

Body composition changes after very low-calorie-ketogenic diet in obesity evaluated by three standardized methods

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Context: A common concern when using low-calorie diets as a treatment for obesity is the reduction in fat-free mass, mostly muscular mass that occurs together with the fat mass loss, and the best methodologies to evaluate body composition changes.

Objective: To evaluate the very low-calorie-ketogenic (VLCK) diet-induced changes in body composition of obese patients and to compare three different methodologies employed to evaluate that changes.

Design: Twenty obese patients followed a VLCK diet for 4 months. Body composition was performed by dual-energy X-ray absorptiometry (DXA), multifrequency bioelectrical impedance (MF-BIA) and air displacement plethysmography (ADP) techniques. Muscular strength was also assessed. Measurements were performed at four points matched with the ketotic phases (basal, maximum ketosis, ketosis declining and out of ketosis).

Results: After 4-months the VLCK diet induced a -20.2 ± 4.5 kg weight loss, at expenses of reductions in fat mass (FM) of -16.5 ± 5.1 kg (DXA), -18.2 ± 5.8 kg (MF-BIA), and -17.7 ± 9.9 kg (ADP). A significant decrease was also observed in the visceral FM. The mild but significant reduction in fat-free mass occurred at maximum ketosis mainly due to changes in the total body water and was recovered thereafter. No changes in muscle strength were observed. A strong correlation was evidenced between the three methods of body composition.

Conclusion: The VLCK diet-induced weight loss was mainly at the expense of FM and visceral mass, preserving muscle mass and strength. From the three employed body composition techniques, the multifrequency bioelectrical impedance seems more convenient in the clinical setting.

Excess fat mass, especially visceral fat, is associated with a variety of pathological conditions such as, hypertension, dyslipidemia, diabetes and even cancer (1–4), as well as with an increase in overall and cardiovascular mor-

talidity (2, 5). Very low calorie diets are commonly used as obesity treatments, however, one of the main concerns in using such diets is the amount of fat-free mass (FFM) (ie, muscular tissue) that is lost together with the fat mass

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Abbreviations:

(FM) (6). This could produce the so-called sarcopenic obesity, ie, the coexistence of an excess of FM and a decline in FFM yielding a near normal body weight (7). Such situation constitutes a double impact on the health of patients, because the reduction in muscle mass and muscle strength is also a cause of cardiometabolic disorders, such as myocardial infarction (MI) and stroke, and other adverse health outcomes (5, 7–9). For such reasons, it is of primary interest to find weight loss strategies that promote preferential loss of FM and preservation of muscle mass and its functional status (ie, muscle strength) (10–12).

Some previous studies have suggested that very low-calorie-ketogenic (VLCK) diets may be effective tools to manage overweight and obesity (10, 11, 13). VLCK diets

are a nutritional intervention that emulates fasting by restricting carbohydrates and fat with a relative increase in protein intake (6). The increased protein content may be partially responsible of the muscle mass preservation (12–14). Importantly, the weight reducing action of these diets is rapid and despite the fact that the ketosis state only lasts 60–90 days in the beginning of the treatment, the weight reduction remains for up to two years (13). Therefore VLCK diets operate by potent mechanisms to induce weight loss, and various body compartments might be altered. To the best of our knowledge, no studies have exhaustively assessed the changes in body composition associated with this type of diet, and variations of muscle strength have been only assessed in athletes (15).

Table 1. Evolution of body composition during the entire study determined by Dual-energy x-ray absorptiometry (DXA), Multi frequency bioelectrical impedance (MF-BIA) and Air-displacement plethysmography (ADP)

Variable	Visit C-1	Visit C-2	Visit C-3	Visit C-4	P Value
Diet time (days)	0	39.2 (8.4)	89.7 (19.1)	123.3 (17.6)	
B-hydroxy-butyrate (mmol/liter)	0.0 (0.1)	1.0 (0.6) ^a	0.7 (0.5) ^{a,b}	0.2 (0.1) ^{b,c}	<0.001
Anthropometric measurements					
Weight (kg)	95.9 (16.3)	84.2 (13.0) ^a	76.6 (11.1) ^{a,b}	75.1 (11.8) ^{a,b}	<0.001
Body mass index (kg/m ²)	35.5 (4.4)	31.2 (3.3) ^a	28.4 (2.6) ^{a,b}	27.8 (2.9) ^{a,b}	<0.001
Waist circumference (cm)	109.4 (12.8)	98.0 (11.0) ^a	90.0 (9.3) ^{a,b}	88.6 (10.1) ^{a,b}	<0.001
DXA					
Fat mass (kg)	42.2 (9.1)	35.0 (7.8) ^a	27.8 (5.7) ^{a,b}	25.7 (5.8) ^{a,b,c}	<0.001
Fat mass percentage (%)	45.6 (5.4)	43.1 (6.1) ^a	37.9 (5.8) ^{a,b}	35.7 (5.9) ^{a,b,c}	<0.001
Visceral fat mass (kg)	2.18 (1.28)	1.46 (0.86) ^a	1.01 (0.58) ^{a,b}	0.95 (0.62) ^{a,b}	<0.001
Visceral fat volume (cm ³)	2313.6 (1358.0)	1551.8 (912.3) ^a	1062.9 (612.9) ^{a,b}	1008.4 (659.2) ^{a,b}	<0.001
Fat-free mass (kg)	52.8 (10.2)	48.5 (9.2) ^a	48.3 (9.1) ^a	49.0 (9.7) ^a	<0.001
Lean body mass (kg)	50.2 (9.9)	46.0 (8.8) ^a	45.8 (8.7) ^a	46.5 (9.3) ^a	<0.001
Bone mineral content (kg)	2.59 (0.49)	2.59 (0.48)	2.57 (0.49) ^{a,b}	2.55 (0.48) ^{a,b}	<0.001
Bone mineral density (g/cm ²)	1.24 (0.13)	1.24 (0.13)	1.24 (0.12)	1.24 (0.13)	0.663
Appendicular lean mass (kg)	23.5 (5.7)	21.4 (5.0) ^a	20.8 (4.6) ^{a,b}	21.0 (4.8) ^a	<0.001
MF-BIA					
Fat mass (kg)	40.0 (10.0)	31.4 (8.4) ^a	23.5 (6.8) ^{a,b}	21.7 (6.6) ^{a,b}	<0.001
Fat mass percentage (%)	41.6 (6.4)	37.3 (7.5) ^a	30.8 (7.5) ^{a,b}	29.0 (7.4) ^{a,b,c}	<0.001
Visceral fat area (cm ²)	154.1 (31.9)	126.8 (30.9) ^a	99.3 (26.7) ^{a,b}	93.3 (28.2) ^{a,b}	<0.001
Fat-free mass (kg)	55.8 (11.0)	52.7 (10.6) ^a	53.0 (10.5) ^a	53.4 (11.0) ^a	<0.001
Total body water (kg)	41.0 (8.1)	38.7 (7.8) ^a	38.9 (7.7) ^a	39.1 (8.1) ^a	<0.001
Intracellular water (kg)	25.5 (5.1)	24.0 (4.9) ^a	24.1 (4.8) ^a	24.2 (5.1) ^a	<0.001
Extracellular water (kg)	15.5 (3.0)	14.6 (2.9) ^a	14.8 (2.9) ^a	14.9 (3.0) ^a	<0.001
Skeletal muscle mass (kg)	31.2 (6.6)	29.3 (6.4) ^a	29.4 (6.3) ^a	29.6 (6.6) ^a	<0.001
Appendicular soft lean mass (kg)	23.0 (5.0)	21.7 (4.7) ^a	21.4 (4.7) ^a	21.3 (4.9) ^a	<0.001
ADP					
Fat mass (kg)	44.0 (11.1)	36.1 (11.4) ^a	27.9 (7.4) ^{a,b}	26.3 (8.5) ^{a,b}	<0.001
Fat mass percentage (%)	45.9 (8.5)	42.6 (9.4)	36.9 (10.3) ^a	34.7 (8.3) ^{a,b}	<0.001
Fat-free mass (kg)	51.8 (12.3)	48.0 (10.8)	48.6 (12.1)	48.8 (9.5)	0.124
Muscle strength					
Handgrip strength (kg)	35.0 (9.9)	35.5 (8.9)	34.3 (11.2)	34.1 (10.5)	0.417
HG / ALM	1.4 (0.2)	1.6 (0.2) ^a	1.6 (0.3) ^a	1.6 (0.2) ^a	0.001
HG / ASLM	1.5 (0.2)	1.6 (0.2)	1.5 (0.3)	1.5 (0.2)	0.056

