was selected to participate in the dietary recall interview (n=1768). Participants who had Type 1 diabetes or were pregnant at each

follow-up were excluded from all analyses. The current analyses used data from 1134 participants who had dietary and risk factor data from baseline, and adult T2D data. All participants gave written informed consent, and local ethics committees approved the study.

T2D and IFG

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

Dietary intake/Diet

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

Other factors

Height and weight were measured in 1980, 2001, 2007 and 2011 and body mass index (BMI) calculated as weight/(height^2) (kg/m^2). The latest available measures were used as adulthood BMI. Baseline serum 25-hydroxyvitamin D (25OHD) levels were measured as previously described[2]. Information on smoking habits was collected during a medical examination in a solitary room. Youth smoking for participants aged <12 years in 1980 was defined on a daily basis between ages 12-18. For those aged 12-18 years in 1980, youth smoking was defined as regular cigarette smoking on a weekly basis (or more often). A physical activity index was calculated as previously described[13]. Briefly, we asked and summed up different variables

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

The Special Turku coronary Risk factor Intervention Project (STRIP)

Participants

The STRIP study is an infancy-onset randomized controlled trial of dietary counseling that aimed to reduce the risk of atherosclerosis. Participants of the study have been closely followed up beginning of age 7 months (between February 1990 and June 1992) to 20 years of age. The details of the STRIP study have been described elsewhere[1536]. Briefly, at baseline (February 1990 to June 1992) the families of 5-month-old infants were recruited from well-baby clinics in Turku, Finland. These families received detailed information about STRIP when their infants were 6 months old. A total of 1062 infants participated in the study (56.5% of the eligible age cohort), and were randomly assigned to a dietary intervention group (N = 540; 256 girls) or a control group (N = 522; 256 girls) when they were aged 7 months. During the study visits, all children met with a nutritionist and a pediatrician or a nurse. The children in the intervention group received individualised dietary counseling at 1- to 3-month intervals until the age of 2 years and biannually thereafter until 20 years of age. The children in the control group came to the study visits biannually until the age of 7 years and annually thereafter until the age of 20 years. During the study visits, the control group received basic health education similar to the education routinely given at Finnish well-baby clinics and by school health care. The study was approved by the Joint Commission on Ethics of the Turku

University and the Turku University Central Hospital. Written informed consent was obtained from the parents in the beginning of the study and from the adolescents at 15 and 18 years of age. The present observational analysis included 420 participants who had height and weight measured at the age of 2 and 20 years, at least one dietary calcium intake assessment between 2 and 20 years of age, and one outcome measure available at age 20.

Impaired glucose metabolism at the age of 20

Fasting venous blood samples were obtained to determine serum glucose and insulin levels. The samples were centrifuged immediately, with 15 µl of the enzyme inhibitor Antagosan added to 0.5-ml serum insulin sample. Samples were stored frozen until analysed. Serum insulin levels were measured with a microparticle enzyme immunoassay (Insulin IMX system reagent, Abbott, [Chicago, IL], interassay coefficient of variation [CV]=6.5%) or with a chemiluminescent microparticle immunoassay (ARCHITECT insulin assay, Abbott, USA, interassay CV=1.8%)[15]. A correction of analytical level between the methods was used[15]. Serum glucose was measured by a hexokinase method (Glucose Olympus System Reagent, Olympus, Ireland, interassay CV 1.8%). Insulin sensitivity was estimated by HOMA-IR (fasting insulin mU/mL×[fasting glucose (mmol/L)/22.5])[16].

IFG was defined as having a fasting glucose ≥5.6 but ≤6.9 mmol/L and T2D as ≥7 mmol/L and diagnosed by a physician[11]. High insulin resistance was defined using a WHO study where HOMA index was at or above the 75th sex-specific percentile[17]. For consistency, high fasting insulin was dichotomized using the 75th sex-specific percentile.

Dietary intake

Diet was obtained annually between ages 2 and 20 years using 4-day food records (consecutive days; at least one weekend day included)[18,19], a method validated in children[20]. The food records were reviewed by a nutritionist for accuracy. The nutrient intakes were calculated using a Micro Nutrica based on the Food and Nutrient Database of the Social Insurance Institution[21]. The mean calcium intake was calculated for each participant from the

age of 2 to 12 (childhood), 13 to 18 (adolescence) and 2 to 20 (long-term) years. The average intakes of fruit and vegetables from the age of 2 to 20 years were also calculated and used in analyses.

