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Effects of 1-Month Very-Low-Calorie Ketogenic Diet on 24-Hour Energy Metabolism and Body Composition in Women With Obesity

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Abstract

Context: Very-low-calorie ketogenic diet (VLCKD) is used for weight loss and management of obesity-related comorbidities.

Objective: We aimed at evaluating the effects of VLCKD on body composition and energy metabolism.

Methods: This prospective outpatient study included 17 women with obesity (mean age 41.6 years; body mass index 37.5 kg/m^2) who followed a 1-month VLCKD (700-800 kcal/day, carbohydrate 11%, fat 46%, protein 43%) at the University Hospital of Pisa. Measurements of 24-hour energy expenditure (24hEE) and substrate oxidation were conducted in a metabolic chamber at day 1 (V1), day 8 (V2), and day 29 (V3). Body composition was assessed by Dual energy X-ray absorptiometry. Twenty-two women with obesity fed a balanced isocaloric diet served as controls.

Results: Compared with controls, carbohydrate oxidation (CarbOx) was lower, whereas fat oxidation (FatOx) and protein oxidation (ProtOx) were higher in the VLCKD group at V1. CarbOx decreased by 65%, while FatOx increased by 11% at V3. The rate of ProtOx was already higher than in controls at V1 and remained stable throughout the study. After 1 month, body weight decreased by 7%, reflecting an 8.8% reduction in fat mass and a 5.6% reduction in lean soft tissue (LST). A 10% decrease in 24hEE and 24-hour sleeping metabolic rate was observed at V3 compared with V1.

Conclusion: VLCKD promotes weight loss in women with obesity. Our findings highlight the shift in energy metabolism towards increased FatOx accompanied by a modest increase in protein oxidation, a decrease in LST and a reduction in EE.

Key Words: ketogenic diet, obesity, energy expenditure, respiratory exchange ratio

Abbreviations: 24hEE, 24-hour energy expenditure; 24hRER, 24-hour respiratory exchange ratio; 24hSMR, 24-hour sleeping metabolic rate; β-OHB, β-hydroxybutyrate; BMI, body mass index; CarbOx, carbohydrate oxidation; DXA, dual-energy X-ray absorptiometry; EE, energy expenditure; FatOx, fat oxidation; FM, fat mass; FFM, fat-free mass; FT3, free triiodothyronine; FT4, free thyroxine; KD, ketogenic diet; LST, lean soft tissue; ProtOx, protein oxidation; REE, resting EE; TSH, thyrotropin; VLCKD, very-low-calorie ketogenic diet.

Obesity is a chronic condition characterized by the accumulation of excess adipose tissue, resulting in a significant public health concern (1). The main goal of obesity treatment is weight loss that may be achieved by lifestyle interventions, pharmacological treatment, or bariatric surgery (2). Currently, the nutritional approach based on calorie restriction, coupled with lifestyle modifications, remains the primary step in the treatment of overweight/obesity (3).

The ketogenic diet (KD) has gained consideration as a weight-loss therapeutic strategy (4-7). The KD, characterized by a low carbohydrate intake (<50 g/day), is a dietary approach based on the shift of energy metabolism from carbohydrates to fatty acids, which are used as the primary fuel, leading to production of ketone bodies. Fat mobilization is triggered upon the attainment of an energy deficit, a process facilitated by the anorectic influence of ketone bodies. The most used variants of KDs in obesity management are identified by 2 types based on the amount of calories introduced: a low-calorie ketogenic diet (LCKD) with a total daily energy intake of 800 to 1200 kcal; and a very-low-calorie ketogenic

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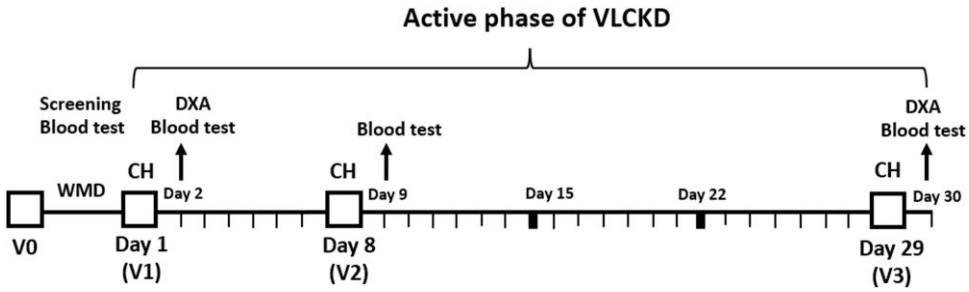

Figure 1. V0: screening interview including screening labs and informed consent. V0-V1: patients were instructed to follow a WMD (50% carbohydrates, 30% fat and 20% protein). Day 1-30: active phase of VLCKD consisting of a very–low-calorie diet (700-800 kcal/day), low in carbohydrate (<30 g daily), and fat (only 20 g per day) with protein ranging between 1.2 and 1.5 g per kg of ideal body weight. Day 1 (V1): CH for assessing the 24-hour energy expenditure on the first day of VLCKD. Day 2: blood test and DXA after an overnight fast. Day 8 (V2): CH for assessing the 24-hour energy expenditure after 1 week of VLCKD. Day 9: Blood test after an overnight fast. Day 29 (V3): CH for assessing the 24-hour energy expenditure after 28 days of VLCKD starting from V1. Day 30: blood test and DXA after an overnight fast.

Abbreviations: CH, whole-room indirect calorimetry; DXA, dual-energy X-ray absorptiometry; WMD, weight-maintaining diet.

diet (VLCKD) consisting of an active phase (<800 kcal/day) characterized by restricted carbohydrate intake and a relative increase in fat (~44%) and protein (~43%). This is followed by a reintroduction phase (800-1500 kcal/day), during which carbohydrates are gradually reintroduced to achieve a balanced macronutrient composition. The final maintenance phase involves a daily calorie intake ranging from 1500 to 2000 kcal, depending on individual patient characteristics (6). Several studies have demonstrated the beneficial effects of the active phase of VLCKD (8-11) and of the reintroduction and long-term maintenance period (ranging from 4 to 12 months) (12-14) on metabolic parameters including glucose, insulin, lipid profile, and liver function (15). In selected patients, the KD is considered a valid alternative to a balanced hypocaloric diet, due to the presumed preservation of lean body mass (16-18), which is the main determinant of energy expenditure (EE) (19-21). To date, very few studies have evaluated the impact of KDs on EE. No significant effects of a 20- to 60-day VLCKD were observed on resting EE (REE) when measured using portable devices over a short period of 10 to 20 minutes (22, 23). During isocaloric feeding following weight loss, reductions in REE and total EE, as assessed by indirect calorimetry and doubly labelled water, varied according to dietary glycemic load, with the smallest decline observed in the very–low-carbohydrate diet, an intermediate reduction in the low glycemic index diet, and the most pronounced decrease in the low-fat diet (24). A slight increase in 24-hour EE (24hEE) was observed after 1 week of an isocaloric KD, as measured using a whole-room indirect calorimeter (25). In this study we aimed at evaluating the effect of 1-month VLCKD on body composition, 24hEE, and substrate oxidation rates in women with obesity.

Materials and Methods

Patients and Study Design

This outpatient prospective intervention study, conducted without random allocation, included 39 women with obesity (aged ≥18 years, body mass index [BMI] > 30 kg/m^2) who were referred to the Obesity and Lipodystrophy Center, University Hospital of Pisa, by general practitioners or community outreach to initiate a weight loss program. All patients received a clinical, biochemical, and instrumental examination (V0) based on current guidelines (2, 26). None of them had major uncontrolled endocrine or metabolic diseases aside from obesity.

