

# Interaction between genetic predisposition to obesity and dietary calcium in relation to subsequent change in body weight and waist circumference<sup>1–4</sup>

Sofus C Larsen, Lars Ångquist, Tarunveer Singh Ahluwalia, Tea Skaaby, Nina Roswall, Anne Tjønneland, Jytte Halkjær, Kim Overvad, Oluf Pedersen, Torben Hansen, Allan Linneberg, Lise Lotte N Husemoen, Ulla Toft, Berit L Heitmann, and Thorkild IA Sørensen

## ABSTRACT

**Background:** Studies indicate an effect of dietary calcium on change in body weight (BW) and waist circumference (WC), but the results are inconsistent. Furthermore, a relation could depend on genetic predisposition to obesity.

**Objective:** The objective was to examine whether genetic predisposition to higher body mass index (BMI), WC, or waist-hip ratio (WHR) interacts with dietary calcium in relation to subsequent annual change in BW ( $\Delta$ BW) and WC ( $\Delta$ WC).

**Design:** The study is based on 7569 individuals from the MONItoring trends and determinants of CARdiovascular disease Study, a sample from the Danish Diet, Cancer and Health Study and the INTER99 study, with information on diet; 54 single-nucleotide polymorphisms (SNPs) associated with BMI, WC, or WHR adjusted for BMI; and potential confounders. The SNPs were combined in 4 scores as indicators of genetic predisposition; all SNPs in a general score and a score for each of 3 phenotype: BMI, WC, and WHR. Linear regression was used to examine the association between calcium intake and  $\Delta$ BW or  $\Delta$ WC adjusted for concurrent  $\Delta$ BW. SNP score  $\times$  calcium interactions were examined by adding product terms to the models.

**Results:** We found a significant  $\Delta$ BW of  $-0.076$  kg ( $P = 0.021$ ; 95% CI:  $-0.140, -0.012$ ) per 1000 mg Ca. No significant association was observed between dietary calcium and  $\Delta$ WC. In the analyses with  $\Delta$ BW as outcome, we found no significant interactions between the developed predisposition scores and calcium. However, we found a significant interaction between a score of 6 WC-associated SNPs and calcium in relation to  $\Delta$ WC. Each risk allele was associated with a  $\Delta$ WC of  $-0.043$  cm ( $P = 0.038$ ; 95% CI:  $-0.083, -0.002$ ) per 1000 mg Ca.

**Conclusions:** Our study suggests that dietary calcium relates weakly to BW loss. We found no evidence of a general association between calcium and  $\Delta$ WC, but calcium may reduce WC among people genetically predisposed to a high WC. However, further replication of this finding is needed. *Am J Clin Nutr* doi: 10.3945/ajcn.113.076596.

## INTRODUCTION

The relation between dietary calcium and weight loss has been the subject of increased interest in recent years, and some studies suggest that a low intake of calcium is related to obesity. In this context, results from observational studies suggest an inverse relation

between dietary calcium and body weight (BW)<sup>5</sup> as well as body fat (1). These results have been confirmed in some randomized intervention studies in which the findings indicate that an adequate calcium intake could help achieve BW and fat loss in obese people (2, 3), possibly by increasing fecal fat excretion (4).

Although these results indicate some effect of calcium on change in BW and body composition, the effect estimates are generally small (5). It is possible that these relatively modest findings may be results of interaction with genes causing genetic subgroups in a population to respond differently to the same dietary factors. In this context, there is some evidence that the obesity-promoting influence of a high-fat diet may depend on genetic predisposition to obesity (6).

In continuation of this, genome-wide association studies (GWAS) have helped to identify several common genetic variants

<sup>1</sup> From the Institute of Preventive Medicine, Bispebjerg and Frederiksberg Hospitals, the Capital Region, Copenhagen, Denmark (SCL, LÅ, BLH, and TIAS); the Novo Nordisk Foundation Center for Basic Metabolic Research, Section on Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark (TSA, OP, TH, and TIAS); the Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark (TS, AL, LLNH, UT, and BLH); the Danish Cancer Society Research Center, Copenhagen, Denmark (NR, AT, and JH); the Section of Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark (KO); the Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark (KO); the National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark (BLH); and the Boden Institute of Obesity, Nutrition, Exercise and Eating Disorders, The University of Sydney, Sydney, Australia (BLH).

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<sup>4</sup> Address correspondence to SC Larsen, Institute of Preventive Medicine, Bispebjerg and Frederiksberg Hospitals, the Capital Region, Copenhagen, Denmark. E-mail: sofus.christian.larsen@regionh.dk.

<sup>5</sup> Abbreviations used: BW, body weight; DCH, Diet, Cancer and Health Study; FFQ, food-frequency questionnaire; GWAS, genome-wide association studies; MONICA, MONItoring trends and determinants of CARdiovascular disease; SNP, single-nucleotide polymorphism; WC, waist circumference; WHR, waist-hip ratio;  $\Delta$ BW, annual change in body weight;  $\Delta$ WC, annual change in waist circumference.

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associated with BMI, waist circumference (WC), or waist-hip ratio (WHR) adjusted for BMI (7–21). It is possible that some of these genetic variants interact with dietary calcium, causing calcium to primarily play a role in BW regulation among those with a genetic predisposition to obesity.

