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Metabolic and hormonal responses to nutritional ketosis differ by sex and metabolic phenotype in adults

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Abstract

Ketogenic diets are increasingly used for weight management and metabolic improvement, but it remains unclear whether nutritional ketosis induces uniform adaptation across metabolic phenotypes and sex. This study examined phenotype- and sex-dependent responses to a 60-day ketogenic diet in adults with normal weight, overweight or obesity. Participants were stratified into six metabolic phenotype–sex groups and assessed at baseline, day 14, day 30 and day 60. Plasma biochemical and hormonal markers, appetite and perceived stress scores, and body composition were analyzed using repeated-measures models. The ketogenic diet treatment increased β-hydroxybutyrate in all groups, confirming nutritional ketosis, but the response differed between groups and was most progressive in obese men. Free fatty acids manifested in a heterogeneous manner, exhibiting distinct time-, phenotype-, and sex-dependent profiles. Glucose decreased after diet initiation, whereas insulin and HOMA-IR improved mainly in overweight and obese participants. Lipid profile changes were variable, while liver enzymes showed a sex-dependent temporal pattern. Appetite score differed by metabolic phenotype, leptin changed according to phenotype and sex, and ghrelin remained unchanged. Self-reported perceived stress scores showed group-specific changes, whereas cortisol changed over time and body mass, fat mass, visceral adipose tissue and subcutaneous adipose tissue decreased. These findings indicate that nutritional ketosis produces heterogeneous adaptive responses shaped by metabolic phenotype and sex.

INTRODUCTION

Overweight and obesity remain major public health challenges, with recent global analyses showing a continuing increase in obesity prevalence worldwide[1]. However, excess body mass does not have the same metabolic meaning in all individuals. Individuals classified within the same BMI category may differ substantially in body fat percentage, fat distribution, lean mass, insulin-related traits, lipid profile and adipose tissue-derived biomarkers. This indicates that BMI alone does not fully capture risk of metabolic disturbances development[2,3]. This heterogeneity is also reflected in differences in insulin-related traits and lipid profiles across obesity phenotypes[4]. In addition, sex-related differences in adipose tissue distribution and insulin resistance, as well as psycho-behavioral factors related to nutritional behavior and stress, may further modify metabolic responses[5,6]. Therefore, an approach that integrates metabolic phenotype and sex may better capture the biological and behavioral diversity of overweight and obesity than BMI classification alone.

Dietary weight-loss interventions remain a key component of obesity and cardiometabolic risk management. Among them, ketogenic diet (KD) and very-low-carbohydrate ketogenic diets (VLCKDs) have gained increasing clinical and public attention. Current diabetes nutrition guidance does not recommend a single universal macronutrient distribution, but recognizes low- and very-low-carbohydrate eating patterns as possible individualized strategies, particularly for improving glycemic control in type 2 diabetes[7,8]. In obesity management, very-low-calorie ketogenic diets have also been proposed as

structured interventions for short-term weight reduction and metabolic improvement[9]. The defining metabolic feature of a KD is nutritional ketosis, commonly reflected by increased circulating BHB (β-hydroxybutyrate), which can be used as an objective marker of dietary adherence and metabolic response[10]. However, KD may influence more than body weight alone, including glucose–insulin homeostasis, lipid metabolism and body composition[11–13].

Importantly, achieving nutritional ketosis does not necessarily imply that these downstream responses are uniform across individuals. Adaptation to KD may also involve appetite regulation, adipose tissue-derived hormones secretion and behavioral factors that influence both adherence and metabolic outcomes. KDs have been suggested to attenuate the compensatory increase in appetite that often accompanies weight loss, although the magnitude of this effect and the involvement of appetite-related hormones such as leptin and ghrelin remain variable across studies[14–17]. In addition, adaptation to a restrictive dietary intervention may involve perceived stress and other psycho-behavioral components, which are increasingly recognized as relevant in obesity phenotyping[6]. Therefore, evaluating responses to the KD solely by body weight or standard metabolic markers may overlook important phenotype- and sex-related differences. Previous studies have shown that KDs are associated with reductions in body weight and adiposity, improvements in glycemic control and insulin-related indices, and changes in lipid profiles, although lipid responses may be variable. KDs have also been reported to attenuate hunger and modify appetite-related hormonal responses, particularly during weight loss[11–17]. However, these responses have rarely been evaluated together within the same intervention and interpreted according to both baseline metabolic phenotype and sex. This limits our understanding of whether responses to nutritional ketosis are uniform or vary according to metabolic phenotype and sex.