Table 1. Inclusion and exclusion criteria of the patients fed VLCKD

Inclusion criteria	Exclusion criteria
BMI > 30 kg/m^2	Type I diabetes mellitus or type II diabetes mellitus treated with insulin
Signing informed consent	Chronic renal disease (estimated glomerular filtration rate <60 mL/min or overt proteinuria)
Agreement to follow the study procedures, including follow-up visits	Use of antidepressants, anticonvulsants, lithium carbonate or neuroleptics for psychiatric comorbidities
	Pregnancy or breastfeeding
	Evidence of alcohol and/or drug abuse Antiobesity medications
	Cancer requiring treatment in the past 5 years
	Other neurological, cardiovascular, liver, respiratory, hematologic, endocrinological, or autoimmune diseases that, in the opinion of the investigator, could influence the study results

Based on physical examination, laboratory tests, and psychological assessment, 17 women were selected for participating in the VLCKD program at V0. Two patients were receiving hypolipemic therapy, 4 patients vitamin D supplementation, 1 patient took metformin, antihypertensive therapy, and hypolipemic therapy, 1 patient was on the contraceptive pill, and 2 patients were receiving L-thyroxine therapy for autoimmune hypothyroidism.

The study design is presented in Fig. 1. Inclusion and exclusion criteria are shown in Table 1. After V0 and until V1, patients were instructed to follow a weight-maintaining diet, specifically an isocaloric balanced diet with a macronutrient composition of 20% protein, 30% fat, and 50% carbohydrates. The individual diet caloric content was determined using specific equations based on body weight, height, and sex (27). At V1 (day 1), while staying inside the whole room indirect calorimeter (metabolic chamber) for 24 hours, patients were fed a VLCKD (breakfast at 8:00 AM, lunch at 12:00, dinner at 19:00) and underwent a 24-hour measurement of

energy metabolism. The proportion of total caloric intake provided at each meal was 29% for breakfast, 43% for lunch, and 28% for dinner. Patients had the option to either consume preassembled products prepared by Kalibra s.r.l. (Cuneo, Italy) or a diet using food supplied by the hospital kitchen according to the dietitian's plan. As a result, 5 patients opted for prepackaged products, while 12 patients chose conventional foods. Patients returned 1 week later (V2, day 8) and 4 weeks later (V3, day 29) for measurement of 24hEE and its components. During the 24-hour stay inside the metabolic chamber on V2 and V3, patients consumed the same diet as at V1. Under free-living conditions from V1 to V3, 12 patients continued following the diet based on self-prepared food supplies as recommended by dietitians, while 5 patients who had chosen assembled products received prepackaged meal boxes provided by the company. From V2 to V3, patients were encouraged to start mild physical activity, such as walking for 30 minutes twice a week.

On days 2 and 30, after exiting the metabolic chamber, a fasting measurement of body composition was performed. Body weight was assessed at each visit (V0, V1, V2, V3). A digital electronic scale was used to assess body weight in light clothing. Standing height without shoes was measured to the nearest 1 cm using a stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters. Classification of overweight and obesity was done according to conventional definitions (28). Fasting blood samples were drawn at 07:30 AM prior to (pre-V1) and after exiting the chamber (V1), and at V2 and V3 upon exiting the metabolic chamber.

Additionally, 22 female patients with age and body weight comparable to the study group, who received the same clinical evaluation, volunteered as controls. These patients were assigned to receive either a lifestyle intervention (n = 7), anti-obesity medications (n = 14), or bariatric surgery (n = 1). Before starting their weight loss program, they underwent a 24-hour energy metabolism assessment while following a balanced isocaloric diet (50% carbohydrates, 30% fat, 20% protein) and received a measurement of body composition using dual-energy X-ray absorptiometry (DXA). This baseline assessment served as a reference for the intervention group on the first day of the VLCKD (V1). Following the initiation of the weight loss program, patients were monitored through periodic evaluations based on their assigned treatment, but no further data were collected for this study.

The study was approved by the Regional Ethics Committee for Clinical Trials of the Tuscany, Region Section: AREA VASTA NORD OVEST (register number 19543). All patients gave their written and informed consent to participate. The procedures employed in the study are in accordance with the 1964 Helsinki Declaration and its later amendments.

Dietary Intervention

On the first day in the metabolic chamber (V1), the patient started the active phase of the VLCKD, which was then continued during the outpatient phase until V3 according to the plan provided by the dietitian. The diet was tailored to each patient based on individual preferences. Adherence to the VLCKD was monitored by weekly telephone calls. During follow-up visits and telephone calls, medical data and changes in physical activity were monitored without evidence of significant variations throughout the study period. The dietary intervention consisted of a very-low-calorie diet (700-800 kcal/day), low in carbohydrates (<30 g daily), derived mainly from vegetables, and fat (20 g per day, obtained primarily from olive oil). The amount of high-biological–value protein was between 1.2 and 1.5 g per kg of ideal body weight. β-Hydroxybutyrate (βOHB) capillary blood detection was performed to confirm ketosis by using a portable device (GlucoMen areo, A. Menarini Diagnostics, Neuss, Germany) at V1 upon exiting the metabolic chamber at 07:30 AM and at V2 and V3 prior to and after exiting the chamber. The threshold value for nutritional ketosis was set at 0.5 mmol/L of βOHB (29). Urinary ketones were analyzed in a morning sample at V1, V2, and V3 following the exit from the chamber. Given the very low caloric nutritional pattern, patients were instructed to take supplement with micronutrients (vitamins, including complex B, C, and E vitamins, minerals, such as potassium, sodium, magnesium, calcium) according to international recommendations (30). Drinking 2 to 2.5 liters of water per day was recommended. Low glycemic index vegetables were indicated to reach the necessary fiber level.

From recruitment until the energy metabolism assessment, the control group was instructed to adhere to a weight-maintaining diet, specifically an isocaloric balanced diet with a macronutrient composition of 20% protein, 30% fat, and 50% carbohydrate. After the 24-hour metabolic chamber session, they were prescribed a hypocaloric, normobalanced diet providing 25 kcal/kg of ideal body weight, which was reassessed during follow-up visits based on their assigned program and weight loss progression.

Whole-Room Indirect Calorimetry

Assessment of 24hEE and 24-hour respiratory exchange ratio (24hRER), an index of substrate oxidation, within the whole-room indirect calorimeter was performed using previously established methodologies (31, 32). In brief, after an overnight fast, patients entered the calorimeter around 7:30 AM and received breakfast at 8:00 AM, lunch at 12:00 PM, and dinner at 7:00 PM. Any unconsumed food was weighed to determine the actual 24-hour energy intake during each session. Patients and controls stayed in the calorimeter for 23.5 hours, during which CO_2 production and O_2 consumption were recorded every minute. The 24hEE was computed using the Lusk formula (19). The chamber air temperature was maintained constant at 23.8 ± 1.4 °C, and the air inflow rate was regulated by a mass flow controller at a fixed rate of 100 L/minute. Air samples from the inlet pipe and chamber were drawn by membrane pumps, dried to a humidity level of <1000 ppm using a gas sample dryer driven by counterflow dry medical air, and then sent to absolute gas analyzers. Radar sensors measured spontaneous physical activity, expressed as the percentage of time when activity was detected (33). The accuracy of the calorimeter system was periodically verified prior to and throughout the study period by monthly combustion of instrument-grade propane on a calibrated scale. The recovery rates of O_2 consumption, CO_2 production, and RER were consistently within 5% of the values expected from stoichiometry. Daytime EE in the inactive, awake state (EE0) was determined as the intercept of the regression line between EE and spontaneous physical activity at 1-minute intervals from 10:00 AM to 1:00 AM, extrapolated to 15 hours (34). Sleeping EE was calculated as the average EE between 11:30 PM and 5:00 AM overnight when subject movement was less