Other factors

Weight was measured using an electronic scale (S10; Soehnle, Murrhardt, Germany) to the nearest 0.1 kg and height using a wall-mounted stadiometer (Harpenden Stadiometer, Holtain, Crymych, U.K.) to the nearest 0.1 cm. BMI was calculated as weight/(height^2) (kg/m^2). Questionnaires were used to assess current regular smoking (once a day or more often), physical activity (metabolic equivalent hours per week of leisure-time physical activity calculated by multiplying weekly mean exercise intensity, duration, and frequency)[22,23], parental history of diabetes, socio-economic status (at least one of the parents had bachelor's degree or higher education when participants were aged 13 months). Latest available values for smoking and physical activity were used in analyses.

Statistical analysis

Mean (standard deviation) and number (%) were used, as appropriate, to describe variables. Univariable and multivariable modified Poisson regression models (using a robust error variance) were used to estimate the relative risk (RR) and 95% confidence intervals (CI) for youth dietary calcium intake and the risk of adult IFG and T2D in YFS and for long-term calcium intake in childhood, adolescent and between childhood and adulthood with the risk of IFG, high HOMA-IR and insulin levels in STRIP. All analyses were stratified by sex. We selected potential confounders based on the biological plausibility of an association of a factor with both the outcome and the exposure of interest, including age, BMI, serum 25OHD levels, parental history of diabetes, fruit and vegetable consumption, physical activity, smoking, socio-economic status (parent's years of education) at baseline and adult BMI in YFS, and BMI at baseline, long-term consumptions of fruit and vegetables, and BMI at age 20 years in the STRIP. We used restricted cubic splines to examine the potential non-linear

associations between calcium intake and outcomes[24]. Non-linearity was tested by comparing the log-likelihood of the new model with that of the linear model. A cut-off of 800 mg/d (the median of recommended intake for youth aged 6-17 years in Finland) was used to estimate the RR (95%CIs) of developing IFG and T2D at different calcium intakes. Missing data were imputed: adulthood BMI (n=13)and long-term calcium intake (n=198) (YFS); current regular smoking (n=157) and parental education (n=66) (STRIP). We assumed all values were missing at random. All analyses were performed in Stata version 15.1 (Stata Corporation, Texas, USA). A two-tailed p value <0.05 was considered statistically significant.

Results

Of the 1134 participants (51% female) in the YFS, 50 developed T2D and 240 developed IFG. **Table 1** shows the comparison of participants' characteristics between females and males in youth and adulthood. At baseline, the mean intake of dietary calcium was 1019 mg/d in females and 1270 mg/d in males; only five participants were taking calcium supplements (<0.5 %). The long-term mean intake was 1160 mg/d for females and 1371 mg/d for males.

We found no evidence of non-linear associations between youth or long-term calcium intake and IFG or T2D in females or males (p for non-linearity>0.05 for all, **Figure 1** and **2**). In unadjusted models, higher youth and long-term (youth to adulthood) dietary calcium intake was associated with increased risk of IFG and T2D among males but these associations were attenuated and no longer statistically significant after adjustment for confounders including youth and adult BMI (**Table 2**). Youth or long-term dietary calcium intake was not associated with IFG or T2D among females (**Table 2**).

Characteristics of participants in the STRIP are given in **Supplemental Table 1**. None developed T2D. Similarly, there was no evidence of non-linear associations between calcium intake and IFG, high HOMA-IR or high insulin levels in females or males (p for non-linearity>0.05 for all). After adjustment for confounders, the associations of calcium intake in

childhood and adolescence, and long-term calcium intake remained non-significant with all studied outcomes (**Supplemental Table 2, 3** and **4**).

