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Original article

Immunomodulatory effect of a very-low-calorie ketogenic diet compared with bariatric surgery and a low-calorie diet in patients with excessive body weight

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SUMMARY

Background & aim: Inflammation and oxidative stress are the most probable mechanistic link between obesity and its co-diseases with cancer among them. The aim of this study was to evaluate whether the nutritional ketosis and weight loss induced by a very-low-calorie ketogenic diet (VLCKD) modulates the inflammatory and oxidative stress profile, compared with a standard, balanced hypocaloric diet (LCD) or bariatric surgery (BS) in patients with obesity.

Methods: The study was performed in 79 patients with overweight or obesity and 32 normal-weight volunteers as the control group. Patients with obesity underwent a weight reduction therapy based on VLCKD, LCD or BS. The quantification of the circulating levels of a multiplexing test of cytokines and carcinogenesis/aging biomarkers, as well as of lipid peroxides and total antioxidant power, was carried out.

Results: First, we observed that pro-inflammatory cytokines increase, while anti-inflammatory cytokines decrease under excessive body weight. Relevantly, when patients underwent weight loss strategies, it was shown that energy-restricted and surgical strategies of weight loss induced changes in circulating cytokine and lipid peroxides. This effect was more notable in patients following the VLCKD than the LCD or BS and it was observed mainly in the ketosis phase of the intervention. Particularly, IL-11, IL-12, IL-2, INF-γ, INF-β, Pentraxin-3 or MMP1 changed after VLCKD. Whereas, APRIL, TWEAK, osteocalcin and IL-28A increased after BS.

Conclusion: As far as we know, this is the first study that evaluate the time-course of cytokines and oxidative stress markers after a VLCKD as compared with a standard LCD and BS. The observed results

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support the immunomodulatory effect of nutritional ketosis induced by a VLCKD synergistically with weight loss as a strategy to improve innate-immunity and to prevent infections and carcinogenesis in patients with obesity.

1. Introduction

Obesity is a worldwide health problem being considered a global epidemic [1–3]. Given that, the prevalence of this metabolic disease is increasing and accelerating the onset of several diseases such as cardiometabolic events, type 2 diabetes and liver steatosis [1–3]. Moreover, obesity is now considered to be the most important modifiable risk factor of cancer, after smoking. In 2016 the IARC working group concluded that there was sufficient evidence for a cancer-preventive effect of avoidance of weight gain for thirteen types of cancers and there was convincing evidence that intentional body-weight loss positively affects the mechanistic links involved in the effect of excess body fat on carcinogenesis [4]. Therefore, it is important to elucidate those weight loss strategies with more potential to prevent and counteract putative neoplastic mechanistic links.

Several mechanisms were proposed to be involved in the effect of excess body fat and carcinogenesis, but the most investigated and the most scientifically supported by experimental studies is the role of dysfunctional adipose tissue observed in the obesity setting [5,6]. Under conditions of obesity, approximately 70–80% of individuals undergo adipose tissue remodeling both structurally and functionally, causing an inflammatory cascade [7]. When this acute inflammation does not subside, a low-grade chronic inflammation ensues [7–9]. Excess visceral fat is a source of cytokines, that creates an inflammatory-oxidative stress axis [10]. These mediators secreted by dysfunctional obese adipose tissue are associated with the onset of obesity comorbidities, including the increased risk of cancer and ageing [5]. Outstandingly, in the new era of COVID-19, obesity again emerged as a relevant risk factor for poor prognosis and high mortality of patients infected with Sars-Cov-2 virus [11]. It was proposed that the relationship between obesity and COVID-19 is promoted by inflammation and oxidative stress induced by the obesity-related dysfunctional adipose tissue [12,13]. Exacerbated inflammatory and oxidative stress biomarkers decrease with dietary intervention and weight loss [14], ameliorating or attenuating metabolic risk factors related to obesity and its comorbidities.

Previous studies from our research group have shown that very-low-calorie ketogenic diet (VLCKD), a type of diet characterized by the restriction of carbohydrates intake to the point of inducing changes in metabolism and the generation of plasma ketone bodies, is effective for the management of obesity and also for the preservation of muscle mass, because weight loss was achieved mainly at the expense of total fat mass and visceral fat [15]. Muscle tissue, an organ that secretes proteins called myokines, is responsible for the improvement of many diseases associated with the inflammatory state [16,17]. In a previous study, it was found that after treatment with VLCKD, myokine levels increased, relating these changes to body composition, especially fat-free mass [18]. Moreover, this nutritional intervention improves the autonomic nervous system activity [19] and is associated with good food control and improvements in the psychological well-being parameters in obese subjects [20]. These beneficial effects of VLCKD could be mediated by epigenetic mechanisms such as was recently demonstrated in an epigenome-wide study [21]. In contrast, bariatric surgery (BS), resulting in rapid and massive weight loss (>20% of total body weight), in addition to producing fat loss, accelerates fat-free mass loss [22]. Fat-free body mass is an important component of body mass loss after BS, especially during the first six months after surgery [23]. Moreover, BS by itself induces metabolic stress, producing a state of inflammation during the first 6 months after the intervention [24,25].

Currently, the role of nutrition to modulate immune system function is been investigated as an adjuvant to conventional therapies in several disease such as cancer [26] and the emergent COVID-19 [27]. It is because, it has been observed that some nutritional components in particular may improve the concentrations of various pro-inflammatory and pro-oxidative biomarkers [28–30]. Thus, in the context of immunonutrition, ketone bodies have been proposed to exert an anti-inflammatory and antioxidant action in the organism and they could lighten age-related diseases or viral infections such as COVID-19 [31,32]. The molecular mechanisms by which ketone bodies prevent oxidative stress and inflammation is pending to be totally elucidated. In this regard, based on the current existing preclinical and clinical studies, it was recently proposed that ketone bodies could promote a moderate mitochondrial stress that induce the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), sirtuins 1 and 3 and AMP-activated kinases which results in an adaptive response that lead to an anti-oxidative and anti-inflammatory state, improved mitochondrial function, cell repair, and regeneration mechanisms [33].

These findings suggest that dietary therapies where ketone bodies are enlarged can reduce inflammation and its negative effects [34] in pathologies such as obesity and its related diseases.

Therefore, the aim of this study was to evaluate whether the nutritional ketosis and weight loss induced by a VLCKD modulates the inflammatory and oxidative stress profile, compared with a standard, balanced hypocaloric diet or BS in patients with obesity. This aim was achieved by quantifying the circulating levels of a number of cytokines and carcinogenesis/ageing biomarkers as well as lipid peroxides and total antioxidant power.

2. Materials and methods

2.1. Patients

The study was performed in 79 patients with overweight or obesity (n = 59 women) and 32 normal-weight volunteers (n = 20 women) who served as the control group. Patients with obesity underwent a weight reduction therapy based on two different energy-restriction programs or BS (Supplementary Fig. 1). Thus, a group of patients completed treatment with a VLCKD (PnK method®). The other group was a subsample of the study for the Reduction of the Metabolic Syndrome in Navarra (RESMENA), Spain, a randomized control trial based on a low-calorie diet (LCD). A third group underwent BS treatment. Written informed consent to participate in the study was obtained before the start of the study in agreement with the Helsinki Declaration and followed national and European Union guidelines. The study was approved by the respective Institutional Ethics Committee for clinical research of Galicia (ref. 2009/076), Navarra (ref. 065/2009) and Andalucia (ref CP1600163).

2.2. Study design

2.2.1. Very low-calorie ketogenic diet (VLCKD)

A cohort of patients subjected to the VLCKD was enrolled in this study. This cohort included 20 patients with obesity (body mass index (BMI) 35.5 ± 4.4; n = 12 women; 47.1 ± 10.2 years) attending the Obesity Unit at the Complejo Hospitalario Universitario de Santiago de Compostela, Spain. The VLCKD was designed according to a commercial weight-loss program (PnK method®), which includes lifestyle and behavioral modification support as described elsewhere [15,35–37]. This method is based on high-biological-value protein preparations obtained from cow's milk, soya, avian eggs, green peas and cereals. Each preparation contained 15 g protein, 4 g carbohydrates, 3 g fat, and 50 mg docosahexaenoic acid, which provided 90–100 kcal of energy [15]. The weight loss program had three phases (Supplementary Fig. 1A). The first phase consisted of a VLCKD (600–800 kcal/day), low in carbohydrates (<50 g/day from vegetables) and lipids (10 g/day from olive oil). The percentage of total energy intake (E%) from macronutrients during this phase was distributed as 57–60 E% from proteins, 14–20 E% from carbohydrates, 23–26 E% from lipids. The amount of high biological-value proteins ranged between 0.8 and 1.2g per kg of ideal body weight to ensure that patients were meeting their minimum bodily requirements and to prevent the loss of lean mass. Throughout this ketogenic phase, supplements of vitamins and minerals such as K, Na, Mg, Ca, and omega-3 fatty acids were provided. When the target amount of weight was lost, the ketogenic phase ended, and patients started a low-calorie diet (800–1500 kcal/day; 43 E% proteins, 30 E% carbohydrates, 26 E% lipids) followed by a maintenance diet of 1500–2000 kcal/day (34 E % proteins, 35 E% carbohydrates, 31 E% lipids). The weight loss program adhered to the 2015 guidelines of the European Food Safety Authority regarding total carbohydrate intake [38].