Table 2. Demographic and anthropometric parameters of patients fed the VLCKD at V1 compared with controls (CON) fed an isocaloric balanced diet

	VLCKD at V1 (n = 17)	CON (n = 22)	*P* value
Age	41.6 ± 15.2 (20; 63)	48.1 ± 11.4 (21; 66)	ns
Body weight (kg)	99.1 ± 17.4 (78.0; 131.0)	96.1 ± 17.0 (74.5; 126.5)	ns
BMI (kg/m^2)	37.5 ± 6.8 (30.9; 50.3)	36.3 ± 6.7 (29.3; 48.3)	ns
FM (kg)	50.6 ± 11.9 (34.7; 75.3)	47.3 ± 10.6 (30.0; 66.6)	ns
LST (kg)	45.8 ± 6.8 (35.3; 65.5)	46.2 ± 4.6 (38.5; 56.2)	ns

Each parameter in the table is expressed as mean ± SD (minimum; maximum). V1: first day of VLCKD while staying inside the metabolic chamber.

Table 3. Calorie intake and ketone bodies concentrations in the study group at V1, V2 and V3 during the VLCKD intervention period

	V1	V2	V3
Prescribed total energy intake (kcal/d)	761.4 ± 29.2	764.4 ± 21.3	768.0 ± 25.4
Actual total energy intake (kcal/day)	740.8 ± 40.1	726.6 ± 75.5	680.7 ± 114.4
Urinary ketone excretion (mg/dL)	0.45 ± 1.5	25.6 ± 28.2[a]	27.1 ± 22.5[a]
β-Hydroxybutyrate (mmol/L)	0.94 ± 1.6	1.8 ± 0.7[a]	2.2 ± 1.3[a]

Data are presented as mean ± SD. Prescribed total energy intake (kcal/d) refers to the amount of energy allocated to patients following a VLCKD during each session (V1, V2, V3) in the metabolic chamber. Actual total energy intake (kcal/day) represents the energy effectively consumed by patients, accounting for any unconsumed food during each session in the metabolic chamber.
Abbreviations: BMI, body mass index; FM, fat mass; LST, lean soft tissue; VLCKD, very-low-calorie ketogenic diet; V1, first day of VLCKD; V2, day 8 of VLCKD; V3, day 29 of VLCKD.
[a]Adjusted $P < .05$ vs V1 by Dunnet's post hoc analysis.

than 1.5% (<0.9 seconds/minute). This value was then extrapolated to a 24-hour period (24-hour sleeping metabolic rate, 24hSMR) to determine the daily minimal energy requirement, excluding any additional EE associated with the cost of being awake (19). The 24hRER was determined as the ratio of 24-hour average CO_2 production to 24-hour average O_2 consumption. Carbohydrate oxidation (CarbOx) and lipid oxidation (FatOx) rates were derived from the 24hRER after adjustment for protein oxidation (ProtOx) rate, estimated from measurement of 24-hour urinary nitrogen excretion (35).

Measurement of Body Composition

DXA (Lunar Prodigy; GE Healthcare) was used to perform the following measures of body composition (36): lean soft tissue (LST), defined as the estimated mass of all nonfat, nonbone mineral molecules in the body, including skeletal muscle mass, nonfat components of adipose tissue, skin, connective tissue, etc; fat mass (FM); appendicular LST (the sum of LSTs of the upper and lower extremities); appendicular fat mass (the sum of FMs of the upper and lower extremities); trunk FM. Body composition assessment using DXA was consistently conducted by the same trained technicians, who were blinded to the intervention group throughout the study, to ensure methodological consistency and minimize interoperator variability.

Assays for Serum Hormones and Metabolic Analytes

Blood samples were drawn after an overnight fast prior to and after V1, at V2 and V3 (upon exiting the metabolic chamber).

Thyrotropin (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) were measured by a specific and sensitive automated chemiluminescent immunoassay (Vitros 3600 System, Ortho-Clinical Diagnostic, Rochester, NY, USA). The normal range was 0.4-4 mUI/L for TSH, 7 to 17 pg/mL for FT4 and 2.7 to 5.7 pg/mL for FT3. Serum insulin, creatinine, total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, and liver enzymes were measured by commercial kits.

Statistical Analysis

A power analysis was conducted to determine the sample size required to detect a clinically significant effect of VLCKD on energy metabolism. This calculation was based on data from a prior study that investigated the impact of a isocaloric KD on EE (25) which reported a mean increase in sleeping EE of approximately 200 kcal/day after 1 week. To detect a more conservative mean increase of 100 kcal/day (SD = 140 kcal) in sleeping EE after 1 week of VLCKD, a sample size of 17 participants was determined to provide a power of >0.80 at a 2-sided a-level of .05, using a paired t-test. All statistical analyses were performed with SAS, version 9.2 (SAS Institute, Cary, NC). *P* values <.05 were considered statistically significant. Normally distributed variables were expressed as mean ± SD, except for variables with skewed distributions, which were expressed as median with interquartile range (IQR). Measurements of TSH concentration were $\log_{10}$ transformed prior to analysis to ensure normality of data distribution. Differences in metabolic measures between the VLCKD group at V1 and controls were evaluated using Student's unpaired t-test. Both 24hEE and 24hSMR were normalized to LST by expressing them per kilogram of LST after verifying that the intercept of the linear regression line was not statistically significantly different from 0.

Differences in body composition measures between V1 and V3 were assessed using Student's paired t-test. Changes in measures of energy metabolism, body weight, ketone bodies and hormone concentrations in the group of patients with VLCKD during the 3 visits (V1, V2, and V3) were analyzed using repeated-measures mixed-model analysis with a first-order autoregressive covariance structure. Changes (Δ) from V1 were expressed as least squares means with SE and analyzed using the Dunnett post hoc test.

Results

Table 2 shows the demographic and anthropometric parameters of patients fed VLCKD at V1 (energy intake shown in Table 3) compared with controls fed an isocaloric balanced diet (actual total intake 1582.4 ± 227.8 kcal/day; carbohydrate intake 787.7 ± 135.4 kcal/day; fat intake 472.5 ± 80.9 kcal/day; protein intake 322.2 ± 49.0 kcal/day). No significant differences in age, body weight, or body composition were observed between the 2 groups (all $P > .3$).

