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# Skim Milk, Whey, and Casein Increase Body Weight and Whey and Casein Increase the Plasma C-Peptide Concentration in Overweight Adolescents[1–4]

Karina Arnberg,[5]* Christian Mølgaard,[5] Kim Fleischer Michaelsen,[5] Signe Marie Jensen,[5] Ellen Trolle,[6] and Anni Larnkjær[5]

[5]The Department of Human Nutrition, Faculty of Science, University of Copenhagen, Frederiksberg; and [6]The National Food Institute, Technical University of Denmark, Søborg, Denmark


## Abstract

In adults, dietary protein seems to induce weight loss and dairy proteins may be insulinotropic. However, the effect of milk proteins in adolescents is unclear. The objective was to test whether milk and milk proteins reduce body weight, waist circumference, homeostatic model assessment, plasma insulin, and insulin secretion estimated as the plasma C-peptide concentration in overweight adolescents. Overweight adolescents ($n$ = 203) aged 12–15 y with a BMI of 25.4 ± 2.3 kg/m$^2$ (mean ± SD) were randomized to 1 L/d of skim milk, whey, casein, or water for 12 wk. All milk drinks contained 35 g protein/L. Before randomization, a subgroup of adolescents ($n$ = 32) was studied for 12 wk before the intervention began as a pretest control group. The effects of the milk-based test drinks were compared with baseline (wk 0), the water group, and the pretest control group. Diet and physical activity were registered. Outcomes were BMI-for-age Z-scores (BAZs), waist circumference, plasma insulin, homeostatic model assessment, and plasma C-peptide. We found no change in BAZ in the pretest control and water groups, whereas it was greater at 12 wk in the skim milk, whey, and casein groups compared with baseline and with the water and pretest control groups. The plasma C-peptide concentration increased from baseline to wk 12 in the whey and casein groups and increments were greater than in the pretest control ($P$ < 0.02). There were no significant changes in plasma C-peptide in the skim milk or water group. These data suggest that high intakes of skim milk, whey, and casein increase BAZs in overweight adolescents and that whey and casein increase insulin secretion. Whether the effect on body weight is primary or secondary to the increased insulin secretion remains to be elucidated. J. Nutr. 142: 2083–2090, 2012.


## Introduction

There is a high prevalence of overweight among children in the Western world and metabolic syndrome is common, with prevalence rates of up to 40% among obese adolescents (1,2). However, little is known about which diets are effective in reducing overweight in this age group. In adults, dietary protein has been found to be effective in the treatment of overweight and high-protein diets have been shown to induce greater weight loss than more conventional diets in overweight and obese adults (3–5).

In the Western world, milk products are an important source of protein. The 2 major milk proteins, whey and casein, constitute 20 and 80%, respectively, of the protein content in milk. In adolescents, dairy food consumption has been inversely associated with central obesity (6) and many epidemiological studies in adults have shown inverse associations between milk intake and risk of metabolic syndrome (7,8). The components responsible for the effects are unclear, but it has been suggested that the protein content in dairy products is more important than calcium for weight loss in overweight adults (9) and in recent years attention has been paid to the potential positive effects of milk proteins on risk markers of metabolic syndrome (10–12).

Dairy proteins contain high amounts of BCAA (leucine, isoleucine, and valine), which in animal and in vitro studies have been shown to reduce weight gain and homeostatic model assessment (HOMA)[7] of insulin resistance, improve cholesterol metabolism, and increase satiety (13,14). In overweight adults, whey protein has been shown to reduce body weight, waist circumference (10), fasting plasma insulin, and insulin resistance

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[3] This trial was registered at clinicaltrials.gov as NCT00785499.
[4] Supplemental Table 1 and Supplemental Figure 1 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: kaar@life.ku.dk.

[7] Abbreviations used: BAZ, BMI-for-age Z-score; BMR, basal metabolic rate; HOMA, homeostatic model assessment; SUN, serum urea nitrogen.


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(10,12). In contrast, our research group previously found an increased fasting plasma insulin concentration after 1 wk of consumption of whey in boys aged 8 y (15) and postprandial studies have shown insulinotropic effects of milk, whey, and casein (16–18).

The fasting plasma insulin concentration in adolescents seems to predict the clustering of risk factors for metabolic syndrome (19) and is positively related to the risk of metabolic syndrome in adulthood (20). However, little is known about the effects of long-term consumption of milk and milk proteins on risk factors for metabolic syndrome in children and adolescents.

Therefore, the aim of the current study was to examine in overweight adolescents the effects of skim milk, whey, and casein on body weight, waist circumference, plasma insulin, HOMA, and the secretion of insulin measured as the plasma C-peptide concentration. Based on results from long-term intervention studies in adults with whey protein (10,12), we hypothesized that a high intake of milk proteins would reduce body weight, waist circumference, plasma insulin, HOMA, and the plasma concentration of C-peptide.

## Methods

*Study design.* The study was a randomized intervention study conducted at the Department of Human Nutrition, University of Copenhagen. The primary aim of the study was to examine the effects of consuming skim milk, whey, and casein drinks on body weight and risk markers of metabolic syndrome in overweight adolescents compared with a pretest control group not consuming test drinks and with the effect of drinking water. The study was approved by the Scientific Ethics Committees of the Capital Region of Denmark.