Patients followed the different steps of the program for up to a maximum period of 4–6 months, although patients remained under medical supervision for the following 12 months [15]. The intervention included an evaluation by the specialist physician conducting the study and an assessment by an expert dietician. All patients underwent a structured program of physical exercise, with external supervision [15].

Throughout the study, the patients completed a maximum of 10 visits with the research team (every 15 ± 2 days); four visits involved a complete physical, anthropometric, and biochemical assessment, while the remaining visits involved control of adherence to the program and evaluation of potential side effects. The four assessment visits were scheduled according to the development of ketosis for each patient as follows: normal level of ketone bodies (baseline), maximum ketosis (1–2 months), reduction of the ketotic approach because of partial reintroduction of normal nutrition (approximately 3 months), no ketosis (4–6 months, end of the study) [15]. The total ketosis state lasted for 60–90 days. In all visits, patients received dietary instructions, individual supportive counseling, and encouragement to exercise on a regular basis using a formal exercise program. The compliance of this advice was not registered. The body weight, body composition, and circulating levels of cytokines and oxidative stress biomarkers were evaluated at baseline (0 months), at maximum ketosis (2–3 months), and at no ketosis (4–6 months).

2.2.2. Low-calorie diet (LCD)

A group of patients with obesity (n = 20, BMI 35.8 ± 4.5; n = 10 women; 49.9 ± 9.3 years) followed a therapy program based on a nutritional intervention controlled by trained dieticians from the Department of Nutrition, Food Sciences and Physiology of the University of Navarra, Spain. Briefly, the study lasted six months in two sequential periods; one intervention period of two months (30% energy restriction, i.e., a reduction of 600–800 kcal/day), during which patients received nutritional assessment every 15 days, followed by a self-control period of four months, during which individuals were advised to follow the lifestyle adopted in the first period (Supplementary Fig. 1B). The energy-restricted diets prescribed were based on the American Heart Association guidelines and included 3–5 meals per day and a macronutrient distribution of 50–55% of the total caloric value from carbohydrates, 15% from proteins and 30% from lipids. Moreover, all participants were asked to maintain their normal physical activity during the study. Anthropometric and body composition measurements and venous blood samples were collected at baseline (week 0), at the end of the diet intervention (endpoint, 2 months), and four months after the end of the treatment (follow-up, 6 months).

2.2.3. Bariatric surgery (BS)

A group of morbidly obese patients (n = 39, BMI 45.6 ± 6.2; n = 37 women; 40.8 ± 10.4 years) underwent BS by laparoscopic techniques at the Hospital Clínico Virgen de la Victoria from Malaga and Complejo Hospitalario Universitario de Santiago de Compostela, Spain.

After surgical intervention, patients were encouraged to follow a special diet that consisted of liquid diet for 1-month, soft diet for 1-month, and subsequently a normal consistency diet providing about 800 kcal/day with a macronutrient distribution of 48–55 E% of the total caloric value from carbohydrates, 23–27 E% from proteins and 21–27 E% from lipids (Supplementary Fig. 1C). Patients subjected to BS were provided with a daily vitamin-mineral supplement, beginning on the day of the surgical procedure, to reduce the risk of developing nutritional deficiencies. The post-surgery nutritional management was according to the Endocrine Society guidelines and it is prescribed in order to prevent complications, weight regain, and progression of obesity-associated comorbidities [39]. Patients involved in this study returned to the clinic for all follow-up visits. The follow-up regimen included visits at baseline, at three and 6 months at which time anthropometric and body composition measurements were performed, and venous blood samples were collected. Body composition data at three visits were available from n = 24 patients included in this group.

2.3. Anthropometric and body composition measurements

All anthropometric measurements were performed with patients wearing only their underwear and after an overnight fast (8–12 h) according to validated procedures. Body weight and height measurements were performed using a wall-mounted stadiometer (Seca 220 scale, Medical Resources, EPI Inc., OH, USA). The BMI was calculated by dividing body weight by the square of the height (kg/m^2). Total body composition was measured by dual-energy X-ray Absorptiometry (GE Healthcare Lunar, Madison, WI, USA) as described elsewhere [15].

2.4. Biochemical analysis

Venous blood samples were collected after a 12-h overnight fast, and ethylenediaminetetraacetic acid-treated plasma and serum were separated from whole blood and immediately frozen at −80 °C until used.

Cytokines plasma levels were quantified using a commercial multiplex enzyme-linked immunosorbent assay (ELISA) kit (Bio-Plex custom assay, Bio-Rad Laboratories, Marnes-la-Coquette, France) according to the manufacturer's instructions. The minimum detectable values specified in the kit were used, where no data was obtained for each cytokine. Under this procedure, the following cytokines were analyzed: April, B cell activator factor

(BAFF), cluster of differentiation (CD)163, CD30, Chitanase, glycoprotein (Gp)130, interferon (IFN)-α2, IFN-β, IFN-γ, interleukin (IL)-2, IL-6R, IL-11, IL-12(p40), IL-12(p70), IL-22, IL-26, IL-28A, IL-29, IL-35, matrix metalloproteinase (MMP)1, MMP3, Osteocalcin, Pentraxin-3, tumor necrosis factor receptor (TNF)-R1, TNF-R2, Thymic stromal lymphopoietin (TSLP) and Tweak.

Among the oxidative stress biomarkers, the levels of malondialdehyde (MDA) and total antioxidative power (AOP) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were evaluated in serum. MDA and AOP were quantified using colorimetric assay kits (OXIS International, Portland, OR, USA). An enzyme immunoassay kit was used for the quantification of 8-OHdG; Japan Institute for the Control of Aging (JaICA), Fukuroi, Japan) in the serum.

Absorbance of each sample was measured in duplicate using a spectrophotometric microplate reader at a wavelength of 450 nm (Versamax Microplate Reader, Associates of Cape Cod Inc., MA, USA).

Ketosis was determined by measuring ketone bodies, specifically β-hydroxybutyrate (β-OHB), in capillary blood using a portable meter (GlucoMen LX Sensor, A. Menarini Diagnostics, Neuss, Germany) before measurements of anthropometric parameters. As with anthropometric and biochemical assessments, all of the determinations of capillary ketonemia were made after an overnight fast of 8–10 h. These measurements were performed daily by each patient during the entire VLCKD, and the corresponding values were reviewed on the machine's memory by the research team for managing adherence. Additionally, β-OHB levels were determined at each complete visit by the physician in charge of the patient. In the LCD and BS groups, ketosis was measured in the three points of analysis by the physician; baseline, endpoint and follow-up.

2.5. Statistical analysis

The sample size of the current study was calculated to detect differences in cytokines levels considering published values of cytokines and standard deviation [40,41]. It was calculated for an $\alpha = 0.05$, and a power (1-β) of 80%. The normal distribution of variables was explored using the Kolmogorov–Smirnov and Shapiro–Wilk tests.

An analysis of variance (ANOVA) or analysis of covariance (ANCOVA) adjusted for age and gender were used to study differences between groups according to adiposity at baseline. A repeated-measures ANOVA were used to study the effects of time-course of the nutritional therapy program and groupings of body composition, biochemical parameters, cytokines and oxidative stress levels in patients with obesity.

Differences respect to baseline (0 months) and between follow-up respect to endpoint were calculated within each weight loss treatment. Differences between weight loss treatments were evaluated with univariant ANCOVA adjusted for age and baseline levels of cytokines. An additional analysis was performed adjusting for initial BMI and body weight loss to compare the weight loss treatments.

The statistical analysis in all variables was performed in patients who had valid data in all three time-points of the treatment follow-up (1: baseline, 2: follow-up, 3: endpoint of the treatment).

Statistical analyses were performed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA) for Windows XP (Microsoft, Redmond, WA, USA). A p value ≤ 0.05 was considered statistically significant.

3. Results

3.1. Differences in circulating levels of cytokines according to adiposity

To evaluate the effect of the weight loss treatments on cytokines, the levels of cytokines were first evaluated relative to excess adiposity (Table 1). Thus, the cohort of excess body weight patients were classified according to their BMI as overweight (BMI 25–29.9), obese (BMI 30–39.9), morbid obese (BMI ≥40) and compared with a group of normal-weight (BMI <25) individuals. In relation to body composition, statistically significant differences were observed between the groups (Table 1, Fig. 1A). Under these conditions, cytokines related to inflammation and carcinogenesis showed increased levels in obesity compared with normal-weight individuals (Table 1), such as April (Fig. 1B), MMP3 (Fig. 1D), TNF-R1, and TNF-R2. By contrast, cytokines which show anti-inflammatory and metabolic homeostasis properties such as GP130, IL-12(p40) (Fig. 1C) and Tweak (Fig. 1E) were higher in normal weight than obese individuals. In relation to the other evaluated cytokines, no statistically significant differences were found between the adiposity-related groups (Table 1). Figure 1 represents those cytokines whose circulating concentration showed differences according to obesity phenotype and also were further modified after weight loss therapies.