Because the response to KD may develop progressively, repeated assessment over time is needed to distinguish early and later changes during the treatment. Therefore, this study investigated changes over time in body composition, metabolic and hormonal markers, appetite, and perceived stress during a 60-day KD intervention. We also examined whether these trajectories differed according to baseline metabolic phenotype and sex. We also examined whether these time-dependent responses differ according to baseline metabolic phenotype and sex.

RESULTS

Participant flow and baseline characteristics

Participant flow throughout the study is presented in Fig. 1. Baseline characteristics of participants included in the longitudinal analyses are shown in Table 1, with additional hormonal and biochemical baseline variables presented in Supplementary Table A.

At baseline, between-group differences were observed mainly in variables related to the predefined metabolic phenotype. As expected, body mass, BMI, body fat percentage, visceral adipose tissue and subcutaneous adipose tissue differed across phenotype groups (all $p < 0.001$), whereas the muscle

mass-to-fat mass ratio was highest in normal-weight participants and lowest in participants with obesity ($p < 0.001$). Baseline insulin ($p = 0.002$) and homeostatic model assessment of insulin resistance (HOMA-IR) ($p = 0.002$) were higher in participants with obesity, particularly in men. Leptin concentrations also differed between groups ($p < 0.001$), with higher values observed in groups with greater adiposity, especially in women. In contrast, age ($p = 0.227$), BHB ($p = 0.755$), glucose ($p = 0.083$), appetite score ($p = 0.625$), and 10-item Perceived Stress Scale (PSS-10) score ($p = 0.230$) did not differ significantly between groups.

Nutritional ketosis was accompanied by heterogeneous lipid substrate and glucose–insulin responses

Changes in ketosis-related and glucose–insulin metabolism parameters are presented in Table 2, and detailed results are presented in Supplementary Table B. The KD induced a marked increase in plasma BHB levels, confirming the development of nutritional ketosis during the intervention. The time-dependent BHB response to KD differed between metabolic phenotype–sex groups, as indicated by a significant time × metabolic phenotype × sex interaction (T×M×S: $F(6, 79.45) = 2.30$, $p = 0.042$. BHB increased significantly from baseline to day 14 in all groups (LEAN_F, $p = 0.016$; LEAN_M, $p = 0.002$; OVERW_F, $p = 0.002$; OVERW_M, $p = 0.032$; OBESE_F, $p < 0.0001$; OBESE_M, $p < 0.0001$). At day 60, BHB remained significantly higher than baseline in lean women, women with overweight and obesity, and men with obesity (all $p \leq 0.0004$), whereas this difference was not significant in lean men and men with overweight. The most progressive response was observed in men with obesity, in whom BHB increased from baseline to day 14 and remained elevated thereafter, with a further significant increase between day 14 and day 60 ($p = 0.02$).

Because the increase in BHB reflects the establishment of nutritional ketosis, we next examined whether this response was accompanied by changes in circulating free fatty acids (FFA), which serve as lipid-derived substrates for hepatic ketogenesis. FFA responses varied across the six metabolic phenotype–sex groups (T×M×S: $F(6, 77.44) = 2.65$, $p = 0.022$). In lean women and women with obesity, FFA levels did not change significantly over time, suggesting a relatively stable circulating FFA profile during the KD treatment. In women with overweight, FFA increased from baseline to day 14 ($p = 0.037$), followed by a marked decrease from day 14 to day 30 ($p < 0.0001$). Among men, FFA levels tended to decline during the later stages of the KD treatment. In lean men, FFA decreased from day 14 and day 30 to day 60 ($p = 0.023$ and $p = 0.044$, respectively). In men with overweight, FFA levels were significantly lower at day 30 and day 60 compared with baseline ($p = 0.0066$ and $p = 0.0031$, respectively) and compared with day 14 ($p = 0.0057$ and $p = 0.0027$, respectively). A similar late decrease was observed in men with obesity, with lower FFA levels at day 30 and day 60 compared with baseline ($p = 0.017$ and $p = 0.039$, respectively) and compared with day 14 ($p = 0.0067$ and $p = 0.019$, respectively).