Participants were recruited from November 2008 to December 2010. By using extractions from the National Danish Civil Registration, invitations were sent by post to all adolescents born in 1995, 1996, 1997, and 1998 and living in the Copenhagen area. The inclusion criteria were age 12–15 y at intervention start, habitual milk and yogurt intake ≤250 mL/d, and an age- and sex-adjusted BMI corresponding to an adult BMI >25 kg/m$^2$ as defined by the International Obesity Task Force (21). The exclusion criteria were smoking, consumption of antibiotics within the last month before the start of the intervention, and chronic diseases defined as those demanding continuous treatment and attention. A total of 349 adolescents responded to the invitations. An initial suitability assessment of the responders was performed by telephone. After the assessment, 131 adolescents were excluded due to excessive milk intake, a BMI that was too low, or chronic diseases; 218 adolescents appeared to fulfill the inclusion criteria and were invited, together with their parents, to an informational meeting at the Department of Human Nutrition. At the meeting, the adolescents and their parents were given information about the study. If a child wished to participate, height and weight were measured and the child was included if the age- and sex-adjusted BMI corresponded to an adult BMI >25. Written consent was obtained from the parents and instructions were given on how to record food intake and physical activity. Fourteen adolescents withdrew, because they thought they could not drink the test drinks and one was excluded due to a BMI that was too low. Thus, 203 adolescents were included in the study (22).

The participants were randomized to drink 1 L/d of one of the test drinks: skim milk, whey, casein, or water. Examinations were conducted before the start of the intervention (wk 0) and after a 12-wk intervention period (wk 12). In addition, a subgroup of adolescents was examined 12 wk prior to the start of the intervention (wk −12), corresponding to a pretest control group with no test drink, and thereafter randomized to the 4 treatment groups.

*Test meals and diet.* The test drinks containing skim milk, whey, and casein were long-life products produced by ARLA Food Ingredients and the water was produced by Jørgensen Engros. The water was packaged in 0.5-L plastic bottles, all of which were provided to the participants at the start of the intervention. The skim milk, whey, and casein drinks were packaged in identical 200-mL milk cartons and coded by ARLA Food Ingredients. At the Department of Human Nutrition, the water and milk-based test drinks were re-coded by a technical assistant who was otherwise not involved in the study by using a letter for each drink. Randomization numbers with the respective test drink letter codes were drawn through an Internet-based platform and stratified by gender. Before the test drinks were provided to the participants and investigators, the test drink letter code was hidden by the technical assistant. The milk-based test drinks were provided to the adolescents at the start of and half-way through the intervention; the blinding was maintained throughout the study for all investigators and participants receiving the milk-based test drinks. Blinding could not be maintained for participants in the water group, because it was obvious to the participants whether they drank water or milk-based test drinks. The test drink letter codes were disclosed to investigators and participants when the statistical analysis had been performed. The composition of the test drinks is shown in **Table 1**. The source of whey in the whey test drink was whey protein isolate. The macro- and micronutrient compositions of the test drinks were analyzed by ARLA Food Ingredients and the amino acid composition was analyzed by the Department of Systems Biology (Technical University of Denmark) (23).

To monitor compliance, participants were instructed to record their consumption of test drinks in a booklet with calendar tick boxes and to count the number of leftover cartons/bottles. Serum urea nitrogen (SUN) was analyzed as a measure of recent protein intake (24) using a kinetic UV assay on Pentra 400 analyzers (Horiba ABX) with intra- and interassay variations of 1.0 and 5.3%, respectively.

All participants were asked to consume their usual diet ad libitum and maintain their typical physical activity levels during the study. The diet was recorded for 4 d (1 weekend day and 3 weekdays) prior to the intervention (wk 0) and just before finishing the intervention (wk 12); for the subgroup, diet was also registered 12 wk before the start of the intervention (wk −12). The participants completed a food diary each day that had precoded, fixed answer possibilities plus a possibility for open answers. The intake was recorded in household measures or portion sizes estimated from photo series. Data were analyzed with GIES software (version 1.000 d, The National Food Institute, DTU Food) and the Danish Food Composition Databank (version 7) (25). The method for dietary registration was validated in adults (26).

*Physical activity.* Physical activity was measured using pedometers for 7 consecutive days at 0 and 12 wk and also at −12 wk for the subgroup (Yamax, SW-200). The participants completed questionnaires daily to register the number of counts.

*Pubertal development.* Pubertal development according to the Tanner stage based on an assessment of pubic hair development in boys and breast stage in girls was determined at 0 wk using self-reported questionnaires (27–29).

*Anthropometry.* The examinations were conducted in the morning after an overnight fast. Weight was recorded to 0.1-kg accuracy on a

**TABLE 1** Nutritional composition of the milk-based test drinks

| Nutritional content | Skim milk | Casein | Whey |
|---|---|---|---|
| Energy, *kJ/100g* | 156 | 136 | 137 |
| Fat, *%* | 0.47 | 0.05 | 0.04 |
| Lactose, *%* | 4.68 | 4.44 | 4.45 |
| Protein, *%* | 3.47 | 3.46 | 3.48 |
| BCAA, *mol%* | | | |
| Isoleucine | 4.77 | 4.71 | 6.23 |
| Leucine | 9.47 | 9.09 | 10.24 |
| Valine | 6.46 | 6.80 | 6.23 |
| Sodium, *%* | 0.03 | 0.14 | 0.01 |
| Phosphorus, *%* | 0.10 | 0.04 | 0.06 |
| Calcium, *%* | 0.12 | 0.06 | 0.01 |

digital scale (Tanita BWB600, Tanita) with participants in underwear and a cotton t-shirt after they had emptied their bladders. Height was measured in triplicate using a wall-mounted digital stadiometer (235 Heightronic Digital Stadiometer, Quick Medical and Measurement Concepts) to the nearest 0.01 cm with participants not wearing shoes. The height and weight measurements were entered into the software program WHO Anthro 2007 (Department of Nutrition, WHO), which calculated gender- and age-specific Z-scores. Waist circumference was measured in triplicate using a nonelastic measuring tape at the umbilicus level. The basal metabolic rate (BMR) was calculated using Schofield's equations (30).

