



## Obesity and Eating Disorders

## Relationship between Ketones, Ghrelin, and, Appetite on Isocaloric Diets with Varying Carbohydrate Quality and Amount: Results from a Randomized Controlled Trial in People with Obesity (CARBFUNC)

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### ABSTRACT

**Background:** Low-carbohydrate high-fat (LCHF) diets may suppress the increase in appetite otherwise seen after diet-induced fat loss. However, studies of diets without severe energy restriction are lacking, and the effects of carbohydrate quality relative to quantity have not been directly compared.

**Objectives:** To evaluate short- (3 mo) and long-term (12 mo) changes in fasting plasma concentrations of total ghrelin,  $\beta$ -hydroxybutyrate ( $\beta$ HB), and subjective feelings of appetite on 3 isocaloric eating patterns within a moderate caloric range (2000–2500 kcal/d) and with varying carbohydrate quality or quantity.

**Methods:** We performed a randomized controlled trial of 193 adults with obesity, comparing eating patterns based on “acellular” carbohydrate sources (e.g., flour-based whole-grain products; comparator arm), “cellular” carbohydrate sources (minimally processed foods with intact cellular structures), or LCHF principles. Outcomes were compared by an intention-to-treat analysis using constrained linear mixed modeling. This trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov) as NCT03401970.

**Results:** Of the 193 adults, 118 (61%) and 57 (30%) completed 3 and 12 mo of follow-up. Throughout the intervention, intakes of protein and energy were similar with all 3 eating patterns, with comparable reductions in body weight (5%–7%) and visceral fat volume (12%–17%) after 12 mo. After 3 mo, ghrelin increased significantly with the acellular (mean: 46 pg/mL; 95% CI: 11, 81) and cellular (mean: 54 pg/mL; 95% CI: 21, 88) diets but not with the LCHF diet (mean: 11 pg/mL; 95% CI: –16, 38). Although  $\beta$ HB increased significantly more with the LCHF diet than with the acellular diet after 3 mo (mean: 0.16 mmol/L; 95% CI: 0.09, 0.24), this did not correspond to a significant group difference in ghrelin (unless the 2 high-carbohydrate groups were combined [mean: –39.6 pg/mL; 95% CI: –76, –3.3]). No significant between-group differences were seen in feelings of hunger.

**Conclusions:** Modestly energy-restricted isocaloric diets differing in carbohydrate cellularity and amount showed no significant differences in fasting total ghrelin or subjective hunger feelings. An increase in ketones with the LCHF diet to 0.3–0.4 mmol/L was insufficient to substantially curb increases in fasting ghrelin during fat loss.

**Keywords:** dietary carbohydrates, appetite, ghrelin, ketosis, obesity, randomized controlled trial

**Abbreviations:** AcAc, acetoacetate; A-HCLF, acellular high-carbohydrate low-fat;  $\beta$ HB,  $\beta$ -hydroxybutyrate; C-HCLF, cellular high-carbohydrate low-fat; cLMM, constrained linear mixed-effects model; E%, energy percent; HCLF, high-carbohydrate low-fat; LCHF, low-carbohydrate high-fat; PAL, physical activity level; RCT, randomized controlled trial; VAS, visual analog scale; VAT, visceral adipose tissue; VLED, very-low-energy diet.

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## Introduction

A major challenge of diet-induced weight loss is the sustained increase in the drive to eat [1], which is likely a result of increased plasma concentrations of the orexigenic hormone ghrelin [2]. Increased hunger feelings during and after weight loss have previously been reported as an adverse effect of weight loss [3] and suggested to contribute to reduced dietary adherence after initial successful weight loss [4, 5]. Therefore, dietary approaches targeting appetite regulation may represent effective treatment strategies for individuals with obesity. Ketogenic diets, such as very-low-energy diets (VLEDs) and ketogenic low-carbohydrate diets, have been shown to suppress the increase in hunger feelings after weight loss [6]. The effect is thought to be mediated by nutritionally induced ketosis resulting from restricted carbohydrate availability [7], with increased plasma concentrations of the ketone bodies  $\beta$ -hydroxybutyrate ( $\beta$ HB) [8] and acetoacetate (AcAc) [6].

Several intervention studies demonstrate that ketogenic diets prevent the weight loss-induced increase in fasting ghrelin concentrations [7, 9–12], suggesting that appetite suppression on such diets may be mediated by attenuated ghrelin secretion. Accordingly, a significantly lower increase in fasting ghrelin concentrations was observed on a low- compared with a high-carbohydrate diet during weight loss maintenance after an initial weight loss program [13], supporting a prolonged suppression of ghrelin secretion after carbohydrate restriction. Although nutritionally induced ketosis through carbohydrate restriction appears to suppress appetite, there is no clear consensus on the exact carbohydrate or  $\beta$ HB threshold to induce ketosis [8]. In addition, only a few studies have investigated the effect of ketogenic diets on weight loss without strict caloric restriction [12, 14–16], and questions remain regarding the ketogenic and appetite-suppressing potential of low-carbohydrate diets with more modest energy restriction and over longer periods of time.

Dietary carbohydrate quality has also been suggested to affect appetite regulation because rates of digestion, absorption, and metabolism vary between different forms of carbohydrates [17]. In recent decades, the availability and consumption of highly processed foods [18], typically those with low carbohydrate quality (i.e., increased added sugars and low fiber), have increased and have been associated with obesity [19]. The intactness of the food matrix and cellular structures in carbohydrate foods, referred to as the degree of “cellularity,” has been suggested to affect appetite [20]. However, the effect of dietary patterns differing in carbohydrate cellularity on appetite regulation remains unexplored. Additionally, although previous short-term studies show that dietary interventions targeting either carbohydrate quality [21] or quantity [7, 9–12, 14, 15] affect subjective appetite and/or ghrelin concentrations, direct comparisons of carbohydrate type and amount are lacking.

Although there is evidence for a ketone-mediated suppression of the increase in fasting ghrelin concentrations and hunger feelings otherwise seen with weight loss [6–9], a consistent effect of ketosis on the postprandial secretion of satiety peptides is not supported [7, 9–11]. For that reason, in this study, we focused on the impact of ketosis on fasting ghrelin and hunger feelings in a 3-arm randomized controlled trial (RCT) with multiple follow-up measurements up to 1 y. We evaluated the

effect of dietary carbohydrates, both quality (degree of cellularity) and quantity, on changes in plasma concentrations of total ghrelin, subjective feelings of appetite, and concentrations of total  $\beta$ HB both in the short term (3 mo) and the long term (12 mo). We hypothesized that concentrations of ghrelin in the fasting state and reported feelings of appetite would show a lesser increase on a low-carbohydrate diet and possibly also on a cellular carbohydrate diet compared with on a more acellular/-processed higher-carbohydrate diet despite only modest isocaloric energy restriction in all diets.