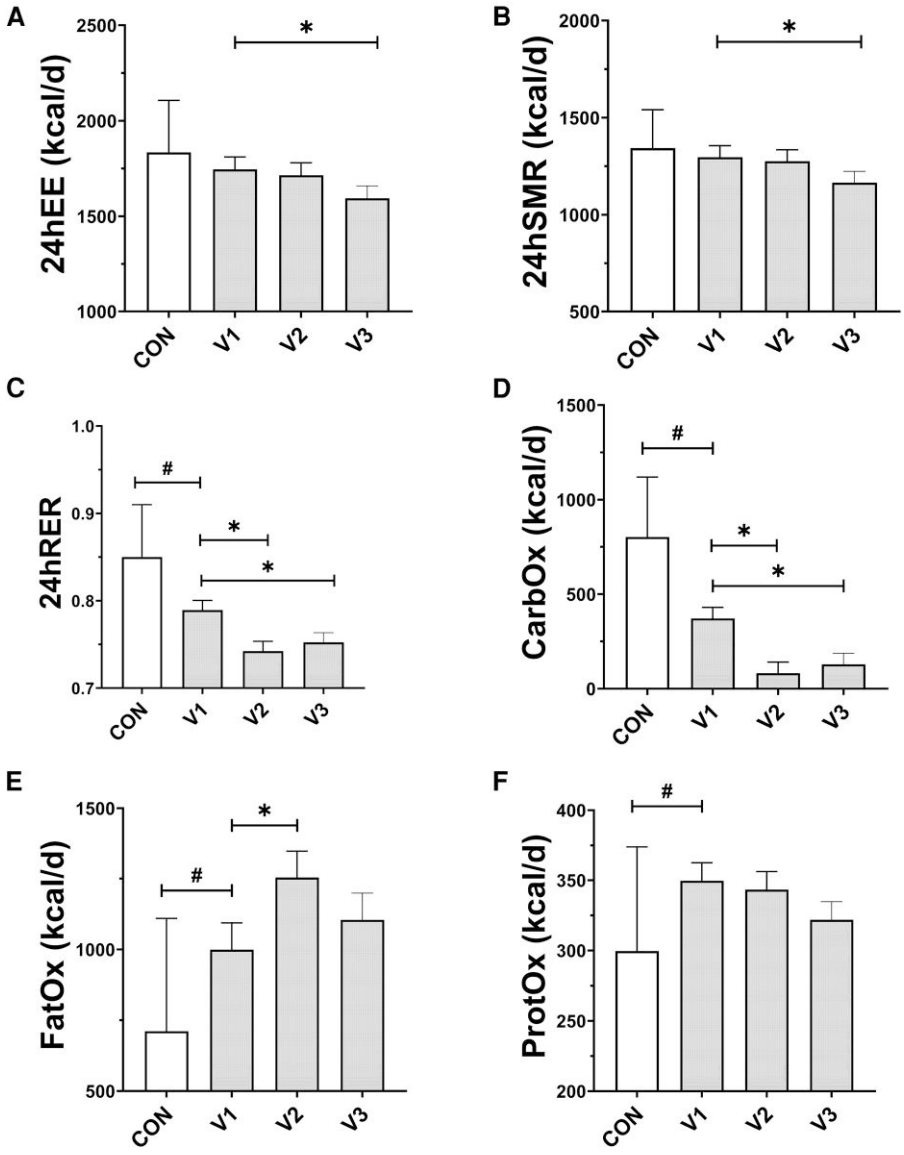

Figure 2. The figure shows the measures of 24hEE (A), 24hSMR (B), 24hRER (C), CarbOx (D), FatOx (E), and ProtOx (F) in the control group (CON) and in patients fed VLCKD at V1, V2, and V3. Metabolic measurements in the control group were conducted only at baseline. These values were used as a reference for the intervention group on the first day of the VLCKD (V1). #Adjusted $P < .05$ vs control by Student's unpaired t-test. *$P < .05$ vs V1 by Dunnet's post hoc analysis. Error bars show the SE of the mean at each visit calculated from the repeated measures mixed model analysis. V1: first day of VLCKD; V2: day 8 of VLCKD; V3: day 29 of VLCKD.

Assessment of Energy Metabolism After 1 Day of VLCKD

No significant difference in 24hEE or 24hSMR was observed between the VLCKD group and controls. As expected from the different nutritional status between the 2 groups, the 24hRER and CarbOx were lower in the VLCKD than in controls, whereas FatOx and ProtOx were higher (Fig. 2). Consistent results were obtained when macronutrient rates were expressed as percentages of 24hEE (Fig. 3).

Assessment of Energy Metabolism at V2 and V3 of the VLCKD Intervention Diet

The actual energy intake, urinary ketone excretion, and βOHB capillary blood levels at each visit are reported in Table 3.

Metabolic parameters during the active phase of VLCKD at each visit are shown in Fig. 2. Compared with V1, a slight decrease in 24hEE and 24hSMR was observed at V2, which became statistically significant at V3, when metabolic rates were approximately 10% lower than in V1. Compared with V1, CarbOx decreased by an average of 78% at V2 and by 65% at V3, and FatOx increased by 25% at V2 and by 11% at V3. The rate of ProtOx remained elevated throughout the active phase of VLCKD, though showing a small decline parallel to the reduction of 24hEE. Consistent with the above data were results obtained by expressing macronutrient oxidation rates as a percentage of 24hEE (Fig. 3). No differences in various measures were observed between patients who consumed self-prepared food supplies and those who chose prepackaged meals.

Changes in Body Composition

The variations of body composition measures are reported in Table 4 and Fig. 4A. Compared with V1, mean body

weight decreased by 3% at V2 and by 7% at V3. At V3, a reduction in FM ($\Delta = -8.8 \pm 3.9\%$, $P < .001$), trunk body fat ($\Delta = -8.2 \pm 8.1\%$, $P = .003$), appendicular FM ($\Delta = -10.0 \pm 7.8\%$, $P = .0002$), LST ($\Delta = -5.6 \pm 3.4\%$, $P = .0001$), and appendicular LST ($\Delta = -6.0 \pm 5.0\%$, $P = .005$) were observed. At V3, FM loss accounted for 62% of the total weight loss, while LST reduction contributed to the remaining 38% (Fig. 4B). No significant differences in 24hEE and 24hSMR between V1 and V3 were observed after normalizing for LST. These findings suggest that the observed reduction in EE at V3 was primarily driven by the decrease in LST.

No differences in body composition measures were observed between patients who consumed self-prepared food supplies and those who opted for prepackaged meals.

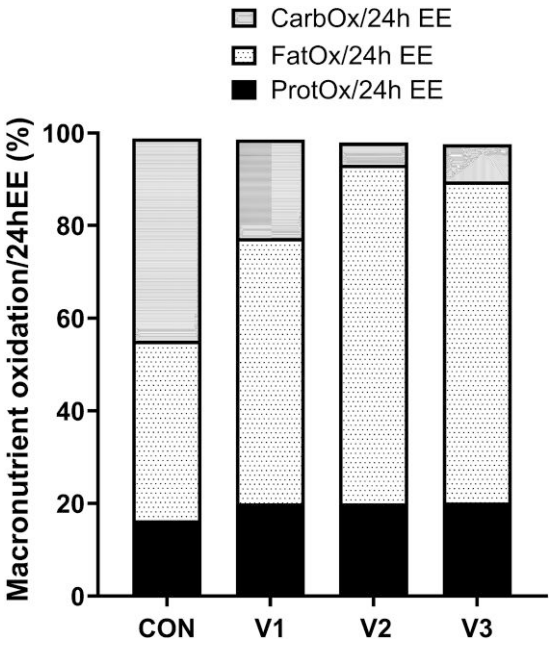

Figure 3. Macronutrient oxidation rates (CarbOx, FatOx, ProtOx) expressed as percentage of 24hEE in the control group (CON) and in the VLCKD group throughout the 1-month dietary intervention period (V1, V2, V3). V1: first day of VLCKD; V2: day 8 of VLCKD; V3: day 29 of VLCKD.

Changes in Serum Hormones and Other Blood Parameters

Compared with pre-V1, serum concentrations of FT3 decreased after 24 hours of VLCKD as did the FT3/FT4 ratio and TSH, whereas no change was observed for FT4 (Fig. 5). Compared with V1, further decreases in serum FT3 concentrations and FT3/fT4 ratio were observed at V2 and V3, whereas serum FT4 concentrations increased significantly during the active phase of VLCKD (Fig. 5). No changes in TSH were observed throughout V1 to V3.

