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Dietary calcium from dairy, body composition and glycaemic control in patients with type 2 diabetes pursuing an energy restricted diet: A parallel group randomised clinical trial

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ABSTRACT

In a 12-week duration parallel group randomised clinical trial, we evaluated the effect of increasing calcium (Ca) intake on body composition and insulin resistance in patients with type 2 diabetes (T2DM). Thirty-six subjects with low habitual Ca intake (<600 mg d^{-1}), consumed low-Ca diet (CD group, 800 mg d^{-1}) or high-Ca fat-free milk diet (MD group, 1500 mg d^{-1}). MD group final anthropometric measures (body weight, BMI, waist circumference, waist-hip ratio, and fat mass) decreased compared with baseline. MD group showed greater decrease in waist circumference compared with CD group. Final fasting glucose decreased in CD group compared with baseline. Both groups reduced glycated haemoglobin. Consumption of high-Ca diet from dairy for 12 weeks was effective in reducing abdominal adiposity, but provided no additional effect on glycaemic control in overweight patients with T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a worldwide public health problem, causing high social and economic costs due to its chronic nature and to the severity of its complications (ADA, 2016). Insulin resistance (IR) characterizes this metabolic disorder and there is usually a relative (rather than absolute) insulin deficiency (ADA, 2016). Therefore, the assessment of insulin sensitivity and β-cell function are useful to identify metabolic abnormalities related to T2DM (Ghasemi et al., 2015).

In overweight patients with T2DM, there are evidences supporting the importance of dietary intervention to prevent the occurrence of metabolic abnormalities (ADA, 2016). Although the traditional dietary intervention does not emphasise the role of micronutrients, dietary calcium (Ca) seems to improve weight loss and glycaemic control in T2DM subjects (Abargouei, Janghorbani, Salehi-Marzijarani, & Esmaillzadeh, 2012; Soares, Murhadi, Kurpad, Ping-Delfos, & Piers, 2012). However, daily Ca intake is low (~500 mg d^{-1}) in industrialized countries, due to the increased consumption of processed foods and reduced consumption of dairy (Imamura et al., 2015).

On the other hand, there is no consensus among authors concerning the effects of Ca supplementation with or without vitamin D, on adiposity, glucose homoeostasis and insulin sensitivity (Ferreira, Torres, & Sanjuliani, 2013; Jones et al., 2013; Nikooyeh et al., 2011; Stancliffe, Thorpe, & Zemel, 2011; Torres, Francischetti, Genelhu, & Sanjuliani, 2010). The beneficial effects of increased Ca intake appear to be most significant in low habitual Ca consumers (less than 600 mg d^{-1}) (Zemel et al., 2009), receiving energy-restricted diet (Abargouei et al., 2012; Stonehouse et al., 2016) and Ca bioavailable supplements such as citrate or fat-free dairy products (Freitas, Martino, Ribeiro, & Alfenas, 2012; Soares, Ping-Delfos, & Ghanbari, 2011). Besides, few studies assessed the influence of dietary Ca on glycaemic control in T2DM subjects, and most (Pittas, Lau, Hu, & Dawson-Hughes, 2007; van Dam, Hu, Rosenberg, Krishnan, & Palmer, 2006; Pittas et al., 2006; Villegas et al., 2009), but not all (Nikooyeh et al., 2011; Tabesh, Azadbakht, Faghihimani, Tabesh, & Esmaillzadeh, 2014), were observational. Therefore, considering the potential beneficial effects of adequate Ca intake on glycaemic control and the few data regarding the usefulness of dietary Ca on T2DM treatment, this study evaluated the effect of increased Ca intake from dairy on body composition and IR in overweight T2DM subjects.

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2. Participants and methods

2.1. Ethics aspects

This study was conducted according to the Declaration of Helsinki guidelines and all procedures involving human participants were approved by the Committee of Ethics in Human Research of the Federal University of Viçosa, Brazil. Written informed consent was obtained from all subjects. The present trial was registered at www.clinicaltrials.gov, as "Dietary Calcium Supplementation, Gut Permeability and Microbiota in Type 2 Diabetics" (ID no. NCT02377076).

2.2. Participants

This study enrolled 36 subjects with T2DM (48.7 ± 8.9 years old and body mass index (BMI) of 30.7 ± 4.5 kg m^{-2}), of both genders, presenting low habitual Ca intake (<600 mg d^{-1}). Power calculations (Mera, Thompson, & Prasad, 1998) indicated that seventeen individuals were necessary to detect 1% change in glycated haemoglobin (HbA1c) ($\alpha = 0.05$; power = 80%) presented by our subjects at baseline.