## Methods

### Participants and study design

Data included in this article are secondary outcome measures from the CARBFUNC study [22], a RCT (clinical trials identifier: NCT03401970) conducted in Bergen, Norway, from January 2018 through March 2021 investigating the effects of dietary carbohydrates on visceral and hepatic fat volume in men and women with obesity.

Study participants had a stable weight (<5% change in body weight within the last 2 mo) and were nonsmoking adults aged 20–55 y with obesity (with a BMI of  $\geq 30$  kg/m<sup>2</sup> and/or a waist circumference of  $\geq 102$  cm for men and 88 cm for women). The study excluded pregnant, breastfeeding, and postmenopausal women and anyone with a habitual alcohol consumption of  $>2$  alcohol units ( $>24$  g/d of pure alcohol) per day, known food allergies, severe diseases (including chronic inflammatory bowel disease), statin or diabetes medication use, and surgical or antibiotics treatment during the past 2 mo.

The study was approved by the Regional Ethics Committee in Western Norway (approval number: 2017/621/REC West) and conducted in accordance with the guidelines in the Declaration of Helsinki. Written informed consent was obtained from all participants before commencement.

### Data collection and randomization

Data collection of appetite measures, ketone bodies, and standard anthropometric measures was performed at 5 time points: at baseline and 3, 6, 9, and 12 mo. The participants arrived at each study visit in the morning after  $\geq 12$  h of fasting, abstaining from the use of alcohol for the previous 24 h, and avoiding any strenuous physical activity for the previous 48 h.

After the baseline data collection, qualified research personnel, not otherwise involved in the study, randomly allocated participants to 1 of the 3 study diets using block randomization with block sizes of 6–9 and stratification by sex (R package blockrand, version 1.5, RStudio, Inc.). Participants and staff were not blinded after randomization owing to the nature of the intervention, except the statistician, who was blinded to the group identities until all measures reported in this article had been analyzed.

### Study interventions

The interventions included 3 study diets, an acellular high-carbohydrate low-fat (A-HCLF) diet, a cellular high-carbohydrate low-fat (C-HCLF) diet, and a low-carbohydrate high-fat (LCHF) diet, as previously described in detail elsewhere [22]. In brief, all 3 diets were planned to be isocaloric and moderately energy-restricted with 2000 kcal/d and 2500 kcal/d for female

and male participants, respectively. The high-carbohydrate low-fat (HCLF) diets had the same macronutrient profile (17 energy percent [E%] protein, 45 E% carbohydrates, and 38 E% fat), which differed from that of the LCHF diet, except for protein intake (17 E% protein, 8 E% carbohydrates, and 75 E% fat). The planned carbohydrate intake on the LCHF diet was 40 and 50 g/d for female and male participants, respectively.

The A-HCLF diet included more refined carbohydrate products, such as store-bought whole-grain bread, fruit juice, pasta, and quick oats, whereas the C-HCLF diet included unrefined or minimally refined carbohydrate foods, such as potatoes, unpolished rice, whole (unground) grains, whole fruits, and rolled oats. For each study diet, an extensive recipe booklet, including diet-specific breakfast, lunch, and dinner recipes, was provided. Of note, the study diets were not eucaloric because tailoring of the recipe booklets to individual energy requirements, rather than being standardized according to sex, was not compatible with the recipe database. In addition to following the assigned study diet, the participants were requested to maintain their usual physical activity level (PAL) throughout the intervention.

### Dietary recordings, intervention adherence, and PAL

Participants recorded their dietary intake for 3 consecutive days every second week in an online dietary recording system ([www.diett.no](http://www.diett.no); operated by Dietika AS) using unique identifiers for the recipes of their choice from the provided recipe booklets. An expanded description of the procedures for nutrient calculation and the collection of consecutive 6-d dietary intake data at baseline is available elsewhere [23]. The participants reported subjective adherence to the assigned study diets every 3 mo, rating dietary adherence from “no adherence” (0%) to “complete adherence” (100%) in 20% increments. In addition, the participants reported physical activity/inactivity for 3 consecutive days between every study visit using the same online system ([diett.no](http://diett.no)) as used for the dietary recordings. The frequency, duration, and intensity of all daily life activities and sports were recorded and used together with their associated metabolic equivalent values [24] to estimate the PAL for each participant.

### Appetite measures

Fasting and postprandial (120 and 240 min after eating a mixed meal) subjective feelings of appetite (hunger, fullness, desire to eat, and prospective food consumption) were measured using a validated 10-cm visual analog scale (VAS). VAS has previously been shown to exhibit a good degree of intrasubject reliability and is therefore suitable for repeated measurements [25].

Postprandial measurements were obtained after intake of a standardized mixed meal: a portion of oatmeal consisting of 80 g of rolled oats, 50 g of a butter-and-oil spread (70% butter and 30% rapeseed oil), 5 g of sugar, and 200 mL of hot water. The meal provided ~700 kcal, 32 E% carbohydrates (56 g), 61 E% fat (47 g), and 5 E% protein (9 g). The meal was consumed within 10 min after the fasting blood samples were drawn. The mixed meal was the same for all participants, regardless of the intervention group. For this reason, the postprandial measurements do not reflect the acute effects of the intervention diets but could reveal changes in postprandial responses due to distinct metabolic adaptations to the different study diets.

Concentrations of total ghrelin in picograms per milliliter were measured in fasting plasma samples using a Human Ghrelin (Total) ELISA kit (EZGRT 89k, Merck Millipore) according to the manufacturer’s instructions. Intra-assay and interassay CVs ranged from 7%–8% and 5%–6%, respectively.

### Ketone bodies

Concentrations of  $\beta$ HB and AcAc (micromole per liter) were measured in fasting plasma samples by adding ion pairs for the analytes and isotope-labeled internal standards to an existing GC tandem MS assay [26]. Within- and between-day CVs for  $\beta$ HB and AcAc ranged from 2%–4%. Concentrations of  $\beta$ HB and AcAc are reported in mmol/L in this article because this unit is more commonly seen in the literature and eases comparisons.

### Anthropometry

Body weight was measured with a class III approved calibrated scale (Seca 877, Seca), and height was measured using a portable stadiometer (Seca 217, Seca). Waist circumference was measured 3 times with a nonelastic tape at the midpoint between the lowest rib and the iliac crest. The mean of the last 2 measurements was recorded. Visceral adipose tissue (VAT) volume (cubic centimeter) was measured at baseline, 6 mo, and 12 mo using CT imaging, as described previously [22].