3.2. Changes in circulating levels of cytokines after the weight loss therapies

Basal characteristics and changes induced by the weight loss treatments in body weight, body composition and biochemical parameters are shown in Supplementary Table 1.

After the effect of the different weight loss treatments, significant changes were observed in several cytokines (Table 2). These changes were statistically significant in circulating levels of April, CD163, CD30, IFN-β, IFN-γ, IL-2, IL-11, IL-12(p40), IL-28A, MMP1, Osteocalcin, Pentraxin-3 and Tweak. Circulating Osteocalcin levels showed an interaction between groups. Also, statistically significant differences were found between studies in circulating MMP3 and Pentraxin-3. In addition to the previous cytokines, statistically significant changes were obtained in the circulating levels of BAFF and TSLP after VLCKD, in IL-29 after LCD, and in Osteocalcin after BS (Table 2, Table 3). The modulation in circulating cytokines induced by VLCKD was particularly evident at maximum ketosis (Table 2). When the analysis of the differences from baseline in cytokine levels between the weight loss treatments was adjusted for baseline BMI and weight loss, statistically significant differences between treatments remained in the changes of IFN-β, IFN-γ and IL-2 (Table 3).

Figure 2 represents the differences from baseline after weight loss treatments in those cytokines that showed differences according to excess adiposity groups. Thus, it can be observed that circulating levels of April increases after the two phases of treatments, obtaining significant changes in BS, but no statistically significant changes was observed after VLCKD and LCD (Fig. 2A). Circulating levels of IL-12(p40) (Fig. 2B) increase after the first phase of weight loss treatments in the three therapies, finding statistically significant differences only in VLCKD and BS. No statistically significant differences were found in MMP3 levels through the treatments, but differences between weight loss therapies were observed, where VLCKD produces a statistically significant decrease in MMP3 levels compared with the other treatments (Fig. 2C). Tweak levels (Fig. 2D) increase through the three therapies, producing a tendency to return to baseline levels in VLCKD and LCD. However, in BS the increase was maintained until the end of the follow-up, obtaining statistically significant differences with respect to the baseline levels in both points.

In addition, other statistically significant changes in circulating cytokine levels were observed and their changes respect to baseline are shown in Fig. 3. CD163 levels tended to decrease after treatments, with a drift to return to baseline levels at the end of the study in BS (Fig. 3A). Circulating CD30 (Fig. 3B), IFN-β (Fig. 3C), IFN-

Table 1
Basal characteristics and cytokines levels of study subjects according to adiposity.

	Normal-weight	Overweight	Obese	Morbidly obese	*P*-value
N	32	8	44	27	
Gender (male/female)	12/20	6/2	13/31	2/25	**<0.001**‡
Age (years)	35.9 ± 9.67	37.9 ± 9.41	45.3 ± 9.88*	41.5 ± 8.10	**0.001**
Height (m)	1.68 ± 0.09	1.66 ± 0.11	1.64 ± 0.09	1.63 ± 0.10	0.252
Body weight (kg)	64.7 ± 9.20	78.1 ± 8.18	96.5 ± 13.1*†	133.1 ± 21.5*†¥	**<0.001**
BMI (kg/m^2)	22.7 ± 1.68	27.9 ± 1.46*	34.7 ± 2.58*†	49.3 ± 8.96*†¥	**<0.001**
FM (kg)[a]	24.0 ± 7.01	27.7 ± 2.50	40.9 ± 9.52*†	60.5 ± 7.83*†¥	**<0.001**
FFM (kg)[a]	47.1 ± 8.99	44.8 ± 6.44	54.4 ± 11.1	54.8 ± 12.8	0.062
April (ng/mL)	37.9 ± 21.4	65.3 ± 40.3	90.4 ± 37.9*	110 ± 64.7*	**<0.001**
BAFF (pg/mL)	3703 ± 1161	3617 ± 1083	4215 ± 1879	3950 ± 2207	0.886
CD163 (ng/mL)	63.3 ± 49.6	34.4 ± 12.4	56.2 ± 33.0	64.0 ± 47.3	0.125
CD30 (pg/mL)	157 ± 66.5	128 ± 43.0	197 ± 99.7	199 ± 136	0.224
Chitanase (pg/mL)	4837 ± 2053	3974 ± 1387	5835 ± 4421	7060 ± 4977	0.142
Gp130 (ng/mL)	38.2 ± 16.3	34.7 ± 19.7	22.2 ± 8.09*	22.4 ± 13.2*	**<0.001**
IFN-α2 (pg/mL)	–	–	14.4 ± 4.40	16.4 ± 4.77	0.228
IFN-β (pg/mL)	30.0 ± 11.1	–	30.4 ± 16.3	32.1 ± 17.7	0.459
IFN-γ (pg/mL)	22.1 ± 39.7	3.15 ± 5.92	12.4 ± 10.9	13.6 ± 14.3	0.279
IL-2 (pg/mL)	–	–	7.39 ± 3.06	8.68 ± 4.96	0.240
IL-6R (pg/mL)	3454 ± 1340	3733 ± 1296	4064 ± 2777	4315 ± 3340	0.793
IL-11 (pg/mL)	3.32 ± 1.41	2.40 ± 0.35	3.90 ± 2.48	3.67 ± 2.17	0.065
IL-12(p40) (pg/mL)	62.8 ± 58.7	23.4 ± 29.7*	24.1 ± 24.5*	27.7 ± 24.0*	**0.006**
IL-12(p70) (pg/mL)	–	–	1.09 ± 0.35	0.97 ± 0.42	0.439
IL-22 (pg/mL)	47.9 ± 147	3.29 ± 6.19	7.98 ± 11.4	5.38 ± 8.25	0.071
IL-26 (pg/mL)	–	–	–	–	–
IL-28A (pg/mL)	–	–	20.3 ± 8.27	17.8 ± 8.89	0.349
IL-29 (pg/mL)	–	–	–	–	–
IL-35 (pg/mL)	–	–	104 ± 84.5	109 ± 147	0.830
MMP1 (pg/mL)	242 ± 385	77.8 ± 87.0	307 ± 470	181 ± 226	0.458
MMP3 (pg/mL)	4991 ± 2876	7885 ± 8224	11015 ± 7815*	6675 ± 5525	**0.025**
Osteocalcin (pg/mL)	1222 ± 610	1165 ± 581	1438 ± 914	1155 ± 1049	0.811
Pentraxin-3 (pg/mL)	955 ± 749	549 ± 218	630 ± 360	839 ± 684	0.302
TNF-R1 (pg/mL)	650 ± 204	599 ± 244	1030 ± 572*	1159 ± 790*†	**0.011**
TNF-R2 (pg/mL)	208 ± 56.4	258 ± 89.8	541 ± 388*	642 ± 601*	**0.003**
TSLP (pg/mL)	59.8 ± 21.3	52.8 ± 29.6	72.8 ± 32.8	62.9 ± 30.2	0.372
Tweak (pg/mL)	174 ± 69.3	158 ± 50.2	221 ± 122	130 ± 76.0¥	**0.013**

Biomarker data is included when levels were detected in a number of samples n ≥ 8. Data show mean ± standard deviation. *P*-value was calculated ANCOVA adjusted for age and gender. ‡ *P*-value was calculated with Chi-square. Asterisk (*) denotes statistically significant differences ($p < 0.05$) in relation to normal-weight individuals. † denotes statistically significant differences ($p < 0.05$) in relation to overweight individuals. ¥ denotes statistically significant differences ($p < 0.05$) in relation to obese individuals.

[a] Body composition data were not obtained from all participants included in the study. BMI, body mass index; FM, fat mass; FFM, fat free mass; BAFF, B cell activator factor; CD, cluster of differentiation; Gp, glycoprotein; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; TNF-R, tumor necrosis factor receptor; TSLP, Thymic stromal lymphopoietin.

γ (Fig. 3D), IL-2 (Fig. 3E), IL-11 (Fig. 3F), MMP1 (Fig. 3H), Osteocalcin (Fig. 3I) and Pentraxin-3 (Fig. 3J) tended to increase after the first phase of weight loss treatments in the three therapies, with a drift to return to baseline levels at the end of the study in VLCKD and LCD, and in CD30 for BS. This increase was statistically significant after VLCKD in circulating levels of IFN-γ and MMP1, and in BS in CD30, IFN-γ and MMP1. Also, getting to find statistically significant differences between the treatments in the differences between follow-up to baseline in the levels of Osteocalcin, and between endpoint to baseline in the levels of IFN-γ, IFN-β, IL-2 and IL-11, and follow-up to endpoint in the levels of IFN-γ and Osteocalcin (Table 3). In the same way IL-28A (Fig. 3G) increase after the first phase of weight loss treatments in the VLCKD and BS, with a decrease in levels at the end of the follow-up in VLCKD.