Subjects were recruited through public advertisements in the town of Viçosa, Minas Gerais, Brazil. Recruitment initiated on 2nd February 2014 and ended on 3rd June 2015 when the required number of subjects for the study was obtained. Eligible subjects were adults of both genders with T2DM treated with only diet or with diet plus oral hypoglycaemic agents, that had BMI between 25.1 and 39.9 kg m^{-2}, had low habitual Ca intake (<600 mg d^{-1}), were between 20 and 59 years of age, had a dietary restraint <14 (Strunkard & Messic, 1985), had light to moderate physical activity levels (PAL) (Pardini et al., 2001), and had T2DM for at least one year and no more than 10 years.

Exclusion criteria were: (1) smoking; (2) use of Ca, vitamin D, zinc (Zn) or magnesium (Mg) supplements or medication that affects the metabolism of these micronutrients; (3) use of drugs (except hypoglycaemic drugs), herbs, or diets for weight loss; (4) on hormone replacement therapy; (5) menopause or post menopause; (6) recent weight gain or loss (±5 kg) over the previous three months; (7) recent change in PAL over the previous 3 months; (8) aversion or intolerance to the shakes provided during the study; (9) alcohol consumption of more than 12 g d^{-1} for women and 24 g d^{-1} for men; (10) eating disorders; (11) endocrine (except T2DM and obesity), kidney, or liver pathology; (12) Ca malabsorption; (13) history of recurrent nephrolithiasis; (14) history of gastric surgery or current gastric disease including gastroparesis; (15) consumption of more than 350 mg d^{-1} of caffeine; (16) pregnancy or lactation; (17) anaemia; and (18) changes in medication type or dosage during the study.

2.3. Study design

This was a 12-week duration, parallel group randomized clinical trial. Participants were initially randomly assigned by simple randomization procedures (computerised random numbers) to high-Ca fat-free milk group (MD) (equivalent to ~3 fat-free milk portions) or low-Ca control group (CD) groups in 1:1 ratio. Participants and data analysts were blinded. An energy restricted diet (restriction of 500 kcal d^{-1}) containing 800 mg of dietary Ca d^{-1} was prescribed. Subjects daily consumed in the laboratory a breakfast shake containing 700 mg d^{-1} (MD) (equivalent to approximately 3 servings of fat-free milk) or 6.4 mg d^{-1} (CD) of Ca. All other meals were consumed in free-living condition in both groups. Participants were instructed to maintain constant PAL and medication use during the study.

Food intake, body composition [fat mass (FM) and fat-free mass (FFM)], anthropometric [body weight, waist circumference (WC), waist-hip ratio (WHR)], and biochemical variables [serum glucose, triglycerides (TG), HbA1c, fructosamine, insulin, HOMA-IR, HOMA2-IR, TyG] were evaluated at baseline and after 12 weeks of intervention (Fig. 1).

2.4. Dietary intervention

Each participant's daily energy requirement was based on the Estimated Energy Requirement (EER; Trumbo, Schlicker, Yates, & Poos, 2002). Then, 500 kcal d^{-1} were subtracted for dietary restriction. Diets were prescribed according to the American Diabetes Association nutrition recommendations (ADA, 2016) and considering the nutritional composition of the breakfast shakes provided during the study. MD and CD prescribed diets presented similar contents of macronutrients, vitamin D, P, Mg, Zn, and dietary fibre. MD prescribed diet contained 1500 mg d^{-1} and CD had 800 mg of Ca d^{-1}. Participants were given meal patterns and one list discriminating the types of foods to help them in their food choices. The foods were grouped in that list considering their energy and Ca content. Participants received individualized nutritional counselling every 2 weeks to increase prescribed diet adherence. An experienced dietitian assessed eating patterns and habitual food intake, exercise and medication patterns. Nutrition counselling was provided to stimulate healthy eating habits, including adequate consumption of dietary fibre, water; besides avoiding alcohol consumption, etc., according to ADA recommendations (ADA, 2016). Energy and nutrient requirements were adjusted according to the nutritional requirement of each subject right before the beginning of the second experimental group. In the washout period, participants were told to maintain their normal diet, which was assessed through three non-consecutive days (two weekdays and a weekend day) food records.