Given this background, the aim of our study was to examine whether genetic predisposition to higher BMI, WC, or WHR in adulthood interacts with dietary intake of calcium in relation to subsequent changes in BW ( $\Delta$ BW; kg/y) and WC ( $\Delta$ WC; cm/y) over a period of 5 y.

## SUBJECTS AND METHODS

The current study is based on data from 3 cohorts, with no possibility of participant overlap. The participants had information on dietary calcium, subsequent changes in anthropometric measurements, and single-nucleotide polymorphisms (SNPs) associated with BMI, WC, or WHR adjusted for BMI as well as information on potential confounders.

### The cohorts

#### MONICA

MONICA (MONItoring trends and determinants of CARdiovascular disease) included a random subset of 4581 men and women born in 1922, 1932, 1942, and 1952 selected from among residents of 11 surrounding municipalities in the former Copenhagen County. Of these, 3608 participated in a health examination during 1982–1983, which included measurement of BW, height, dietary intake, and blood sampling. Five years later during 1987–1988, a second invitation was sent to all living participants; 2987 participated in both the first and the second health examinations (22). A total of 1852 participants completed a 7-d food record in 1982–1983 (23): 1578 had complete information on covariates and repeated measures of BW and 1426 had information on genetic variants. For this study, we further excluded participants with prevalent cancer ( $n = 16$ ), cardiovascular disease ( $n = 61$ ), or self-reported diabetes ( $n = 20$ ). Hence, the final study population consisted of 1329 participants.

#### The Diet Cancer and Health Study

During 1993–1997, a total of 160,725 Danish men and women living in Copenhagen and Aarhus were invited to participate. The criteria for invitation were age between 50 and 64 y, born in Denmark, and no diagnosis of cancer registered in the Danish Cancer Registry. A total of 57,053 (35%) accepted the invitation. All participants filled in a lifestyle questionnaire and a 192 item semiquantitative food-frequency questionnaire (FFQ) to assess the average intake of foods over the past 12 mo (24). The follow-up survey took place from 1999 to 2002 and included self-administered questionnaires about diet, lifestyle, and anthropometric measures. Data used in the current study are based on 2 samples: 1200 BW gainers and 1209 from a random sample. Additional inclusion criteria were as follows: age  $<60$  y at baseline and  $<65$  y at follow-up; no cancer, cardiovascular disease, or diabetes at baseline and follow-up; stable smoking habits; and an annual BW gain of  $\leq 5$  kg/y. BW gainers were defined as those individuals who experienced the greatest degree of annual BW gain during follow-up. They were identified by using the residuals from a regression model of annual BW

change on baseline values of age, BW, and height; smoking status (current, former, or never-smokers); and follow-up time. A total of 600 male and 600 female BW gainers were selected. In addition, a random subcohort of similar size was sampled based on the total eligible cohort; the overlap of BW gainers with this subcohort was small ( $n = 79$ ); hence, the random sample ( $n = 1130$ ) nearly equaled the number of BW gainers (25). Of these 2330 individuals, we had information on genes, dietary intake,  $\Delta$ BW, and potential confounders on 2167 individuals (2128 in the analysis of  $\Delta$ WC). However, 278 of the 2330 had information only on *FTO* (rs9939609).

#### INTER99

INTER99 is a population-based randomized controlled trial (CT00289237, clinicaltrials.gov) that was initiated in 1999. An age- and sex-stratified random sample of 13,016 men and women born in 1939–1940, 1944–1945, 1949–1950, 1954–1955, 1959–1960, 1964–1965, and 1969–1970 living in 11 municipalities in the former Copenhagen County was drawn from the Civil Registration System and invited for a health examination. A total of 12,934 persons were eligible for invitation, 6784 of whom participated. The health examination included a self-administered questionnaire, a physical examination, and various blood tests. Dietary intake was assessed through a validated FFQ (26). The study design and methods were described in detail elsewhere (27, 28). After 5 y (2004), the baseline examination program was repeated, and all participants from the baseline examination were re-invited (29). We had information on diet, genes, and baseline and follow-up anthropometric measures as well as information on potential confounders for a total of 4574 participants. For the current study, we further excluded participants with prevalent cancer ( $n = 87$ ), cardiovascular disease ( $n = 320$ ), or self-reported diabetes ( $n = 94$ ); we ended up with 4073 participants (3536 participants in the analysis of  $\Delta$ WC). All procedures in the 3 studies were conducted in accordance with the Helsinki Declaration, and all participants provided written informed consent.

#### Anthropometric measurements

In MONICA and INTER99, height was measured to the nearest 0.5 cm and BW to the nearest 0.1 kg. We lacked measures of WC on enough of the participants to include this measure in the analysis of the MONICA participants. In INTER99, WC was measured horizontally midway between the lower rib margin and the iliac crest to the nearest 1 cm. A similar procedure was used in the Diet, Cancer and Health Study (DCH) for the baseline measures during 1993–1997. Regarding the follow-up measures, during 1999–2002, participants in the DCH received a self-administered questionnaire and reported their BW (kg) and WC (cm) measured at the level of the umbilicus by using an enclosed paper measuring tape. A validation study was performed on 408 participants to compare measures of WC obtained by technicians and by self-report, which showed that the self-reported WC at the level of the umbilicus was highly correlated with the technician-measured WC at the natural WC. Spearman's correlation coefficient was 0.87 in men and 0.88 in women (30).