3.3. Changes in circulating levels of oxidative stress biomarkers after the weight loss therapies

Oxidative stress markers were evaluated after VLCKD or BS. No statistically significant changes were observed following the weight loss treatments in AOP or 8-OHdG, but a statistically significant increase was observed in MDA in patients following the VLCKD (Table 2). Moreover, an interaction was observed in MDA between both weight loss treatments (Table 2) which was translated into an increase after VLCKD and a decrease after BS respect to baseline. These differences were statistically significant (Table 3).

Importantly, a positive correlation was observed between circulating levels of ketone bodies (β-OHB) at the 2–3 months of the interventions and MDA ($r = 0.56$; $p = 0.001$) (Fig. 4A) and AOP ($r = 0.47$; $p = 0.009$) levels at endpoint (Fig. 4B). Also, ketone bodies at the 2–3 months of intervention were correlated with the changes at 2–3 months with respect to baseline induced by the intervention in MDA ($r = 0.37$; $p = 0.042$) and AOP ($r = 0.40$; $p = 0.029$), and the changes at 4–6 months with respect to at baseline in MDA ($r = 0.72$; $p = <0.001$) and AOP ($r = 0.37$; $p = 0.047$).

4. Discussion

The current study demonstrated that when patients with obesity underwent energy-restricted or surgical weight loss strategies, inflammatory and oxidative stress biomarkers are improved. It was reached by performing a multiplexing assay that included 27 cytokines together with the analysis of lipid peroxidation markers in plasma. Relevantly, the effect was more notable in patients following the VLCKD than the LCD or BS. As far as we know, this is the first study that evaluated the time-course of cytokines after a VLCKD as compared with a standard LCD and BS. The observed results support the need for additional long-size, longitudinal studies to elucidate the immunomodulatory effect of nutritional ketosis induced by a VLCKD in the management of obesity as a strategy to improve innate-inmmunity and to prevent infections and carcinogenesis risk in patients with obesity.

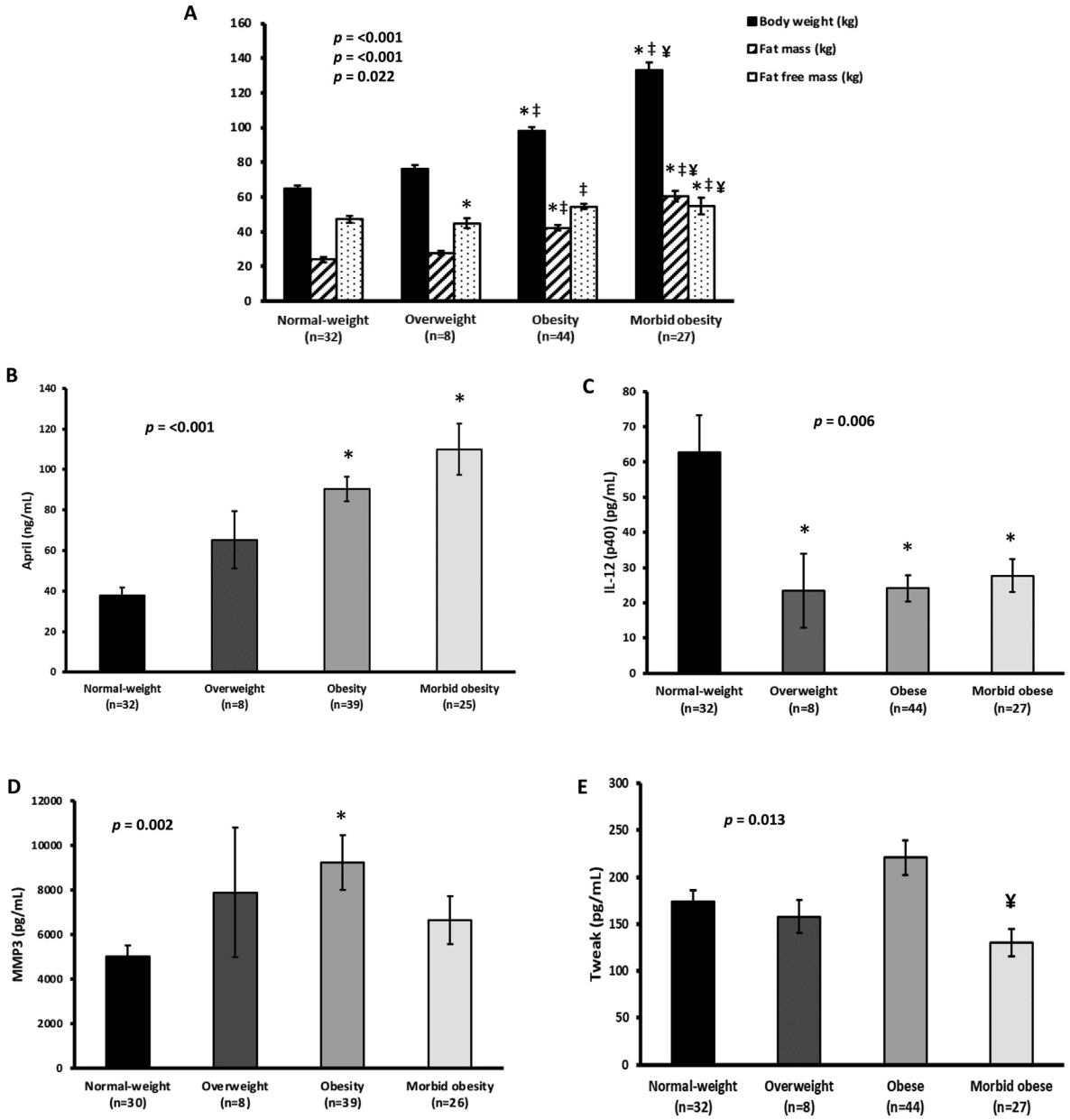

Fig. 1. Comparison of body composition and circulating levels of cytokines according to adiposity at baseline. A) Differences in body weight, fat mass and fat free mass between normal-weight, overweight, obese and morbid obese individuals. B) Differences in April levels between normal-weight, overweight, obese and morbid obese individuals. C) Differences in interkeukin-12(p40) (IL-12(p40)) levels between normal-weight, overweight, obese and morbid obese individuals. D) Differences in matrix metalloproteinase-3 (MMP3) levels between normal-weight, overweight, obese and morbid obese individuals. E) Differences in Tweak levels between normal-weight, overweight, obese and morbid obese individuals. The data are presented as the mean (SE). *P*-value is calculated with ANOVA or ANCOVA adjusted for age and gender, as applicable. Asterisk (*) denotes statistically significant ($p < 0.05$) differences in relation to normal-weight individuals. ‡ denotes statistically significant ($p < 0.05$) differences in relation to overweight individuals. ¥ denotes statistically significant ($p < 0.05$) differences in relation to obesity individuals.

Recent research has proposed that excess fat mass, mainly visceral fat, secretes a type of molecule called cytokines that contribute to the inflammatory process [10,42,43]. These pro-inflammatory molecules can decrease with dietary weight loss treatments in people with obesity [14]. In this way, by reducing the inflammatory state, the risk factors associated with obesity can decrease and even be eliminated. In recent years, certain components of diet have been shown to change in cytokine and oxidative stress levels, exerting an anti-inflammatory effect in the context of what is now known as immunonutrition [28–30]. Among the effect of dietary factors, nutritional ketosis has emerged as a modulator of immune system since ketone bodies acts as signaling effectors [32,44]. In the current study we found a relevant effect of weight loss therapies on several cytokines and oxidative stress markers. Despite the comparable weight loss induced by BS and VLCKD, a more relevant effect was observed in patients who follow the VLCKD, suggesting that ketone bodies production induced by this ketogenic diet could be the main factor associated with the improvement in the inflammation observed. A prove of this hypothesis was that the effect of the VLCKD treatment was more evident in the acute phase of the nutritional intervention where patients showed next to 3 mM of circulating ketone bodies. The current results were reached by performing a multiplexing assay which allows to analyze immunity responses in human diseases in

Table 2
Changes induced by the weight loss treatments in cytokines and oxidative stress biomarkers.

