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

Feitong Wu, PhD[1], Markus Juonala, MD, PhD[2,3,4], Katja Pahkala, PhD[5,6], Marie-Jeanne Buscot, PhD[1], Matthew A. Sabin, MD, PhD[7], Niina Pitkänen, PhD[5], Tapani Rönnemaa, MD, PhD[2], Antti Jula, MD, PhD[8], Terho Lehtimäki, MD, PhD[9], Nina Hutri-Kähönen, MD, PhD[10], Mika Kähönen, MD, PhD[11], Tomi Laitinen, MD, PhD[12], Jorma S.A.Viikari, MD, PhD[2], Olli T. Raitakari, MD, PhD[5,13*], Costan G. Magnussen, PhD[1, 5*]

[1] Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia.
[2] Department of Medicine, University of Turku, Turku, Finland.
[3]Division of Medicine, Turku University Hospital, Turku, Finland.
[4]Murdoch Children's Research Institute, Parkville, Victoria, Australia.
[5] Research Centre of Applied and Preventive Cardiovascular Medicine; University of Turku, Turku, Finland.
[6]Paavo Nurmi Centre, Sports & Exercise Medicine Unit, Department of Physical Activity and Health, University of Turku, Turku, Finland.
[7]Murdoch Children's Research Institute, Royal Children's Hospital, and Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia.
[8]National Institute for Health and Welfare, Turku, Finland.
[9] Department of Clinical Chemistry, Fimlab Laboratories and Faculty of Medicine and Health Technology, Finnish Cardiovascular Research Center - Tampere, Tampere University, Tampere, Finland.
[10] Department of Pediatrics, University of Tampere and Tampere University Hospital, Tampere, Finland.
[11] Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland.
[12] Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital and University of Eastern Finland, Kuopio, Finland.
[13]Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland.
[*]**These authors contributed equally to this work.**

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

Costan G. Magnussen, PhD

Menzies Institute for Medical Research

University of Tasmania

Private Bag 23, Hobart, 7000 Tasmania, Australia.

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

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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).

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

Main outcome measures Adult impaired fasting glucose (IFG) 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). Higher youth and long-term dietary calcium intake was not associated with the risk of IFG or T2D among females or males after adjustment for confounders including youth and adult BMI.

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) and T2D in a study among Finns with a generally high calcium intake.

Methods

Participants

Participants were from the prospective Cardiovascular Risk in Young Finns Study (YFS), which 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.

Statistical analysis

Mean (standard deviation) and number (%) were used, as appropriate, to describe variables. We compared baseline characteristics between participants who participated the baseline dietary recall interview and those who did not, and between participants with complete data and those lost to follow-up (or with incomplete baseline characteristics). Univariable and multivariable modified Poisson regression models (using a robust error variance)(15) were used to estimate the relative risk (RR) and 95% confidence intervals (CI) for youth and long-term dietary calcium intake and the risk of adult IFG and T2D. 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. The association of tertiles of long-term dietary calcium intake with the risk of adult IFG and T2D was further examined using above mentioned method. We used restricted cubic splines to examine the potential non-linear associations between calcium intake and outcomes(16). 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. 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).

Discussion

Using data from a cohort 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 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 higher dietary calcium intake might not confer an increased risk of developing impaired glucose metabolism or T2D in a population with calcium intake much higher than the recommended level (but lower than the tolerable upper intake level).

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(18). In line, recent epidemiological studies have found a positive association between increased serum calcium levels and the risk of T2D in adults(5,19-22). The conflicting evidence may be due to the differences in serum calcium levels of the studied population as the association between serum calcium concentration and the risk of T2D may differ by calcium levels(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(23). 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(24,25). 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(25). 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(24). 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(3,26). 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. Moreover, a 6-month small RCT (n=95) showed that daily supplementation of calcium (1,200 mg calcium carbonate) in combination with vitamin D (2,000-6,000 IU/d cholecalciferol) improved insulin sensitivity in middle-aged adults with prediabetes and low vitamin D status(27). However, future research is needed to clarify whether this benefit is due to calcium or vitamin D.

Methodological considerations and limitations

The strength of this study is the analysis using data from a cohort with long-term follow-up in a population-based sample, enabling the examination of childhood factors with adult health outcomes. However, this study has limitations. 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). 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. While the statistical power for IFG appears to be sufficient, studies of similar settings but larger sample size are needed to confirm our findings about T2D before any potential risk of high calcium intake could be ruled out. Although no T2D patients were reported at baseline, we could not determine baseline status of IFG because fasting glucose levels were not measured. Nevertheless, the rate of IFG at baseline is likely very low because

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

Conclusions and policy implications

In conclusion, dietary calcium intake in youth and between youth and adulthood is not associated with the risk of IFG or T2D in adulthood in a population with calcium intake much higher than the recommended level (but lower than the tolerable upper intake levels). 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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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.

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 (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).