### Statistical analyses

The outcome measurements reported in this study are the between-group differences in absolute and relative change scores of fasting plasma concentrations of total ghrelin (picograms per milliliter), fasting and postprandial subjective feelings of appetite (centimeter), total  $\beta$ HB (millimoles per liter), AcAc (millimoles per liter), VAT volume (cubic centimeter), and body weight (kilogram). The results presented here are derived from an intention-to-treat analysis using constrained linear mixed-effects models (cLMMs) including all randomly assigned participants ( $n = 192$ ).

The statistical analyses were conducted with R v3.6.1 (<https://www.r-project.org>), data transformation and exploration were performed with the tidyverse packages (<https://tidyverse.tidyverse.org>), and plots were made using the ggplot2 package v3.3.5. Continuous study outcomes were analyzed using baseline-adjusted cLMMs from within- and between-group comparisons, performed with the nlme package v3.1-140 in R v3.6.1. In the planned comparisons showing absolute or relative between-group differences, the A-HCLF diet was defined as the reference group (comparator arm). Before analyzing responses in relative terms, values were transformed using the natural logarithm [27]. Further details of the statistical procedures using cLMMs are available elsewhere [22].

As part of the model validation procedure, the Shapiro-Wilk test for normality, the D’Agostino test for skewness, and graphical tools (boxplots, quantile-quantile plots, and histograms) were used to assess the distribution of standardized residuals.

Data are presented as raw, unadjusted means SDs, or mean score differences (with or without absolute/relative effect estimates [95% CIs]). The distribution of data points from different measurements is shown using violin plots in [Supplemental Figure 1](#). All inferential tests were 2-tailed, with a nominal  $\alpha$  level of 0.05. Raw  $P$  values were not adjusted for multiple testing

because a general adjustment method for mixed modeling of repeated measurements has not yet been developed [28].

The sample size was calculated for the primary outcome (between-group differences in relative change scores of VAT) in the CARBFUNC study [22] but not for the secondary outcomes included in this manuscript. We chose not to conduct post hoc analyses because this approach is considered flawed [29].

Because linear mixed-effects modeling efficiently deals with data sets containing missing outcome values and may serve as an optimal estimator in trials of repeated outcome measures with a large portion of missing data [30–33], we did not prespecify any other strategy for dealing with potential intermittent missing data or missing data resulting from dropouts. For example, we did not conduct multiple imputations before mixed modeling because this has shown to add no obvious benefits compared with a standard mixed model approach without imputed values [30–33].

Exploratory linear regression–determined associations were analyzed between continuous variables with linear regression models using the “lm” function in the R stats package v3.6.1. We performed split sample analyses and interaction analyses to estimate within-group associations and between-group differences, respectively. We obtained 2 different effect sizes from these models: 1) standardized regression coefficients (95% CIs) and 2) partial Cohen’s  $f^2$ . A Cohen’s  $f^2$  of  $\geq 0.02$  (2%),  $\geq 0.15$  (15%), and  $\geq 0.35$  (35%) represents a weak, moderate, and strong association, respectively [34–36].

In figures showing changes from baseline, the relative data are presented as sympercents (s%), which are additive and symmetric percentage differences on the 100 log<sub>e</sub> scale, calculated as the difference between the natural logs of two numbers multiplied by 100, i.e.,  $100 \times \ln(a) - 100 \times \ln(b)$  [37].

## Results

Among the 193 randomly assigned participants, 1 participant withdrew consent, resulting in available data from 192 (53%

**TABLE 1**

Baseline characteristics of participants by group included in the intention-to-treat analysis<sup>1</sup>.

	Randomly assigned participants <sup>2</sup>			
	All (n = 192)	A-HCLF (n = 67)	C-HCLF (n = 62)	LCHF (n = 63)
Age, y	41.6 ± 8.8	41.4 ± 8.8	42.3 ± 8.6	41.2 ± 8.8
Body weight, kg	111 ± 19	111 ± 19	114 ± 17	108 ± 18
Height, m	1.74 ± 0.09	1.74 ± 0.10	1.74 ± 0.09	1.73 ± 0.08
BMI, kg/m <sup>2</sup>	36.7 ± 4.8	36.4 ± 4.3	37.7 ± 5.1	35.9 ± 4.7
WC, cm	117 ± 12	116 ± 11	120 ± 13	115 ± 12
PAL <sup>3</sup>	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.2	1.6 ± 0.3

A-HCLF, acellular high-carbohydrate low-fat; C-HCLF, cellular high-carbohydrate low-fat; LCHF, low-carbohydrate high-fat; PAL, physical activity level; WC, waist circumference.

<sup>1</sup> Values are means ± SDs.

<sup>2</sup> All randomly assigned participants, excluding 1 participant who withdrew consent (n = 192).

<sup>3</sup> Calculated PAL based on estimated energy expenditure for self-reported activity and their associated metabolic equivalent values divided by 24 h.

women) (Supplemental Figure 2). Baseline characteristics by diet group are shown in Table 1 (67, 62, and 63 participants in the A-HCLF, C-HCLF, and LCHF diet groups, respectively). One hundred-eighteen (62%) participants completed 3 mo of follow-up, and 57 (30%) participants completed 12 mo of follow-up. The overall dropout rate was higher among women than among men at both 3 (44% compared with 33%) and 12 mo (76% compared with 64%), independent of diet allocation. Thirty-four, 37, and 47 participants completed 3 mo and 14, 22, and 21 participants completed 12 mo on the A-HCLF, C-HCLF, and LCHF diet, respectively (Supplemental Figure 2).

## Dietary adherence and maintenance of PAL

Dietary recordings indicated considerable adherence to the assigned study diets and estimated that mean PALs were similar at 1.5–1.6 throughout the intervention across groups. The mean energy intake was close to the target in all 3 groups at 2000 kcal/d for the women at 3 mo (1923–1981 kcal/d) and 12 mo (1820–2064 kcal/d) and 2500 kcal/d for men at 3 mo (2316–2585 kcal/d) and 12 mo (2568–2844 kcal/d). Energy intake did not differ between the groups at any time point, except for a difference in change scores between the C-HCLF and A-HCLF groups at 12 mo (mean: –290 kcal/d [95% CI: –562, –18.8]) because energy intake on the C-HCLF diet was significantly reduced from baseline.