*Blood analysis.* Blood samples were taken on each examination day (−12, 0, and 12 wk). Fasting plasma glucose was analyzed using the ABX Pentra Glucose HK CP kit (Horiba ABX) on Pentra 400 analyzers. The intra- and interassay variations were 0.7 and 2.5%, respectively. Both fasting plasma insulin and HOMA correlate with insulin sensitivity (31,32) and therefore we used both measures in this paper. Plasma insulin concentrations were analyzed by a solid-phase, 2-site, chemiluminescent immunometric assay using IMMULITE 1000 analyzers (Siemens) with intra-and interassay variations of 2.7 and 7.4%, respectively. The chemiluminescent immunometric assay had a cross-reactivity of 8.5% with proinsulin. HOMA was calculated as [glucose (mmol/L) × insulin (pmol/L)]/135. Plasma insulin values below the detection limit of 14.4 were set at 7.2 pmol/L ($n$ = 1 at −12 wk, $n$ = 10 at 0 wk, and $n$ = 7 at 12 wk). Plasma C-peptide concentrations were analyzed by a solid-phase, competitive, chemiluminescent enzyme immunoassay using IMMULITE 1000 analyzers (Siemens) with intra- and interassay variations of 5.4 and 8.0%, respectively.

*Statistical analysis.* $P < 0.05$ was considered significant and values in the text are presented as mean ± SD. Differences in characteristics between the 4 test drink groups at 0 wk were tested by the Kruskal-Wallis and chi-squared tests and post hoc pairwise comparisons were performed with the significance level adjusted for multiple testing. The paired $t$ test and Wilcoxon's Signed Rank test were used to compare changes within the groups during the 12-wk period for normally and not normally distributed variables, respectively. A Spearman correlation test was performed to assess the correlation between the intake of test drinks estimated from compliance booklets and the intake estimated by counting leftover cartons/bottles. Intake estimated from the number of leftover cartons/bottles was used if the diary was missing. Compliance was calculated as the percentage of planned intake. The primary outcome variables were BMI-for-age Z-score (BAZ), waist circumference, plasma insulin, HOMA, and plasma C-peptide. To assess the effects of skim milk, whey, and casein compared with the effect of drinking water and with a pretest control group not drinking the test drinks, a mixed linear model was fitted by xtmixed in STATA using a random intercept and slope model, i.e., each individual was allowed to have a random level and a random change in the outcome during the study period. The model included the predictors: time [−12 wk; 12 wk], intervention time [0; 12 wk], and the intervention group × intervention time interaction. For all individuals, a general time effect (called "time") was assumed, representing the underlying change happening over time with no intervention, i.e., as in the pretest control group. Furthermore, an intervention time effect (called "intervention time") was included for each intervention group (intervention time × group interaction) during the intervention period only, representing the additional change happening over time due to the intervention. For a given intervention group, the effect of intervention time corresponded to the effect of the test drink compared with the effect of no test drink in the pretest control group. The interaction term was used to assess the effect of each test drink compared with the effect of drinking water using the latter as the reference group in the intervention group variable. A second model also included the covariates gender, Tanner stage, and age. The gender × test drink interaction was tested and, if nonsignificant, the interaction term was removed from the model. To assess whether the effect of the test drinks on plasma insulin was mediated via changes in body size, a final model was constructed with plasma insulin, HOMA, and plasma C-peptide as outcome variables and inclusion of BAZ as covariate. Because the repeated-measurement model is based on available case analysis, data on all participants starting the intervention were used and shown in tables. Plasma insulin, HOMA, and plasma C-peptide were log-transformed before fitting the mixed linear models. All analyses were performed using STATA 11.0 (StataCorp). The figures were performed using the open source statistical programming environment R version 2.15.0 (33).

*Study size and power calculation.* We decided to recruit a sample of 200 adolescents with an expected drop-out of 10%. This sample size was selected to determine a difference of 0.4 SD, which was assumed to correspond to a weight difference of 1 kg, with a significance level of 0.05 and a power of 80% for each treatment comparison.

## Results

*Participants.* In total, 203 adolescents were randomly assigned to the 4 treatment groups (**Supplemental Fig. 1**). Ten participants withdrew before the start of the intervention. Thus, 193 adolescents (62% girls) were examined. Twenty withdrew during the study period, so 173 completed the study. At wk 0, the age of the 193 adolescents was 13.2 ± 0.7 y. The majority were Caucasian (95%). Some weighed less in a fasting state on the examination day than when weighed at the informational meeting held in the afternoon 1–2 wk prior to the examination. Therefore, at the start of the intervention, 19 adolescents had a BMI below the cutoff value corresponding to an adult BMI ≤25 kg/m$^2$. The remaining participants were above the cutoff value, i.e., 152 adolescents had a BMI corresponding to an adult BMI in the range of 25.1–29.9 and 22 had a BMI corresponding to an adult BMI ≥30 kg/m$^2$. At wk 0, the 4 test drink groups did not significantly differ in age, gender, weight, Tanner stage, or height (**Table 2**). There were no differences between the 4 groups in the primary outcomes BAZ, waist circumference, fasting plasma insulin, HOMA, and plasma C-peptide. Of the 193 adolescents, a subgroup of 32 was examined 12 wk prior to the start of the intervention (time −12 wk). Descriptive data of the subgroup are shown in Table 2.