Discussion

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

Important findings and possible explanations

Findings for the association between calcium intake and risk of T2D in adults have been contradictory[5-8]. Overall, participants in previous studies had a low to moderate average intake of calcium with the authors of these works concluding that increased calcium intake was not, or was inversely, associated with T2D. For example, Lorenzo et al. found that an increased serum calcium level but not dietary calcium intake was associated with increased risk of T2D in adults during a mean follow-up of 5.2 years (mean calcium intake=942 mg/d; aged 40-69 years)[5]. In contrast, the Nurses' Health Study showed that women (aged 30-55 years, mean calcium intake =731 mg/d) in the highest category of calcium intake (>1200 mg/d) from all sources had 21% lower risk of developing T2D compared with those in the lowest category (≤600 mg/d)[6]. However, the association of dietary calcium intake with T2D is similar to our findings in females in the fully adjusted model. Importantly, the analyses in the Nurses' Health Study were stratified by pre-specified cut-offs, which risk missing important associations. For example, it is unclear whether the association is linear and if not, where and how the association changes particularly in those with high calcium intake. In the Shanghai

Women's Health Study, similar findings were observed (high calcium intake associated with lower risk of T2D) when data were analysed by fifths of calcium intake[7]. However, the average intake of calcium was low (median=466 mg/d). The median calcium intake of the highest fifth in the study was only 650 mg/d; much lower than the recommended level for adults. Therefore, these previous findings might not apply to populations with higher average dietary calcium intake.

Although the exact mechanisms for the association between calcium and T2D remain unclear, those supporting a favourable role of calcium suggest an adverse effect of low serum calcium concentration on insulin secretion and other insulin actions[8]. In contrast, increased serum calcium levels were associated with decreased insulin sensitivity but not insulin secretion in elderly men, even in participants with normal glucose and normal levels of serum calcium[25]. In line, recent epidemiological studies have found a positive association between increased serum calcium levels and the risk of T2D in adults[5,26-29]. The conflicting evidence may be due to the differences in serum calcium levels of the studied population. For example, it has been shown that increased serum calcium concentration was only inversely associated with the risk of T2D among those with calcium levels >2.38 mmol/l[5]. In addition, a higher serum calcium level may not reflect high calcium intake but rather an indicator of hyperparathyroidism, which might be attributed to long-term insulin insufficiency or insulin resistance, leading to increased risk of T2D[30]. Future studies should consider the potential threshold effect of calcium intake or serum calcium levels on T2D and related outcomes considering the impact of serum parathyroid hormone levels.

Only a few randomised controlled trials (RCT) have examined the effect of calcium supplementation on T2D in adults and the results were also conflicting[31,32]. In 20 nondiabetic patients with essential hypertension, calcium supplementation of 1,500 mg/d vs. placebo for 8 weeks improved insulin sensitivity but did not affect fasting glycemia[32]. However, a 2-by-2 factorial-design RCT of 92 adults found no effect of twice-daily 400 mg calcium supplementation (calcium + vitamin D or vitamin D placebo) vs. no calcium (calcium placebo

+ vitamin D or vitamin D placebo) for 16 weeks on pancreatic β cell function, acute insulin response, insulin sensitivity, or measures of glycemia[31]. Of note, participants in the control group of the smaller RCT were maintained on a low calcium intake (≈500 mg/d) while participants in the larger study had a moderate calcium intake at baseline (mean= 976 mg/d). These data suggest calcium supplementation might only be effective at reducing the risk of T2D among those with low calcium intake. Importantly, it is suggested that calcium supplementation but not high intake of dietary calcium increases the risk of cardiovascular diseases[3]. However, our ability of examining calcium supplement is limited due to the low rate of supplement (<0.5% in youth and 8% in adulthood in the YFS) and this should be examined in future research in people with high rate of calcium supplementation.

Methodological considerations and limitations

The strength of this study is the analysis using data from two independent cohorts with long-term follow-up in a population-based sample, enabling the examination of childhood factors with adult health outcomes. Moreover, the STRIP is an infancy-onset study, minimising the potential influence of age and age-related factors. The dietary calcium intake in the STRIP was assessed annually using a well-validated method, providing an accurate measurement of the long-term calcium intake. In the YFS, youth dietary calcium intake was measured by the 48-hour recall method, which captures limited intra-individual variability. However, the long-term calcium intake was based on four time points (two time points using food frequency questionnaire), partly overcoming this limitation. Moreover, we had a small number of T2D patients and participants with very low calcium intake (only 5% <800 mg/d for the long-term intake in the YFS and <10% in the STRIP). Therefore, we could not rule out the possible association between calcium intake and T2D in those with very low calcium intake. Our total sample size is relatively small and studies of similar settings but larger sample size are needed to confirm our findings before any potential risk of high calcium intake could be ruled out. We had participants lost to follow-up in both cohorts but we have previously shown that these

samples are representative of the original cohorts[19,33,34]. Moreover, multiple imputation was used to account for missing data. Lastly, the STRIP is a dietary intervention study and thus half of the sample had a healthier diet in terms of e.g. quality of dietary fat compared to the control group. Nevertheless, there was no association between the intervention and calcium intake, suggesting that our results are likely to be generalisable to a general population.