2.5. Breakfast shakes

Twelve shake types (six for MD and six for CD experimental group) were developed for consumption at breakfast to add variety to diet. Shake flavours (frozen fruit pulps or chocolate powder) were the same for both groups. They presented similar macronutrient, vitamin D, sodium, and dietary fibre contents, differing mainly in their Ca content (Table 1). Shakes were also visually very similar, ensuring participants remained unaware of group differences and the purpose of the intervention. High-Ca shakes contained fat-free milk powder (Itambé® enriched with iron, vitamins A, C, and D, and Ca) reconstituted in water (250 mL). To ensure similarity to high-Ca shakes, low-Ca shakes contained whey protein (BemVital®, Diacom), sucrose, sodium chloride (Cisne®), and a powder supplement containing iron (iron chelate) and vitamins A (retinol acetate), C (ascorbic acid), and D3 (cholecalciferol). The supplement was prepared by a certified compounding pharmacy. Shakes were prepared mixing all the ingredients in a blender right before ingestion. Breakfast shake flavours were offered in random order, according with study group.

Subjects daily consumed a shake in the laboratory for 12 consecutive weeks. In case any subject eventually could not come to the laboratory, the shake was consumed in their homes/jobs.

2.6. Food intake assessment

Habitual Ca consumption was assessed at baseline using a quantitative food frequency questionnaire (QFFQ) (Ribeiro & Cardoso, 2002). Food intake at baseline and after 12 weeks of each

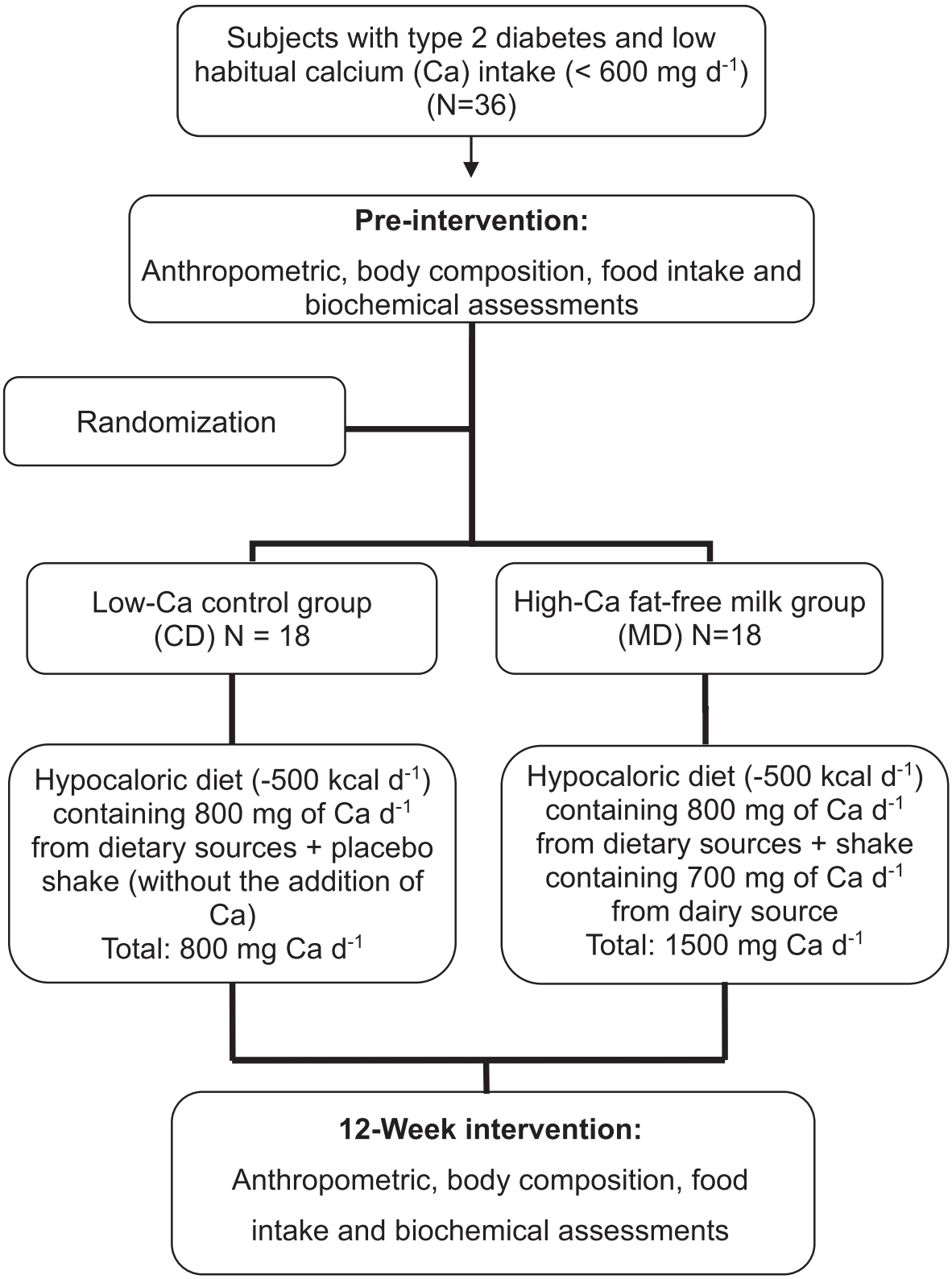

Fig. 1. Study design.