As intended, the reported carbohydrate intake differed substantially between the diets, with a mean intake (± SD) in the LCHF diet group of 59 ± 14 g/d at 3 mo and 86 ± 53 g/d at 12 mo (corresponding to 11–15 E%), compared with 245–252 g/d and 218–225 g/d in the A-HCLF and C-HCLF diet groups, respectively (corresponding to 41–43 E%).

Despite large reductions in carbohydrate intake on the LCHF diet both at 3 and 12 mo, mean fiber intake (± SD) was 17 ± 5 g/d and 19 ± 6 g/d at 3 and 12 mo, respectively, compared with 20 ± 6 g/d at baseline. Fiber intake increased substantially from baseline to 3 mo on the A-HCLF diet to 33 ± 8 g/d and doubled on the C-HCLF diet to 43 ± 11 g/d, remaining >30 g/d on both HCLF diets throughout the intervention.

As intended, protein intake did not differ between the groups, remaining constant at 16–17 E% throughout the intervention. Fat intake substantially increased on the LCHF diet and decreased marginally on the HCLF diet compared with baseline. More detailed information concerning dietary intake during the CARBFUNC trial is provided elsewhere [22].

Reported adherence to the study diets after 3 mo differed significantly between the LCHF (mean ± SD: 80 ± 23%) and A-HCLF diets (71 ± 21%) but not between the C-HCLF (78 ± 15%) and A-HCLF diets. At 6 and 9 mo, the reported adherence remained  $\geq 70\%$  across groups, with no significant differences. After 12 mo, adherence to the A-HCLF, C-HCLF, and LCHF diets was 70 ± 13%, 67 ± 22%, and 63 ± 26%, respectively.

## Body weight and composition

Changes in body weight, VAT volume, and body composition have previously been described [22]. In brief, there were no significant between-group differences in change scores for body weight or VAT at any time point. Body weight decreased significantly after 3 mo on the A-HCLF (mean –4% [95% CI: –5, –3]), C-HCLF (mean –5% [95% CI: –6, –4]), and LCHF (mean –6% [95% CI: –7, –5]) diets and remained significantly



decreased after 12 mo by  $-5\%$  [95% CI:  $-8, -2$ ],  $-6\%$  [95% CI:  $-8, -4$ ], and  $-7\%$  [95% CI:  $-9, -5$ ], respectively. VAT volume, measured at baseline and 6 and 12 months of follow-up, was significantly reduced on all 3 study diets at 6 mo by 14–18%, which was largely maintained at 12 mo (12–17%).

### Ketosis

At 3 mo, there was a significant group difference in plasma  $\beta$ HB concentrations between the LCHF diet and the comparator A-HCLF diet (Figure 1, Table 2). On the LCHF diet, the concentration of  $\beta$ HB significantly increased from baseline (mean  $\pm$  SD:  $0.06 \pm 0.07$  mmol/L) to 3 mo ( $0.25 \pm 0.25$  mmol/L), whereas  $\beta$ HB concentrations did not increase on the HCLF diets (Supplemental Table 1). On the LCHF diet, 34% of the participants achieved  $\beta$ HB concentrations of  $\geq 0.30$  mmol/L, an often-considered threshold for nutritionally induced ketosis [8]. On both the A-HCLF and C-HCLF diets, 3% of the participants achieved  $\beta$ HB concentrations of  $\geq 0.30$  mmol/L at 3 mo. After 3 mo,  $\beta$ HB gradually decreased on the LCHF diet to (mean  $\pm$  SD)  $0.08 \pm 0.06$  mmol/L at 12 mo and was no longer significantly different from the comparator group or baseline levels. The same patterns were observed for changes in AcAc concentrations on the LCHF and HCLF diets (Table 2, Supplemental Table 1).

### Total ghrelin

We found no significant between-group differences in fasting plasma concentrations of total ghrelin at any time point (Figure 2, Table 2), except when the 2 HCLF diets combined were compared with the LCHF diet at 3 mo ( $-39.6$  pg/mL [ $-76, -3.3$ ]) (absolute model-adjusted mean change score [95% CI] from the cLMM, data not shown). From baseline to 3 mo, fasting plasma ghrelin increased significantly on the A-HCLF (mean: 46 pg/mL; 95% CI: 11, 81) and C-HCLF diets (mean: 54 pg/mL; 95% CI: 21, 88) (Supplemental Table 1) but not on the LCHF diet (mean: 11 pg/mL; 95% CI:  $-16, 38$ ). At 6 and 9 mo, all groups had a significant increase in fasting plasma concentrations of ghrelin compared with baseline (Supplemental Table 1). By 12 mo, fasting plasma concentrations of ghrelin remained significantly increased on the A-HCLF (mean: 51 pg/mL; 95% CI: 13, 88) and C-HCLF diets (mean: 42 pg/mL; 95% CI: 5, 78), and

change scores from baseline did not differ on the LCHF diet (mean: 24 pg/mL; 95% CI:  $-2, 51$ ) (Supplemental Table 1).

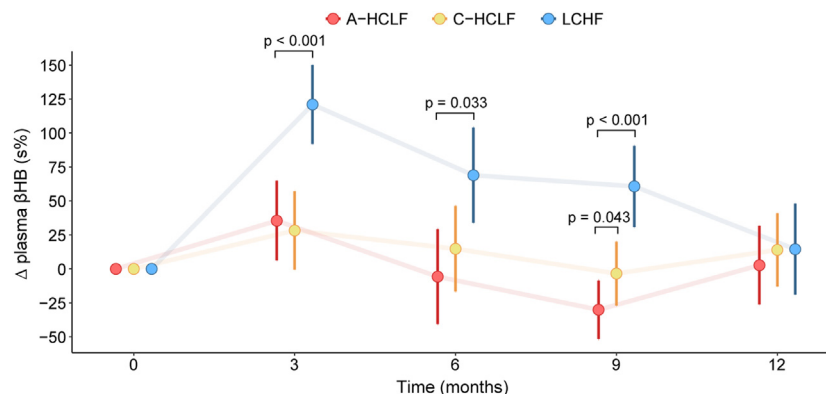
To explore to what extent plasma ketone concentrations predicted plasma ghrelin, we correlated changes in  $\beta$ HB and ghrelin at 3, 6, 9, and 12 mo. As expected, no correlations were seen within the A-HCLF and C-HCLF groups (Table 3), whereas greater increases in  $\beta$ HB by 3 mo on the LCHF diet correlated weakly with lower increases in ghrelin concentrations (Cohen's  $f^2 = 0.216$ ,  $P = 0.155$ ) (Table 3, Figure 3).