Conclusions and policy implications

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

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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)**

Data are mean (standard deviation) unless otherwise stated.

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

[a] IFG cut-off is 5.6 mmol/L.

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

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

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	
Youth calcium		n	RR (95% CI)[a]	n	RR (95% CI)[a]
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 (1.93, 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.

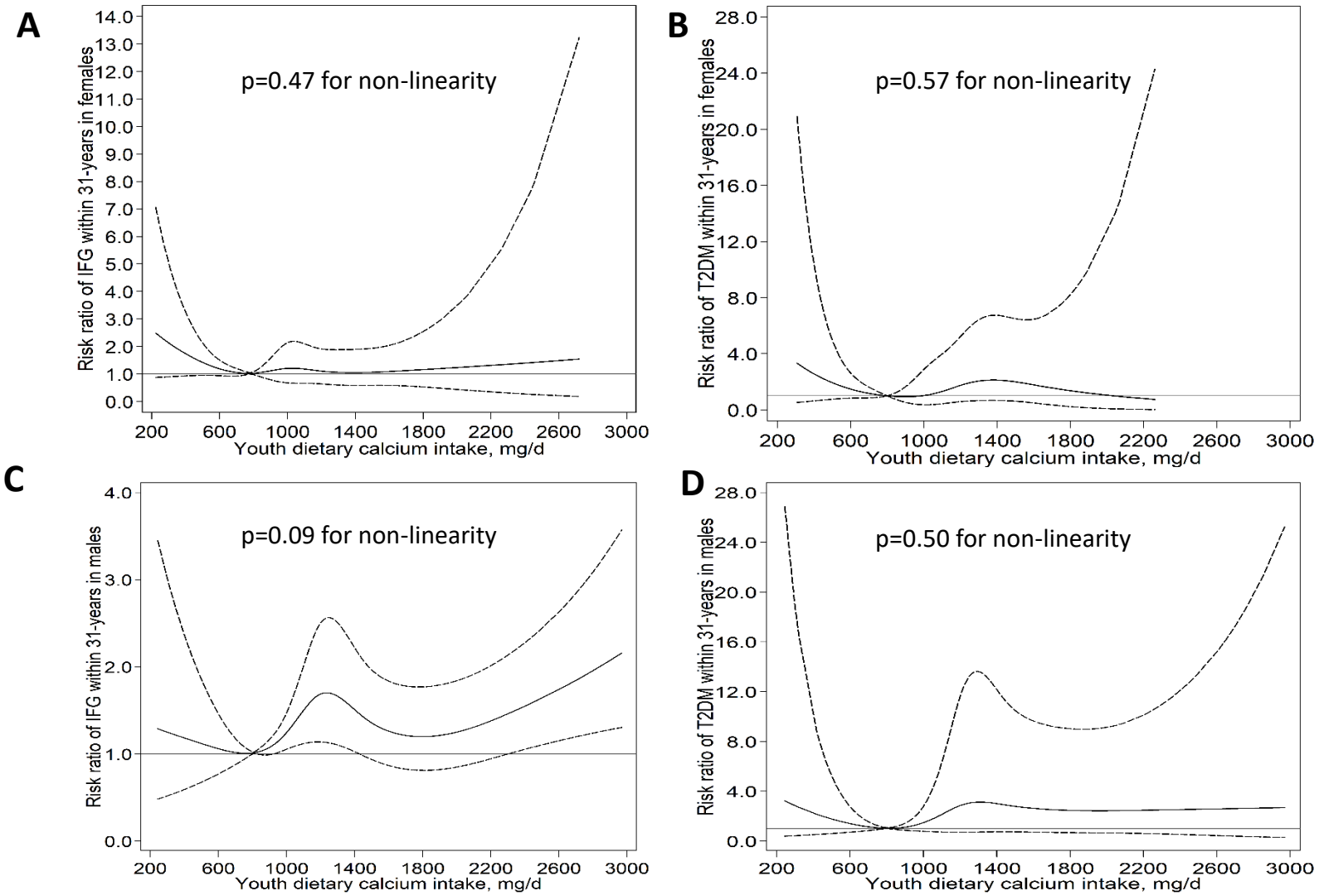
Bold denotes statistical significance, $p<0.05$.