Biomarkers	VLCKD (n = 20)			LCD (n = 20)			BS (n = 39)			ANOVA *p*-value		
	Baseline	Endpoint	Follow-up	Baseline	Endpoint	Follow-up	Baseline	Endpoint	Follow-up			
	(0 months)	(2–3 months)	(4–6 months)	(0 months)	(2–3 months)	(4–6 months)	(0 months)	(2–3 months)	(4–6 months)	Time	Study	Time* Study
April (n = 17; n = 16; n = 26)	89.3 ± 35.1	93.8 ± 29.7	96.7 ± 35.6	88.7 ± 31.5	90.7 ± 31110	90.6 ± 34.6	104.5 ± 66.8	123.4 ± 79.4	129.2 ± 77.5‡*	**0.039**	0.136	0.171
BAFF (n = 17; n = 16; n = 26)	3913 ± 1472	3815 ± 1435	3552 ± 1402‡*	4299 ± 1327	3980 ± 1228	4117 ± 1995	4025 ± 2284	4000 ± 1981	4084 ± 2231	0.511	0.779	0.755
CD163 (n = 17; n = 16; n = 26)	51.1 ± 21.7	43.4 ± 22.4	40.8 ± 19.9‡*	68.3 ± 30.5	57.8 ± 24.6‡	56.2 ± 26.4‡*	62.2 ± 49.0	58.2 ± 39.6	62.0 ± 35.4	**0.047**	0.200	0.328
CD30 (n = 17; n = 16; n = 26)	191 ± 88.6	238 ± 185	239 ± 240	178 ± 80.9	204 ± 120	166 ± 59.8	198 ± 132	290 ± 274	284 ± 154‡*	**0.018**	0.266	0.056
Chitanase (n = 17; n = 16; n = 26)	6186 ± 5432	4767 ± 1942	4415 ± 2230	6084 ± 3563	5452 ± 2757	5310 ± 2969	6591 ± 5167	6940 ± 4811	6590 ± 3660	0.125	0.290	0.388
Gp130 (n = 15; n = 16; n = 26)	21.8 ± 59.1	26.4 ± 14.2	24.0 ± 8.2	23.7 ± 7.1	22.6 ± 7.1	21.3 ± 6.6	20.3 ± 13.0	20.8 ± 9.9	21.9 ± 9.1	0.706	0.479	0.276
IFN-α2 (n = 9; n = 0 -; n = 8)	15.07 ± 4.32	26.67 ± 19.66	24.90 ± 22.36	–	–	–	15.33 ± 5.62	15.30 ± 2.19	16.40 ± 4.35	–	–	–
IFN-β (n = 11; n = 13; n = 22)	33.60 ± 16.20	48.80 ± 24.20‡	44.28 ± 17.86‡*	30.40 ± 16.30	31.04 ± 19.60	29.11 ± 15.90	35.14 ± 17.53	37.57 ± 21.98	41.43 ± 24.01	**0.017**	0.250	0.099
IFN-γ (n = 12; n = 10; n = 16)	16.50 ± 10.53	38.78 ± 21.39‡	20.35 ± 13.35†*	14.60 ± 6.80	19.32 ± 5.71	16.56 ± 8.68	21.79 ± 11.22	26.72 ± 15.03‡	30.10 ± 16.44‡*	**0.006**	0.122	0.241
IL-2 (n = 14; n = 13; n = 19)	7.13 ± 3.34	22.65 ± 13.72‡	11.73 ± 7.68‡†*	6.85 ± 2.14	13.19 ± 15.46	8.48 ± 2.76‡*	9.08 ± 3.96	11.76 ± 4.95‡	12.27 ± 5.04‡*	**<0.001**	0.090	0.283
IL-6R (n = 17; n = 16; n = 26)	4436 ± 3801	4973 ± 3059	3817 ± 1158	4181 ± 1237	4215 ± 1045	3895 ± 1230	3993 ± 3316	3840 ± 1734	3738 ± 1535	0.282	0.575	0.282
IL-11 (n = 17; n = 16; n = 23)	3.32 ± 1.75	5.59 ± 3.71‡	4.39 ± 1.99‡†*	2.97 ± 0.94	4.00 ± 1.62‡	3.50 ± 1.36‡*	4.34 ± 3.32	4.74 ± 2.75	6.05 ± 5.58	**0.004**	0.209	0.412
IL-12(p40) (n = 12; n = 10; n = 21)	30.42 ± 28.59	50.14 ± 36.15‡	40.51 ± 35.51†*	25.25 ± 20.08	32.64 ± 20.81	29.84 ± 19.41	40.26 ± 20.31	54.16 ± 32.46‡	50.95 ± 29.57‡*	**0.011**	0.142	0.711
IL-12(p70) (n = 10; n = 6; n = 6)	1.19 ± 0.40	1.41 ± 0.64	1.16 ± 0.35	0.91 ± 0.13	0.97 ± 0.44	1.05 ± 0.30	0.96 ± 0.28	0.99 ± 0.42	0.96 ± 0.29	0.534	0.173	0.470
IL-22 (n = 6; n = 7; n = 7)	15.61 ± 2.90	22.84 ± 17.57	18.41 ± 5.34	16.40 ± 6.76	18.66 ± 8.95	19.29 ± 10.72	16.49 ± 9.26	19.18 ± 8.89	18.64 ± 10.68	0.071	0.980	0.970
IL-26 (n = 16; n = 15; n = 20)	27.70 ± 18.66	29.57 ± 17.93	27.57 ± 18.18	25.69 ± 16.69	27.67 ± 18.29	25.34 ± 16.88	30.07 ± 14.13	36.38 ± 20.15	36.67 ± 24.92	0.376	0.340	0.344
IL-28A (n = 13; n = 10; n = 14)	18.37 ± 7.30	23.05 ± 11.77	21.11 ± 10.16	20.28 ± 9.19	19.82 ± 7.34	21.76 ± 8.44	20.02 ± 8.50	22.62 ± 10.01	25.10 ± 9.54‡*	**0.017**	0.805	0.487
IL-29 (n = 14; n = 11; n = 3)	39.69 ± 28.30	57.42 ± 50.38	43.24 ± 23.49	46.18 ± 24.26	57.36 ± 30.89	60.76 ± 34.08‡*	–	–	–	–	–	–
IL-35 (n = 10; n = 9; n = 11)	91.32 ± 69.98	145.60 ± 123.68	115.81 ± 99.27	97.99 ± 103.38	117.82 ± 164.75	132.20 ± 188.70	133.26 ± 148.12	150.38 ± 135.76	139.46 ± 105.44	0.099	0.879	0.655
MMP1 (n = 15; n = 13; n = 13)	318 ± 320	611 ± 569‡	415 ± 357*	194 ± 75.15	261 ± 111	249 ± 117	268 ± 222	417 ± 245‡	407 ± 257‡*	**<0.001**	0.120	0.289
MMP3 (n = 16; n = 16; n = 25)	12745 ± 10139	10377 ± 5408	8528 ± 3679	11539 ± 7393	11654 ± 8152	11648 ± 7894	6766 ± 5652	6859 ± 3631	6684 ± 3985	0.090	**0.017**	0.074
Osteocalcin (n = 17; n = 16; n = 25)	1515 ± 959	1843 ± 1459	1775 ± 1236	1477 ± 1480	1661 ± 1586	1556 ± 1165	1323 ± 1089	1751 ± 1146‡	2183 ± 1299‡†*	**<0.001**	0.880	**0.006**
Pentraxin-3 (n = 17; n = 16; n = 26)	627 ± 426	1567 ± 1617‡	922 ± 593‡*	488 ± 185	750 ± 481‡	631 ± 313‡*	904 ± 662	1239 ± 751‡	1430 ± 1155‡*	**<0.001**	**0.029**	0.123
TNF-R1(n = 17; n = 16; n = 26)	890 ± 506	965 ± 641	755 ± 465	973 ± 650	911 ± 559	844 ± 405	1208 ± 809	1245 ± 701	1242 ± 667	0.244	0.078	0.432
TNF-R2 (n = 17; n = 16; n = 26)	539 ± 423	656 ± 511	438 ± 309	538 ± 323	541 ± 360	475 ± 314	655 ± 627	752 ± 563	710 ± 494	0.513	0.280	0.433
TSLP (n = 17; n = 16; n = 25)	77.42 ± 36.72	96.24 ± 44.39	76.59 ± 26.12†*	69.40 ± 31.94	75.59 ± 30.53	73.93 ± 37.24	61.95 ± 30.21	68.59 ± 32.20	72.84 ± 39.56	0.155	0.275	0.334
Tweak (n = 17; n = 16; n = 26)	214 ± 132	265 ± 148	236 ± 140	177 ± 102	225 ± 124	194 ± 89.1	160 ± 83.41	183 ± 100	200 ± 100‡*	**0.008**	0.210	0.556
MDA (n = 19; n = 0; n = 12)	0.68 ± 0.08	0.70 ± 0.08	0.75 ± 0.11‡*	–	–	–	0.70 ± 0.15	0.63 ± 0.07	0.64 ± 0.12	0.754	0.101	**0.003**
AOP (n = 19; n = 0; n = 12)	0.53 ± 0.21	0.59 ± 0.24	0.52 ± 0.12	–	–	–	0.38 ± 0.14	0.38 ± 0.18	0.33 ± 0.09	0.381	**0.001**	0.604
8-OHdG (n = 18; n = 0; n = 12)	1.89 ± 0.34	1.88 ± 0.87	2.06 ± 0.63	–	–	–	2.04 ± 0.49	2.10 ± 0.73	2.10 ± 0.58	0.332	0.495	0.627

Data represent mean ± standard deviation. *Statistically significant ($p < 0.05$) changes across time evaluated by a repeated-measures ANOVA within each weight loss treatment. ‡Statistically significant ($p < 0.05$) differences respect to baseline (0 months) evaluated by Student's *t*-test within each weight loss treatment. †Statistically significant ($p < 0.05$) differences respect to endpoint (2–3 months) evaluated by Student's *t*-test within each weight loss treatment. All biomarkers are measured in pg/mL except April, CD163, Gp130 and 8-OHdG that are in ng/mL and MDA, AOP that are in mM. VLCK, very-low-calorie ketogenic diet; LCD, low-calorie diet; BS, bariatric surgery; BAFF, B cell activator factor; CD, cluster of differentiation; Gp, glycoprotein; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; TNF-R, tumor necrosis factor receptor; TSLP, Thymic stromal lymphopoietin; MDA, malondialdehyde; AOP, total antioxidative power; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

Table 3
Comparison of differences in cytokines levels and oxidative stress biomarkers during time course weight loss between very-low-calorie ketogenic diet, low-calorie diet and bariatric surgery.