Compared with V1, a significant reduction in total cholesterol, low-density lipoprotein, high-density lipoprotein, triglycerides, and insulin levels was observed at V3 (Table 5). No significant changes in serum creatinine, liver enzymes, and electrolytes were detected.

Discussion

In this prospective outpatient study, VLCKD proved effective in promoting weight loss, with an average reduction of 7% over a 1-month period of caloric restriction and low carbohydrate intake. This relevant reduction in body weight was associated with an improvement of patients' metabolic status, as demonstrated by the significant reduction of serum lipids and insulin.

In a study involving individuals with obesity undergoing VLCKD, REE was measured at 1 to 2 months by O_2 consumption, as assessed by a portable desktop metabolic system. Despite a 12% body weight loss, no significant reduction in REE was observed compared with baseline values (23). A randomized controlled trial in subjects with overweight compared the effects of a 20-day low-carbohydrate KD to those of a Mediterranean diet: no significant difference in REE was observed, as assessed by measuring oxygen uptake and carbon dioxide production using a ventilated hood (22). Similarly, a study on male soccer players being randomized to a VLCKD or a Western diet found no difference in REE between the 2 dietary regimens (37). At variance with previous reports, our study, investigating a group of women with obesity administered a VLCKD for a 1-month period, demonstrates a clear-cut decrease in 24EE and 24hSMR. In this context, it is important to highlight that our study utilized a whole room indirect calorimetry, which allows for accurate measurement of 24hEE and its individual components.

Table 4. Changes in body weight and body composition during the VLCKD intervention period at V1, V2, and V3

	V1	V2	V3
Body weight (kg)	99.1 ± 17.3 (78.0; 131.0)	96.1 ± 17.0[a] (74.5; 126.5)	92.1 ± 16.5[a] (73.0; 122.5)
BMI (kg/m^2)	37.5 ± 6.8 (30.9; 50.3)	36.3 ± 6.7[a] (29.3; 48.3)	34.7 ± 6.5[a] (28.4; 47.9)
FM (kg)	50.6 ± 11.9 (34.7; 75.3)	—	46.2 ± 11.9[b] (32.5; 70.5)
TBF (kg)	25.3 ± 6.5 (18.2; 41.1)	—	23.5 ± 7.5[b] (15.9; 40.4)
AFM (kg)	24.3 ± 7.0 (15.6; 39.6)	—	21.8 ± 6.4[b] (14.5; 37.1)
LST (kg)	45.8 ± 6.8 (35.3; 65.5)	—	43.1 ± 5.9[b] (32.5; 59.7)
ALST (kg)	21.9 ± 3.9 (15.8; 29.4)	—	20.5 ± 2.5[b] (14.9; 24.5)

Data are presented as mean ± SD (minimum; maximum).
Abbreviations: AFM, appendicular fat mass; ALST, appendicular lean soft tissue; BMI, body mass index; FM, fat mass; LST, lean soft tissue; TBF, trunk body fat; V1, first day of VLCKD; V2, day 8 of VLCKD; V3, day 29 of VLCKD.
[a]Adjusted $P < .05$ vs V1 by Dunnet's post hoc analysis.
[b]$P < .05$ vs V1 by Student's paired t-test.

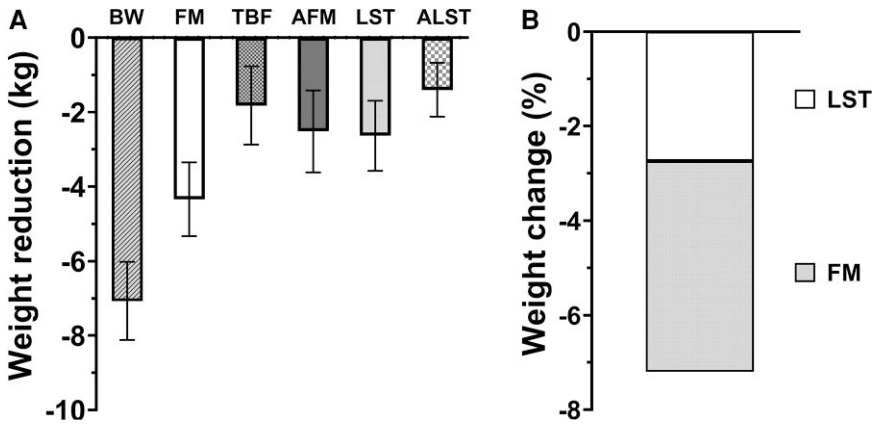

Figure 4. (A) Changes in body composition parameters from V1 are expressed as absolute values. Data are presented as mean ± SD. (B) Effect of weight loss induced by VLCKD on the proportion of total weight lost derived from FM and LST.

Abbreviations: ALST, appendicular lean soft tissue; AFM, appendicular fat mass; BW, body weight; FM, fat mass; LST, lean soft tissue; TBF, trunk body fat.

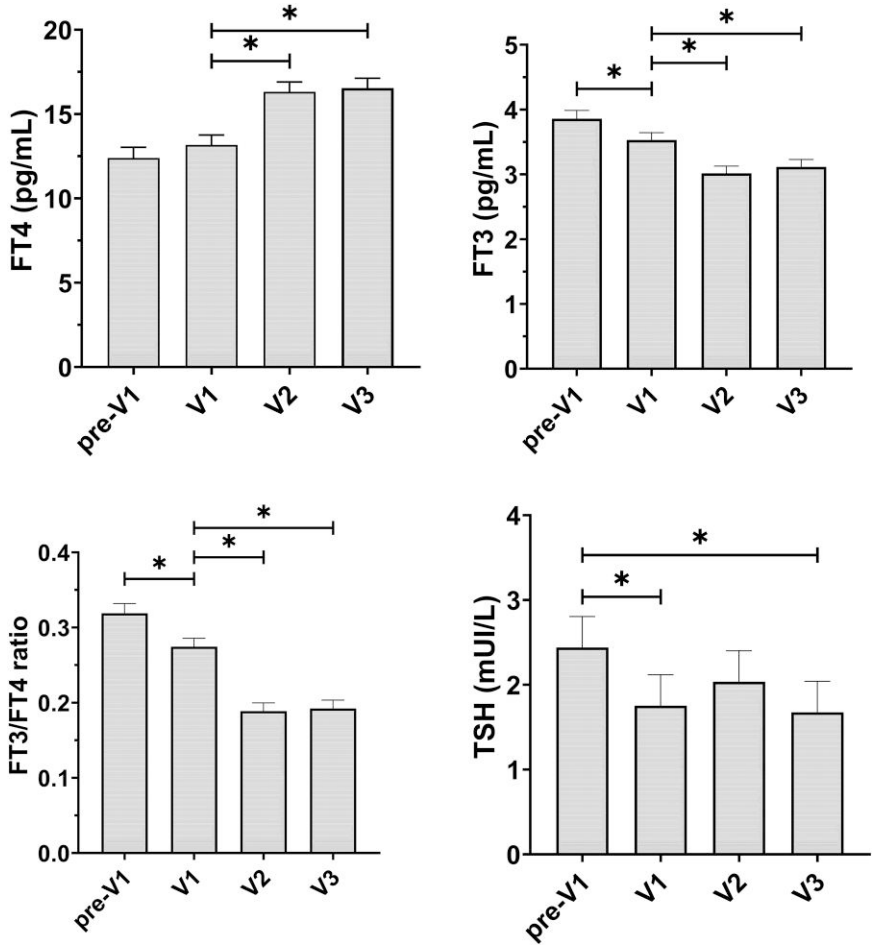

Figure 5. Serum thyroid hormone and TSH concentrations prior to and following the start of VLCKD. Fasting blood samples were drawn at 07:30 AM prior to (pre-V1) and after exiting the chamber (V1), and at V2 and V3 upon exiting the metabolic chamber. At pre-V1, the measurement of TSH, FT4 and FT3 was available in 10 patients. *Adjusted $P < .05$ by Dunnet's post hoc analysis. Error bars show the SE of the least-squares mean at each visit calculated using the repeated measures mixed model analysis. V1: first day of VLCKD; V2: day 8 of VLCKD; V3: day 29 of VLCKD.