experimental group was assessed using three non-consecutive days (two weekdays and a weekend day) food records. Participants were trained to keep free-feeding dietary records at baseline to increase data reliability. Each dietary record was reviewed with the participants to ensure accuracy and completeness. Moreover, a food portion photo album was used to improve data quality (Monteiro, Pfrimer, Tremeschin, Molina, & Chiarello, 2007). The amounts of foods registered in household measures were converted into grams for energy intake, macronutrients, Ca, P, Mg, Zn, and dietary fibre intake analyses using DietPro, version 5.1i (July, 2015; Agromídia Software Sistemas Viçosa, Minas Gerais, Brazil). A single dietitian analysed the food records. The revised Goldberg method was used to

Table 1
Ingredients and nutrient composition of the breakfast meals according to study groups.[a]

Ingredients and nutrient composition	Study group	
	MD	CD
Ingredients (1 serving of 500 mL)		
Fat-free milk powder (g)	47.0	0.0
Sugar (g)	0.0	23.5
Whey protein (g)	0.0	18.02
Sodium chloride (g)	0.0	0.7
Micronutrient supplement (mg)	0.0	4.0
Frozen fruit pulps or chocolate powder (g)	100 or 10	100 or 10
Water (mL)	250	250
Nutrient composition (mean ± SD) of the twelve shake types		
Energy (kcal)	197.8 ± 11.1	198.0 ± 11.1
Carbohydrate (g)	31.9 ± 2.9	31.9 ± 2.9
Fibre (g)	1.9 ± 2.2	1.9 ± 2.2
Protein (g)	17.1 ± 0.2	17.1 ± 0.2
Fat (g)	0.1 ± 0.3	0.1 ± 0.3
Calcium (mg)	710.5 ± 3.7	6.4 ± 3.7***
Iron (mg)	10.0 ± 0.1	10.1 ± 0.1
Phosphorus (mg)	799.5 ± 7.8	13.7 ± 7.4***
Mg (mg)	58.9 ± 3.9	7.8 ± 3.9***
Sodium (mg)	280.9 ± 3.3	280.9 ± 3.3
Zinc (mg)	1.91 ± 0.1	0.12 ± 0.1***
Vitamin A (mcg)	423.0 ± 0.0	423.0 ± 0.0
Vitamin C (mg)	163.8 ± 243.3	163.8 ± 243.3
Vitamin D (mcg)	3.5 ± 0.0	3.5 ± 0.0

[a] Abbreviations are: MD, high-calcium fat-free milk diet; CD, low-calcium control diet. Ingredients and nutrient composition were calculated by DietPro® software or according to food labels. The micronutrient powder supplement containing iron (iron chelate) and vitamins A (retinol acetate), C (ascorbic acid), and D3 (cholecalciferol) was prepared by a certified compounding pharmacy. Shakes presented similar flavours (frozen fruit pulps or chocolate powder), total of six different flavours. All data were parametric; ***$P < 0.001$ by Student's t test.

categorise misreported reported energy intake (rEI) (WHO, 2000). Participants were classified as underreporters, acceptable reporters, or overreporters using the ratio of rEI to total energy expenditure (TEE). TEE was calculated from the product of basal metabolic rate (FAO/WHO/UNU, 1985) and PAL. PAL was assumed to be 1.55 (light activity) for all subjects (FAO/WHO/UNU, 1985). A 95% confidence interval (CI) was calculated, and individuals who fell outside of the CI 95% were classified as under or overreporters and were excluded from statistical analysis.

2.7. Anthropometric and body composition measurements

Participants fasted overnight. All measurements were assessed while the participants were barefoot and wearing light clothing. Participants abstained from strenuous exercise, as well as caffeine and alcohol consumption 48 h prior to these assessments. Women were not in the menstrual period.

Body weight was assessed using an electronic platform scale (Model 2096 PP, Toledo, São Bernardo do Campo, São Paulo, Brazil) with a capacity for 150 kg and precision of 50 g. Height was measured using a stadiometer with a scale of 0–220 cm, precision 0.1 cm (SECA 206, Seca, São Paulo, São Paulo, Brazil). Both measurements were performed according to Jellife (1968). Body mass index (BMI) was calculated from the ratio of weight (kg) to height squared (m^2). WC and hip circumference (HipC) were measured using a flexible inelastic tape. WC was measured at the midpoint between the lowest rib and the iliac crest with a precision of 0.1 cm (Wang, Manson, Buring, Lee, & Sesso, 2008), and HipC was measured at the largest circumference between the anterior iliac crest and the greater trochanter (WHO, 2011). WHR was calculated by dividing WC by HipC.