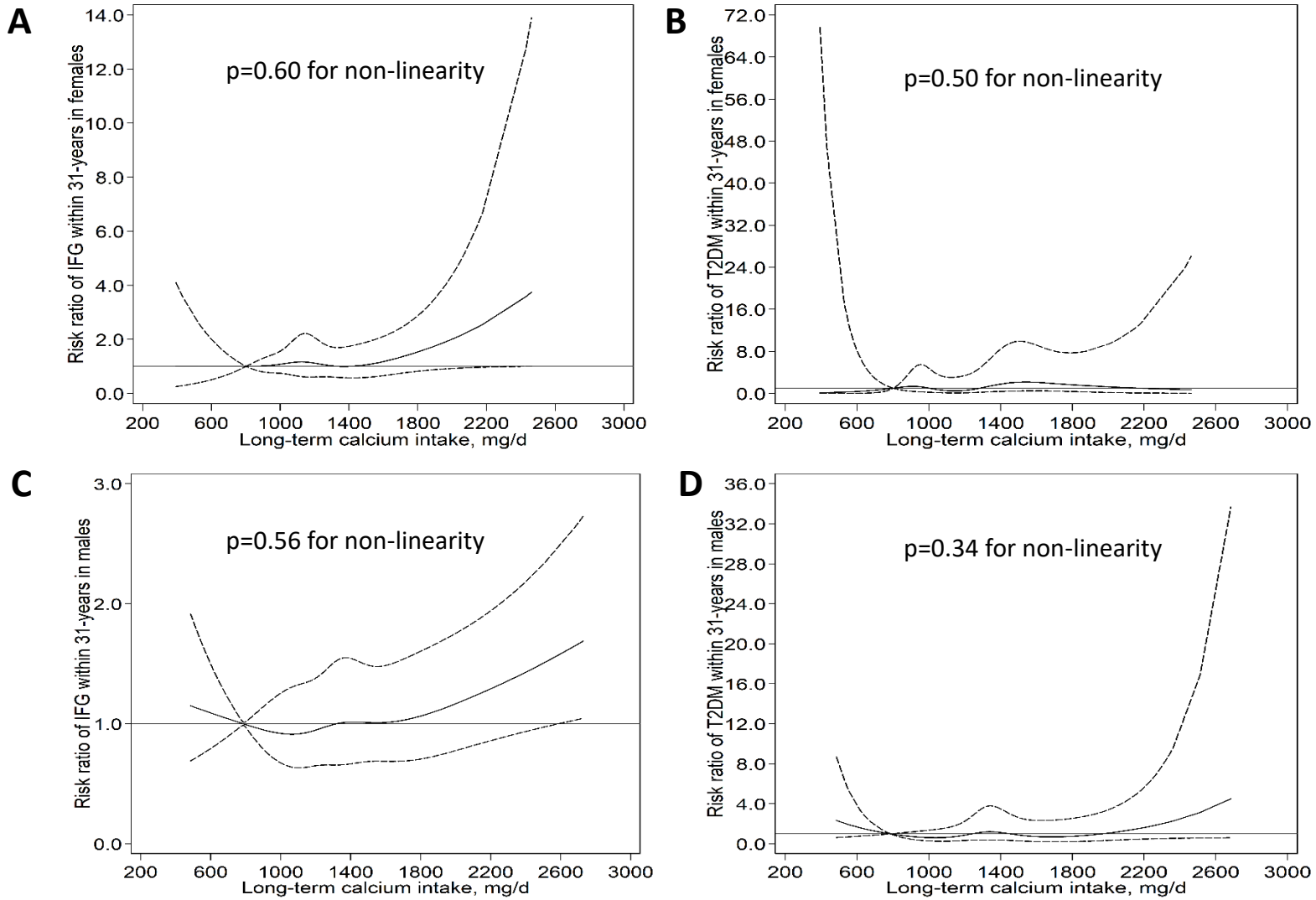
Model 1, unadjusted; Model 2, adjusted for age and childhood and adulthood body mass index; Model 3, model 2 + baseline serum 25OHD levels, parental history of diabetes, fruit and vegetable consumption, physical activity, smoking, and socioeconomic status (parental education years).