In individuals fed a VLCKD, the restricted carbohydrate availability promotes an increased dependence on lipids and proteins as energy sources (38-40), driven by a reduction of insulin and by an increase in glucagon (41). These metabolic changes enhance liver glycogenolysis (42), an energy-costly process, and may initially lead to a transient rise in REE (25, 40, 43). This metabolic shift also raises the circulating levels of nonesterified fatty acids (44, 45). An increased FatOx in the liver stimulates ketogenesis (46), which may contribute to the initial increase in REE (47, 48). Indeed, using an

Table 5. Changes in serum levels of lipids and insulin during the VLCKD intervention period at V1 and V3

	V1	V3
Total cholesterol (mg/dL)	202.3 ± 23.3 (149.0; 246.0)	164.7 ± 25.4[a] (140.0; 204.0)
LDL (mg/dL)	117.3 ± 25.3 (93.0; 180.0)	96.3 ± 29.2[a] (49.0; 169.0)
HDL (mg/dL)	60.9 ± 18.1 (29.0; 100.0)	50.1 ± 14.7[a] (27.0; 87.0)
Triglycerides (mg/dL)	143.8 ± 80.3 (45.0; 386.0)	102.4 ± 32.8[a] (53.0; 171.0)
Insulin (mUI/L)	18.3 ± 13.6 (3.7; 49.5)	8.6 ± 6.1[a] (4.2; 19.0)

Data are expressed as mean with SD (minimum; maximum).
Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLCKD, very-low-calorie ketogenic diet; V1: first day of VLCKD; V3: day 29 of VLCKD.
[a]$P < .05$ vs V1 by Student's paired t-test.

isocaloric KD, Hall et al (25) reported a slight increase in EE after 1 week of intervention.

In our study, we did not see any preservation of EE in our calorie restricted KD, with EE declining over time and culminating in a significant reduction after 1 month. The overall state of negative energy balance, accompanied by a reduction in LST and decreased circulating thyroid hormone levels, may contribute to the observed decrease in EE. Additionally, reduced insulin secretion per se may lead to an adaptive suppression of EE (41). Meanwhile, the increase in FatOx, liver ketogenesis, and ketone oxidation, mainly in the brain and skeletal muscle, leads to a reduced whole-body respiratory exchange ratio (40, 49). As assessed by measurement of body composition, our study shows a reduction in fat stores, due to increased lipolysis, that reflects the utilization of lipids and ketone bodies as preferred energy sources. Several studies have evaluated the impact of VLCKD on body composition, but the effect on the LST is still a matter of debate. In 20 patients with obesity who lost 12% of body weight after 40 days of VLCKD, the decrease in FM and fat-free mass (FFM) from baseline was about 17% and 8%, respectively (16). Similarly, after a 2 month-period of VLCKD, patients with obesity showed a 23% reduction of FM and 7% FFM (50). A recent metanalysis (15) evaluating the effects of VLCKD on body weight and composition across 14 studies (including noncontrolled, controlled, and randomized trials) demonstrated a significant mean reduction of 11 kg in FM and about 3 kg in FFM.

Our findings highlight the relevant impact of a VLCKD on body composition. The observed reduction in body weight (on average 7%) after 1 month was accounted for by a 62% loss in fat body mass, primarily due to a 25% increase in FatOx, which already occurred 1 week after the initiation of VLCKD. More importantly, our results reveal a smaller yet notable 5.6% decrease in LST, which accounted for 38% body weight loss. This latter result is in line with the reduction reported in patients with obesity treated with antiobesity medications (retatrutide, semaglutide and tirzepatide) who showed a FFM decrease of 25% to 39% of their total weight loss (51).

When liver glycogen stores are depleted, gluconeogenesis from proteins becomes the primary source of plasma glucose. The need for glucose derived from proteolysis is partially limited by ketone bodies, somewhat replacing glucose as fuel for the brain (52). The infusion of βOHB is indeed associated with lower leucine oxidation and higher protein synthesis in skeletal muscle (53). Notably, our findings reveal that patients on a VLCKD increased ProtOx rates within the first 24 hours compared with those on an isocaloric balanced diet. This elevation persisted throughout the 1-month period of the diet, despite an overall reduction in 24hEE. These results are consistent with previous findings by Hall et al (25), who demonstrated an increase in ProtOx after 4 weeks of a KD, though isocaloric. To further explore the underlying mechanisms of LST loss during VLCKD, we can estimate nitrogen balance and its relationship with body composition changes. Our patients exhibited a urinary nitrogen excretion of 12.5 g/day. Assuming a fecal nitrogen loss of 3 g/day (comprising both endogenous and exogenous nitrogen loss) (54-56), the total daily nitrogen loss is approximately 15.5 g. With a median daily nitrogen intake of 13.1 g, this results in a predicted negative nitrogen balance of 2.4 g/day. Over a 1-month period, this would equate to a total nitrogen deficit of 72 g, corresponding to 444 g of dried protein. Given that that protein content in LST is about 20% and water accounts for 75% (57), we estimate that the LST loss attributable to ProtOx in our cohort is around 2 kg. The remaining LST reduction, bringing the total observed loss to 2.6 kg, is likely due to glycogen depletion (and its associated water loss) as well as a decrease in extracellular fluid, driven by increased renal sodium excretion associated with urinary 3-hydroxybutyrate excretion (58, 59).

VLCKD not only increases circulating ketones but also influences the concentrations of many other energy substrates and hormones, including thyroid hormone. Several studies have shown that a reduction of carbohydrate intake is followed by a reduction in serum T3 and/or an increase in serum T4 (60-63). Our study indicates that a VLCKD is associated with an early and sustained reduction in the FT3/FT4 ratio. All these findings align with earlier pivotal studies demonstrating that starvation leads to decreased serum T3 concentrations and elevated levels of reverse T3 (64). This phenomenon can be attributed to a reduction in type 1 iodothyronine deiodinase activity in peripheral tissues (65), which, under conditions of caloric deprivation, leads to a diminished conversion of T4 to its bioactive hormone, T3.

The VLCKD is considered safe in this setting because, despite common concerns about its potential side effects, the patients in the study did not experience any significant adverse events. Specifically, no issues such as lethargy, halitosis, migraines, hypotension, constipation, gastrointestinal discomfort, dehydration, electrolyte imbalances, or changes in renal function were observed. The absence of these side effects suggests good tolerability of the diet. These findings align with existing evidence indicating that, when properly supervised, VLCKD is a safe dietary intervention (66).

Our study has some limitations. The study design did not permit strict monitoring of adherence to the KD outside the clinical setting. However, measurements of blood and urinary ketone bodies, consistently taken prior to entering the metabolic chamber, indicated good compliance with the dietary intervention. Although both the intervention group and controls were instructed to follow an isocaloric balanced diet before their first entry into the metabolic chamber, adherence verification was limited by the outpatient setting, and potential carryover effects from the participants' habitual diet prior to the study cannot be ruled out. The study design is not a case–control intervention; therefore, causality cannot be definitively established, and the observed effects may not be solely attributable to VLCKD. Including male patients and incorporating additional hormonal measurements could offer deeper insights into the mechanisms underlying the metabolic effects of VLCKD. A follow-up reassessment after the active phase of VLCKD will be crucial to determine whether metabolic adaptations occur during the reintroduction and maintenance phases.