Body composition was assessed using a Prodigy densitometer (GE Lunar Medical Systems, Milwaukee, WI, USA). The scanner was calibrated daily against the standard calibration block supplied by the manufacturer to control for possible baseline drift. The participants laid supine on the bed and were scanned from head to toe. The scanner uses a narrow fan beam (4.5°) parallel to the longitudinal axis of the body. Scans were analysed using EnCoreTM, version 13.5. The manufacturer's algorithms provide a three-compartment analysis consisting of non-bone lean tissue mass, FM, and bone mineral content (BMC) ash. FFM was defined as the sum of lean tissue mass and BMC. Data from BMC were not shown. In our laboratory, the within coefficients of variation for the measurement of percentage fat mass (%FM) and percentage fat-free mass (%FFM) in five participants, measured twice (with repositioning), were 1.9% and 2.0%, respectively (data not shown).

2.8. Biochemical assays

Venous blood samples were obtained after 12 h of overnight fasting. Serum glucose and serum triglycerides levels were measured by enzymatic colorimetric assay (BS200, Mindray, Diagnostic Laboratory Instrument Inc., Shenzhen, Guangdong, China). The HbA1c was measured by an ion-exchange high performance liquid chromatography (HPLC). Serum fructosamine levels were measured by a colorimetric nitroblue tetrazolium assay (Technicon Co., Oakland, California, United States of America or EUA). Serum insulin levels were measured with an electrochemiluminescence immunoassay (Elecsys Modular-E-170, Roche Diagnostics Systems, Burgess Hill, West Sussex, England).

IR was assessed by: (i) HOMA-IR (homoeostasis model assessment of insulin resistance), calculated according to the formula.

$$\text{Glycaemia (mmol)} \times \text{Insulin}\left(\text{uU mL}^{-1}\right)/22.5 \quad (1)$$

(Matthews et al., 1985); (ii) HOMA2-IR, calculated by HOMA Calculator software (available at https://www.dtu.ox.ac.uk/homacalculator/); (iii) TyG, a product of fasting glucose and serum triglycerides, calculated according to the formula:

$$\text{Ln triglyceridaemia}\left(\text{mg dL}^{-1}\right) \times \text{glycaemia}\left(\text{mg dL}^{-1}\right)/2 \quad (2)$$

(Simental-Mendía, Rodríguez-Morán, & Guerrero-Romero, 2008).

2.9. Statistical analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences for Windows, version 20.0 (IBM). All variables were examined for normality of distribution according to the Shapiro–Wilk test at 5% significance. Data are expressed as means and standard deviations, unless otherwise indicated. Bartlett test, at 1% significance, was applied to assess the homogeneity of the residual variances. Data within groups were analysed using paired t-test or Wilcoxon rank sum test, pairing results from the same individual before (baseline) and after 12 weeks of intervention (CD or MD group), considering P values ≤ 0.05 as significant. Data on changes from the baseline over the 12 weeks of the intervention (deltas, i.e., the final value minus the baseline value) were compared between the groups using the t-test or Mann–Whitney test, with Bonferroni correction for multiple comparisons. The criterion of significance was $P < 0.025$, two tailed.

3. Results and discussion

This study was designed to determine whether a high-Ca intervention could be effective in promoting glycaemic control in subjects with T2DM. There is a critical gap in the literature since only a few clinical trials involved T2DM subjects.

A total of 68 subjects were contacted and 40 were eligible according to the inclusion and exclusion criteria, but two refused to participate. Thirty-eight subjects were therefore initially included in the study, but two dropped out after enrolment due to personal reasons (one from each group); thirty-six subjects completed the study (the numbers of men/women were 6/12 and 9/9 in MD and CD groups, respectively). Mean age was 48.9 ± 9.6 years for MD group and 48.4 ± 8.3 years for CD group. This study had a power of 80% to detect a reduction of 1% in HbA1c, considering a standard deviation of 2.2%. Anthropometric and body composition measurements, biochemical variables, and food intake did not differ between groups at baseline (Table 2).

Although the breakfast shakes containing the amount of Ca tested in the study were consumed in the laboratory, subjects received diet prescriptions, which were consumed under free living conditions. The analyses of the dietary records obtained during the study indicated that the prescribed energy restriction was not followed by the subjects as expected. However, Ca intake increased after the MD intervention compared with baseline and with the CD group ($P < 0.01$). Body weight, BMI, and WHR decreased in the MD group ($P < 0.05$), but not in the CD group ($P > 0.05$). WC and FM decreased, while FFM increased in both groups after 12 week intervention compared with baseline.