Data are presented as mean (standard deviation). HG: Handgrip strength; ALM: Appendicular lean mass (DXA); ASLM: Appendicular soft lean mass (MF-BIA). P value = repeated measures ANOVA for differences between measurement times (Visits C-1, C-2, C-3, C-4). Tukey's adjustment was used for multiple comparisons: ^a $P < 0.05$ in comparison with Visit C-1; ^b $P < 0.05$ in comparison with Visit C-2; ^c $P < 0.05$ in comparison with Visit C-3.

Hence, the two main objectives of this study were to assess the changes in body composition and muscle strength promoted by a VLCK diet in the treatment of obese patients and to compare three different methodologies employed to evaluate body composition. To achieve this, body composition was evaluated by three potent and well-validated techniques (dual-energy X-ray absorptiometry (DXA), multifrequency bioelectrical impedance analysis (MF-BIA), and air displacement plethysmography (ADP) at different stages during the weight reduction process induced by a VLCK diet.

Materials and Methods

Study design

This study was a nutritional intervention clinical trial, open, uncontrolled, prospective for 4 months, and performed in a single center.

Study population

The patients attending the Obesity Unit at the Complejo Hospitalario Universitario of Santiago de Compostela, Spain, to receive treatment for obesity were consecutively invited to participate in this study.

The inclusion criteria were, age 18–65 years, body mass index (BMI) ≥ 30 kg/m², stable body weight in the previous 3 months, desire to lose weight, and history of failed dietary efforts. The main exclusion criteria were diabetes mellitus, obesity induced by other endocrine disorders or by drugs, and participation in any active weight loss program in the previous 3 months. In addition, those patients with previous bariatric surgery, known or suspected abuse of narcotics or alcohol, severe depression or any other psychiatric disease, severe hepatic insufficiency, any type of renal insufficiency or gout episodes, nephrolithiasis, neoplasia, previous events of cardiovascular or cerebrovascular disease, uncontrolled hypertension, orthostatic hypotension, and hydroelectrolytic or electrocardiographic (ECG) alterations, were excluded. Females who were pregnant, breast-feeding, or intending to become pregnant, and those with child-bearing potential and not using adequate contraceptive methods, were also excluded. Apart from obesity and metabolic syndrome, participants were generally healthy individuals.

The study protocol was in accordance with the Declaration of Helsinki and was approved by the Ethics Committee for Clinical Research of Galicia, Santiago de Compostela, Spain (registry 2010/119). Participants gave informed consent before any intervention related to the study. Participants received no monetary incentive.

Nutritional intervention

All the patients followed a VLCK diet according to a commercial weight loss program (PNK method), which includes lifestyle and behavioral modification support. The intervention included an evaluation by the specialist physician conducting the study, an assessment by an expert dietician. All patients underwent an structured program of physical exercise, with external supervision (16). This method is based on a high-biological-value

protein preparations obtained from cow-milk, soya, avian eggs, green-peas and cereals. Each preparation contained 15g protein, 4g carbohydrates, 3g fat, and 50 mg docosahexaenoic acid, and provided 90–100 kcal (16).