In conclusion, the current study confirms the efficacy of a VLCKD in promoting significant weight loss and improving metabolic status of women with obesity. Our findings obtained using a whole room indirect calorimetry (metabolic chamber) highlight a shift in energy metabolism towards increased FatOx, driven by reduced carbohydrate intake and enhanced ketone utilization. However, patients on VLCKD experienced increased ProtOx as early as the first 24 hours, which persisted throughout the 1-month dietary period. This phenomenon may contribute to the reduction of LST and the subsequent decrease in EE, potentially indicating metabolic adaptation. These findings underscore the need for caution in selecting patients who may benefit from this diet, as well as the importance of supporting measures (multidisciplinary care, controlled clinical settings, and a tailored approach to physical activity) to mitigate the chances of long-term weight regain.

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Author Contributions

A.B., P.P., and F.S. designed the study protocol, interpreted data and wrote the manuscript. P.F., R.J., D.T, and V.A. performed nutritional counseling during follow-up visits. C.P. and M.P. performed laboratory tests. C.B., E.V., M.P., G.S., L.C., J.K., and A.L. assisted with the interpretation of the data and revised the manuscript. All authors read, critically revised the draft and approved the final manuscript. A.B. and F.S. have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosures

The authors declare no conflict of interest.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Information

ClinicalTrials.gov, identifier NCT06252077 (registered February 6, 2024).

References

1. Janssen F, Bardoutsos A, Vidra N. Obesity prevalence in the long-term future in 18 European countries and in the USA. *Obes Facts*. 2020;13(5):514-527.
2. Yumuk V, Tsigos C, Fried M, *et al*. European guidelines for obesity management in adults. *Obes Facts*. 2015;8(6):402-424.
3. Hassapidou M, Vlassopoulos A, Kalliostra M, *et al*. European association for the study of obesity position statement on medical nutrition therapy for the management of overweight and obesity in adults developed in collaboration with the European federation of the associations of dietitians. *Obes Facts*. 2023;16(1):11-28.
4. Ryan DH. Guidelines for obesity management. *Endocrinol Metab Clin North Am*. 2016;45(3):501-510.
5. Abbasi J. Interest in the ketogenic diet grows for weight loss and type 2 diabetes. *JAMA*. 2018;319(3):215-217.
6. Caprio M, Infante M, Moriconi E, *et al*. Very-low-calorie ketogenic diet (VLCKD) in the management of metabolic diseases: systematic review and consensus statement from the Italian society of endocrinology (SIE). *J Endocrinol Invest*. 2019;42(11):1365-1386.
7. Sukkar SG, Muscaritoli M. A clinical perspective of low carbohydrate ketogenic diets: a narrative review. *Front Nutr*. 2021;8:642628.
8. Merra G, Gratteri S, De Lorenzo A, *et al*. Effects of very-low-calorie diet on body composition, metabolic state, and genes expression: a randomized double-blind placebo-controlled trial. *Eur Rev Med Pharmacol Sci*. 2017;21(2):329-345.
9. Moriconi E, Camajani E, Fabbri A, Lenzi A, Caprio M. Very-low-calorie ketogenic diet as a safe and valuable tool for long-term glycemic management in patients with obesity and type 2 diabetes. *Nutrients*. 2021;13(3):758.
10. Bruci A, Tuccinardi D, Tozzi R, *et al*. Very low-calorie ketogenic diet: a safe and effective tool for weight loss in patients with obesity and mild kidney failure. *Nutrients*. 2020;12(2):333.
11. Barrea L, Caprio M, Camajani E, *et al*. Ketogenic nutritional therapy (KeNuT)-a multi-step dietary model with meal replacements for the management of obesity and its related metabolic disorders: a consensus statement from the working group of the Club of the Italian Society of Endocrinology (SIE)-diet therapies in endocrinology and metabolism. *J Endocrinol Invest*. 2024;47(3):487-500.
12. Moreno B, Crujeiras AB, Bellido D, Sajoux I, Casanueva FF. Obesity treatment by very low-calorie-ketogenic diet at two years: reduction in visceral fat and on the burden of disease. *Endocrine*. 2016;54(3):681-690.
13. Perticone M, Maio R, Sciacqua A, *et al*. Ketogenic diet-induced weight loss is associated with an increase in vitamin D levels in obese adults. *Molecules*. 2019;24(13):2499.
14. Sajoux I, Lorenzo PM, Gomez-Arbelaez D, *et al*. Effect of a very-low-calorie ketogenic diet on circulating myokine levels