Anthropometric and body composition measurements did not differ between groups at the end of the study ($P > 0.05$) (Table 2). There was a greater reduction in the MD group WC over 12 weeks from the baseline (delta) compared with CD group ($P = 0.004$) (Table 3). Our results suggest that moderate energy restriction associated with Ca intake of ~1200 mg d^{-1} during 12 weeks enhanced central adiposity reduction (assessed by WC measurement) in overweight subjects with T2DM. Body weight, BMI, and FM decrease, and FFM increase were similar between groups, which suggest redistribution of body fat in the MD group. This is a relevant result, since central adiposity has been associated to beta-cell failure and inflammation, exacerbating T2DM micro and macrovascular complications (Laakso & Kuusisto, 2014).

Similar results were observed in another randomised parallel clinical trial involving 39 obese subjects, who consumed an energy restricted diet (−800 kcal d^{-1}) containing high Ca (1200–1300 mg d^{-1}) compared with low-Ca diet (<500 mg d^{-1}) over 16 weeks (Torres et al., 2010). Weight loss and glycaemic

Table 3
Anthropometric, body composition, and biochemical data changes from baseline (deltas), according to study group.[a]

Parameter	MD group (N = 18)		CD group (N = 18)	
	Mean Δ	SD	MeanΔ	SD
Anthropometry and body composition				
Body weight (kg)	−4.6	7	−4.8	14.1
BMI (kg m^{-2})	−1.8	2.9	−1.9	5,7
Waist circumference (cm)	−4.8	2.7	−3.1	1.9*
Waist-hip ratio (WHR)	−0.03	0.02	−0.02	0.02
Fat mass (%)	−1.6	6.6	−1.1	1.7
Fat-free mass (%)	1.4	6.4	1.1	1.8
Biochemical variables				
Triglycerides (mg dL^{-1})	−25.4	75.9	−10.9	56.3
Fructosamine (μmol L^{-1})	−7.7	35.4	−8.5	49.5
Hb1Ac (%)	−0.4	1.4	−0.6	0.8
Fasting insulin (μUI mL^{-1})	−0.4	4.3	1.0	5.5
Fasting glycaemia (mg dL^{-1})	−1.9	34.3	−15.6	33.5
HOMA-IR	−0.2	1.2	0.0	2.2
HOMA-IR 2	−0.1	0.5	0.0	0.8
TyG index	−0.1	0.5	0.0	0.1

[a] Abbreviations are: MD group, high-calcium fat-free milk group; CD group, low-calcium group; Hb1Ac: glycated haemoglobin; HOMA-IR, homoeostasis model assessment of insulin resistance, TyG index, triglyceride-glucose index. Except for body weight, fat mass, serum triglycerides and fasting glycaemia, variables were parametric; *$P < 0.025$. P-value was estimated by paired t-test or Wilcoxon rank sum (both with Bonferroni correction for multiple comparisons).

Table 2
Anthropometric, body composition, biochemical and food intake data at baseline and after 12-week intervention, according to study group.[a]

Characteristics	Baseline	Final	Baseline	Final
	MD group (N = 18)	MD group (N = 18)	CD group (N = 18)	CD group (N = 18)
Anthropometry and body composition				
Body weight (kg)	80.8 ± 15.6	76.1 ± 16.2*	85.6 ± 17.1	80.7 ± 24.6
BMI (kg m^{-2})	30.4 ± 4.7	28.6 ± 4*	31.0 ± 4.9	30.4 ± 4.8
Waist circumference (cm)	98.3 ± 10.8	93.5 ± 11*	101 ± 11.2	97.9 ± 10.6*
Waist-hip ratio (WHR)	0.98 ± 0.06	0.95 ± 0.07*	0.99 ± 0.05	0.97 ± 0.06
Fat mass (%)	38.8 ± 7.8	37.2 ± 8.5*	36.5 ± 9.1	35.4 ± 8.8*
Fat-free mass (%)	59.1 ± 7.5	60.5 ± 8.1*	61.2 ± 8.4	62.2 ± 8.1*
Biochemical variables				
Triglycerides (mg dL^{-1})	175.6 ± 97.6	150.2 ± 80.1	134.2 ± 71.1	123.2 ± 57.2
Fructosamine (μmol L^{-1})	310.5 ± 80.4	300.2 ± 94.5	324.3 ± 89.6	315.8 ± 77.8
Hb1Ac (%)	7.6 ± 1.9	7.2 ± 2.4*	8.3 ± 2.5	7.7 ± 2.1*
Fasting insulin (μUI mL^{-1})	9.4 ± 3.8	8.9 ± 4.6	7.8 ± 3.8	8.8 ± 5.0
Fasting glycaemia (mg dL^{-1})	145.1 ± 54.1	143.2 ± 67.0	163.6 ± 79.0	148.0 ± 56.3*
HOMA-IR	3.4 ± 1.8	3.1 ± 1.7	2.9 ± 1.9	2.9 ± 1.6
HOMA2-IR	1.4 ± 0.5	1.3 ± 0.6	1.2 ± 0.6	1.3 ± 0.7
TyG index	2.2 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.1
Food intake				
Energy (kcal d^{-1})	1867.1 ± 530.5	1759.2 ± 427.5	1989.7 ± 471.7	1890.9 ± 340.9
Carbohydrates (g d^{-1})	251.5 ± 92.1	244.2 ± 58.4	259 ± 66	220.1 ± 28.6
Proteins (g d^{-1})	69.2 ± 18.8	82.3 ± 19*	69.5 ± 20.9	82.7 ± 22.7
Total fat (g d^{-1})	66.4 ± 27.2	48 ± 16.9	66.4 ± 27.1	56.5 ± 38.9
Calcium (mg d^{-1})	414.6 ± 202.4	1193.5 ± 146.1*	462.2 ± 157.1	478.8 ± 201.1