When investigating the relationship between changes in weight and ghrelin concentrations, we found that greater weight loss by 3 and 12 mo corresponded to a greater increase in ghrelin across all groups (3 mo: Cohen's  $f^2 = -0.393$ ,  $P = 0.001$ ; 12 mo:  $-0.452$ ,  $P = 0.001$ ) and also within the C-HCLF group ((3 mo:  $-0.399$ ,  $P = 0.030$ ; 12 mo:  $-0.781$ ,  $P < 0.001$ ). However, we only observed correlations between weight loss and ghrelin concentrations after 3 mo but not 12 mo in the LCHF group (3 mo: Cohen's  $f^2 = -0.524$ ,  $P < 0.001$ ; 12 mo:  $-0.378$ ,  $P = 0.104$ ) and neither after 3 nor 12 mo in the A-HCLF group (3 mo:  $-0.393$ ,  $P = 0.062$ ; 12 mo:  $-0.312$ ,  $P = 0.294$ ). No statistically significant group differences were observed (Table 3, Figure 3). In addition, increased VAT loss across all groups by 6 and 12 mo corresponded to a greater increase in ghrelin (Cohen's  $f^2 = -0.256$ ,  $P = 0.046$ ;  $-0.336$ ,  $P = 0.032$ , respectively) (Table 3).

Although changes in ghrelin correlated with changes in body weight and VAT volume, there were no significant correlations between changes in total energy intake from baseline and changes in ghrelin at any of the follow-up time points (Table 3).

### Subjective feelings of appetite

In the fasting state, there were no between-group differences in the subjective feelings of hunger, desire to eat, or prospective food consumption at any time point (Figure 4, Supplemental Table 2). Only feelings of fullness significantly differed between the LCHF and A-HCLF diets in changes from baseline to 6 mo ( $-1.34$  cm [ $-2.31, -0.37$ ]), 9 mo ( $-1.36$  cm [ $-2.29, -0.42$ ]) and 12 mo ( $-0.99$  [ $-1.90, -0.083$ ]) (absolute model-adjusted mean change scores [95% CI] from the cLMMs) (Figure 4), decreasing slightly on the LCHF diet and increasing on the A-HCLF diet (Supplemental Table 3). Because no clear differences in feelings of hunger, desire to eat or prospective food



**FIGURE 1.** Relative changes from baseline in fasting plasma concentrations of  $\beta$ -hydroxybutyrate ( $\beta$ HB) (millimoles per litre) for the acellular high-carbohydrate low-fat (A-HCLF), cellular high-carbohydrate low-fat (C-HCLF), and low-carbohydrate high-fat diet (LCHF) diets (intention to treat). Changes are shown in sympercents (s%) (95% CIs).  $P$  values of  $< 0.05$  from the constrained linear mixed-effects models are shown for the difference between indicated groups in change from baseline to each follow-up time point.

**TABLE 2**

Fasting plasma concentrations of total ghrelin,  $\beta$ -hydroxybutyrate, and acetoacetate during the intervention showing between-group differences in absolute change scores<sup>1</sup>.

Variable	Baseline <sup>2</sup>	3 mo <sup>2</sup>	6 mo <sup>2</sup>	9 mo <sup>2</sup>	12 mo <sup>2</sup>	Change score 3 mo <sup>3</sup>	P value <sup>3</sup>	Change score 12 mo <sup>4</sup>	P value <sup>4</sup>
Total ghrelin (pg/mL)									
A-HCLF <sup>1</sup>	321 (203)	357 (193)	362 (212)	349 (208)	315 (182)	—	—	—	—
C-HCLF	292 (156)	324 (166)	341 (173)	308 (165)	318 (166)	8.39 (−40.1, 56.9)	0.734	−9.11 (−61.7, 43.4)	0.733
LCHF	312 (162)	339 (183)	347 (177)	355 (228)	335 (195)	−35.2 (−79.0, 8.63)	0.115	−26.3 (−72.1, 19.5)	0.259
$\beta$ -hydroxybutyrate (mmol/L)									
A-HCLF	0.06 (0.08)	0.08 (0.11)	0.06 (0.08)	0.03 (0.01)	0.05 (0.03)	—	—	—	—
C-HCLF	0.06 (0.06)	0.08 (0.08)	0.07 (0.05)	0.06 (0.05)	0.07 (0.08)	−0.01 (−0.05, 0.04)	0.707	0.02 (−0.02, 0.05)	0.419
LCHF	0.06 (0.07)	0.25 (0.25)	0.16 (0.23)	0.11 (0.09)	0.07 (0.06)	0.16 (0.09, 0.24)	<0.001	0.02 (−0.01, 0.05)	0.146
Acetoacetate (mmol/L)									
A-HCLF	0.04 (0.03)	0.05 (0.04)	0.03 (0.03)	0.02 (0.01)	0.03 (0.02)	—	—	—	—
C-HCLF	0.04 (0.03)	0.05 (0.05)	0.04 (0.02)	0.03 (0.02)	0.05 (0.05)	0.03 (−0.02, 0.02)	0.756	0.01 (−0.01, 0.03)	0.425
LCHF	0.04 (0.04)	0.12 (0.11)	0.08 (0.08)	0.07 (0.05)	0.05 (0.03)	0.07 (0.04, 0.11)	<0.001	0.01 (−0.01, 0.02)	0.294

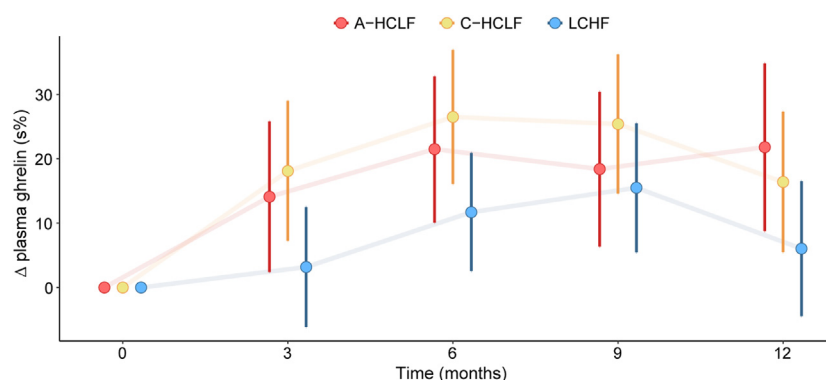
A-HCLF, acellular high-carbohydrate low-fat; C-HCLF, cellular high-carbohydrate low-fat; LCHF, low-carbohydrate high-fat.

<sup>1</sup> Data from measurements of blood samples were analyzed with constrained linear mixed-effects models (intention to treat). In the between-group comparisons, the A-HCLF intervention was defined as the comparator arm.

<sup>2</sup> Values are arithmetic means (SDs) of measurements at baseline and after 3, 6, 9, and 12 mo of follow-up.

