Interaction between genetic predisposition to obesity and dietary calcium in relation to subsequent change in body weight and waist circumference

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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 (ΔBW) and WC (ΔWC).

Design: The study was 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 phenotypes: BMI, WC, and WHR. Linear regression was used to examine the association between calcium intake and ΔBW or ΔWC adjusted for concurrent ΔBW. SNP score × calcium interactions were examined by adding product terms to the models.

Results: We found a significant ΔBW 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 ΔWC. In the analyses with ΔBW 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 ΔWC. Each risk allele was associated with a ΔWC 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 ΔWC, 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 2014;99:957–65.

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) 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...
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 (ΔBW; kg/y) and WC (ΔWC; 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 CArdio-vascular 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 reported their BW (kg) and WC (cm) measured at the level of the umbilicus by using an enclosed technician-measured WC at the nearest 0.5 cm and BW to the nearest 0.1 kg. We lacked measures of WC obtained by techni-
for DCH, and during 1999–2001 and 2004–2006 for INTER99. From this we calculated ΔBW and ΔWC 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: ≥4 h/wk, including, for example, walking, cycling, construction work, bowling, and table tennis; 3) regular hard activity ≥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 ≥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 ΔBW and ΔWC 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 ΔBW, the analysis with ΔWC as outcome was further adjusted for concurrent ΔBW. 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 tests and \( I^2 \) values. \( I^2 \) values indicate 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, 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 body weight change; \( \Delta WC \), annual waist circumference change.

#### Table 1

<table>
<thead>
<tr>
<th></th>
<th>MONICA (n = 1329)</th>
<th>DCH (n = 2167)</th>
<th>INTER99 (n = 4073)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>1148 (573–2118)</td>
<td>1006 (4837–1953)</td>
<td>796 (314–1619)</td>
</tr>
<tr>
<td>Age at baseline (y)</td>
<td>50.5 (30.6–61.1)</td>
<td>53.0 (50.0–58.0)</td>
<td>45.1 (34.7–59.8)</td>
</tr>
<tr>
<td>Sex, female (%)</td>
<td>51.6</td>
<td>49.4</td>
<td>51.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 (156–184)</td>
<td>171 (158–186)</td>
<td>172 (158–188)</td>
</tr>
<tr>
<td>BW (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>69.0 (52.0–93.0)</td>
<td>77.1 (56.8–104.4)</td>
<td>75.5 (55–104)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>70.0 (51.6–94.7)</td>
<td>82.0 (58.0–110.0)</td>
<td>76.5 (55.6–105.0)</td>
</tr>
<tr>
<td>( \Delta BW )</td>
<td>0.22 (−1.04 to 1.61)</td>
<td>1.01 (−1.05 to 2.39)</td>
<td>0.20 (−1.41 to 1.75)</td>
</tr>
<tr>
<td>WC (cm)**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>—</td>
<td>90 (70–112)</td>
<td>85 (67–107)</td>
</tr>
<tr>
<td>Follow-up</td>
<td>—</td>
<td>98 (76–121)</td>
<td>88 (69–110)</td>
</tr>
<tr>
<td>( \Delta WC )</td>
<td>1.37 (−0.78 to 4.44)</td>
<td>0.55 (−1.27 to 2.46)</td>
<td></td>
</tr>
<tr>
<td>SNP-based variables4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNP score (BMI)</td>
<td>29 (23–35)</td>
<td>29 (23–35)</td>
<td>29 (23–34)</td>
</tr>
<tr>
<td>SNP score (WC)</td>
<td>3 (1–6)</td>
<td>3 (1–6)</td>
<td>3 (1–6)</td>
</tr>
<tr>
<td>SNP score (WHR)</td>
<td>18 (14–23)</td>
<td>18 (14–22)</td>
<td>18 (14–23)</td>
</tr>
<tr>
<td>SNP score (complete)</td>
<td>48 (41–56)</td>
<td>48 (41–56)</td>
<td>48 (41–56)</td>
</tr>
<tr>
<td>Adjustment variables</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking, never-smokers (%)</td>
<td>27.0</td>
<td>41.4</td>
<td>40.1</td>
</tr>
<tr>
<td>Education, ≤primary school (%)</td>
<td>35.7</td>
<td>30.1</td>
<td>26.2</td>
</tr>
<tr>
<td>Physical activity, most sedentary group (%)</td>
<td>21.4</td>
<td>9.5</td>
<td>11.5</td>
</tr>
<tr>
<td>Menopausal status, postmenopausal (%)</td>
<td>42.4</td>
<td>55.6</td>
<td>27.5</td>
</tr>
<tr>
<td>Total energy intake, excluding alcohol (MJ/d)</td>
<td>8.5 (4.8–13.9)</td>
<td>8.2 (4.9–13.4)</td>
<td>9.0 (4.9–15.1)</td>
</tr>
<tr>
<td>Alcohol (MJ/d)</td>
<td>0.4 (0–1.8)</td>
<td>0.4 (0–1.9)</td>
<td>0.3 (0–1.5)</td>
</tr>
</tbody>
</table>

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1. 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 body weight change; \( \Delta WC \), annual waist circumference change.
2. Median; 5th–95th percentiles in parentheses (all such values).
3. \( \Delta WC \): 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 \( \Delta WC \).
4. 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 ΔBW (kg) and ΔWC (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 ΔBW or ΔWC in any of the 3 cohorts. However, in the meta-analysis, we found a significant ΔBW of −0.076 kg ($P = 0.021; \text{95\% CI:} −0.140, −0.012$).

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 ΔBW and ΔWC, 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 ΔBW 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 ΔWC 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 ΔWC of −0.043 cm ($P = 0.038; \text{95\% CI:} −0.083, −0.002$) per 1000-mg higher calcium intake (Figure 2).

Finally, for exploratory purposes, we investigated the association between the individual SNPs and ΔBW (see Supplemental Table 4 under “Supplemental data” in the online issue) and ΔWC (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 ΔBW (see Supplemental Table 6 under “Supplemental data” in the online issue) and ΔWC (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 ΔWC. In the analyses with ΔBW 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 ΔWC. 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$^1$ |
| | Crude $\beta$ (95% CI)$^2$ | % Weight | Adjusted $\beta$ (95% CI)$^3$ | % Weight$^4$ |
| 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 circumference (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 |

$^1$DCH, Diet, Cancer and Health Study; MONICA, MONItoring trends and determinants of CARdiovascular disease.

$^2$Adjusted for baseline outcome and height.

$^3$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 was adjusted for concurrent weight change.

$^4$The effect estimates from the individual cohorts were weighted by the inverses of their variance.
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 calcium interactions is also minor. Therefore, it is possible that a population of 7569 individuals might be too small to discover potential SNP 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 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 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 1](https://academic.oup.com/ajcn/article-abstract/99/4/957/4637877)
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 × 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. With the use of 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 ΔBW. 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 ΔBW. 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 ΔWC, given the lack of knowledge about the mechanisms these genetic variants are involved in. Moreover, the association with ΔWC adjusted for ΔBW implies that greater ΔWC 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 ΔBW depends on genetic predisposition to higher BMI, WC, or WHR. Furthermore, we found no evidence of an association between dietary calcium and ΔWC 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...
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, LA, 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 LA¨; 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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