The group-specific FFA response was accompanied by changes in glucose–insulin homeostasis, indicating a broader metabolic shift during adaptation to the KD. Plasma glucose decreased during the

intervention, with lower mean values observed after diet initiation, particularly at day 14. Plasma glucose changed over time and differed according to metabolic phenotype (T: $F(3, 79.03) = 13.80$, $p < 0.001$; M: $F(2, 28.51) = 4.59$, $p = 0.019$). Mean glucose levels decreased after diet initiation, particularly at day 14, while the significant metabolic phenotype effect indicates overall differences in glucose levels between phenotype groups across the KD treatment. However, no higher-order interaction was observed, suggesting that the time course of glucose change was not clearly dependent on the combined effect of metabolic phenotype and sex.

In contrast, insulin showed a more phenotype-dependent response. The reduction in insulin was most evident in overweight and obese groups, whereas changes in lean participants were smaller and less consistent. This was reflected by significant time × metabolic phenotype (T×M: $F(6, 79.81) = 4.23$, $p < 0.001$) and metabolic phenotype × sex effects (M×S: $F(2, 28.18) = 5.23$, $p = 0.012$). Consistently, HOMA-IR decreased over time in a metabolic phenotype-dependent manner, with the clearest reductions observed in participants with overweight and obesity (T×M: $F(6, 80.27) = 4.00$, $p = 0.001$). This indicates an improvement in the insulin resistance-related index during the KD treatment, particularly in groups with higher baseline HOMA-IR.

Finally, we examined glucagon as a hormonal component of the metabolic adaptation to carbohydrate restriction. Compared with the more phenotype-specific responses observed above, glucagon showed a less differentiated pattern across metabolic phenotypes. Glucagon levels changed over time, with lower mean values observed at day 14 in most groups and a partial return toward baseline values at later time points (T: $F(3, 76.40) = 10.96$, $p < 0.001$). In addition, glucagon levels were higher in men than in women across the KD treatment (S: $F(1, 27.92) = 5.53$, $p = 0.026$). However, no higher-order interaction was observed, indicating that the glucagon response was not strongly dependent on the combined effect of time, metabolic phenotype and sex.

Appetite-related responses during KD treatment differ by baseline adiposity status and sex

We next assessed appetite score and appetite-related hormones as potential downstream responses to the metabolic adaptation described above (Table 4, and detailed results are presented in Supplementary Table D).

Appetite score differed according to metabolic phenotype, without a higher-order interaction with time or sex (M: LR $\chi^2(2) = 29.45$, $p < 0.001$). Overall, participants with lean and overweight phenotype tended to report higher appetite scores during the KD treatment than those with obesity. This indicates that the subjective appetite response differed primarily by baseline metabolic phenotype ($p < 0.001$), rather than following a uniform time-dependent pattern related to the duration of KD exposure.

The leptin response to KD over time depended on metabolic phenotype and also differed according to the combined effect of metabolic phenotype and sex (T×M: $F(6, 77.16) = 2.78$, $p = 0.017$; M×S: $F(2,$

27.31) = 3.80, p = 0.035). Leptin levels were generally higher in women and in participants with greater body mass, consistent with the significant metabolic phenotype × sex effect (p = 0.035), while changes over time differed according to metabolic phenotype (p = 0.017). Thus, leptin appeared to reflect both baseline adiposity and the metabolic response to the KD treatment. In contrast, ghrelin did not show significant changes during the KD treatment. This suggest that, within this panel, the appetite-related hormonal response was driven mainly by leptin rather than by ghrelin.

Perceived stress changed in a group-specific manner, with limited concordance across stress biomarkers

Next we assessed whether the metabolic and appetite-related responses described above were accompanied by changes in perceived stress and stress-related biomarkers (Table 5, and detailed results are presented in Supplementary Table E).