<sup>3</sup> Absolute model-adjusted between-group change scores (95% CIs) from baseline to 3 mo and P values from the constrained linear mixed-effects models.

<sup>4</sup> Absolute model-adjusted between-group change scores (95% CIs) from baseline to 12 mo and P values from the constrained linear mixed-effects models.



**FIGURE 2.** Relative changes from baseline in fasting plasma concentrations of ghrelin (picograms per milliliter) for the acellular high-carbohydrate low-fat (A-HCLF), cellular high-carbohydrate low-fat (C-HCLF), and low-carbohydrate high-fat diet (LCHF) diets (intention to treat). Changes are shown in sympercents (s%) (95% CIs).

consumption were found in the fasting state, we further investigated the postprandial responses after consumption of the standardized mixed meal to potentially reveal other tonic effects of the diet interventions. Postprandially, we only observed between-group differences in ratings of prospective food consumption, which significantly differed between the LCHF and the A-HCLF diets (mean:  $-1.28$  cm [95% CI:  $-2.32$ ,  $-0.25$ ]) after 120 min at 3 mo (Supplemental Table 2), decreasing slightly on the A-HCLF diet and increasing on the LCHF diet.

Within groups, feelings of hunger in the fasting state significantly increased from baseline to 3 mo on the LCHF (mean:  $0.63$  cm [95% CI:  $0.004$ ,  $1.25$ ]) and C-HCLF diets ( $1.05$  cm [0.38, 1.73]) (Supplemental Table 3). Feelings of hunger remained significantly increased throughout the study on both diets, whereas the increase in hunger feelings on the A-HCLF diet was only significant at 6 mo (mean:  $0.95$  cm [95% CI:  $0.063$ ,  $1.83$ ]). Desire to eat in the fasting state increased significantly on the LCHF diet at 6, 9, and 12 mo (Supplemental Table 3). The analyses of within-group changes in postprandial feelings of appetite

showed no clear/consistent patterns throughout the intervention (Supplemental Table 3).

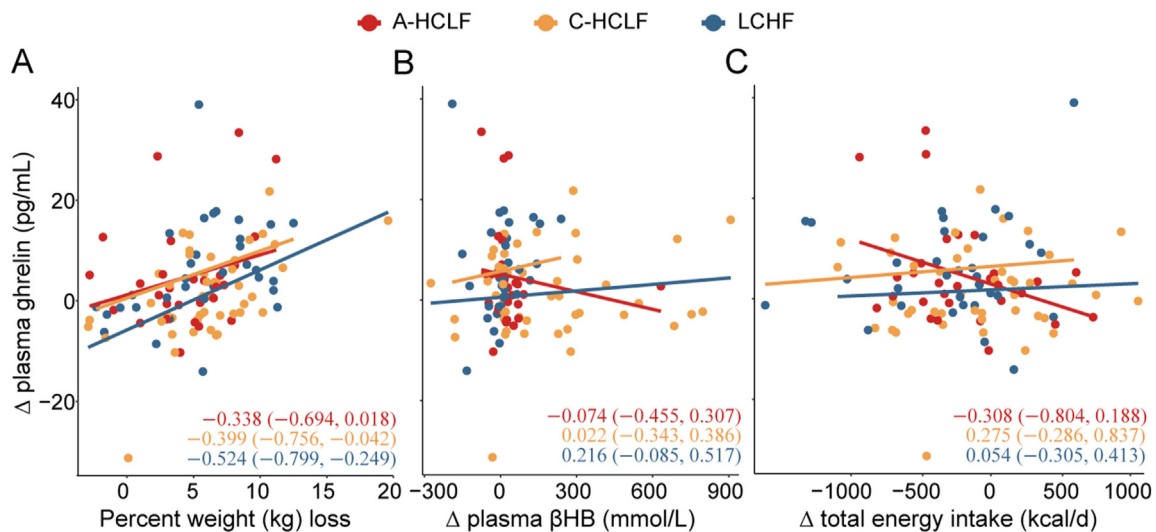
## Discussion

In a 3-arm RCT of adults with obesity lasting for 12 mo, we explored the effects of modest energy-restricted isocaloric diets on fasting plasma concentrations of total ghrelin, ketone bodies ( $\beta$ HB in the fasting state), and subjective appetite. We hypothesized that a higher concentration of  $\beta$ HB induced by an LCHF diet would be associated with lower total ghrelin concentrations and subjective hunger feelings. However, we found no significant differences in total ghrelin concentration and subjective hunger feelings in the short term (3 mo) or the long term (12 mo) on diets with different quality (cellularity) or quantity of dietary carbohydrates. At 3 mo, there was a significant group difference in plasma concentrations of  $\beta$ HB between diets differing in carbohydrate content. However,  $\beta$ HB concentrations gradually regressed toward baseline levels on the LCHF diet parallel with a

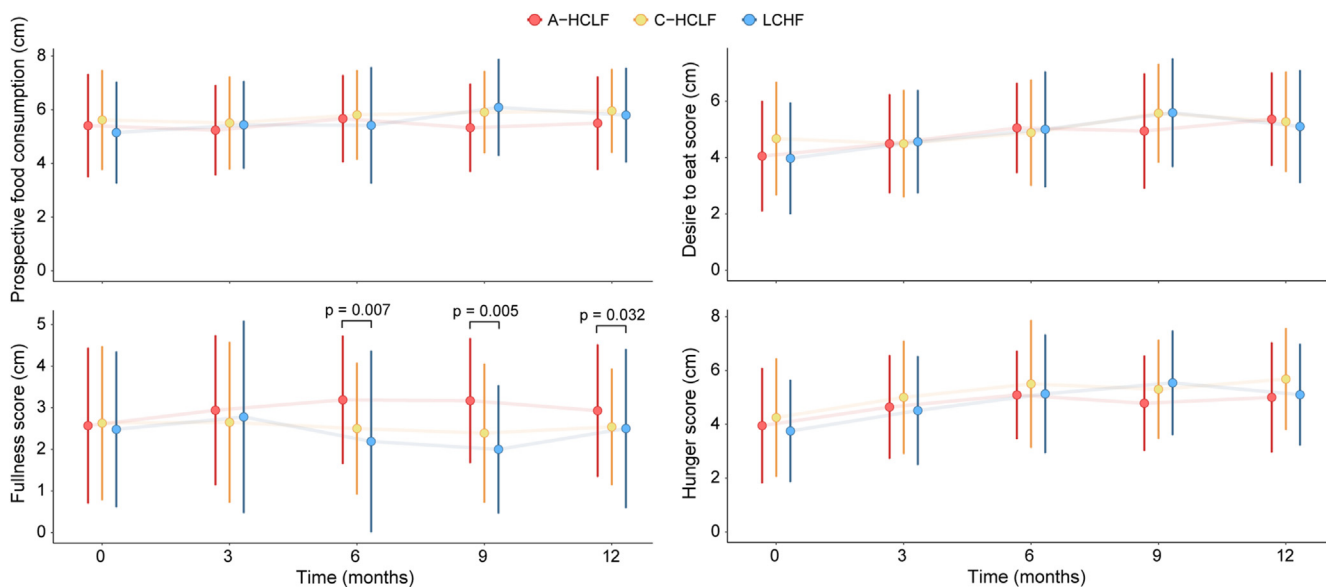
**TABLE 3**Associations between relative changes in fasting plasma concentrations of total ghrelin and other variables of interest after 3, 6, 9, and 12 months of follow-up<sup>1</sup>.