[a] Abbreviations are: MD group, high-calcium fat-free milk group; CD group, low-calcium group; Hb1Ac: glycated haemoglobin; HOMA-IR: homoeostasis model assessment of insulin resistance; TyG index: triglyceride-glucose index. Data are means ± standard deviation. Baseline characteristics did not differ between groups:*, intra-group statistical difference ($P < 0.05$); **, intergroup statistical difference in the final moment ($P < 0.05$). Except from carbohydrate intake, serum triglycerides and fasting glycaemia, other variables were parametric. Baseline and final (12-week intervention) data were analysed using paired t-test or Wilcoxon test; between groups data were analysed using Student t test or Mann–Whitney test.

control were similar in both groups, but the high-Ca diet promoted greater abdominal fat reduction (assessed through WC measurement; Torres et al., 2010). The mechanism suggested for this reduction in central adiposity is not completely clear, though a reduction in cortisol production has been suggested. High-Ca diets suppress 1,25-dihydroxyvitamin D production, which increases 11 beta-hydroxysteroid dehydrogenase type 1, an enzyme that converts inactive cortisone to active cortisol (Soares et al., 2012). Consequently, the decrease in cortisol production from adipose tissue may contribute to the preferential loss of visceral adiposity by high-Ca diets (Soares et al., 2012).

Similar weight loss was observed in overweight subjects (N = 49) who received energy restricted diets (−500 kcal d^{-1}) with low (700 mg d^{-1}) or high-Ca content (1400 mg d^{-1}) for 12 weeks, in a randomised parallel clinical trial (Jones et al., 2013). Although weight loss did not differ between experimental groups, high-Ca diet modestly reduced appetite, suggesting the occurrence of a long-term beneficial effect (Jones et al., 2013). No changes in WC were observed in both groups, possibly due to high habitual Ca intake in the control group (943 ± 127 mg d^{-1}) and in the dairy/Ca group (884 ± 82 mg d^{-1}) (Jones et al., 2013). Zemel et al. (2009) indicate that the benefits of increased dietary Ca consumption has been observed only in low habitual Ca consumers (<700 mg d^{-1}).

HbA1c decreased in both groups ($P = 0.007$ for MD group and CD group). Fasting glucose decreased only in the CD group ($P = 0.05$) after 12 weeks of intervention (Table 2). Other biochemical parameters and their deltas (changes from baseline) did not differ from baseline and between groups ($P > 0.05$) (Tables 2 and 3). Contrary to our results, some authors observed greater glycaemic control and/or decrease in IR after the consumption of high-Ca diets (Nikooyeh et al., 2011; Stancliffe et al., 2011). In metabolic syndrome obese subjects (N = 40), the consumption of 3.5 portions per day of whole dairy products (~1000 mg of Ca d^{-1}) reduced fasting insulin levels compared with the consumption of 0.5 portion per day of whole dairy products (~150 mg of Ca d^{-1}), over 12 weeks (Stancliffe et al., 2011). Insulin sensitivity (measured by the HOMA-IR) increased after the high-dairy consumption (Stancliffe et al., 2011). In our study, HOMA-IR remained unchanged in both groups. We believe that the differences in these results are due to the characteristics presented by subjects at baseline. Stancliffe et al. (2011) selected subjects with metabolic syndrome and normal glycaemia. Our subjects presented hyperglycaemia at baseline, and were therefore probably less responsive to high-Ca diet due to their worse metabolic condition. In that study, high-dairy consumption also reduced oxidative stress and inflammation (Stancliffe et al., 2011), parameters that were not assessed in our study. We also evaluated IR by measuring the TyG index, which did not change between groups. This index seems to predict the development of cardiovascular events (Sánchez-Íñigo, Navarro-González, Fernández-Montero, Pastrana-Delgado, & Martínez, 2016) and it has been considered superior than HOMA-IR to predict IR (Vasques et al., 2011).