PSS-10 score showed a group-specific temporal response, indicating that changes in perceived stress depended on the combined effect of time, metabolic phenotype and sex (T×M×S: F(6, 78.31) = 4.99, p < 0.001). Based on post hoc comparisons, PSS-10 scores increased after KD initiation in woman with lean and obese phenotypes, and men with obesity, with higher values maintained at later time points compared with baseline (p < 0.05, Table 5). In lean men, a different pattern was observed, with lower PSS-10 scores at day 60 compared with earlier time points. In woman with overweight, PSS-10 scores did not change significantly across the KD treatment. Thus, the perceived stress response was not uniform across the study groups, but varied according to metabolic phenotype and sex. To complement the subjective assessment of perceived stress, we also examined circulating cortisol and noradrenaline levels as stress-related biomarkers. Cortisol changed over time and differed according to metabolic phenotype, but without a higher-order interaction with sex (T: F(3, 76.05) = 7.97, p < 0.001; M: F(2, 26.76) = 3.37, p = 0.049). Analysis of the group means showed an early increase from baseline to day 14 in lean woman and men, men with overweight and obesity, followed by lower values at later time points, suggesting a transient stress-related response during the early adaptation phase. Noradrenaline did not show significant changes during KD treatment. Thus, among the stress-related biomarkers analyzed in this panel, the response was more evident for cortisol than for noradrenaline.

Body composition changes during ketogenic diet treatment

To complement the biochemical, appetite-d and stress-related responses described above, we next assessed whether the KD treatment was accompanied by measurable changes in body composition. Body composition outcomes were analyzed as percentage changes from baseline, allowing comparison of the relative magnitude of change between metabolic phenotype–sex groups. Changes in body, fat, lean masses, VAT and SAT are presented in Table 6, and detailed results are presented in Supplementary Table F.

Body mass decreased across the KD treatment in all groups, with the magnitude of change depending on both metabolic phenotype and sex. This was reflected by significant time interval × metabolic phenotype (T×M: $F(4, 49.97) = 3.06$, $p = 0.025$) and time interval × sex effects (T×S: $F(2, 49.97) = 3.49$, $p = 0.038$). Overall, body mass reduction became more pronounced at later time intervals, particularly by day 60.

Fat mass also decreased during the KD treatment, with a sex-dependent temporal pattern (T×S: $F(2, 50.99) = 9.69$, $p < 0.001$). The direction of change was consistent across groups, but the magnitude of fat mass reduction differed between women and men over time. Lean mass showed a significant effect of time interval, without significant metabolic phenotype- or sex-dependent interactions (T: $F(2, 49.64) = 135.26$, $p < 0.001$). This indicates that lean mass changed during the KD treatment, but the pattern of change was not strongly modified by baseline metabolic phenotype or sex.

VAT decreased during the KD treatment, with a sex-dependent temporal response (T×S: $F(2, 49.21) = 4.95$, $p = 0.011$). The magnitude of VAT reduction varied between groups, but no higher-order interaction with metabolic phenotype and sex was observed. SAT also decreased during the KD treatment. The relative change in SAT differed according to time interval, metabolic phenotype and sex, but without a significant interaction between these factors (T: $F(2, 50.33) = 30.69$, $p < 0.001$; M: $F(2, 27.21) = 7.95$, $p = 0.002$; S: $F(1, 27.21) = 7.69$, $p = 0.010$). Thus, SAT reduction was a consistent component of the body composition response, although its magnitude differed between metabolic phenotypes and between women and men.

To further explore whether the main metabolic, hormonal and body composition responses were related to each other, selected Pearson correlation analyses were performed for early changes from baseline to day 14 and final changes from baseline to day 60. The full set of selected correlations is presented in the Supplementary Table G. Among the analyzed associations, the only significant final-response correlation was observed between percentage change in fat mass and change in leptin levels from baseline to day 60. Correlation analysis showed a significant moderate negative association between Δfat mass (%) and Δleptin ($r = -0.473$, 95% CI: −0.723 to − 0.113, $R^2 = 0.223$, $p = 0.013$, $n = 27$), indicating that the leptin response was related to the magnitude of body fat change during the KD treatment (Fig. 2).

DISCUSSION

The main finding of the present study is that a 60-day KD induced nutritional ketosis across all metabolic phenotype–sex groups. However, the accompanying adaptive responses did not follow a uniform pattern and involved metabolic, hormonal, appetite-related, stress-related and body composition changes. This observation is consistent with previous human studies and meta-analyses showing that KD or VLCKDs can reduce body weight in individuals with overweight or obesity. However, the magnitude and direction of these responses vary between studies and participant subgroups[9,11,18].

In the present study, nutritional ketosis was accompanied by changes in glucose–insulin homeostasis, lipid substrate availability and body composition, including reductions in body mass and adiposity-related compartments. Previous studies have similarly reported that KD or VLCKDs may reduce body weight and fat mass, including visceral adiposity[19,20].