Variable	0–3 mo		0–6 mo		0–9 mo		0–12 mo	
	Est. (95% CI)	Cohen's $f^2$	Est. (95% CI)	Cohen's $f^2$	Est. (95% CI)	Cohen's $f^2$	Est. (95% CI)	Cohen's $f^2$
$\Delta$ Body weight (kg)								
All	−0.393 (−0.577, −0.209)	17.08	−0.364 (−0.623, −0.106)	12.36	−0.479 (−0.764, −0.194)	20.63	−0.452 (−0.718, −0.187)	22.89
A-HCLF	−0.338 (−0.694, 0.018)	14.08	−0.418 (−0.907, 0.071)	20.52	−0.052 (−0.631, 0.527)	0.27	−0.312 (−0.936, 0.311)	11.04
C-HCLF	−0.399 (−0.756, −0.042)	16.72	−0.638 (−1.114, −0.162)	41.36	−0.805 (−1.348, −0.261)	53.77	−0.781 (−1.167, −0.396)	100.67
LCHF	−0.524 (−0.799, −0.249)	34.37	−0.270 (−0.680, 0.140)	7.37	−0.613 (−1.021, −0.206)	52.23	−0.378 (−0.842, 0.086)	16.27
$\Delta$ VAT (cm <sup>3</sup> )								
All	—	—	−0.256 (−0.508, −0.004)	6.78	—	—	−0.336 (−0.642, −0.030)	9.75
A-HCLF	—	—	−0.264 (−0.616, 0.089)	16.95	—	—	−0.332 (−0.931, 0.267)	13.53
C-HCLF	—	—	−0.299 (−0.860, 0.262)	6.56	—	—	−0.653 (−1.201, −0.105)	37.23
LCHF	—	—	−0.282 (−0.745, 0.181)	6.92	—	—	−0.253 (−0.733, 0.228)	6.78
$\Delta$ $\beta$ HB (mmol/L)								
All	0.022 (−0.170, 0.214)	0.05	−0.083 (−0.305, 0.139)	0.97	0.198 (−0.070, 0.466)	4.00	0.049 (−0.222, 0.321)	0.26
A-HCLF	−0.074 (−0.455, 0.307)	0.58	0.0967 (−0.242, 0.435)	2.93	0.373 (−0.114, 0.860)	19.24	0.424 (−0.148, 0.996)	24.18
C-HCLF	0.022 (−0.343, 0.386)	0.05	0.020 (−0.465, 0.504)	0.04	0.248 (−0.222, 0.717)	6.81	0.130 (−0.339, 0.600)	1.88
LCHF	0.216 (−0.085, 0.517)	4.86	−0.154 (−0.593, 0.285)	2.40	0.245 (−0.248, 0.737)	5.69	−0.325 (−0.780, 0.130)	12.51
$\Delta$ Energy (kcal/d)								
All	−0.018 (−0.271, 0.236)	0.02	−0.019 (−0.331, 0.292)	0.03	0.122 (−0.286, 0.529)	0.80	−0.457 (−0.977, 0.064)	7.67
A-HCLF	−0.308 (−0.804, 0.188)	6.00	0.142 (−0.486, 0.770)	1.55	0.395 (−0.810, 1.600)	0.49	−0.541 (−1.327, 0.246)	26.87
C-HCLF	0.275 (−0.286, 0.837)	3.23	−0.095 (−0.617, 0.426)	0.87	0.241 (−0.530, 1.012)	0.52	−0.204 (−1.514, 1.105)	0.80
LCHF	0.054 (−0.305, 0.413)	0.22	−0.021 (−0.546, 0.505)	0.03	−0.082 (−0.683, 0.519)	0.77	−0.382 (−1.020, 0.257)	11.75

A-HCLF, acellular high-carbohydrate low-fat;  $\beta$ HB,  $\beta$ -hydroxybutyrate; C-HCLF, cellular high-carbohydrate low-fat; LCHF, low-carbohydrate high-fat; VAT, visceral adipose tissue.<sup>1</sup> Associations between relative changes (sympercent) from baseline to 3, 6, 9, and 12 mo in fasting plasma concentration of ghrelin (picograms per milliliter) and relative changes (sympercent) in body weight (kilogram), VAT (cubic centimeter), fasting plasma concentration of  $\beta$ HB (millimoles per liter), or energy intake (kilocalories per day).



**FIGURE 3.** Associations between changes in fasting plasma concentrations of ghrelin (picograms per milliliter) from baseline to 3 mo, and changes in (A) body weight (kilogram), (B) fasting plasma concentration of  $\beta$ -hydroxybutyrate ( $\beta$ HB) (millimoles per liter), or (C) energy intake (kilocalories per day). Standardized regression coefficients with 95% CIs from linear regression models are shown for each group. A-HCLF, acellular high-carbohydrate low-fat; C-HCLF, cellular high-carbohydrate low-fat; LCHF, low-carbohydrate high-fat.



**FIGURE 4.** Subjective feelings of appetite (prospective food consumption, desire to eat, fullness, and hunger) assessed using a visual analog scale in the fasting state from baseline to each follow-up time point up to 12 mo. *P* values of <0.05 from the constrained linear mixed-effects models are shown for the difference between indicated groups in change from baseline to each follow-up time point. A-HCLF, acellular high-carbohydrate low-fat; C-HCLF, cellular high-carbohydrate low-fat; LCHF, low-carbohydrate high-fat.

slight increase in carbohydrate intake (from 11 to 15 E%), resulting in no observed between-group differences in the long term. Weight loss (and fat loss) was comparable between the 3 study diets both in the short term (4–6%) and in the long term (5–7%).