compared with the effect of bariatric surgery or a low-calorie diet in patients with obesity. *Nutrients*. 2019;11(10):2368.
15. Muscogiuri G, El Ghoch M, Colao A, Hassapidou M, Yumuk V, Busetto L. European guidelines for obesity management in adults with a very low-calorie ketogenic diet: a systematic review and meta-analysis. *Obes Facts*. 2021;14(2):222-245.
16. Gomez-Arbelaez D, Bellido D, Castro AI, *et al.* Body composition changes after very-low-calorie ketogenic diet in obesity evaluated by 3 standardized methods. *J Clin Endocrinol Metab*. 2017;102(2): 488-498.
17. Paoli A, Mancin L, Giacona MC, Bianco A, Caprio M. Effects of a ketogenic diet in overweight women with polycystic ovary syndrome. *J Transl Med*. 2020;18(1):104.
18. Camajani E, Feraco A, Proietti S, *et al.* Very low calorie ketogenic diet combined with physical interval training for preserving muscle mass during weight loss in sarcopenic obesity: a pilot study. *Front Nutr*. 2022;9:955024.
19. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. *J Clin Invest*. 1986; 78(6):1568-1578.
20. Basolo A, Votruba SB, Heinitz S, Krakoff J, Piaggi P. Deviations in energy sensing predict long-term weight change in overweight native Americans. *Metabolism*. 2018;82:65-71.
21. Basolo A, Bechi Genzano S, Piaggi P, Krakoff J, Santini F. Energy balance and control of body weight: possible effects of meal timing and circadian rhythm dysregulation. *Nutrients*. 2021;13(9):3276.
22. Rubini A, Bosco G, Lodi A, *et al.* Effects of twenty days of the ketogenic diet on metabolic and respiratory parameters in healthy subjects. *Lung*. 2015;193(6):939-945.
23. Gomez-Arbelaez D, Crujeiras AB, Castro AI, *et al.* Resting metabolic rate of obese patients under very low calorie ketogenic diet. *Nutr Metab (Lond)*. 2018;15:18.
24. Ebbeling CB, Swain JF, Feldman HA, *et al.* Effects of dietary composition on energy expenditure during weight-loss maintenance. *JAMA*. 2012;307(24):2627-2634.
25. Hall KD, Chen KY, Guo J, *et al.* Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Am J Clin Nutr*. 2016;104(2):324-333.
26. Pasquali R, Casanueva F, Haluzik M, *et al.* European Society of Endocrinology Clinical Practice Guideline: endocrine work-up in obesity. *Eur J Endocrinol*. 2020;182(1):G1-G32.
27. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*. 1990;51(2):241-247.
28. Bray GA, Heisel WE, Afshin A, *et al.* The science of obesity management: an endocrine society scientific statement. *Endocr Rev*. 2018;39(2):79-132.
29. Gershuni VM, Yan SL, Medici V. Nutritional ketosis for weight management and reversal of metabolic syndrome. *Curr Nutr Rep*. 2018;7(3):97-106.
30. Muscogiuri G, Barrea L, Laudisio D, *et al.* The management of very low-calorie ketogenic diet in obesity outpatient clinic: a practical guide. *J Transl Med*. 2019;17(1):356.
31. Basolo A, Begaye B, Hollstein T, *et al.* Effects of short-term fasting and different overfeeding diets on thyroid hormones in healthy humans. *Thyroid*. 2019;29(9):1209-1219.
32. Bandini G, Landi A, Santini F, Basolo A, Marracci M, Piaggi P. Sensitivity analysis of whole-room indirect calorimeters at the steady-state condition. *IEEE Trans Instrum Meas*. 2023;72:1-8.
33. Schutz Y, Ravussin E, Diethelm R, Jequier E. Spontaneous physical activity measured by radar in obese and control subject studied in a respiration chamber. *Int J Obes*. 1982;6(1):23-28.
34. Piaggi P, Krakoff J, Bogardus C, Thearle MS. Lower "awake and fed thermogenesis" predicts future weight gain in subjects with abdominal adiposity. *Diabetes*. 2013;62(12):4043-4051.
35. Abbott WG, Howard BV, Christin L, *et al.* Short-term energy balance: relationship with protein, carbohydrate, and fat balances. *Am J Physiol*. 1988;255(3 Pt 1):E332-E337.
36. Heymsfield SB, Brown J, Ramirez S, Prado CM, Tinsley GM, Gonzalez MC. Are lean body mass and fat-free mass the same or different body components? A critical perspective. *Adv Nutr*. 2024;15(12):100335.
37. Antonio Paoli A, Mancin L, Caprio M, *et al.* Effects of 30 days of ketogenic diet on body composition, muscle strength, muscle area, metabolism, and performance in semi-professional soccer players. *J Int Soc Sports Nutr*. 2021;18(1):62.
38. Cahill GF Jr. Starvation in man. *N Engl J Med*. 1970;282(12): 668-675.
39. Cahill GF Jr. Fuel metabolism in starvation. *Annu Rev Nutr*. 2006;26:1-22.
40. Fernández-Verdejo R, Mey JT, Ravussin E. Effects of ketone bodies on energy expenditure, substrate utilization, and energy intake in humans. *J Lipid Res*. 2023;64(10):100442.
41. Müller MJ, Enderle J, Pourhassan M, *et al.* Metabolic adaptation to caloric restriction and subsequent refeeding: the Minnesota starvation experiment revisited. *Am J Clin Nutr*. 2015;102(4):807-819.
42. Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol*. 2017;13(10):572-587.
43. Veldhorst MA, Westerterp-Plantenga MS, Westerterp KR. Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am J Clin Nutr*. 2009;90(3):519-526.
44. Fernández-Verdejo R, Castro-Sepulveda M, Gutiérrez-Pino J, *et al.* Direct relationship between metabolic flexibility measured during glucose clamp and prolonged fast in men. *Obesity (Silver Spring)*. 2020;28(6):1110-1116.
45. Bergman BC, Cornier MA, Horton TJ, Bessesen DH. Effects of fasting on insulin action and glucose kinetics in lean and obese men and women. *Am J Physiol Endocrinol Metab*. 2007;293(4): E1103-E1111.
46. Ruppert PMM, Kersten S. Mechanisms of hepatic fatty acid oxidation and ketogenesis during fasting. *Trends Endocrinol Metab*. 2024;35(2):107-124.
47. Mikkelsen KH, Seifert T, Secher NH, Grøndal T, van Hall G. Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-β-hydroxybutyratemia in postabsorptive healthy males. *J Clin Endocrinol Metab*. 2015;100(2): 636-643.
48. Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF Jr. Brain metabolism during fasting. *J Clin Invest*. 1967;46(10):1589-1595.
49. Schutz Y, Ravussin E. Respiratory quotients lower than 0.70 in ketogenic diets. *Am J Clin Nutr*. 1980;33(6):1317-1319.
50. Moreno B, Bellido D, Sajoux I, *et al.* Comparison of a very low-calorie-ketogenic diet with a standard low-calorie diet in the treatment of obesity. *Endocrine*. 2014;47(3):793-805.
51. Conte C, Hall KD, Klein S. Is weight loss-induced muscle mass loss clinically relevant? *JAMA*. 2024;332(1):9-10.
52. Ibrahim M, Ayoub D, Wasselin T, *et al.* Alterations in rat adipose tissue transcriptome and proteome in response to prolonged fasting. *Biol Chem*. 2020;401(3):389-405.
53. Nair KS, Welle SL, Halliday D, Campbell RG. Effect of beta-hydroxybutyrate on whole-body leucine kinetics and fractional mixed skeletal muscle protein synthesis in humans. *J Clin Invest*. 1988;82(1):198-205.
54. Rose C, Parker A, Jefferson B, Cartmell E. The characterization of feces and urine: a review of the literature to inform advanced treatment technology. *Crit Rev Environ Sci Technol*. 2015;45(17): 1827-1879.
55. Scrimshaw NS, Hussein MA, Murray E, Rand WM, Young VR. Protein requirements of man: variations in obligatory urinary and fecal nitrogen losses in young men. *J Nutr*. 1972;102(12):1595-1604.
56. Nicol BM, Phillips PG. Endogenous nitrogen excretion and utilization of dietary protein. *Br J Nutr*. 1976;35(2):181-193.
57. Listrat A, Lebret B, Louveau I, *et al.* How muscle structure and composition influence meat and flesh quality. *ScientificWorldJournal*. 2016;2016:3182746.

58. Kolanowski J, Bodson A, Desmecht P, Bemelmans S, Stein F, Crabbe J. On the relationship between ketonuria and natriuresis during fasting and upon refeeding in obese patients. *Eur J Clin Invest*. 1978;8(5):277-282.
59. Rothman DL, Magnusson I, Katz LD, Shulman RG, Shulman GI. Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. *Science*. 1991;254(5031):573-576.
60. Volek JS, Sharman MJ, Love DM, *et al*. Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism*. 2002;51(7):864-870.
61. Phinney SD, Bistrian BR, Evans WJ, Gervino E, Blackburn GL. The human metabolic response to chronic ketosis without caloric restriction: preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism*. 1983;32(8):769-776.
62. Bisschop PH, Sauerwein HP, Endert E, Romijn JA. Isocaloric carbohydrate deprivation induces protein catabolism despite a low T3-syndrome in healthy men. *Clin Endocrinol (Oxf)*. 2001;54(1):75-80.
63. Rinaldi R, De Nucci S, Castellana F, *et al*. The effects of eight weeks' very low-calorie ketogenic diet (VLCKD) on liver health in subjects affected by overweight and obesity. *Nutrients*. 2023;15(4):825.
64. Spaulding SW, Chopra IJ, Sherwin RS, Lyall SS. Effect of caloric restriction and dietary composition of serum T3 and reverse T3 in man. *J Clin Endocrinol Metab*. 1976;42(1):197-200.
65. Santini F, Chopra IJ. A radioimmunoassay of rat type I iodothyronine 5'-monodeiodinase. *Endocrinology*. 1992;131(6):2521-2526.
66. Barrea L, Verde L, Vetrani C, *et al*. VLCKD: a real time safety study in obesity. *J Transl Med*. 2022;20(1):23.