*Dietary changes and compliance.* The compliance booklet was missing for 14 participants and of those, 2 in the whey group did not count leftover cartons. An analysis of the SUN concentration was not possible in 3 participants at 12 wk. There was a good correlation between the consumption of test drinks as indicated in the compliance booklets and the reported numbers of leftover cartons/bottles ($\rho = 0.78$; $P < 0.001$). The mean intake of the test drinks expressed as the percentage of planned intake was 95% for water, 92% for skim milk, 91% for casein, and 87% for whey. In the water group, there were no changes in SUN at 12 wk compared with 0 wk ($P = 0.80$); in the pretest control group, SUN did not differ at 0 wk compared with −12 wk ($P = 0.34$). However, SUN increased from 0 to 12 wk in the casein ($P < 0.001$) and skim milk ($P < 0.001$) groups but not in the whey group ($P = 0.06$) (**Supplemental Table 1**). There were no changes in SUN in the water group ($P = 0.76$) compared with the pretest control group, whereas the change in SUN was greater in the casein ($P < 0.001$) and skim milk ($P = 0.002$) groups during the 12-wk intervention period than in the pretest control group. The increase in SUN was greater during the 12-wk intervention period in the casein ($P < 0.001$), skim milk ($P < 0.001$), and whey ($P = 0.041$) groups compared with the water group.

Energy intake ($P = 0.09$), fat energy percentage ($P = 0.23$), and protein energy percentage ($P = 0.58$) did not differ between

**TABLE 2** Characteristics of test drink groups at 0 wk and pretest control group at −12 wk[1]

| | Pretest control (n = 32) | Water (n = 50) | Casein (n = 47) | Skim milk (n = 48) | Whey (n = 48) |
|---|---|---|---|---|---|
| Girls, % | 62.5 | 64.0[a] | 61.7[a] | 64.6[a] | 58.3[a] |
| Age, y | 13.7 ± 0.6 | 13.2 ± 0.7[a] | 13.2 ± 0.7[a] | 13.2 ± 0.8[a] | 13.0 ± 0.7[a] |
| Tanner stage 1:2:3:4:5, n | 0:2:9:13:8 | 3:7:20:16:4[a] | 2:10:22:8:5[a] | 2:9:17:18:2[a] | 0:12:16:17:3[a] |
| Weight, kg | 69.8 ± 7.8 | 66.9 ± 8.5[a] | 66.0 ± 9.0[a] | 66.0 ± 10.1[a] | 66.2 ± 10.7[a] |
| Height, cm | 165.5 ± 8.3 | 162.9 ± 7.6[a] | 162.3 ± 7.7[a] | 162.4 ± 7.5[a] | 162.8 ± 8.7[a] |
| BMI, $kg/m^2$ | 25.5 ± 2.2 | 25.2 ± 2.3[a] | 25.0 ± 2.2[a] | 24.9 ± 2.5[a] | 24.8 ± 2.4[a] |
| BAZ | 1.77 ± 0.43 | 1.82 ± 0.45[a] | 1.79 ± 0.46[a] | 1.75 ± 0.49[a] | 1.79 ± 0.47[a] |
| Plasma insulin, pmol/L | 72.9 ± 41.1 | 85.4 ± 67.7[a] | 70.0 ± 51.9[a] | 77.3 ± 52.3[a] | 71.5 ± 35.8[a] |
| Plasma C-peptide, pmol/L | 700 ± 223 | 695 ± 247[a] | 649 ± 271[a] | 715 ± 279[a] | 658 ± 209[a] |

[1] Values are mean ± SD except for categorical data. In the test drink groups, means without a common letter differ at the 0-wk measure, $P < 0.05$. BAZ, BMI-for-age Z-score.

the 4 test drink groups at 0 wk. The energy intake had fallen from 0 to 12 wk ($P = 0.008$) in the water group and this decrease during the intervention period differed from that of the pretest control group ($P = 0.011$). The change in energy intake did not differ during the intervention period for the skim milk ($P = 0.56$), whey ($P = 0.20$), or casein ($P = 0.70$) group compared with the pretest control group. However, the skim milk and casein groups had a greater energy intake during the intervention period compared with the water group ($P = 0.023$ and $P = 0.001$, respectively) (**Table 3**). At the start of the intervention, 49% of the adolescents reported an energy intake below their BMR, whereas this percentage fell to 46% at 12 wk.

*Physical activity.* One child had missing data at 0 wk. At 12 wk, one participant had missing data and one was excluded due to poor registration. At 0 wk, the number of steps was 10,020 ± 3940 (mean ± SD). The number of steps did not significantly differ during the intervention period for any of the milk-based test drink groups compared with the water or pretest control group.