On the basis of these measures, we calculated changes in BW and WC as the difference between measures during 1987–1988 and 1993–1994 for MONICA, during 1993–1997 and 1999–2002

for DCH, and during 1999–2001 and 2004–2006 for INTER99. From this we calculated  $\Delta BW$  and  $\Delta WC$  in each cohort by dividing the derived differences with the individual follow-up time in years.

### Assessment of dietary intake

During 1982–1983, all MONICA participants were given thorough verbal and written instruction on how to complete a 7-d food record within a 3-wk period. Information on average household weights of 19 frequently consumed foods (eg, weight of a slice of bread, glass of milk) was provided. The entries were in grams, estimated as accurately as possible or preferably weighed (23).

DCH participants completed an FFQ during 1993–1997, and INTER99 participants completed a nearly identical questionnaire during 1999–2001. The FFQs consisted of 192/198 items for which the participants were asked about their average intake of different foods and beverages. Articles on the development and validation of these questionnaires were published previously (26,31,32).

Daily intakes of foods and nutrients were calculated for each participant by means of the software program DANKOST (33) in MONICA and FoodCalc (34) in DCH and INTER99. Both DANKOST and FoodCalc are based on the official Danish food-composition tables (<http://www.foodcomp.dk>). From this information, we calculated the participants' daily intake of calcium and their total daily energy intake. Both dietary calcium and total energy intakes were included in the analysis as continuous variables (mg/d and MJ/d, respectively).

### Questionnaire data

All participants reported whether they had never smoked, were ex-smokers, or were current smokers. Likewise, information was gathered about consumption of alcohol in all 3 cohorts. Alcohol was included in the analysis as a continuous variable (MJ/d). Information on physical activity and other lifestyle factors was obtained from questionnaires in all 3 cohorts. In MONICA, all participants were asked to classify themselves into 1 of 4 groups: 1) almost completely inactive: sedentary activities such as reading, watching television, and going to the movies; 2) some physical activity:  $\geq 4$  h/wk, including, for example, walking, cycling, construction work, bowling, and table tennis; 3) regular hard activity  $\geq 3$  h/wk, including, for example, swimming, tennis, badminton, or heavy gardening; and 4) hard activity: elite sports such as swimming, soccer, badminton, or long-distance running several times a week. In the DCH, the questionnaire was used to obtain information on duration and types of physical activity. From this information, the validated Cambridge Physical Activity Index was calculated by combining occupational physical activity with time spent on cycling and sports in summer and winter (35). Participants were then divided into 4 physical activity categories (inactive, moderately inactive, moderately active, or active). In INTER99, information on physical activity was based on 2 questions on commuting physical activity and leisure-time physical activity. From these 2 questions, overall physical activity was calculated by summing responses on commuting physical activity (converted into min/wk by using a 5-d working week) and a leisure-time physical activity

variable (converted into min/wk) (29). From this variable, overall physical activity was grouped into 4 categories  $<2$ , 2–3.9, 4–6.9, and  $\geq 7$  h/wk. Education was assessed with questions about years of regular schooling in all 3 cohorts and classified with respect to having a school education above or below primary school (7 y). Finally, we included information on the participants' age and sex, and the women reported whether they had entered menopause.

### SNP selection and genotyping

Through a review of GWAS, we found 63 SNPs that were consistently associated with BMI, WC, or WHR (7–21). In the current study, we included SNPs that were available in all 3 cohorts. Hence, we ended up with a total of 54 SNPs (*see* Supplemental Table 1 under “Supplemental data” in the online issue). In the MONICA cohort, the SNPs were genotyped with the KASPar SNP Genotyping method (KBioscience). They had an average genotyping success rate of 98.3% (minimum: 95.8%).

Likewise, in the DCH, all the included SNPs were genotyped with the KASPar SNP Genotyping method. They had an average genotyping success rate of 97.8%; 185 replicate samples had a success rate  $>98\%$  and an error rate  $<0.5\%$ . For the INTER99 study, all the SNPs were successfully genotyped by using either the KASPar method (52 SNPs), or through Human Cardio-metabo bead chip array (2 SNPs; rs7138803 and rs7647305) with the use of Illumina Hi-Scan technology and GenomeStudio software (<http://www.illumina.com/systems/hiscan.ilmn>). The average genotyping success rate for the INTER99 study was 96.7% (minimum: 94.7%).

### Genetic predisposition scores

For each individual, the 54 SNPs were coded as 0, 1, or 2 according to the number of obesity-associated risk alleles. Genetic predisposition to obesity was illustrated through 4 SNP scores. In addition to a score consisting of all 54 SNPs (MONICA,  $n = 842$ ; DCH,  $n = 1194$ ; INTER99,  $n = 1973$ ), we developed 3 phenotype-specific scores to reduce random variation, 1 by adding up the risk alleles of 33 BMI-associated SNPs (MONICA,  $n = 989$ ; DCH,  $n = 1438$ ; INTER99,  $n = 2511$ ), 1 by adding up the risk alleles of 6 WC-associated SNPs (MONICA,  $n = 1250$ ; DCH,  $n = 1805$ ; INTER99,  $n = 3381$ ), and 1 by adding up risk alleles of 18 WHR-associated SNPs (MONICA,  $n = 1133$ ; DCH,  $n = 1547$ ; INTER99,  $n = 3086$ ) (*see* Supplemental Table 1 under “Supplemental data” in the online issue). Higher scores indicated higher genetic predisposition to these specific traits.