The weight loss program has five steps (Supplemental Figure 1) and adheres to the most recent guidelines of 2015 EFSA on total carbohydrate intake (17). The first three steps consist of a VLCK diet (600–800 kcal/d), low in carbohydrates (<50g (26–30g) daily from vegetables) and lipids (only 10g of olive oil per day). The amount of high-biological-value proteins ranged between 0.8 and 1.2g per each kg of ideal body weight, to ensure meeting the minimal body requirements and to prevent the loss of lean mass. In step 1, the patients ate high-biological-value protein preparations five times a day, and vegetables with low glycemic indexes. In step 2, one of the protein servings was substituted by a natural protein (eg, meat and fish) either at lunch or at dinner. In step 3, a second serving of low fat natural protein substituted for the second serving of biological protein preparation. Throughout these ketogenic phases, supplements of vitamins and minerals, such as K, Na, Mg, Ca, and omega-3 fatty acids, were provided in accordance to international recommendations (18). These three steps were maintained until the patient lost the target amount of weight, ideally 80%. Hence, the ketogenic steps were variable in time depending on the individual and the weight loss target.

In steps 4 and 5, the ketogenic phases were ended by the physician in charge of the patient based on the amount of weight lost, and the patient started a low-calorie diet (800–1500 kcal/d). At this point, the patients underwent a progressive incorporation of different food groups and participated in a program of alimentary re-education to guarantee the long-term maintenance of the weight loss. The maintenance diet, consisted of an eating plan balanced in carbohydrates, protein, and fat. Depending on the individual the calories consumed ranged between 1500 and 2000 kcal/d, and the target was to maintain the weight lost and promote healthy life styles.

During the present study, patients followed the different steps of the method until reach the target weight or up to a maximum of 4 months of follow-up, although patients remained under medical supervision for the following months.

Schedule of visits

Throughout the study the patients completed a maximum of 10 visits with the research team (every 15 ± 2 days), of which 4 were for a complete physical, anthropometric and biochemical assessment, and the remaining visits were to control adherence and evaluation of potential side effects. The 4 complete visits were made according to the evolution of each patient through the steps of ketosis as follows: Visit C-1 (baseline), normal level of ketone bodies; Visit C-2, maximum ketosis; Visit C-3, reduction of ketotic approach because of partial reintroduction of normal nutrition; Visit C-4, no ketosis (Supplemental Figure 1). The total ketosis state lasted for 60–90 days only. In all the visits, patients received dietary instructions, individual supportive counsel, and encouragement to exercise on a regular basis using a formal exercise program. Additionally, a program of telephone reinforcement calls was instituted, and a phone number was provided to all participants to address any concern.

Anthropometric assessment

All anthropometric measurements were undertaken after an overnight fast (8 to 10 hours), under resting conditions, in duplicate, and performed by well-trained health workers. Participant's body weights were measured to the nearest 0.1 kg on the same calibrated electronic device (Seca 220 scale, Medical Resources, EPI Inc OH, USA), in underwear and without shoes. BMI was calculated by dividing body weight by the square of height ($\text{BMI} = \text{weight (kg)} / \text{height (m)}^2$). Waist circumference was recorded with a standard flexible nonelastic metric tape at the middle point between the lower edge of the ribs and the iliac anterior spine. This measurement was made at the end of a normal expiration while the subject stood upright.

Handgrip strength

Muscular strength was measured with a Jamar handgrip dynamometer (Lafayette Instruments Company, Lafayette, USA). After a brief demonstration and verbal instructions, the test was done in the standing position with the wrist in the neutral position and the elbow flexed to 90 degrees. Patients were given verbal encouragement to squeeze as hard as possible and apply maximal effort for at least 3 seconds. Two trials were allowed in the dominant limb and the highest score was recorded as peak grip strength (kg). Considering possible influences in the muscular strength of changes in body composition, handgrip strength (HG) was divided by appendicular lean mass (ALM) determined by DXA (HG/ALM) and by appendicular soft lean mass (ASLM) determined by MF-BIA (HG/ASLM).

Total body composition and selective visceral fat mass analysis

Dual-energy X-ray Absorptiometry (DXA)

Total body mass

Total body composition was firstly measured by iDXA (GE Healthcare Lunar, Madison, USA). Daily quality control (QC) scans were acquired during the study period. No hardware or software changes were made during the course of the trial. Subjects were scanned using standard imaging and positioning protocols, while wearing only light clothing. For the present study, the values of bone mineral density (BMD), lean body mass and fat mass (FM) that are directly measured by the GE Lunar Body Composition Software option were used. Some derivative values, such as bone mineral content, regional lean mass, appendicular lean mass (ALM), fat free mass (FFM), and fat mass percentage (FM%), as well as android and ginoïd fat (%) were also calculated. The android/ginoïd ratio was automatically generated and analyzed using enCORE software version 13.6. For measuring android fat, a region of interest was automatically defined as the caudal limit placed at the top of the iliac crest and its high was set to 20% of the distance from the top of the iliac crest to the base of the skull to define the cephalic limit of abdominal subcutaneous adipose tissue. The scanner was calibrated daily against the calibration block supplied by the manufacturer.

Visceral fat mass

Visceral fat was calculated using a newly developed software (Core Scan® GE Healthcare, Madison, USA), which was validated against computed tomography (CT) in patient population

with a wide range of BMI (19, 20). Visceral fat mass data from DXA were transformed into adipose tissue volume using a constant correction factor (0.94 g/cm^3).

Multifrequency bioelectrical impedance

Multifrequency bioelectrical impedance **MF-BIA (In Body 720, Biospace Inc., Tokyo, Japan)** was also used for determining body composition. FM, FM%, FFM, total body water (TBW), intra- and extracellular water, skeletal muscle mass, and appendicular soft lean mass (ASLM). This noninvasive technology employs eight contact electrodes, which are positioned on the palm and thumb of each hand and on the front part of the feet and on the heels. Additionally, multifrequency bioelectrical impedance uses the body's electrical properties and the opposition to the flow of an electric current by different body tissues. The analyzer measured resistance at specific frequencies (1, 5, 50, 250, 500 and 1000 kHz) and reactance at specific frequencies (5, 50, and 250 kHz). The participants were examined while lightly dressed, and the examination took less than two minutes and required only a standing position. The validity of this technology has been documented in previous studies (21, 22). Visceral fat area values were also calculated in cm^2 by MF-BiA. Importantly, these values are significantly correlated with those generated by CT (22, 23). The calculation of the different body compartments was performed according to the manufacturer's instructions (In Body 720, Biospace Inc.)