Despite significant and similar weight loss on diets with comparable energy intakes after 3 mo, total ghrelin concentrations were only significantly increased on the HCLF diets and not on the LCHF diet. The lack of increase on the LCHF diet after 3 mo is in line with several other studies reporting a suppression of ghrelin secretion during weight loss on ketogenic diets [7, 11, 12, 38]. Interestingly, we observed this lack of increase in

ghrelin concentrations despite a lower  $\beta$ HB level (mean  $\pm$  SD:  $0.25 \pm 0.25$  mmol/L) on the LCHF diet after 3 mo compared with previous studies. Previous studies report suppression of ghrelin secretion at  $\beta$ HB levels of  $1.24 \pm 0.82$  mmol/L and  $0.48 \pm 0.07$  mmol/L achieved after 13–16% weight loss on 8-wk VLEDs [7, 38]. The relatively modest increase in  $\beta$ HB on the LCHF diet in our study was likely related to the higher energy intake, with more moderate weight loss, compared with many other studies of diet-induced fat loss. In an 8-wk trial among individuals with obesity, Martins et al. [38] found an inverse correlation between fasting  $\beta$ HB and ghrelin concentrations on a VLED (550–660 kcal/d) inducing significant increases in  $\beta$ HB (from  $0.21 \pm 0.25$



to  $1.24 \pm 0.82$  mmol/L). A reasonable assumption may be that higher levels of  $\beta$ HB than those observed in our study may have resulted in greater suppression of total ghrelin secretion because we only observed a weak correlation between  $\beta$ HB and ghrelin concentrations in our LCHF group. Consistent with this, ghrelin concentrations on the LCHF diet increased when the participants no longer had elevated  $\beta$ HB levels.

Although there was no significant increase in ghrelin concentration on the LCHF diet at 3 mo, suggesting a smaller increase in hunger on the LCHF diet, this was not observed for subjective feelings of appetite in our study. The increase in fasting subjective hunger feelings compared with baseline was significant for both the LCHF and C-HCLF diets at all follow-up time points, whereas the A-HCLF group showed no such increase. On the contrary, we observed an increase in feelings of fullness on the A-HCLF diet, which significantly differed from the LCHF diet at 6, 9, and 12 mo. Dietary factors associated with satiety and feelings of fullness, such as overall food volume and fiber intake [39], were highest on the C-HCLF diet. However, dietary energy density, which was found to increase fullness but not hunger, was highest on the LCHF diet in a recent systematic review and meta-analysis of clinical trials [40]. The overall lack of an association between subjective feelings of appetite and plasma ghrelin concentrations, diet volume, fiber intake, and energy density may be attributed to the complexity of appetite control, resulting from interactions between homeostatic (internal energy balance) and hedonic (reward-related) regulation [41]. In addition, even greater power may have been required to observe these associations.

Although the reported mean carbohydrate intake on the LCHF diet reached 11 E% after 3 mo, corresponding to 59 g/d, this was sufficient to differentially affect the  $\beta$ HB level compared with the A-HCLF diet despite the relatively high total energy intake. Previously suggested threshold levels for carbohydrate intake to induce ketosis vary and include 65–180 g/d [42],  $\leq 50$  g/d, or  $\leq 8$ –10 E% [6, 43, 44] and  $< 100$  g/d [8]. The suggested threshold levels for appetite suppression range from 0.3–0.5 mmol/L [6] to 1.48 mmol/L [14].

However, these suggested threshold levels were not reached. The  $\beta$ HB levels on the HCLF diets after an overnight fast varied from 0.03 to 0.08 mmol/L throughout the study, providing our LCHF group with reference values for  $\beta$ HB levels induced by overnight fasting alone without carbohydrate restriction. In comparison, Gibson et al. [6] previously suggested that the  $\beta$ HB concentration induced by overnight fasting alone is  $\sim 0.10$  mmol/L. Unlike the present study, previous studies investigating the effect of ketogenic diets on appetite have tended to be single-arm trials with no control group [3, 9, 10, 45]. Further, the measurements of fasting  $\beta$ HB concentrations both at baseline and at follow-up vary substantially between studies, as exemplified by  $\beta$ HB concentrations of 0.07 mmol/L and 0.28 mmol/L at baseline [7, 45] and 0.48 mmol/L and 1.6 mmol/L [7, 9] after 8–9 wk on VLEDs. These variations highlight the importance of including a comparator group following a nonketogenic diet, such as our A-HCLF group, for internal validity. The comparability of studies investigating the suggested appetite suppression of ketogenic diets is further challenged by the fact that many studies only report ketosis as a dichotomous variable (i.e., yes/no) and do not report specific levels of ketosis biomarkers [12].

The strengths of our study include a direct comparison of carbohydrate type and amount on diets with similar and only

modest energy restrictions to assess effects on appetite. In addition, the study diets were isocaloric, with similar intakes of both energy and protein, which might otherwise explain at least some of the previously observed effects on ghrelin and appetite. We also included repeated measures of both subjective (VAS score) and objective (plasma concentrations of total ghrelin) appetite markers as well as precise quantification and reporting of ketones ( $\beta$ HB and AcAc) by GC tandem MS.

Our study also has limitations. First, the rebound of carbohydrate intake on the LCHF diet over time undermined our ability to robustly evaluate the long-term effect on ketones, ghrelin, and subjective appetite. Second, the objective assessment of appetite was limited to the measurement of total ghrelin, whereas the measurement of acylated ghrelin may have been more precise. Third, the high dropout rate not only increases the chance of biased or imprecise effect estimates and limits the generalizability of the study but also demonstrates the challenge of investigating the effect of long-term dietary interventions. Finally, the outcome measures included in the present analysis were among several other secondary outcomes, raising the risk of selective and biased reporting. Nevertheless, the hypothesized relationship between ketones, ghrelin, and appetite was explicitly stated in previous reviews before our analyses [8].

In conclusion, in an RCT of men and women with obesity, we examined the effects of isocaloric, moderately energy-restricted diets on secondary outcomes related to appetite regulation. We found no significant differences in plasma concentrations of total ghrelin and subjective hunger feelings in the short (3 mo) or long term (12 mo), comparing a diet based on acellular carbohydrate sources to a cellular carbohydrate or low-carbohydrate diet. All 3 diets induced similar fat loss and increased reported feelings of hunger. Our findings suggest that the previously observed appetite-suppressive effect of ketones on a carbohydrate-restricted or VLEDs is not achieved without a carbohydrate restriction that induces sufficient ketosis (e.g.,  $< 50$  g/d).

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## Author disclosures

The authors report no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://doi.org/10.1016/j.tjnut.2022.12.030>.

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