### Statistical analyses

Linear regression was used to examine the association between dietary calcium and subsequent  $\Delta BW$  and  $\Delta WC$  with adjustment for baseline measures of outcome, age, sex, height, smoking status, education level, physical activity, menopausal status, total energy intake, and alcohol consumption. To assess associations independent of  $\Delta BW$ , the analysis with  $\Delta WC$  as outcome was further adjusted for concurrent  $\Delta BW$ . The same procedure was used in models with the 4 SNP scores as exposures.

Furthermore, to examine whether genetic predisposition modifies the association between calcium consumption and  $\Delta$ BW or  $\Delta$ WC, the statistical interaction was examined by correspondingly adding an SNP score variable as well as an SNP score  $\times$  calcium product term to the model.

To combine results from the individual cohorts, we performed both fixed- and random-effects meta-analyses. The effect-estimates from the individual cohorts were weighted by the inverses of their variances. Heterogeneity between the studies was assessed by  $Q$  test and  $I^2$  values.  $I^2$  indicates the amount of total variation explained by between-study variation (36).  $I^2$  values were evaluated according to the following categories: no heterogeneity,  $I^2 = 0$ –25%; moderate heterogeneity,  $I^2 = 25$ –50%; significant heterogeneity,  $I^2 = 50$ –75%; and extreme heterogeneity,  $I^2 = 75$ –100% (37). Because the  $Q$  tests showed no significant heterogeneity and the calculated  $I^2$  values were all  $<25\%$ , we only presented results from the fixed-effect models. Furthermore, results from the random-effects models were almost identical to those from the fixed-effect models.

As a final point, significant interactions were further assessed by calculating the association between calcium and outcome of interest in strata of SNP scores. In addition to the analyses with

SNP scores, for exploratory purposes, single-SNP interaction analyses were also conducted with respect to the 54 individual SNPs, with adjustment for multiple testing by using the Bonferroni method. In the analysis using SNP scores,  $P$  values  $\leq 0.05$  were regarded as statistically significant. All analyses were performed by using the statistical software package Stata 12 (StataCorp LP; www.stata.com).

## RESULTS

### Characteristics of the study population

For the current study, we had information on 7569 individuals, 1329 of whom were from MONICA, 2167 were from the DCH, and 4073 were from the INTER99 cohort. Anthropometric measures, calcium intake, SNP scores, and covariates in MONICA, DCH, and INTER99 are shown in **Table 1**. The table shows the highest baseline median calcium consumption among the MONICA participants (1148 mg/d; range: 573–2118 mg/d) and the lowest among the INTER99 participants (796 mg/d; range: 314–1619 mg/d). In relation to measures of BW and WC,

**TABLE 1**

Information on calcium intake, anthropometric measures, genetic predisposition scores, and covariates in MONICA, DCH, and INTER99<sup>1</sup>

	MONICA ( <i>n</i> = 1329)	DCH ( <i>n</i> = 2167)	INTER99 ( <i>n</i> = 4073)
<b>Basic variables</b>			
Calcium (mg/d)	1148 (573–2118) <sup>2</sup>	1006 (4837–1953)	796 (314–1619)
Age at baseline (y)	50.5 (30.6–61.1)	53.0 (50.0–58.0)	45.1 (34.7–59.8)
Sex, female (%)	51.6	49.4	51.4
Height (cm)	169 (156–184)	171 (158–186)	172 (158–188)
<b>BW (kg)</b>			
Baseline	69.0 (52.0–93.0)	77.1 (56.8–104.4)	75.5 (55–104)
Follow-up	70.0 (51.6–94.7)	82.0 (58.0–110.0)	76.5 (55.6–105.0)
$\Delta$ BW	0.22 (–1.04–1.61)	1.01 (–1.05–2.39)	0.20 (–1.41–1.75)
<b>WC (cm)<sup>3</sup></b>			
Baseline	—	90 (70–112)	85 (67–107)
Follow-up	—	98 (76–121)	88 (69–110)
$\Delta$ WC	—	1.37 (–0.78–4.44)	0.55 (–1.27–2.46)
<b>SNP-based variables<sup>4</sup></b>			
SNP score (BMI)	29 (23–35)	29 (23–35)	29 (23–34)
SNP score (WC)	3 (1–6)	3 (1–6)	3 (1–6)
SNP score (WHR)	18 (14–23)	18 (14–22)	18 (14–23)
SNP score (complete)	48 (41–56)	48 (41–56)	48 (41–56)
<b>Adjustment variables</b>			
Smoking, never-smokers (%)	27.0	41.4	40.1
Education, $\leq$ primary school (%)	35.7	30.1	26.2
Physical activity, most sedentary group (%)	21.4	9.5	11.5
Menopausal status, postmenopausal (%)	42.4	55.6	27.5
Total energy intake, excluding alcohol (MJ/d)	8.5 (4.8–13.9)	8.2 (4.9–13.4)	9.0 (4.9–15.1)
Alcohol (MJ/d)	0.4 (0–1.8)	0.4 (0–1.9)	0.3 (0–1.5)

<sup>1</sup> BW, body weight; DCH, Diet, Cancer and Health Study; MONICA, MONItoring trends and determinants of Cardiovascular disease; SNP, single nucleotide polymorphism; WC, waist circumference; WHR, waist-hip ratio;  $\Delta$ BW, annual BW change;  $\Delta$ WC, annual WC change.