*Changes in anthropometry.* Weight and BMI increased from 0 to 12 wk for the skim milk (both $P < 0.001$), whey (both $P < 0.001$), casein (both $P < 0.001$), and water ($P < 0.001$ and $P = 0.010$, respectively) groups and weight and BMI increased from −12 to 0 wk in the pretest control group ($P < 0.001$ and $P = 0.042$, respectively) (**Fig. 1**; **Table 4**). The BAZ increased from 0 to 12 wk only in the skim milk ($P = 0.008$), whey ($P < 0.001$), and casein ($P < 0.001$) groups and the increments were significantly greater during the intervention period compared with the pretest control and water groups (Fig. 1). The increase in weight was greater during the intervention period in the skim milk ($P = 0.032$), whey ($P = 0.011$), and casein ($P < 0.001$) groups than in the group drinking water. The increase in weight

**TABLE 3** Dietary intakes of overweight adolescents in the pretest control group and in those consuming water, casein, skim milk, or whey beverages for 12 wk[1]

| | −12 wk | 0 wk | 12 wk | Change in period[2] | n at −12, 0, and 12 wk |
|---|---|---|---|---|---|
| Energy, kJ/d | | | | | |
| Pretest control | 6890 ± 2520 | 7070 ± 2630 | — | 180 ± 2330 | 32, 32, – |
| Water | — | 7620 ± 2100[a] | 6700 ± 2450 | −920 ± 2330*,# | –, 50, 50 |
| Casein | — | 7190 ± 2190[a] | 8040 ± 2570 | 460 ± 2480** | –, 47, 36 |
| Skim milk | — | 6980 ± 2390[a] | 7360 ± 2130 | 100 ± 1980** | –, 48, 44 |
| Whey[3] | — | 8310 ± 2870[a] | 7510 ± 1960 | −730 ± 2800 | –, 48, 41 |
| Protein, %E | | | | | |
| Pretest control | 15.0 ± 2.6 | 15.4 ± 2.5 | — | 0.5 ± 2.6 | 32, 32, – |
| Water | — | 15.3 ± 2.2[a] | 15.5 ± 2.2 | 0.2 ± 3.1 | –, 50, 50 |
| Casein | — | 14.8 ± 2.5[a] | 19.4 ± 3.5 | 4.8 ± 3.0*,#,** | –, 47, 36 |
| Skim milk | — | 14.7 ± 2.4[a] | 20.2 ± 3.7 | 5.5 ± 3.6*,#,** | –, 48, 44 |
| Whey[3] | — | 14.8 ± 2.8[a] | 19.9 ± 3.3 | 4.9 ± 2.9*,#,** | –, 48, 41 |
| Fat intake, %E | | | | | |
| Pretest control | 35.1 ± 7.4 | 35.4 ± 7.6 | — | 0.2 ± 7.1 | 32, 32, – |
| Water | — | 36.6 ± 5.3[a] | 35.2 ± 5.5 | −1.4 ± 5.8 | –, 50, 50 |
| Casein | — | 34.4 ± 6.4[a] | 29.5 ± 5.1 | −4.1 ± 5.5*,#,** | –, 47, 36 |
| Skim milk | — | 36.1 ± 5.6[a] | 29.9 ± 5.1 | −6.6 ± 6.5*,#,** | –, 48, 44 |
| Whey[3] | — | 37.0 ± 5.6[a] | 30.8 ± 5.5 | −7.0 ± 6.1*,#,** | –, 48, 41 |

[1] Values are mean ± SD or n. Data at 12 wk include the intake of test drinks based on compliance registration. *Different from baseline within group, $P < 0.05$; #different from pretest control group, $P < 0.05$; **different from water group, $P < 0.05$. In the test drink groups, means without a common letter differ at the 0-wk measure, $P < 0.05$. Dash indicates that no measurements were taken. %E, energy percentage.
[2] For test drinks, calculated as the change from 0 to 12 wk. For the pretest control group, calculated as the change from −12 to 0 wk.
[3] Compliance data were missing for 2 participants.