Figure Legend

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

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


A
p=0.47 for non-linearity
Risk ratio of IFG within 31-years in females
Youth dietary calcium intake, mg/d
B
p=0.57 for non-linearity
Risk ratio of T2DM within 31-years in females
Youth dietary calcium intake, mg/d
C
p=0.09 for non-linearity
Risk ratio of IFG within 31-years in males
Youth dietary calcium intake, mg/d
D
p=0.50 for non-linearity
Risk ratio of T2DM within 31-years in males
Youth dietary calcium intake, mg/d


A
p=0.60 for non-linearity
Risk ratio of IFG within 31-years in females
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
200
600
1000
1400
1800
2200
2600
3000
Long-term calcium intake, mg/d
B
p=0.50 for non-linearity
Risk ratio of T2DM within 31-years in females
0.0
8.0
16.0
24.0
32.0
40.0
48.0
56.0
64.0
72.0
200
600
1000
1400
1800
2200
2600
3000
Long-term calcium intake, mg/d
C
p=0.56 for non-linearity
Risk ratio of IFG within 31-years in males
0.0
1.0
2.0
3.0
200
600
1000
1400
1800
2200
2600
3000
Long-term calcium intake, mg/d
D
p=0.34 for non-linearity
Risk ratio of T2DM within 31-years in males
0.0
4.0
8.0
12.0
16.0
20.0
24.0
28.0
32.0
36.0
200
600
1000
1400
1800
2200
2600
3000
Long-term calcium intake, mg/d

Supplemental Table 1 Characteristics of participants by sex in the STRIP

	Females (n=216)	Males (n=204)
BMI (kg/m^2)		
2 years old	16.4 (1.4)	16.5 (1.3)
13 years old	19.6 (3.3)	19.1 (3.0)
20 years old	22.8 (4.2)	23.0 (3.6)
Average calcium intake (mg/d)		
Childhood (2-12 years old)	**1003 (186)**	**1067 (174)**
Adolescence (13-18 years old)	**1106 (324)**	**1349 (359)**
Long-term (2-20 years old)	**1037 (208)**	**1177 (217)**
Average consumption of fruits, 2-20 years old (g/d)	**188 (76)**	**173 (80)**
Average consumption of vegetables, 2-20 years old (g/d)	**182 (45)**	**203 (55)**
Current smoking (yes)	25 (21)	27 (19)
Physical activity (MET/week)	23 (21)	26 (25)
Parental diabetes (yes), n (%)	23 (11)	24 (12)
Parental education, n (%) [a]	133 (72)	118 (70)
IFG, n (%)	9 (4.2)	10 (4.9)
Fasting glucose (mmol/L)	**4.76 (0.42)**	**4.93 (0.42)**
HOMA-IR	1.59 (1.02)	1.68 (2.08)
Fasting insulin level (mIU/L)	7.38 (4.24)	7.37 (6.22)

BMI, body mass index; MET, metabolic equivalent; IFG, impaired fasting glucose.
n=263 for current smoking; 354 for parent education.
Values are mean (standard deviation) unless otherwise stated.
[a] at least one of the parents had bachelor's degree or higher education (master or PhD).
Bold denotes significant difference between females and males, $p<0.05$.