<sup>2</sup> Medians; 5th–95th percentiles in parentheses (all such values).

<sup>3</sup> DCH: *n* = 2165 for baseline, *n* = 2130 for follow-up, and *n* = 2128 for  $\Delta$ WC. INTER99: *n* = 4067 for baseline, *n* = 3540 for follow-up, and *n* = 3536 for  $\Delta$ WC.

<sup>4</sup> Sum of BMI-, WC-, or WHR-associated risk alleles. MONICA: *n* = 989 for SNP score (BMI), *n* = 1250 for SNP score (WC), *n* = 1133 for SNP score (WHR), and *n* = 842 for SNP score (complete). DCH: *n* = 1438 for SNP score (BMI), *n* = 1805 for SNP score (WC), *n* = 1547 for SNP score (WHR), and *n* = 1194 for SNP score (complete). INTER99: *n* = 2511 for SNP score (BMI), *n* = 3381 for SNP score (WC), *n* = 3086 for SNP score (WHR), and *n* = 1973 for SNP score (complete).

both the baseline values and annual gains were highest among the DCH participants, which reflected the case-cohort design. The genetic predisposition scores were nearly identical in terms of medians and 5th–95th percentiles in all 3 cohorts. The included SNPs are presented elsewhere (*see* Supplemental Table 1 under “Supplemental data” in the online issue) along with information on which obesity traits they have been found to be associated with in GWAS.

### Dietary calcium and change in BW and WC

The association between dietary calcium on change in anthropometric measures is presented in **Table 2**. The table shows  $\Delta$ BW (kg) and  $\Delta$ WC (cm) per 1000-mg higher calcium intake in the 3 cohorts. After adjusting for potential confounders, we found no statically significant association between dietary calcium and  $\Delta$ BW or  $\Delta$ WC in any of the 3 cohorts. However, in the meta-analysis, we found a significant  $\Delta$ BW of  $-0.076$  kg ( $P = 0.021$ ; 95% CI:  $-0.140, -0.012$ ).

### Genetic predisposition scores in relation to BW and WC

We further investigated the association between the 4 SNP scores and BW, WC, and WHR at baseline (*see* Supplemental Table 2 under “Supplemental data” in the online issue) and the association of scores to subsequent annual changes in these measures (*see* Supplemental Table 3 under “Supplemental data” in the online issue). Because the 4 scores are based on SNPs identified through cross-sectional GWAS, we generally observed statistically significant associations with both BW and WC at baseline, with the exception of the WHR score. However, when we analyzed the associations between the 4 SNP scores and  $\Delta$ BW and  $\Delta$ WC, neither the crude nor the adjusted models showed any statistically significant associations.

### Interaction between genetic predisposition scores and dietary calcium

Regarding SNP score  $\times$  calcium interactions, we found no significant results in relation to  $\Delta$ BW when using the total score of all 54 adiposity-associated SNPs or when using the phenotype-specific scores (**Figure 1**). Likewise, in the analysis with  $\Delta$ WC as the outcome, we found no significant interaction when

we used the score of all 54 SNPs. However, we found a significant interaction in INTER99 using the score of 6 WC-associated SNPs. This interaction was not significant in the DCH, but the direction of the association was the same. In the meta-analysis of the 2 cohorts, we found that each additional risk allele was associated with a  $\Delta$ WC of  $-0.043$  cm ( $P = 0.038$ ; 95% CI:  $-0.083, -0.002$ ) per 1000-mg higher calcium intake (**Figure 2**). We further examined this interaction by looking at the association between calcium and  $\Delta$ WC in tertiles of the WC score. However, none of the tertiles showed a significant association between calcium and  $\Delta$ WC (**Figure 3**).

Finally, for exploratory purposes, we investigated the association between the individual SNPs and  $\Delta$ BW (*see* Supplemental Table 4 under “Supplemental data” in the online issue) and  $\Delta$ WC (*see* Supplemental Table 5 under “Supplemental data” in the online issue) as well as the interaction between the individual SNPs and dietary calcium in relation to  $\Delta$ BW (*see* Supplemental Table 6 under “Supplemental data” in the online issue) and  $\Delta$ WC (*see* Supplemental Table 7 under “Supplemental data” in the online issue). However, after we adjusted for multiple testing, none of the results were statistically significant.

## DISCUSSION

In the meta-analysis of the 3 cohorts, we found a statistically significant association between calcium and BW loss, but we found no significant association between dietary calcium and  $\Delta$ WC. In the analyses with  $\Delta$ BW as outcome, we found no statistically significant interactions between the developed SNP scores and dietary calcium. However, we found a significant interaction between a score of 6 WC-associated SNPs and dietary calcium in relation to  $\Delta$ WC. In the meta-analysis, each additional risk allele was associated with a greater WC loss with higher calcium intake.

Our study had many strengths, including detailed measures of dietary information collected either with 7-d food records or validated FFQs, genetic information on 54 SNPs selected based on their consistent association with obesity-related traits, repeated measures of BW and WC, and information on potential confounders.