Other authors observed reductions in Hb1Ac and fasting glycaemia in overweight subjects with T2DM (N = 30) who received vitamin D-fortified (500 UI of vitamin D), or vitamin D and Ca-fortified (500 UI of vitamin D and 150 mg of Ca d^{-1}) yogurt drink, or placebo (no vitamin D and 150 mg of Ca d^{-1}) (Nikooyeh et al., 2011). Daily intake of a vitamin D-fortified yogurt drink, either with or without added Ca, improved glycaemic status in patients with diabetes. That result suggests that dietary Ca was not capable of improving glycaemic control (Nikooyeh et al., 2011). However, it is difficult to isolate the vitamin D effects, considering that vitamin D is crucial in regulating Ca absorption in the small intestine (Kopic & Geibel, 2013). Besides, 35% of the subjects were vitamin D deficient at baseline (Nikooyeh et al., 2011), and Ca intake did not differ between groups at baseline and after interventions (Nikooyeh et al., 2011). In our study, fasting glucose decreased only in the CD group, while HbA1c reduced in both groups. HbA1c has greater pre-analytical stability, greater association with cardiovascular diseases and T2DM complications, and less day-to-day perturbations than fasting glucose (ADA, 2016). So, in our study high- and low-Ca diets promoted similar improvements on glycaemic control. Considering that WC reduction favours an enhancement on insulin sensitivity (ADA, 2016; Soares et al., 2011, 2012), it is possible that the reduction in central fat observed in our study could improve the glycaemic profile after a longer period.

A potential mechanism by which high-Ca intake improves glycaemic control is its effects on weight loss and adiposity reduction. Increased calcitriol produced in response to low-Ca diets stimulates Ca^{2+} influx in human adipocytes and thereby promotes adiposity (Soares et al., 2011, 2012). On the other hand, an adequate Ca intake may indirectly enhance calcitriol status, reducing the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (Kopic & Geibel, 2013). Vitamin D enhances insulin sensitivity and improves β-cell function (Kopic & Geibel, 2013). Ca is regulator of glucose-induced insulin release and it plays a critical role in muscle contraction (Gilon, Chae, Rutter, & Ravier, 2014). That mineral also modulates the interaction of insulin with its receptor, increasing insulin sensitivity (Gilon et al., 2014).

The main limitation of our study is that we did not assess visceral and subcutaneous fat contents, which could confirm Ca effect on body fat redistribution. The small sample size of our study limited the statistical power to conduct a multivariate statistical analysis. However, the randomization process was carefully conducted by us. Because of that, the intervention groups (MD and CD) presented similar baseline body composition, besides clinical, biochemical, and anthropometric characteristics. It is also possible that other components of fat-free milk contributed to a synergistic effect after the consumption of MD diet. Milk proteins and bioactive peptides seem to increase satiety, thermogenesis, and lean mass loss (Acheson et al., 2011; Soares et al., 2012). Leucine favours fat oxidation, and recovery of muscle protein synthesis, thus avoiding FFM loss (Longland, Oikawa, Mitchell, Devries, & Phillips, 2016). That amino acid has also been associated with greater insulin sensitivity and lower oxidative stress (Hirahatake, Slavin, Maki, & Adams, 2014). Other milk minerals such as Zn and Mg play a key role on insulin action. Zn is involved in the synthesis, storage and secretion of insulin, as well as maintaining the insulin hexameric conformational integrity (Yahya, Yahya, & Saqib, 2011). Mg is essential for insulin secretion and it acts as a cofactor of various enzymes involved in carbohydrates metabolism (Yahya et al., 2011).

4. Conclusion

The consumption of energy-restricted diet with high-Ca compared with low-Ca content derived from fat-free milk (~1200 mg d^{-1}) over 12 weeks was more effective in reducing abdominal fat in subjects with T2DM. Dietary intervention improved glycaemic control, independently of Ca content. The findings from this study are novel, and have potentially important clinical implications, since few previous clinical trials assessed Ca supplementation in patients with T2DM. However, long-term studies involving subjects with T2DM are needed to better understand the effects of high-Ca intake on glycaemic profile, since the reduction in central adiposity may favour insulin sensitivity.

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