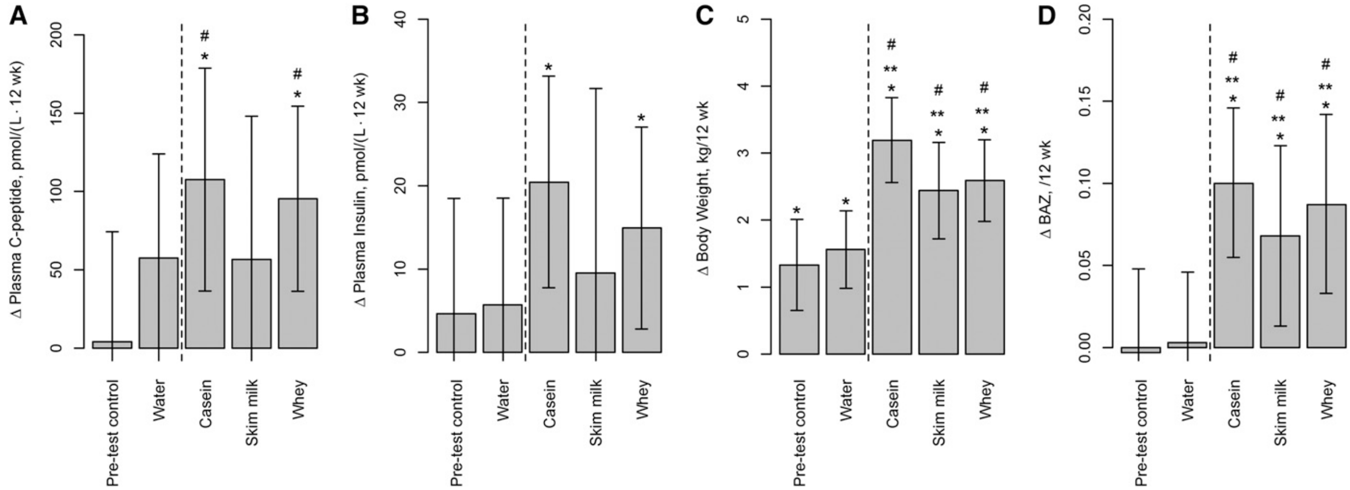


**FIGURE 1** Changes in plasma C-peptide concentrations (*A*), plasma insulin concentrations (*B*), body weight (*C*), and BAZ (*D*) in the pretest control group and in those consuming water, casein, skim milk, or whey beverages for 12 wk. Values are mean changes and 95% CI, $n$ = 32 (pretest control), 50 (water), 36 (casein), 44 (skim milk), and 43 (whey). One blood sample for analysis of plasma C-peptide and insulin concentrations was missing from the water and skim milk groups. *Different from baseline within a group, $P < 0.05$; #different from the pretest control group, $P < 0.05$; **different from water group, $P < 0.05$. BAZ, BMI-for-age Z-score.

was also greater in the skim milk ($P$ = 0.001), whey ($P$ < 0.001), and casein ($P$ < 0.001) groups than in the pretest control group. The increase in BMI was greater during the intervention period in the skim milk ($P$ = 0.022), whey ($P$ = 0.009), and casein ($P$ = 0.002) groups than in the group drinking water. The increase in BMI during the intervention was also greater in the skim milk ($P$ = 0.001), whey ($P$ < 0.001), and casein ($P$ < 0.001) groups than in the pretest control group. The increases in weight and BMI in the water group did not significantly differ from those in the pretest control group. The results were the same when we adjusted for gender, Tanner stage, and age and there were no interactions between gender and test drink group.

Waist circumference increased from 0 to 12 wk in the skim milk ($P$ = 0.001), whey ($P$ = 0.003), and casein ($P$ = 0.007) groups and from −12 to 0 wk ($P$ = 0.015) in the pretest control group. Waist circumference did not change from 0 to 12 wk in the water group. There were no differences in the increase in waist circumference or in the increase in the waist:height ratio for any of the milk-based test drink groups during the intervention period compared with the water and pretest control groups.

*Changes in plasma insulin, glucose, HOMA, and C-peptide.* No blood samples were obtained at 12 wk in 1 boy from the skim milk group and 1 boy from the water group. Fasting plasma insulin increased by 21% in the whey group ($P$ = 0.029) and 29% in the casein group ($P$ = 0.006) from wk 0 to 12. The plasma C-peptide concentration increased from wk 0 to 12 by 14% in the whey group ($P$ = 0.005) and 17% in the casein group ($P$ = 0.009) (Fig. 1). HOMA increased by 23% within the whey group ($P$ = 0.021) and 32% in the casein group ($P$ = 0.006) from wk 0 to 12. The plasma glucose concentration increased from wk 0 to 12 in the whey group only ($P$ = 0.017) (Table 4).

The increments in plasma insulin and HOMA over the intervention period were not greater in the casein group than in the pretest control group (both $P$ = 0.07) and in the models including BAZ, the effects of casein on plasma insulin and HOMA were attenuated ($P$ > 0.1). There were no significant differences during the 12-wk intervention period in plasma insulin, HOMA, or plasma C-peptide for any of the milk-based test drink groups compared with the group drinking water. The plasma C-peptide concentration was greater in the whey ($P$ = 0.016) and casein groups ($P$ = 0.017) during the intervention period compared with the pretest control group. Also, in the models adjusted by gender, Tanner stage, and age, the increment in plasma C-peptide during the intervention period was higher in the whey ($P$ = 0.021) and casein groups ($P$ = 0.022) than in the pretest control group. There were no significant interactions between gender and test drink. BAZ was a positive predictor of plasma insulin, HOMA, and plasma C-peptide ($P$ < 0.001).

Adjusted by the BAZ, the increment in plasma C-peptide during the intervention period remained higher in the whey ($P$ = 0.026) and casein groups ($P$ = 0.034) than in the pretest control group. Plasma C-peptide did not change during the intervention period in the skim milk ($P$ = 0.15) or water groups ($P$ = 0.16) compared with the pretest control group.

## Discussion

In the present study, we have shown that high intakes of skim milk, whey, and casein for 12 wk increase the BAZ in overweight adolescents. Also, we have shown that whey and casein increase the insulin secretion measured by the concentration of plasma C-peptide. To our knowledge, no other studies have examined the effect of whey and casein on body weight or fasting plasma insulin in overweight adolescents.