However, our study also had some limitations. Although our dietary data were collected through validated methods, both

**TABLE 2**  
Annual change in body weight and waist circumference per 1000-mg higher calcium intake in the 3 cohorts<sup>1</sup>

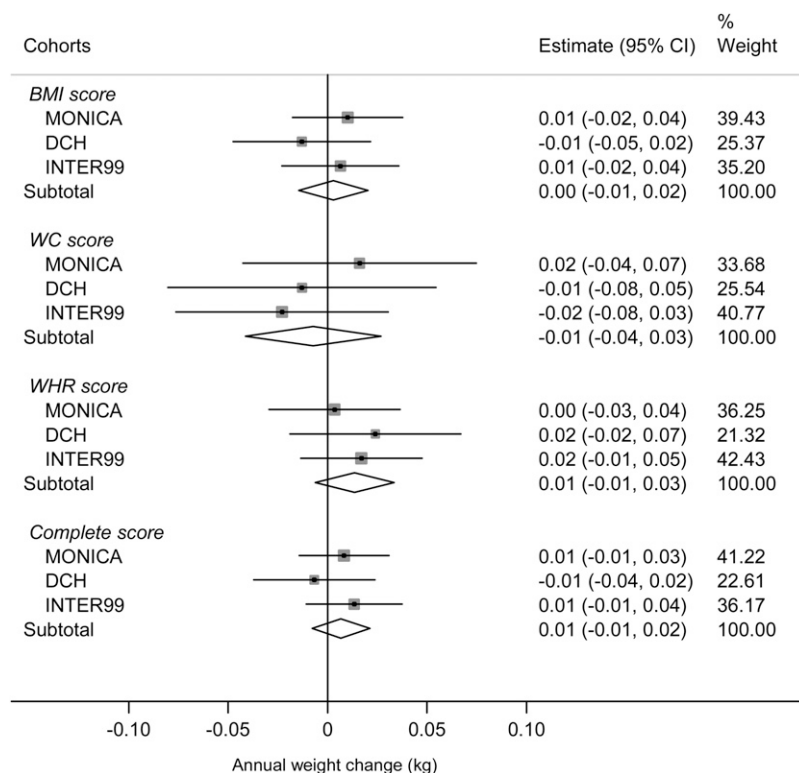
	Crude $\beta$ (95% CI) <sup>2</sup>	% Weight	Adjusted $\beta$ (95% CI) <sup>3</sup>	% Weight <sup>4</sup>
Annual change in body weight (kg)				
MONICA ( $n = 1329$ )	$-0.07$ ( $-0.162, 0.022$ )	29.05	$-0.114$ ( $-0.238, 0.009$ )	26.86
DCH ( $n = 2167$ )	$-0.104$ ( $-0.202, -0.006$ )	24.45	$-0.074$ ( $-0.201, 0.054$ )	25.08
INTER99 ( $n = 4073$ )	$0.010$ ( $-0.083, 0.063$ )	45.49	$-0.055$ ( $-0.147, 0.037$ )	48.06
Overall ( $n = 7569$ )	$-0.051$ ( $-0.082, -0.002$ )	100	$-0.076$ ( $-0.140, -0.012$ )	100
Annual change in waist (cm)				
DCH ( $n = 2128$ )	$-0.090$ ( $-0.208, 0.028$ )	37.79	$-0.105$ ( $-0.256, 0.047$ )	22.12
INTER99 ( $n = 3536$ )	$-0.014$ ( $-0.106, 0.078$ )	62.21	$0.011$ ( $-0.092, 0.070$ )	77.88
Overall ( $n = 5664$ )	$-0.043$ ( $-0.115, 0.030$ )	100	$-0.032$ ( $-0.103, 0.039$ )	100

<sup>1</sup> DCH, Diet, Cancer and Health Study; MONICA, MONItoring trends and determinants of CARdiovascular disease.

<sup>2</sup> Adjusted for baseline outcome and height.

<sup>3</sup> Adjusted for baseline outcome, height, sex, smoking status, total energy consumption, physical activity, education, and menopausal status for women. Analysis with change in waist circumference adjusted for concurrent weight change.

<sup>4</sup> The effect estimates from the individual cohorts were weighted by the inverses of their variance.



**FIGURE 1.** Interaction between genetic predisposition scores and dietary calcium in relation to change in body weight, expressed as annual weight change for each additional risk allele per 1000-mg higher calcium intake. The study-specific single nucleotide polymorphism score  $\times$  calcium interaction estimates were calculated by using standard linear regression, and the corresponding meta-analysis results were derived by using a fixed-effect approach, whereby the effect estimates from the individual cohorts were weighted by the inverses of their variances (% by weight). The results were adjusted for baseline weight, height, sex, smoking status, total energy consumption, alcohol consumption, physical activity, education, and menopausal status for women. BMI score, sum of BMI-associated risk alleles; DCH, Diet, Cancer and Health Study; MONICA, MONItoring trends and determinants of CArdiovascular disease; WC score, sum of waist circumference-associated risk alleles; WHR score, sum of waist-hip ratio-associated risk alleles.