Biomarkers	Difference Endpoint to Baseline			*p*-value Study	Difference Follow-up to Baseline			*p*-value Study	Difference Follow-up to Endpoint			*p*-value Study
	VLCKD	LCD	BS		VLCKD	LCD	BS		VLCKD	LCD	BS	
April	4.47 ± 31.54	1.96 ± 13.32	13.78 ± 57.25	0.184	4.35 ± 24.17	2.86 ± 18.35	24.67 ± 55.59†	**0.040**	2.89 ± 28.92	−0.04 ± 18.10	2.17 ± 41.50	0.961
BAFF	−98.20 ± 900	−319 ± 616	−88.79 ± 1211	0.697	−853 ± 1635	−201 ± 1072	38.72 ± 2479	0.381	−263 ± 759	137 ± 1091	−33.50 ± 2414	0.669
CD163	−7.57 ± 23.41	−10.46 ± 17.46	−4.62 ± 27.95	0.768	−16.15 ± 25.67	−8.22 ± 14.96	0.14 ± 36.79	0.076	−2.64 ± 17.85	−1.62 ± 8.83	5.67 ± 26.77	0.717
CD30	46.92 ± 135	25.50 ± 105	73.92 ± 217	0.609	17.30 ± 184	−10.45 ± 51.84	86.22 ± 116	0.128	0.76 ± 130	−37.48 ± 90.74	12.81 ± 231	0.868
Chitanase	−1419 ± 5981	−631 ± 2211	175 ± 2829	**0.047**	−2094 ± 5722	−685 ± 1689	−113 ± 3465	0.055	−352 ± 2236	−142 ± 2191	−207 ± 3672	0.709
Gp130	4.55 ± 15.34	−1.07 ± 4.99	−0.67 ± 11.08	0.170	0.69 ± 6.76	−1.11 ± 5.67	0.66 ± 12.24	0.546	−2.39 ± 16.50	−1.31 ± 4.94	4.33 ± 19.75	0.636
IFN-α2	11.60 ± 17.77	1.84 ± 3.34	0.28 ± 5.78	0.067	7.51 ± 19.25	–	1.07 ± 3.85	0.434	−2.30 ± 7.23	–	−1.32 ± 8.39	0.488
IFN-β	15.90 ± 18.79	0.65 ± 11.10†	1.00 ± 13.63†	**0.006***	9.87 ± 10.23	0.47 ± 7.97	6.58 ± 16.77	0.184	−4.83 ± 12.78	−1.94 ± 8.53	5.09 ± 19.63	0.572
IFN-γ	22.28 ± 19.74	4.72 ± 7.76†	4.03 ± 7.32†	**<0.001***	4.94 ± 10.87	1.75 ± 3.37	10.01 ± 13.99	0.655	−22.30 ± 21.82	−2.43 ± 7.76†	3.11 ± 10.48†	**<0.001***
IL-2	15.53 ± 13.53	6.34 ± 15.37	2.02 ± 4.63†	**0.005***	4.37 ± 6.06	1.79 ± 1.89	3.30 ± 4.56	0.334	−14.09 ± 18.01	−4.71 ± 15.69	0.75 ± 5.44	0.067
IL-6R	538 ± 4908	34.40 ± 835	−274 ± 2425	0.213	−593 ± 3439	−122 ± 824	−361 ± 2608	0.810	−1156 ± 3681	−320 ± 851	544 ± 2863	0.325
IL-11	2.28 ± 2.98	1.03 ± 1.05	0.39 ± 1.73†	**0.027**	1.12 ± 1.43	0.64 ± 0.93	1.71 ± 4.01	0.761	−1.20 ± 2.30	−0.50 ± 1.00	1.12 ± 3.99	0.130
IL-12p40	19.72 ± 28.97	7.39 ± 14.69	11.75 ± 25.88	0.263	10.01 ± 21.50	4.84 ± 7.63	10.98 ± 20.20	0.772	−9.06 ± 15.46	−1.85 ± 14.03	−0.25 ± 27.82	0.423
IL-12p70	0.22 ± 0.48	−0.06 ± 0.47	0.03 ± 0.26	0.352	−0.004 ± 0.32	0.07 ± 0.29	0.005 ± 0.21	0.884	−0.25 ± 0.68	0.07 ± 0.16	−0.03 ± 0.21	0.548
IL-22	3.16 ± 17.72	2.26 ± 8.32	2.39 ± 4.36	0.923	3.86 ± 4.12	2.60 ± 6.54	−2.44 ± 13.80	0.522	−7.16 ± 13.67	0.62 ± 6.09	12.68 ± 37.52	0.332
IL-26	1.87 ± 6.50	1.97 ± 6.00	4.61 ± 13.25	0.256	0.96 ± 9.83	−0.44 ± 3.41	6.24 ± 23.97	0.188	−1.97 ± 7.54	−2.32 ± 5.27	0.97 ± 12.22	0.779
IL-28A	4.68 ± 8.65	−0.46 ± 6.41	3.30 ± 7.90	0.360	3.08 ± 6.98	0.61 ± 7.24	5.95 ± 8.17	0.533	−1.93 ± 8.42	1.94 ± 6.01	3.26 ± 7.35	0.255
IL-29	17.73 ± 49.27	11.18 ± 16.89	−8.13 ± 21.73	0.612	1.89 ± 19.67	16.42 ± 17.85	−2.39 ± 13.53†	**0.047**	−17.30 ± 44.56	3.81 ± 21.00	5.28 ± 31.68	0.380
IL-35	54.28 ± 75.40	19.83 ± 69.62	15.22 ± 54.87	0.283	24.84 ± 41.54	34.40 ± 81.94	6.20 ± 67.79	0.912	−29.79 ± 52.14	14.38 ± 31.24	−10.92 ± 36.88	0.094
MMP1	293 ± 440	66.24 ± 99.35	135 ± 199	0.139	−41.83 ± 465	49.78 ± 94.39	139 ± 172	0.430	−196 ± 351	−11.36 ± 115	−3.45 ± 198	0.091
MMP3	−2368 ± 9328	115 ± 4452	111 ± 4234	0.140	−3574 ± 7641	269 ± 2774†	−81.37 ± 5065	**0.020**	−1849 ± 2929	−5.99 ± 4236†	233 ± 3552†	0.285
Osteocalcin	328 ± 912	184 ± 394	384 ± 906	0.890	303 ± 809	92.38 ± 554‡	864 ± 833†	**0.006**	−67.98 ± 1082	−105 ± 550	749 ± 1478	**0.030**
Pentraxin-3	939 ± 1568	263 ± 347	300 ± 628	0.069	329 ± 596	162 ± 195	500 ± 767	0.680	−645 ± 1462	−120 ± 281	246 ± 873	0.080
TNF-R1	75.47 ± 629	−62.87 ± 200	−3.28 ± 513	0.579	−189 ± 466	−127 ± 332	34.74 ± 732	**0.019**	−210 ± 608	−66.75 ± 242	11.55 ± 493	0.340
TNF-R2	118 ± 595	2.60 ± 126	57.37 ± 403	0.666	−112 ± 417	−58.18 ± 146	55.34 ± 487	0.226	−218 ± 421	−65.75 ± 125	−18.05 ± 389	0.242
TSLP	18.82 ± 42.36	6.20 ± 20.03	6.35 ± 21.33	0.140	1.52 ± 26.52	4.52 ± 17.79	10.89 ± 28.27	0.876	−19.65 ± 34.03	−1.67 ± 23.04	5.06 ± 30.88	0.059
Tweak	51.24 ± 103	47.91 ± 90.49	19.60 ± 75.25	0.472	7.79 ± 78.78	14.73 ± 33.92	39.80 ± 84.52	0.414	−29.69 ± 67.93	−30.61 ± 79.66	28.41 ± 87.52	0.083
MDA	0.02 ± 0.08	–	−0.07 ± 0.14	**0.031**	0.07 ± 0.12	–	−0.06 ± 0.10	**0.003**	0.05 ± 0.12	–	0.007 ± 0.10	**0.031**
AOP	0.06 ± 0.24	–	0.004 ± 0.12	0.435	−0.01 ± 0.23	–	−0.05 ± 0.10	0.604	−0.08 ± 0.21	–	−0.05 ± 0.13	0.759
8-OHdG	−0.02 ± 0.80	–	0.05 ± 0.40	0.778	0.17 ± 0.72	–	0.05 ± 0.33	0.627	0.18 ± 0.70	–	0.0008 ± 0.40	0.422