Air displacement plethysmography

The last technique employed to determine body composition in the current study was air displacement plethysmography ADP (BodPod, Life Measurements Instruments, Concord, Canada), that is accepted as a convenient alternative to the water immersion method of assessing body composition. The standard BodPod protocol was followed (24), and weekly QC tests were performed during the study period with a second calibration conducted immediately prior to the measurement of each participant. ADP determines body volume using Boyle's law of the pressure/volume relationship. Therefore, body volume is equivalent to the decrease of volume in the chamber with the entrance of the patient under isothermal conditions. The participants were instructed to wear a swimming suit tight to the body and a swim cap during the test to diminish accumulated air, and avoiding volume discrepancies. Thoracic gas volume was measured by connecting the subject to a breathing circuit. The process was repeated until a consistent measurement was obtained. Body density was calculated as mass divided by volume and corrected for lung volume. The Siri formula was used to calculate FM, FM% and FFM (24, 25).

Determination of levels of ketone bodies

Ketosis was determined by measuring ketone bodies, specifically B-hydroxy-butyrate (B-OHB), in capillary blood using a portable meter (GlucoMen LX Sensor, A. Menarini Diagnostics, Neuss, Germany) before measurements of anthropometric parameters. As with anthropometric assessments, all the determinations of capillary ketonemia were made after an overnight fast of 8 to 10 hours. These measurements were performed daily by each patient during the entire VLCK diet, and the corresponding values were reviewed on the machine memory by the research team for controlling adherence. Additionally, B-OHB levels were determined at each complete visit by the physician in charge of

the patient. The measurements reported as “low value” (≤ 0.2 mmol/l) by the meter were assumed as zero for purposes of statistical analyses.

Statistical analysis

The data are presented as mean (standard deviation). All statistical analyses were carried out using Stata statistical software, release 12.0 (Stata Corporation, College Station, TX, USA). A $P < .05$ was considered statistically significant. Changes in the different variables of interest from the baseline and throughout the study visits were analyzed following a repeated measures design. A repeated measures analysis of variance (ANOVA) test was used to evaluate differences between different measurement times, followed by post hoc analysis with Tukey's adjustment for multiple comparisons. In addition, linear regression analyses were used to evaluate the accuracy of MF-BIA and ADP in comparison to DXA because DXA is considered the reference technique in the estimation of body composition in clinical research (26). Finally, the Bland-Altman approach was also used to assess the accuracy of MF-BIA and ADP against DXA in the estimation of FM%.

Results

Baseline characteristics of participants

Initially 23 participants were recruited into the study, but 3 dropped out voluntarily during the first week of the intervention for reasons unrelated to diet, and therefore were excluded from analysis. The 20 patients that completed the study exhibited the following baseline characteristics: mean age 47.2 ± 10.2 years, BMI 35.5 ± 4.4 , and waist circumference 109.4 ± 12.8 cm, 12 (60%) were women (Supplemental Table 1). Other baseline characteristics and their corresponding changes during the study are presented in Table 1.

Changes in anthropometric parameters and body composition

Although the patients underwent a total of 10 visits, the complete body composition analyses were synchronized with the ketone levels in four visits (Table 1 and Figure 1). Visit C1 was the baseline visit, before starting the diet with no ketosis (0.0 ± 0.1 mmol/L) and a body weight (bw) of 95.9 ± 16.3 kg. Visit C2 was at the time of maximum level of ketosis (1.0 ± 0.6 mmol/L) with a bw of 84.2 ± 18.0 kg. At Visit C3 (after 89.7 ± 19.1 day of VLCK), patients started the return to a normal diet and showed a reduction in ketone levels (0.7 ± 0.5 mmol/L) and a bw of 76.6 ± 11.1 kg. Finally at Visit C4 the patients were out of ketosis (0.2 ± 0.1 mmol/L) and showed a bw of 75.1 ± 11.8 kg. All weights were statistically different from baseline levels ($P < .05$; Table 1 and Figure 1).

The severe reduction in body weight was mainly due to FM reduction, assessed by DXA scan, the -20.2 kg of

weight reduction at the end of the study were in large part due to the -16.5 kg reduction in FM. When FM component was assessed by MF-BIA, the result was very similar -18.2 kg and was further corroborated by the ADP analysis -17.7 kg (Figure 2A), without statistical differences among them. It was very remarkable for three methods of evaluating body composition that operate through different principles to yield such similar results. FM represents nearly 85% of the total weight losses achieved across the study.

As visceral fat is physiologically and clinically more relevant than total FM, especial emphasis was placed on its analysis. The VLCK diet lead to a significant reduction in visceral fat that can be seen in assessment by either new DXA software (-1.2 ± 0.7 kg) or by MF-BIA (-60.8 ± 20.7 cm²) (Figure 2B-C). Therefore, when evaluated by different methods, the VLCK diet induced a significant body weight reduction by targeting total FM and visceral FM (Figure 2A-C).

The concern regarding the possible loss in muscular mass was focused by a strict analysis of the FFM compartment using DXA, MF-BIA and ADP, and mild but significant reductions in relation to baseline were observed (Figure 3A). The greatest decrease in FFM occurred at the visit of maximum ketosis (Visit C-2), with partial recovery thereafter (Figure 3A). The FFM losses determined by the different techniques at this point were: -4.2 ± 1.8 kg (DXA), -3.1 ± 1.5 kg (MF-BIA), and -3.7 ± 7.6 kg (ADP). At the end of the study (Visit C-4) these FFM