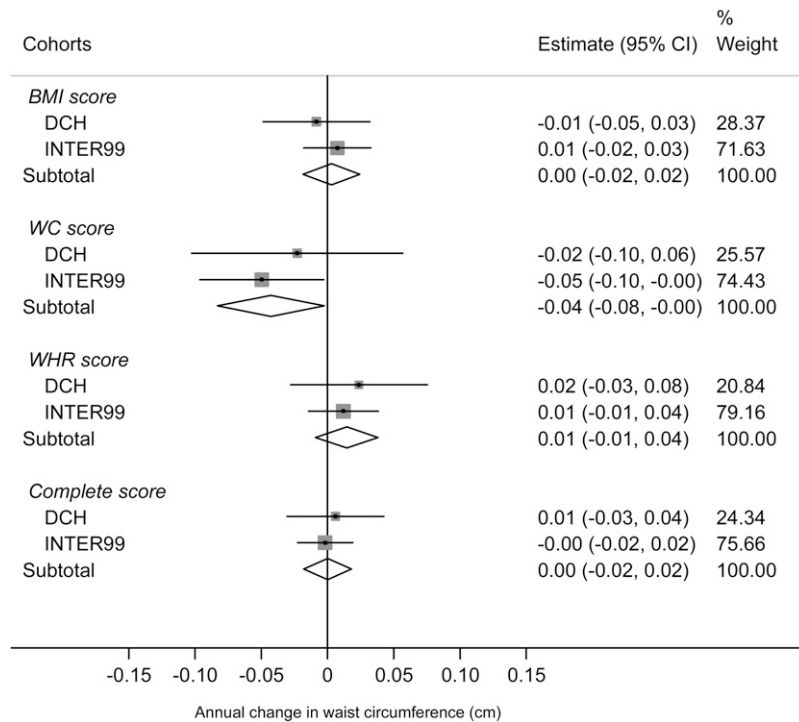
FFQs and 7-d food records are subject to some inaccuracies. Hence, measurement error related to dietary intake may have biased the results toward null. Furthermore, because we get most of our dietary calcium from dairy products we cannot exclude that other components of these products are responsible for the observed association in our study. In particular, the protein content of dairy products and their amino acid composition could have played a role in the observed BW loss (38). Moreover, studies of dietary factors and studies on genetic variants in relation to obesity have generally shown relatively weak associations. Hence, it is likely that the size of any SNP  $\times$  calcium interactions is also minor. Therefore, it is possible that a population of 7569 individuals might be too small to discover potential SNP  $\times$  calcium interactions. Consequently, it is possible that we overlooked some associations because of a lack of statistical power. However, the generally quite narrow CIs suggest that it is less likely that we overlooked any noteworthy associations. In addition, the size of the statistically significant associations observed in the current study was very modest, which indicated that we had sufficient power to measure associations so small that any public health relevance is questionable.

Moreover, when results are replicated, it is clearly important that the cohorts are similar in terms of both design and population. Hence, the lack of consistent findings in our study may have been because of heterogeneity between the 3 cohorts, both in relation to the enrolled participants and the design of the studies. In this context, the DCH participants included in this

study consisted of both a random sample and a sample of BW gainers. To get the largest possible sample size, we chose to include both groups. However, it is possible that BW gainers react differently to calcium than a random sample, which could have influenced our results. Hence, as a precaution, we also performed the analyses of BW gainers and the random sample separately and found no statistically significant difference in these results. In addition, INTER99 was a multifactorial lifestyle intervention in which the intervention group received a lifestyle counseling talk focusing on smoking, physical activity, diet, and alcohol. Thus, it is possible that the intervention affected the results in INTER99. However, as a sensitivity analysis, we adjusted for baseline intervention status, which did not change the tendencies in the reported results.

Finally, to assess interaction between calcium intake and overall genetic predisposition to obesity, we developed 4 predisposition scores based on all 54 included SNPs or the sum of BMI-, WC-, and WHR-associated SNPs. A basic assumption when doing this is consistency between the direction of the main effect of each SNP and the direction of the SNP  $\times$  calcium interaction effect. This assumption, however, may be too simplistic, and the limited findings with respect to the scores could potentially be explained by this. However, this approach has been successful when used to investigate gene  $\times$  environment interaction in relation to obesity in other studies (39,40).

The observed association between calcium and BW loss is in line with the results from several other studies, which, despite



**FIGURE 2.** Interaction between genetic predisposition scores and dietary calcium in relation to change in waist circumference, expressed as annual waist change for each additional risk allele per 1000-mg higher calcium intake. The study-specific single nucleotide polymorphism score  $\times$  calcium interaction estimates were calculated by using standard linear regression, and corresponding meta-analysis results were derived by using a fixed-effect approach, whereby the effect estimates from the individual cohorts were weighted by the inverses of their variances (% by weight). The results were adjusted for baseline measure of waist circumference, height, sex, smoking status, total energy consumption, alcohol consumption, physical activity, education, menopausal status for women, and concurrent change in body weight. BMI score, sum of BMI-associated risk alleles; DCH, Diet, Cancer and Health Study; WC score, sum of waist circumference-associated risk alleles; WHR score, sum of waist-hip ratio-associated risk alleles.

some inconsistency, indicates an inverse relation between calcium intake and BW gain. This applies to observational studies that have found a greater BW and fat loss with higher intakes of calcium or dairy product (1) and to randomized studies that have reported that calcium supplementation generates a small but statistically significant BW loss in overweight and obese individuals (5).