We were surprised to see an increase in the BAZ following skim milk, whey, and casein consumption given that intervention studies in overweight and obese adults have shown that both dairy products and whey protein reduce body weight (10,34) and other studies have shown no changes in body weight (12,35,36). Also, regarding the increased plasma C-peptide concentration, our results are in contrast to recent, longer term, intervention studies in overweight adults that showed a lowering effect on fasting plasma insulin concentrations and HOMA following whey consumption (10,12). There are several possible explanations for the inconsistency between the findings. First, other studies have used iso-caloric control meals rich in carbohydrates (10,12,36) that may adversely affect both weight development and insulin compared with our control groups, where no extra energy was consumed. Thus, our study provides

**TABLE 4** Body composition, HOMA insulin resistance, and plasma glucose concentrations of overweight adolescents in the pretest control group and in those consuming water, casein, skim milk, or whey beverages for 12 wk[1]

| | −12 wk | 0 wk | 12 wk | Change in period[2] | *n* at −12, 0, and 12 wk |
|---|---|---|---|---|---|
| BMI, *kg/m²* | | | | | |
| Pretest control | 25.5 ± 2.2 | 25.7 ± 2.0 | — | 0.2 ± 0.7* | 32, 32, – |
| Water | — | 25.2 ± 2.3[a] | 25.5 ± 2.4 | 0.3 ± 0.8* | –, 50, 50 |
| Casein | — | 25.0 ± 2.2[a] | 26.0 ± 2.2 | 0.8 ± 0.7*,#,** | –, 47, 36 |
| Skim milk | — | 24.9 ± 2.5[a] | 25.8 ± 2.8 | 0.6 ± 0.9*,#,** | –, 48, 44 |
| Whey | — | 24.8 ± 2.4[a] | 25.5 ± 2.5 | 0.7 ± 0.8*,#,** | –, 48, 43 |
| Waist, *cm* | | | | | |
| Pretest control | 83.1 ± 5.4 | 84.7 ± 5.6 | — | 1.6 ± 3.6* | 32, 32, – |
| Water | — | 85.3 ± 6.2[a] | 86.0 ± 6.7 | 0.7 ± 3.7 | –, 50, 50 |
| Casein | — | 86.7 ± 7.4[a] | 89.2 ± 7.8 | 1.5 ± 3.1* | –, 47, 36 |
| Skim milk | — | 84.7 ± 7.7[a] | 87.6 ± 8.6 | 2.2 ± 4.2* | –, 48, 44 |
| Whey | — | 83.7 ± 7.9[a] | 85.3 ± 8.6 | 2.1 ± 4.3* | –, 48, 43 |
| Waist:height ratio | | | | | |
| Pretest control | 0.50 ± 0.04 | 0.51 ± 0.04 | — | 0.01 ± 0.02 | 32, 32, – |
| Water | — | 0.52 ± 0.04[a] | 0.53 ± 0.04 | 0.00 ± 0.02 | –, 50, 50 |
| Casein | — | 0.53 ± 0.05[a] | 0.54 ± 0.05 | 0.01 ± 0.02 | –, 47, 36 |
| Skim milk | — | 0.52 ± 0.04[a] | 0.54 ± 0.05 | 0.01 ± 0.03* | –, 48, 44 |
| Whey | — | 0.51 ± 0.05[a] | 0.52 ± 0.05 | 0.01 ± 0.03* | –, 48, 43 |
| Plasma glucose, *mmol/L* | | | | | |
| Pretest control | 5.10 ± 0.34 | 5.11 ± 0.36 | — | 0.01 ± 0.26 | 32, 32, – |
| Water[3] | — | 5.18 ± 0.32[a] | 5.18 ± 0.33 | 0.01 ± 0.30 | –, 50, 49 |
| Casein | — | 5.17 ± 0.34[a] | 5.30 ± 0.29 | 0.09 ± 0.34 | –, 47, 36 |
| Skim milk[3] | — | 5.20 ± 0.35[a] | 5.23 ± 0.38 | 0.01 ± 0.24 | –, 48, 43 |
| Whey | — | 5.16 ± 0.22[a] | 5.23 ± 0.30 | 0.09 ± 0.23* | –, 48, 43 |
| HOMA | | | | | |
| Pretest control | 2.80 ± 1.64 | 3.00 ± 1.84 | — | 0.20 ± 1.50 | 32, 32, – |
| Water[3] | — | 3.31 ± 2.74[a] | 3.54 ± 2.62 | 0.24 ± 1.84 | –, 50, 49 |
| Casein | — | 2.71 ± 2.05[a] | 3.70 ± 2.32 | 0.87 ± 1.63* | –, 47, 36 |
| Skim milk[3] | — | 3.04 ± 2.12[a] | 3.57 ± 4.24 | 0.44 ± 3.29 | –, 48, 43 |
| Whey | — | 2.76 ± 1.45[a] | 3.44 ± 2.24 | 0.64 ± 1.65* | –, 48, 43 |

[1] Values are mean ± SD or *n*. *Different from baseline within group, $P < 0.05$; #different from pretest control group, $P < 0.05$; **different from water group, $P < 0.05$. Dash indicates that no measurements were taken. HOMA, homeostatic model assessment.
[2] For test drinks, calculated as the change from 0 to 12 wk; for the pretest control, calculated as the change from −12 to 0 wk.
[3] One blood sample was not obtained at 12 wk.