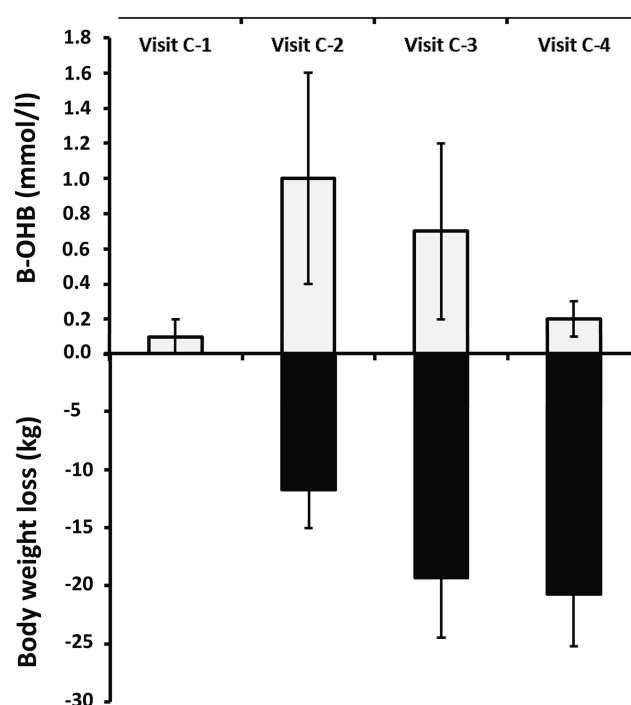


Figure 1. Changes in total body weight and its relationship with levels of ketone bodies

losses had diminished to -3.7 ± 2.0 kg, -2.4 ± 1.8 kg, and -3.0 ± 8.0 kg, respectively.

By discriminating the components of the FFM compartment determined by DXA, it was observed that these variations were mainly due to changes in the lean mass, while bone mineral content remained unchanged from baseline (0.003 ± 0.066 kg Visit C-2; -0.018 ± 0.066 kg Visit C-3; -0.028 ± 0.066 kg Visit C-4; $P > .05$). As DXA technique is unable to discriminate the composition of lean mass, the question was raised as to the observed reductions of lean mass were at the expense of muscle mass or body water content. Therefore, further analysis was performed by multifrequency bioelectrical impedance that is able to discriminate these two variables. Remarkably, the measurements done by MF-BIA showed that the initial loss of FFM at Visit C-2 (-3.1 ± 1.5 kg) was mostly due to

total body water (-2.3 ± 1.1 kg), both intra (-1.5 ± 0.7 kg) and extracellular (-0.8 ± 0.5 kg) (Figure 3B), probably due to the intense diuresis that occurs in the first phase of any VLCK diet. In subsequent visits a slight recovery of intra- and extracellular water was observed, which followed a similar to than that observed with the total FFM. This means that reductions attributable to muscle mass were, depending on the method employed, around 1 kg throughout the 4-months study. Only 5% of FFM from total of 20.2 kg of weight lost.

The observation that VLCK diet severely reduced FM preserving muscle mass was reinforced by the maintenance of its physiological action (ie, muscle strength). Despite a slight reduction in ALM and ASLM determined by DXA and MF-BIA, respectively, crude hand grip strength (HG) remained unchanged during the study (Table 1).

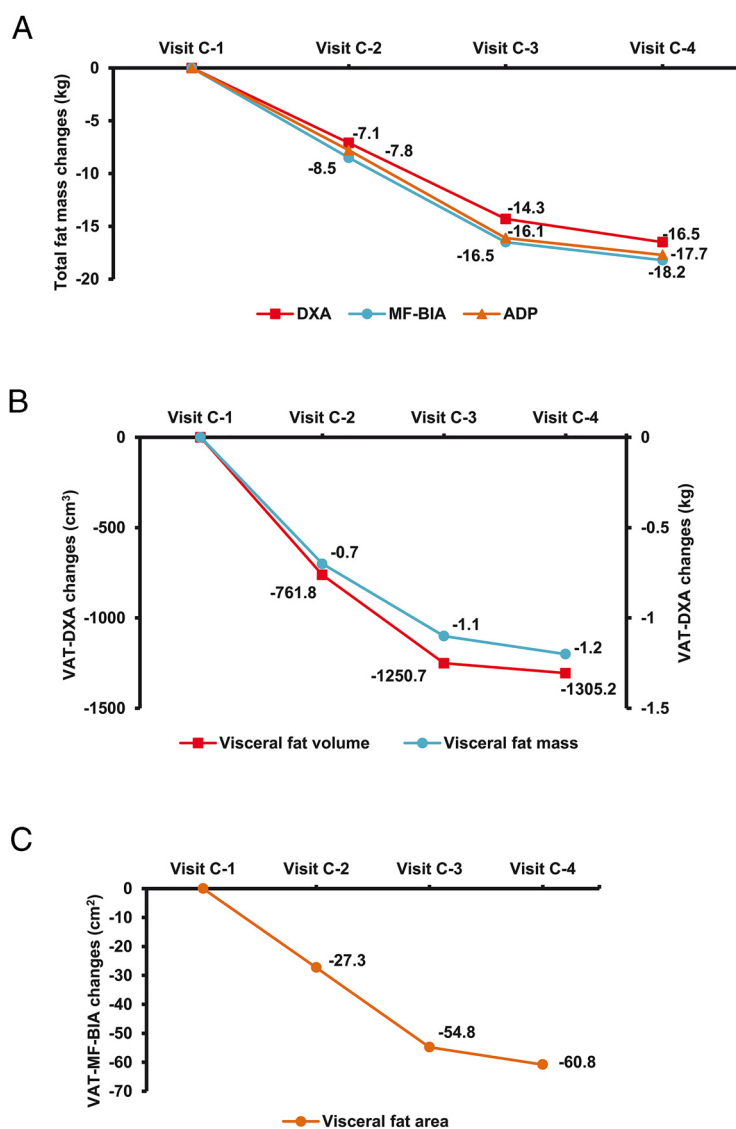


Figure 2. A. Changes in total fat mass in comparison to baseline determined by Dual-energy X-ray absorptiometry (DXA); Multifrequency bioelectrical impedance (MF-BIA); and Air-displacement plethysmography (ADP). B-C. Changes in visceral fat tissue in comparison to baseline determined by Dual-energy X-ray absorptiometry (DXA) and Multifrequency bioelectrical impedance (MF-BIA), respectively.

Even more, HG/ALM and HG/ASLM showed a moderate increase in comparison to baseline (Figure 3C).