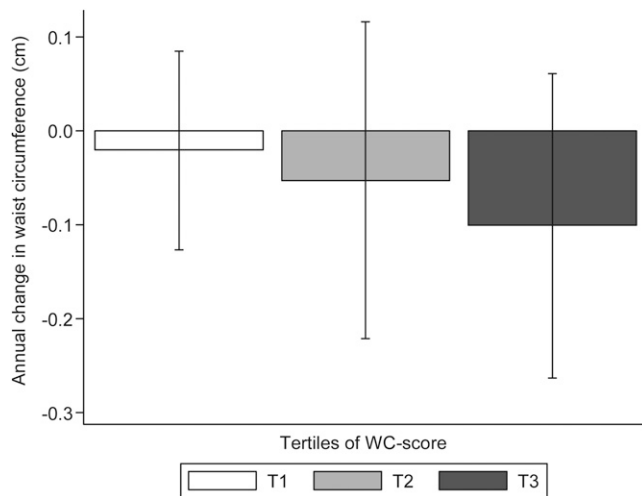
The studies addressing the effects of calcium intake and adiposity measures did not examine interactions with obesity-associated genetic variants (4, 5). Most studies that have looked at gene  $\times$  environment interactions in relation to obesity have focused on single genes. One of the most widely described is the interaction between *FTO* and physical activity in relation to obesity (41). However, in addition to this, many recently published studies have successfully included genetic predisposition to obesity through different genetic predisposition scores. Using this procedure, one study suggested that there is an interaction between a score of 32 BMI-associated loci and sugar-sweetened beverages in relation to BMI, with data from 3 different cohorts ( $n = 33,097$ ) (40), and between a score of 12 BMI-associated loci and physical activity in relation to obesity among 20,430 participants (39). In our study, we could not find an interaction between a score of 33 BMI-associated SNPs and calcium in relation to  $\Delta$ BW. However, we did find a small but statistically significant interaction between a score of 6 WC-associated SNPs and calcium in relation to subsequent WC loss. This could indicate that the sum of certain obesity-associated risk alleles is more sensitive to environmental influences than are the individual variants.

Several biological mechanisms have been proposed that may explain the observed association between calcium and  $\Delta$ BW.

Some authors suggest that calcium may bind as insoluble soap and reduce fat absorption and increase fecal fat excretion (4). Others have suggested that a high dietary calcium intake results in the suppression of 1,25-vitamin D concentrations, which in turn results in reduced intracellular calcium concentrations, which is believed to stimulate lipolysis and inhibit lipogenesis in adipocytes (5). It would be highly speculative to make suggestions about exactly what mechanisms might underlie the observed interaction between the developed score of 6 WC-associated SNPs and calcium in relation to  $\Delta$ WC, given the lack of knowledge about the mechanisms these genetic variants are involved in. Moreover, the association with  $\Delta$ WC adjusted for  $\Delta$ BW implies that greater  $\Delta$ WC must be accompanied by less change elsewhere in the body, and where the mechanisms operate therefore remains unclear.

As a final point, the participants included in this study were not necessarily representative of the general Danish population. In particular, this applies to participants from the DCH sample, half of whom were a select group of BW gainers. Thus, the generalizability of our results might be limited.

In conclusion, our study suggests a weak association between dietary calcium and subsequent BW loss. However, we found no evidence that the association between calcium and  $\Delta$ BW depends on genetic predisposition to higher BMI, WC, or WHR. Furthermore, we found no evidence of an association between dietary calcium and  $\Delta$ WC in general, but there was some evidence that a score composed of 6 WC-associated SNPs interacted with dietary calcium in relation to development in WC. This could indicate that calcium primarily relates to WC loss among people



**FIGURE 3.** Annual change in WC per 1000-mg higher calcium intake, expressed as tertiles of the WC-associated single nucleotide polymorphism score. Study-specific estimates (not presented) were calculated by using standard linear regression, and corresponding meta-analysis results were derived by using a fixed-effect approach, whereby the effect estimates from the individual cohorts were weighted by the inverses of their variances. The results were adjusted for baseline measure of WC, height, sex, smoking status, total energy consumption, alcohol consumption, physical activity, education, menopausal status for women, and concurrent change in body weight. Participants per tertile (range of risk alleles): T1,  $n = 2676$  (score: 0–3); T2,  $n = 1215$  (score: 4); and T3,  $n = 1265$  (score: 5–9). T, tertile; WC, waist circumference.

who are genetically predisposed to having a high WC. However, the association was weak and not strongly statistically significant. Hence, replication of the finding is needed before any firm conclusions can be drawn relating dietary calcium to change in WC. Finally, the associations observed in this study were generally small. Thus, any major public health relevance of these findings remains questionable.

The authors' responsibilities were as follows—TIAS, BLH, and SCL: conceived the study; SCL, LÄ, BLH, and TIAS: designed the study; SCL: wrote the manuscript, prepared the tables and figures, and conducted the statistical analyses under the supervision of TIAS, BLH, and LÄ; and TS, AL, LLNH, UT, NR, KO, AT, JH, TSA, OP, and TH: helped acquire the data and interpret the results and provided comments on the manuscript. All authors read and approved the final manuscript. None of the authors had a financial or personal conflict of interest.

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