information about the effect of supplementing with skim milk, whey, or casein, whereas the other studies provide information about the effect of exchanging carbohydrate-rich drinks with milk, whey, or casein. Second, the age of our study population was different, which may also explain the inconsistency. In adults, high-protein diets seem to induce weight loss compared with low-protein diets (3,5), whereas during infancy, high-protein diets have been shown to induce greater weight gain than low-protein diets (37). This indicates that high-protein diets may exert different effects on body weight during the life span. Because many of our adolescents were close to the puberty growth spurt, it is likely that the effect of dietary protein on weight changes in our population is more comparable with the effects in infants, who are also in a period of rapid growth velocity. High-protein diets have been suggested to induce weight gain during periods of growth via insulinotropic effects (38). Insulin is a growth factor, because it binds to insulin-like-growth factor-I receptors, which promote cell replication in connective and musculoskeletal tissue (39). Thus, the milk proteins may have increased insulin secretion in our adolescents, which thereby may have increased the weight gain. On the other hand, obesity is also thought to impair the insulin signaling cascade and thus cause insulin resistance and increased insulin secretion (40). Therefore, the increased plasma C-peptide concentration in the whey and casein groups may also have been mediated via the increased BAZ. We included BAZ in the model with plasma insulin, HOMA, and C-peptide as outcomes, which attenuated the effect of whey and casein, but there remained a significantly increased effect on plasma C-peptide. This indicates that the increase in BAZ may partly explain the increase in the plasma C-peptide concentration in the whey and casein groups. Abdominal adiposity is thought to be closely linked with insulin resistance (41,42). However, none of the milk-based test drinks had a significant effect on the changes in waist circumference. Thus, the present study does not support that the insulin-stimulating effects of whey and casein were caused by an increased amount of visceral adipose tissue.

The increased BAZ in the skim milk, whey, and casein groups may also have been due to an imbalance between energy intake and expenditure. During the intervention, the adolescents drinking skim milk, whey, and casein did not change their physical activity level and there was no change in energy intake despite an increase in the BAZ. There are several possible explanations for this finding. A large proportion of the participants reported energy intakes below their BMR, indicating under-reporting as seen in other studies of overweight adolescents (43). There was no change in the BAZ and hence no weight loss in the water group despite a decreased energy intake at 12 wk; therefore, it is likely that all the groups underestimated the total energy intake at 12 wk to a greater extent than at 0 wk. This is consistent with studies that validated dietary records in adults and showed higher amounts of dietary under-reporting on the second occasion compared with the first occasion (44). If so, the increase in the BAZ in the skim milk, whey, and casein groups in our study may be due to increased energy intake and hence a lack of downregulation of the habitual energy intake. This is in contrast to studies showing satiating effects of high-protein diets (45). However, it may be that protein from liquid does not provide the same satiating effect as protein from solid foods. This is supported by several studies showing that energy arising from carbohydrates in liquids is not compensated for by a reduction in solid food consumption (46–48).

In all analyses, the effects of whey and casein were more significant for plasma C-peptide compared with plasma insulin despite a high correlation between these variables (data not shown). Plasma C-peptide may be a better marker of insulin secretion than the fasting plasma insulin concentration, because C-peptide is less exposed to first-pass metabolism by the liver and therefore has a longer half-life (49,50). The plasma insulin concentrations and HOMA insulin resistance increased within the whey and casein groups; however, we cannot conclude whether the increased insulin resistance is primary or simply reflects the hyperinsulinemia. The increased plasma C-peptide concentration supports an increase in endogen insulin secretion and hence that hyperinsulinemia is primary and insulin resistance is caused by it. On the other hand, the increase in plasma glucose within the whey group indicates that insulin resistance may be primary to hyperinsulinemia.

We did not observe any significant changes in the plasma C-peptide concentration in the skim milk group despite an increase in the BAZ. An explanation could be that a fasting insulinotropic effect of the milk proteins exists only when separating the protein fractions. This seems unlikely, however, because several studies have shown insulinotropic effects of milk after shorter term consumption (16,51,52). The increased plasma C-peptide concentrations of the whey and casein groups are in agreement with previous findings of postprandial insulinotropic effects (17,18). Also, we previously showed that intake of whey for 7 d increased fasting plasma insulin and HOMA in healthy 8-y olds, but no effects were seen for casein (15). The high content of BCAA has been suggested to cause the insulinotropic properties of whey and casein (13,53). However, the content was nearly identical in all the milk-based test drinks. In our previous study in 8-y-old boys, we found increased plasma insulin concentrations in a milk group compared with a meat group consuming the same amount of protein; there were no differences in blood concentrations of BCAA (51), however, indicating that the insulin-stimulating effect may be explained by other mechanisms.

The strength of this study is the randomized intervention design conducted in a large sample of overweight adolescents. We had a low dropout rate of 10% and both the reported compliance and the biochemical measure showed that compliance with beverage consumption was high. The study was conducted in adolescents with low habitual milk intakes and the results therefore mainly apply to this selected group. However, because low milk intakes have been associated with higher risk of central adiposity and metabolic syndrome (6,8), our data are unique; they provide information on the effects of increasing the intake of skim milk and milk proteins in individuals with habitual low milk intakes. A limitation of the current study is that the subgroup followed 12 wk prior to the start of the intervention was approximately one-half year older than the mean age of the test drink groups. However, the adolescents in the subgroup were subsequently randomized into the 4 test drink groups, and these groups did not significantly differ in age or Tanner stage. We also performed analyses with adjustments for age and Tanner stage, which did not remarkably change the results. Also, 95% of the adolescents were Caucasian and the results may therefore only apply to this selected ethnic group.

In conclusion, the results from this study show that skim milk, whey, and casein increase BAZ and that whey and casein increase insulin secretion measured as the plasma C-peptide concentration in fasting conditions and after consumption for a 12-wk period in overweight adolescents. Thus, skim milk, whey, and casein in high amounts do not seem to be beneficial in terms of reducing the risk of metabolic syndrome in overweight adolescents. We cannot conclude from the current study whether the increased BAZ is primary or secondary to the increased insulin secretion, nor can we conclude whether whey and casein primarily induce hyperinsulinemia or insulin resistance.


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