Estimation of body composition between different methods

Finally, the accuracy of MF-BIA and ADP in the estimation of body composition was studied in relation to DXA. As shown in Table 2, the unadjusted regression coefficients for FM, FM% and FFM were consistently higher for MF-BIA in comparison to ADP throughout the study. Concretely, regression coefficients of MF-BIA were high ($R^2 > 0.8$) for FM and FFM, while those regression

coefficients for FM% were slightly lower ($R^2 > 0.7$). However, most of the regression coefficients using ADP were lower ($R^2 < 0.7$) for FM, FM% and FFM. A similar pattern was observed when adjusting for age and sex. The regression coefficients for both MF-BIA and ADP decreased with weight loss.

The results of the Bland-Altman approach in regards to the FM% are shown in Figure 4. MF-BIA underestimates the FM% during all visits, although with increasing body fat there is a trend towards a better agreement (Figure 4A). This negative slope is significant in Visits C-2 ($P = .015$), C-3 ($P = .003$), and C-4 ($P = .005$). Importantly, MF-BIA

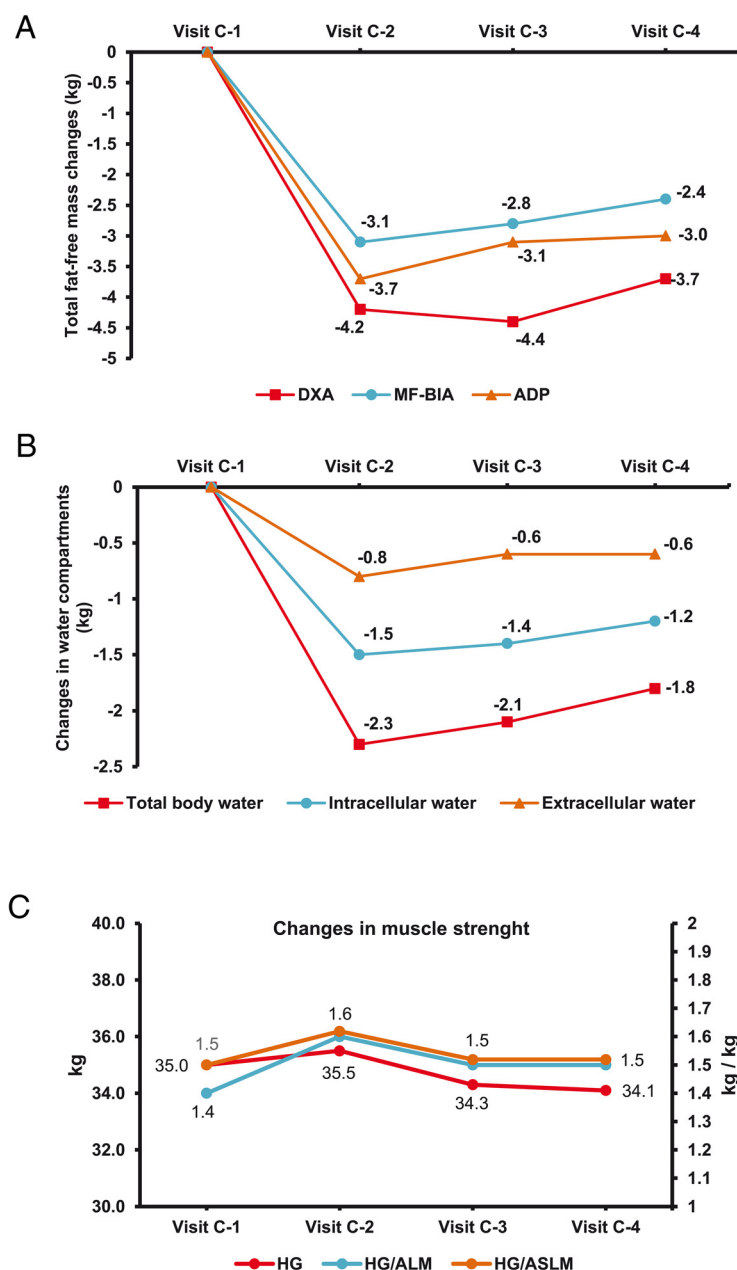


Figure 3. A. Changes in total fat-free mass in comparison to baseline determined by Dual-energy X-ray absorptiometry (DXA); Multifrequency bioelectrical impedance (MF-BIA); and Air-displacement plethysmography (ADP). B. Changes in water compartments evaluated by MF-BIA. C. Changes in muscle strength evaluated by Handgrip Dynamometer. HG: Handgrip strength; ALM: Appendicular lean mass; ASLM: Appendicular soft lean mass.

Table 2. Body composition measured during the entire study in comparison with Dual-energy x-ray absorptiometry (DXA), Multi frequency bioelectrical impedance (MF-BIA) and Air-displacement plethysmography (ADP)

		Visit C-1			Visit C-2			Visit C-3			Visit C-4		
Method	Variable	R ²	95% CI	P Value	R ²	95% CI	P Value	R ²	95% CI	P Value	R ²	95% CI	P Value
Model 1: Unadjusted													
MF-BIA	Fat mass (kg)	0.89	0.80 – 0.99	<0.001	0.88	0.76 – 1.01	<0.001	0.79	0.64 – 0.93	<0.001	0.82	0.68 – 0.97	<0.001
	Fat mass percentage (%)	0.79	0.64 – 0.94	<0.001	0.76	0.63 – 0.89	<0.001	0.73	0.61 – 0.85	<0.001	0.75	0.63 – 0.87	<0.001
	Fat-free mass (kg)	0.91	0.80 – 1.01	<0.001	0.84	0.75 – 0.94	<0.001	0.84	0.75 – 0.93	<0.001	0.86	0.79 – 0.94	<0.001
ADP	Fat mass (kg)	0.74	0.56 – 0.92	<0.001	0.61	0.47 – 0.76	<0.001	0.52	0.24 – 0.80	0.001	0.49	0.26 – 0.72	<0.001
	Fat mass percentage (%)	0.50	0.31 – 0.69	<0.001	0.49	0.28 – 0.70	<0.001	0.34	0.12 – 0.56	0.004	0.37	0.07 – 0.66	0.017
	Fat-free mass (kg)	0.76	0.60 – 0.93	<0.001	0.72	0.50 – 0.95	<0.001	0.67	0.51 – 0.84	<0.001	0.82	0.52 – 1.12	<0.001
Model 2: Adjusted by age and sex													
MF-BIA	Fat mass (kg)	0.90	0.81 – 1.00	<0.001	0.95	0.84 – 1.05	<0.001	0.86	0.73 – 0.99	<0.001	0.86	0.74 – 0.98	<0.001
	Fat mass percentage (%)	0.77	0.57 – 0.96	<0.001	0.81	0.62 – 1.00	<0.001	0.73	0.57 – 0.89	<0.001	0.76	0.59 – 0.93	<0.001
	Fat-free mass (kg)	0.89	0.71 – 1.07	<0.001	0.84	0.66 – 1.02	<0.001	0.79	0.63 – 0.94	<0.001	0.82	0.67 – 0.97	<0.001
ADP	Fat mass (kg)	0.75	0.55 – 0.94	<0.001	0.61	0.46 – 0.76	<0.001	0.59	0.26 – 0.91	0.001	0.55	0.27 – 0.82	0.001
	Fat mass percentage (%)	0.40	0.20 – 0.61	0.001	0.35	0.15 – 0.56	0.002	0.15	– 0.12 – 0.42	0.271	0.36	0.08 – 0.63	0.013
	Fat-free mass (kg)	0.57	0.37 – 0.76	<0.001	0.37	0.07 – 0.66	0.016	0.42	0.17 – 0.66	0.002	0.45	0.19 – 0.71	0.002