Data represent mean ± standard deviation. *P*-value is calculated with ANCOVA adjusted by age and baseline levels of cytokines. * Statistically significant ($p < 0.05$) differences evaluated by ANCOVA adjusted by baseline body mass index (BMI) and body weight loss. † Statistically significant ($p < 0.05$) differences in relation to very-low-calorie ketogenic diet (VLCKD) and ‡ statistically significant ($p < 0.05$) differences in relation to bariatric surgery (BS). Baseline (0 months), Endpoint (2–3 months) and Follow-up (4–6 months). All biomarkers are measured in pg/mL except April, CD163, Gp130 and 8-OHdG that are in ng/mL and MDA, AOP that are in mM. LCD, low-calorie diet; BAFF, B cell activator factor; CD, cluster of differentiation; Gp, glycoprotein; IFN, interferon; IL, interleukin; MMP, matrix metalloproteinase; TNF-R, tumor necrosis factor receptor; TSLP, Thymic stromal lymphopoietin; MDA, malondialdehyde; AOP, total antioxidative power; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

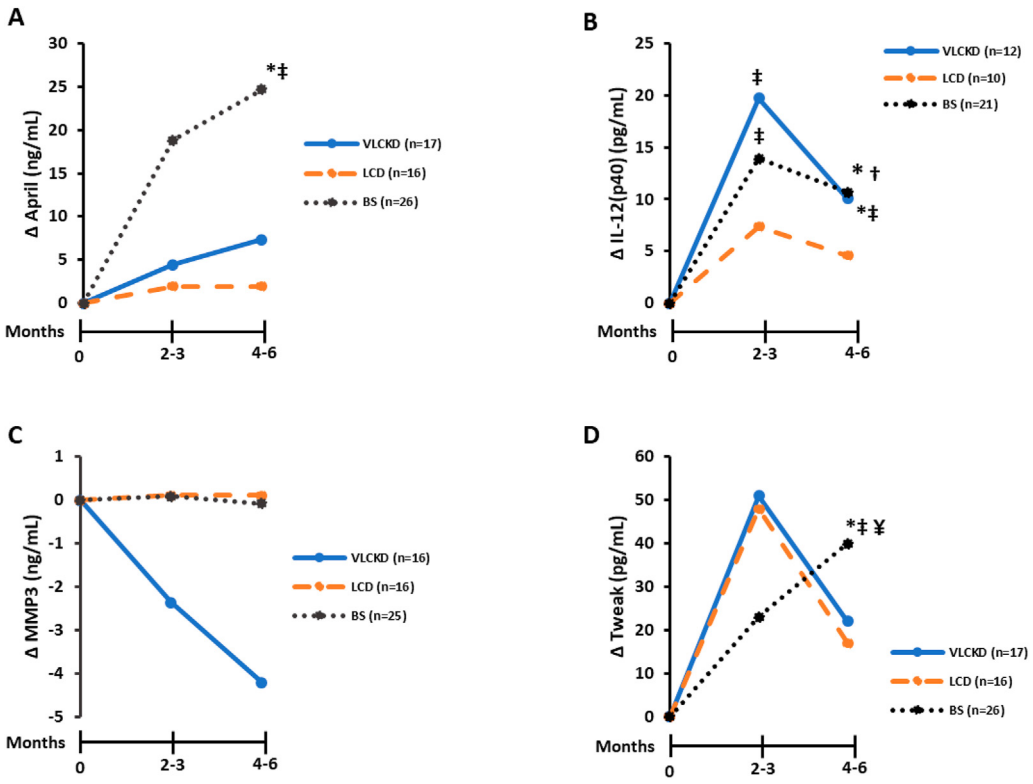

Fig. 2. Changes in circulating levels of cytokines during very-low-calorie ketogenic diet (VLCKD), a low-calorie diet (LCD) or bariatric surgery (BS). A) Changes compared to baseline in April during the weight loss treatments. B) Changes compared to baseline in circulating interleukin-12(p40) (IL-12(p40)) levels during the weight loss treatments. C) Changes compared to baseline in circulating metalloproteinase-3 (MMP3) levels during the weight loss treatments. D) Changes compared to baseline in circulating Tweak levels during the weight loss treatments. Data show differences compared to baseline during the time-course of the intervention. Statistically significant differences were evaluated with repeated-measures ANCOVA adjusted for age and baseline levels of cytokines. * Statistically significant ($p < 0.05$) differences over the duration of the nutritional program (from 0 to 4–6 months), ‡ statistically significant ($p < 0.05$) differences in relation to baseline, † statistically significant ($p < 0.05$) differences in relation to 2–3 months and ¥ statistically significant ($p < 0.05$) differences between treatments compared to baseline.

a comprehensive way [45]. Particularly, levels of IL-11, IL-12, IL-2, INF-γ, INF-β, Pentraxin-3 or MMP1 changed after VLCKD. Most of them showed differential levels between obesity and normal-weight. After weight loss, levels of these cytokines were restored to levels similar to that observed in normal-weight patients, suggesting an improvement in innate-immunity and inflammatory responses as it was previously observed for example in IFN-γ which increases after weight loss and exercise in obese individuals [46] or Pentraxin-3 which represents a powerful biomarker of inflammatory status inversely associated with BMI and increase after chronic exercise [47,48]. Also, previous studies have shown increased levels of MMP1 with obesity [49].

On the other hand, patients underwent BS showed an increase in April, Tweak, osteocalcin and IL-28A. All of them are proinflammatory factors that orchestrates inflammation, fibrosis and tissue remodelling such as Tweak [50,51], or are involved in carcinogenesis such as April [52]. By contrast, osteocalcin [53] and IL-28A [54] were proposed to have beneficial metabolic and anti-inflammatory properties. These results agree with a potential low-grade metabolic stress induced by BS in the first phase of the intervention as it was previous proposed [24,25,55–58] that is improved in the long-term by the increase in beneficial metabolic factors [59].

Obesity is associated with a disturbed inflammatory and oxidative status, mainly promoted by a dysfunctional adipose tissue that secretes inflammatory proteins and promote the production of reactive oxygen species (ROS) by disturbances in mitochondria and endothelium reticulum [60]. This status was proposed to be the main mechanistic link of obesity and its comorbidities [61]. It was observed that after weight loss treatments, the inflammatory and oxidative status in patients with obesity improved [14,62]. In this regard, it should be expected to found improvements in oxidative stress biomarkers in association with body weight states. Surprisingly, in this study, an increase in lipid peroxide biomarker was observed during the time-course of the VLCKD while a decrease respect to baseline was observed after BS. Moreover, levels of MDA but also AOP at the follow-up of the interventions correlated with circulating levels of β-OHB induced during the acute phase of weight loss treatments. These results could be counterintuitive, however previous studies have evidenced that low-carbohydrate diet in humans enhanced ROS defence capacity by increased oxidative metabolism [63]. Moreover, interventions such as caloric restriction, glucose restriction or moderate physical activity enhanced mitochondrial activity with a concomitant increase in ROS production that promote and adaptive response, termed mitohormesis, increasing antioxidant defences and then improving metabolic health and lifespan [64]. In this study we were unable to detect a statistically significant increase in AOP, probably due to the small sample size, even though, the positive correlation with circulating ketone bodies could demonstrate the beneficial effect of the nutritional ketosis induced by the VLCKD on antioxidant defences.

Importantly, on inflammatory markers and oxidative stress biomarkers, the effect was mainly observed after VLCKD, while BS showed a slow effect mainly in the more acute phase (3 months after surgical). It suggests that, despite the important weight loss induced by VLCKD or BS, the metabolic status induced by both interventions in a short-term is different and it is probable that the nutritional ketosis induced during de acute phase of VLCKD could protect patients with obesity from other diseases such as type 2