Supplemental Table 2 Associations of childhood (2-12 years old) dietary calcium intake with IFG, insulin resistance and insulin levels in adult females and males in the STRIP

	Outcomes	Females		Males	
		n	RR (95% CI)[a]	n	RR (95% CI)[a]
Model 1	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.63 (0.30, 1.31)	10	0.93 (0.54, 1.60)
Model 2	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.55 (0.29, 1.05)	10	0.99 (0.51, 1.92)
Model 3	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.69 (0.41, 1.14)	10	0.99 (0.53, 1.87)
Model 1	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.15 (0.85, 1.54)	49	1.00 (0.76, 1.32)
Model 2	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.07 (0.79, 1.44)	49	1.07 (0.80, 1.42)
Model 3	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.12 (0.85, 1.47)	49	1.12 (0.86, 1.47)
Model 1	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.16 (0.87, 1.55)	48	0.95 (0.71, 1.26)
Model 2	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.05 (0.79, 1.41)	48	1.01 (0.75, 1.37)
Model 3	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.07 (0.81, 1.42)	48	1.07 (0.80, 1.42)

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

[a] relative risk for every standard deviation (186 mg/d for females and 174 mg/d for males) increase in calcium intake.

Model 1, unadjusted; Model 2, adjusted for body mass index at 2 and 20 years old; Model 3, model 2 + average consumptions of fruit and vegetables from 2 to 20 years old.

Supplemental Table 3 Associations of adolescent (13-18 years old) dietary calcium intake with IFG, insulin resistance and insulin levels in adult females and males in the STRIP

	Outcomes	Females		Males	
		n	RR (95% CI)[a]	n	RR (95% CI)[a]
Model 1	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.67 (0.36, 1.25)	10	0.87 (0.40, 1.89)
Model 2	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.67 (0.34, 1.33)	10	0.95 (0.50, 1.82)
Model 3	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.77 (0.37 1.57)	10	0.94 (0.46, 1.93)
Model 1	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.02 (0.77, 1.36)	49	0.85 (0.67, 1.07)
Model 2	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.16 (0.88, 1.54)	49	0.88 (0.70, 1.12)
Model 3	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.20 (0.90, 1.60)	49	0.95 (0.74, 1.23)
Model 1	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	0.95 (0.72, 1.26)	48	**0.75 (0.59, 0.94)**
Model 2	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.08 (0.82, 1.42)	48	0.80 (0.63, 1.01)
Model 3	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.11 (0.84, 1.46)	48	0.85 (0.65, 1.10)

Abbreviations: RR, relative risk; CI, confidence interval; NFG, normal fasting glucose; IFG, impaired fasting glucose (cut-off 5.6 mmol/L).
Bold denotes statistical significance, $p<0.05$.
[a] relative risk for every standard deviation (324 mg/d for females and 359 mg/d for males) increase in calcium intake.
Model 1, unadjusted; Model 2, adjusted for body mass index at 2 and 20 years old; Model 3, model 2 + average consumptions of fruit and vegetables from 2 to 20 years old.

Supplemental Table 4 Association of long-term (2-20 years old) dietary calcium intake with IFG, insulin resistance and insulin levels in adult females and males in the STRIP

	Outcomes	Females		Males	
		n	RR (95% CI)[a]	n	RR (95% CI)[a]
Model 1	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.62 (0.30, 1.27)	10	0.83 (0.44, 1.58)
Model 2	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.58 (0.28, 1.20)	10	0.93 (0.4249 1.78)
Model 3	NFG	207	1.00 (Ref)	194	1.00 (Ref)
	IFG	9	0.70 (0.42, 1.17)	10	0.93 (0.50, 1.73)
Model 1	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.09 (0.82, 1.46)	49	0.90 (0.70, 1.16)
Model 2	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.09 (0.80, 1.46)	49	0.99 (0.78, 1.26)
Model 3	Low HOMA-IR	165	1.00 (Ref)	155	1.00 (Ref)
	High HOMA-IR	51	1.15 (0.86, 1.53)	49	1.07 (0.84, 1.37)
Model 1	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.07 (0.80, 1.41)	48	0.80 (0.62, 1.04)
Model 2	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.05 (0.78, 1.40)	48	0.89 (0.69, 1.14)
Model 3	Low insulin level	164	1.00 (Ref)	156	1.00 (Ref)
	High insulin level	52	1.08 (0.80, 1.46)	48	0.96 (0.74, 1.27)

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

[a] relative risk for every standard deviation (208 mg/d for females and 217 mg/d for males) increase in calcium intake.

Model 1, unadjusted; Model 2, adjusted for body mass index at 2 and 20 years old; Model 3, model 2 + average consumptions of fruit and vegetables from 2 to 20 years old.