Correlation coefficients (R^2), confidence intervals (95%CI), and P values are given for each of the methods compared with DXA.

had a consistent variability of about 5% in determining FM% when compared with DXA. On the other hand, the concordance between DXA and ADP is shown in (figure 4B). In Visits C-1 ($P = .005$), C-2 ($P = .010$) and C-3 ($P = .004$) significant negative slopes were observed, indicating underestimation of ADP at lower levels of FM%, but it seemed to overestimate FM% with increasing body fat. During Visit C-4 a similar pattern was observed although the slope does not reach statistical significance ($P = .093$). During all visits there was a high variability in the FM% determined by ADP, reaching values of up to 20% in comparison with DXA.

Discussion

In the present study the effects on body composition and muscle strength induced by a VLCK diet (Pnk Method®) in obese patients during an intervention period of up to 4 months was determined. To the best of our knowledge this is the first work assessing body composition under severe weight loss by using three different highly sophisticated and widely validated techniques such as Dual-energy X-ray Absorptiometry (DXA), Multifrequency Bioelectrical Impedance (MF-BIA), and Air displacement Plethysmography (ADP); which allowed an accurate evaluation of the body changes during dieting. The main findings of the

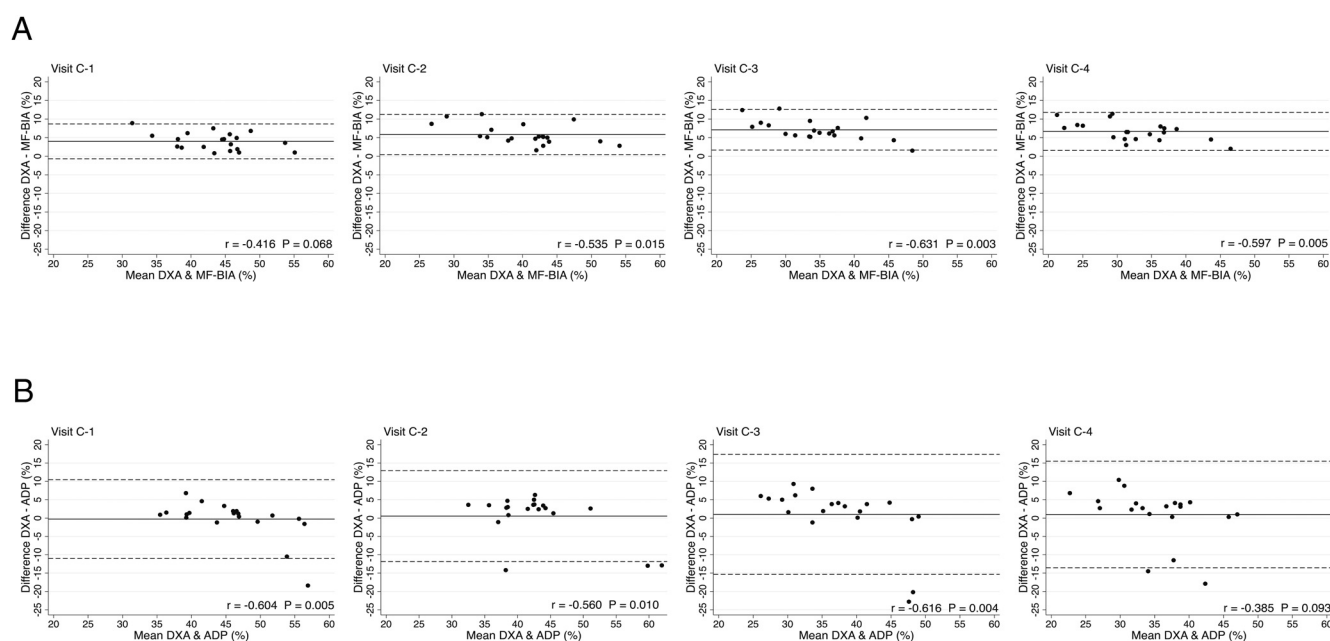


Figure 4. Bland–Altman analysis plotted from fat mass percentage in comparison with Dual-energy X-ray absorptiometry (DXA). A. Agreement Dual-energy X-ray absorptiometry (DXA) –Multi frequency bioelectrical impedance (MF-BIA). B. Agreement Dual-energy X-ray absorptiometry (DXA) –Air-displacement plethysmography (ADP).

present work were: 1) significant weight loss throughout the entire study, that was mostly explained by reductions in total FM and visceral fat tissue (VF); 2) a mild initial loss of FFM followed by a partial subsequent recovery of this compartment, which was principally due to changes in body water; 3) adequate preservation of muscle strength during the course of the diet; and 4) that the less expensive and more convenient technique of MF-BIA showed an acceptable agreement with DXA in estimating body composition.