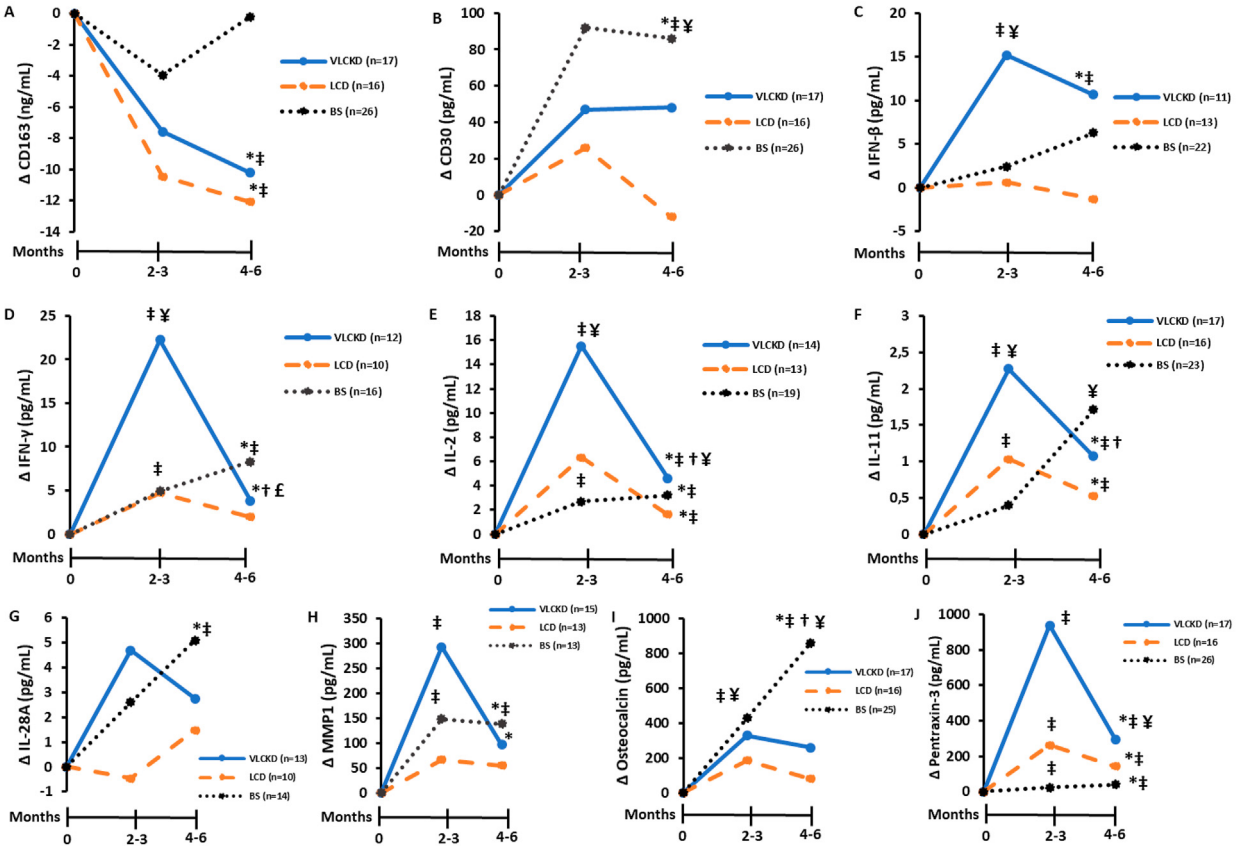

Fig. 3. Changes in circulating levels of cytokines during very-low-calorie ketogenic diet (VLCKD), a low-calorie diet (LCD) or bariatric surgery (BS). A) Changes compared to baseline in cluster of differentiation 163 (CD163) during the weight loss treatments. B) Changes compared to baseline in cluster of differentiation 30 (CD30) during the weight loss treatments. C) Changes compared to baseline in circulating interferon-β (IFN-β) levels during the weight loss treatments. D) Changes compared to baseline in inferferon-γ (IFN-γ) during the weight loss treatments. E) Changes compared to baseline in circulating interleukin-2 (IL-2) levels during the weight loss treatments. F) Changes compared to baseline in circulating interleukin-11 (IL-11) levels during the weight loss treatments. G) Changes compared to baseline in circulating interleukin-28A (IL-28A) levels during the weight loss treatments. H) Changes compared to baseline in metalloproteinase-1 (MMP1) during the weight loss treatments. I) Changes compared to baseline in circulating Osteocalcin levels during the weight loss treatments. J) Changes compared to baseline in circulating Pentraxin-3 levels during the weight loss treatments. Data show differences compared to baseline during the time-course of the intervention. Statistically significant differences were evaluated with repeated-measures ANCOVA adjusted for age and baseline levels of cytokines. *Statistically significant ($p < 0.05$) differences over the duration of the nutritional program (from 0 to 4–6 months), ‡ statistically significant ($p < 0.05$) differences in relation to baseline, † statistically significant ($p < 0.05$) differences in relation to 2–3 months. ¥ statistically significant ($p < 0.05$) differences between treatments compared to baseline and £ statistically significant ($p < 0.05$) differences between treatments compared to endpoint.

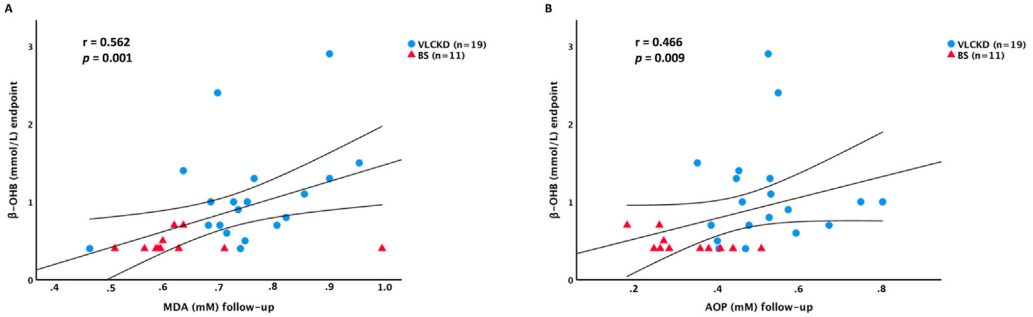

Fig. 4. Association between circulating levels of ketone bodies at the 2–3 months of the interventions and oxidative stress biomarkers at follow-up. Scatterplot representing the association between β-hydroxybutyrate (β-OHB) circulating levels at the 2–3 months and (A) malondialdehyde (MDA) levels at follow-up and (B) total antioxidative power (AOP) levels at follow-up. The center line represents the linear regression trendline. The lines above and below the center line represent the upper and lower bounds of the 95% confidence interval around the trendline. r, correlation coefficient evaluated by the Rho Spearman test; *p*, *p*-value.

diabetes [65], cardiovascular disease [66], cancer [67–70] and the newly emerged infection COVID-19 [55]. In line with this proposal, it was previously hypothesized that a VLCKD could represent an elective therapy to counteract inflammation-related dermatological diseases such as psoriasis, which shows an important inflammatory component [71]. Moreover, beneficial effect on inflammation associated to obesity was previously observed even after a short period of VLCKD follow-up [72].

The small sample size could be a limitation in this study. However, patients were evaluated in three points during a longitudinal

procedure which allowed to carry out a paired analysis considering each patient as his own control. This approach increased the statistical power of the study. In addition, differences in the inflammatory and oxidative stress parameters were detected after a short clinical course, such as 2–3 months in the more acute phases of the weight loss treatments, which reinforce the findings. On the other hand, the difference in the number of males and females could alter the results, especially from a hormonal point of view and it could be considered other limitation of this study. However, the number of women included in this study was higher than men particularly in VLCKD and BS groups. It suggests that the results can be comparable between both weight loss treatments. Even thought, it highlights the need to perform further longitudinal studies with a more homogeneous gender distribution to explore the potential effect of hormonal status on this topic. Other limitation of this study could be that the three groups start from different baseline BMI. It could suggest that the results are not independent of simple weight loss, and one could not conclude that the difference is related to nutritional ketosis. However, the changes in BMI induced by the VLCKD (−12.4% of BMI) and BS (−14.0% of BMI) during the most acute phase of the interventions are comparable. The most relevant difference between VLCKD and BS is focused on the nutritional ketosis induced during the most acute phase of the VLCKD, but not after BS. At the end of the interventions, the induced body weight loss was higher after BS (35% of body weight loss) than after VLCKD (20% of body weight loss). With this difference in the induced body weight loss, higher effect on cytokine levels would be expected after BS than after VLCKD. As this was not the case, the results reinforce that nutritional ketosis could induce an anti-inflammatory and antioxidative effect synergistically with body weight loss. Moreover, when differences between groups were further evaluated after adjusting for baseline BMI and weight loss, the statistical significance remained in relevant cytokines of innate immune system such as INF-γ, INF-β and IL2.

In conclusion, this study demonstrates differential effects of weight lowering therapeutic approaches on remodelling the inflammatory and oxidative status of patients with obesity, being a VLCKD the most effective, while patients that underwent BS were in an acute metabolic stress status during the first three months. Differences between VLCKD and BS could be explained by the nutritional ketosis induced during the ketogenic diet, besides of the effect of weight loss. Further longitudinal studies are needed to demonstrate the effect of nutritional ketosis or therapies that induce an increase in ketone bodies of patients with obesity to improve the immune function and oxidative stress status and counteract the risk of obesity-related diseases such as infections, cancer and ageing.

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Author contributions

PML, IS and AGI, performed experiments and analysed data; PML and ABC wrote the manuscript; IS and DG-A, MAZ, IA, AIC, JB performed the recruitment and following of the patients; MPP, FJT and JAM contributed to the interpretation of data and discussion; FFC and ABC obtained funding, designed and coordinated research. FFC and ABC are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. All authors read and approved the final manuscript.

Conflict of Interest

D. G-A., A.I.C., A.B.C. and F.F.C. received advisory board fees and/or research grants from Pronokal Protein Supplies, Spain. I.S. is the Medical Director of Pronokal, Spain.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.05.007.

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