The VLCK diet was employed because its ability to produce a rapid and well-tolerated weight loss with a ketogenic phase that lasts 60–90 days and a final result of 20 kg of weight reduction at 4 months. The rapid reduction in weight is the probable explanation of the positive effects of this dieting approach that are evident 1 and 2 years later (12, 13). The VLCK diet was used to obtain a model with four different stages; a basal stage with obese body weight and no ketosis, the second stage with extreme ketosis and important body weight loss, a third stage with body weight loss and ketosis declining and a fourth state with weight loss and no ketosis. Body composition was studied with the three techniques in each of these stages.

The accurate measurement of body composition changes is relevant to assess the contribution of the diet intervention, not only on total body weight but in the changes produced in FM, FFM, VF, and total body water (25, 27). To obtain such information, multicompartmental models that integrate information obtained from a single measurement (body density, total mineral mass, total body water) may be used to reduce the number of assumptions made on the stability of body compartments (28). These models are of limited application in the clinical practice as they do not provide immediate results, are expensive and require advanced analytical expertise (29, 30). For such reasons the three more widely used body composition analysis techniques were used in the present work. DXA is the most validated and used technique to analyze body composition in obese patients and is based on the attenuation of a low energy X ray beam depending on the tissue density and chemical composition. DXA is considered the gold standard technique by most groups working on body composition and it was used as the reference method in the present work. Bioelectrical impedance techniques are low cost and readily available and rely on the use of population-specific equations for assess intracellular and extracellular water distribution. The multifrequency bioelectrical impedance (MF-BIA) system here employed is a recently developed version not based on statistical population data and capable of accurately assessing subjects with different body shapes and also obese subjects. Finally, AD-plethysmography (ADP) measures

body density and is employed better than other more complex systems for measuring body density, such as underwater weighing, providing comparable results for obese subjects. Therefore, the use of three validated methods that employ different principles was relevant for evaluating patients in different stages of a body weight reduction program.

Previous studies have shown that ketogenic diets preferably reduce the total FM in obese patients (10–13). However, the precise distribution of these losses has not been determined. In this study we confirmed that the diet reduces total FM and specifically visceral adipose tissue, which has a greater impact in predicting cardio-metabolic complications associated with obesity than the total volume of body adiposity (2, 31).

On the other hand, maintaining muscle mass and its functionality (ie, muscle strength) has an important role in preventing weight regain, maintenance of physical functions, improving cardio-metabolic risk factors, and in the reduction of cardiovascular outcomes (5, 7–9, 32, 33). It is commonly assumed, and stated in several textbooks on obesity, that weight loss is associated with an important loss of muscle mass that evolves in parallel with the fat reduction. Some dietary guidelines have even suggested that diets that induce rapid weight loss, such as VLCK diets, create a greater energy deficit and contain lower amounts of protein, and therefore increase the risk of reductions in muscle mass compared to other interventions with more gradual weight loss (34, 35). In this paper it was shown that the reductions observed in lean mass were mostly due to body water loss, both intra- and extracellular. The combined information of DXA and MF-BIA allowed to such differentiation (28, 36–38). The loss attributable to muscle mass was minimal (around 1 kg), and an absolute preservation of hand grip (HG) strength was observed, a remarkable fact considering that the patients have experienced a weight reduction of circa 20 kg.

Various mechanisms may explain the variations in body water. For example, glycogen depletion induced by VLCK diets could cause a significant increase in diuresis, since glycogen is usually stored together with water (39, 40). Water loss might also be associated with ketonuria, because ketone bodies increase the renal sodium and water loss as a result (39, 41). These assumptions seem to be feasible considering that the peak of water loss coincides with the phase of maximum ketosis. However, the mechanisms explaining the diuresis observed with VLCK and with most hypocaloric diets are not known at present (30). Contrarily to previous observations (42, 43), DXA analysis evidenced a maintenance in BMD in the present study.

Although in most clinical settings BMI and waist circumference are used because they are inexpensive and con-

venient, it is evident that they are not able to precisely determine excess fat mass and its loss under treatment (44). More precise techniques to assess body composition are needed in specialized clinical settings and for research purposes. Therefore, another target of this work was to compare the accuracy of the information provided by the, more expensive and less convenient DXA, currently considered the gold standard, with the less expensive and convenient MF-BIA, as well as with the ADP which is only used in highly specialized centers because of its high cost (45). The results obtained showed that MF-BIA correlates very well with DXA, although with a tendency to slightly underestimate the FM%. These results are consistent with previous works that found that multifrequency bioelectrical impedance may overestimate the FFM, and thus produce an underestimation of the FM and FM% (45). MF-BIA provided highly relevant information about the water compartments under dieting. On the other hand, the ADP instrument showed a lower correlation with DXA and a greater variability in estimating the FM%. Compared to DXA, ADP underestimates the FM% in thinner patients, and overestimates the FM% in those patients with a higher body fat. The three techniques correlated remarkably well, although the less expensive one, MF-BIA, performed with high precision.

An important strength of this study was the use of three different techniques for determining body composition in different settings, ie, obesity and no ketosis, important reduction in body weight with high ketosis and finally strong reduction in body weight without ketosis. The tight control of adherence by daily measurement of B-OHB is another significant strength of this work. A potential limitation of our study could be the sample size; however, as each subject underwent 4 evaluations and results compare with themselves, this adds statistical power to the study and a real difference between the experimental points.

In conclusion, to the best of our knowledge, this is the first study that exhaustively assessed body composition changes during the weight reduction process induced by a VLCK diet. This assessment was performed using three complementary techniques: dual-energy X-ray absorptiometry (DXA), multifrequency bioelectrical impedance (MF-BIA), and air displacement plethysmography (ADP). The VLCK diet induced a significant weight loss that was achieved mainly at the expense of total FM, and also visceral fat, with a maximum conservation of muscle mass and muscle strength. Additionally, multifrequency bioelectrical impedance positioned as an effective and convenient alternative for measuring body composition in clinical practice due to its minimal burden for the patient, ease of operation, low cost and high accuracy.

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