The appetite-related findings also align with the broader literature suggesting that ketosis may attenuate the compensatory increase in appetite typically observed during weight loss. However, our results indicate that this response was not uniform across metabolic phenotypes. Leptin, rather than ghrelin, was the main appetite-related hormone showing a clear KD-related pattern. This is consistent with previous reports linking ketosis, weight loss and appetite-regulating hormones, although published findings remain heterogeneous across study designs[14,17,21,22].

Finally, the group-specific changes in perceived stress and cortisol suggest that adaptation to a KD should not be interpreted only as a metabolic process, but also as a behavioral and physiological response to a restrictive dietary intervention. Evidence on KD or VLCKDs and stress-related biomarkers in humans remains limited. However, available studies suggest that carbohydrate restriction and VLCKD may influence cortisol and hypothalamus-pituary-adrenal axis-related responses in humans under some conditions[14,23]. These findings suggest that adaptation to KD may also reflect individual differences in physiological and psychological adjustment to a restrictive dietary intervention.

The increase in plasma BHB confirms that the KD treatment effectively induced nutritional ketosis. Blood BHB is widely used as an objective marker of nutritional ketosis and adherence during KD treatment [10]. In the present study, the most progressive increase in BHB was observed in men with obesity. A similar induction of ketosis has previously been reported in men with obesity during a VLCKD[21]. Although our study further indicates that the time course of this response may differ between metabolic phenotype–sex groups. In contrast to BHB, FFA followed different trajectories over time across the six metabolic phenotype–sex groups, rather than showing a uniform increase during the intervention. This is biologically plausible because circulating FFA levels reflect not only adipose tissue lipolysis, but also β-oxidation, ketogenesis and peripheral tissue utilization[24]. These findings suggest that achieving nutritional ketosis does not necessarily imply a uniform circulating FFA response. To summarize, the plasma BHB reflects successful ketogenesis, whereas circulating FFA may represent a dynamic balance between lipid mobilization, hepatic conversion to ketone bodies, and tissue uptake or oxidation.

Because nutritional ketosis reflects a shift from carbohydrate-dependent metabolism, we next interpreted the glucose–insulin response as a central component of adaptation to the KD. In the present study, plasma glucose decreased after diet initiation, with the most evident reduction observed at day 14. Similar reductions in glycemic parameters have been reported in previous KD or VLCKD studies, particularly in individuals with overweight, obesity or type 2 diabetes[11,25–27]. In contrast to glucose, insulin and HOMA-IR showed a more phenotype-dependent pattern. The clearest reductions were observed in participants with overweight and obesity, who also had higher baseline insulin and HOMA-IR values. This is consistent with previous studies showing that KD or VLCKD treatments may improve

insulin-related indices. These improvements appear to be most evident in individuals with excess body mass or baseline glycemic and insulin dysregulation[25,26,28,29]. Therefore, the reductions in insulin and HOMA-IR in our study should be interpreted as improvements in insulin-resistance-related indices rather than direct evidence of improved insulin sensitivity. Glucagon showed a less differentiated pattern than the other ketosis- and glucose–insulin-related parameters. Its levels changed over time and were higher in men than in women. However, the absence of a higher-order interaction indicates that this response was not strongly dependent on the combined effect of time, metabolic phenotype and sex. Glucagon regulation during carbohydrate restriction may depend not only on carbohydrate availability, but also on insulin, protein intake, amino acid metabolism, and the overall hormonal context. Previous studies with participants have shown that carbohydrate-reduced diets can modify glucagon responses[30]. Moreover, the glucagon responses may differ between men and women under metabolic challenge conditions[31]. However, our findings suggest that, in this KD treatment, glucagon was a less phenotype-specific marker of ketogenic adaptation than BHB, FFA, insulin or HOMA-IR.

Because KDs substantially alter lipid substrate use, lipid profiles and liver enzyme levels were analyzed as secondary biochemical markers of cardiometabolic and hepatic adaptation. In the present study, the lipid profile did not follow a single uniform pattern. HDL-C increased or returned toward higher values by the end of the KD treatment. While triglycerides showed a more sex-dependent pattern, with more pronounced temporal changes in men. In contrast, total cholesterol and LDL-C showed variable responses across metabolic phenotype–sex groups, indicating that the lipid response to the KD was heterogeneous. This is in line with previous reports showing that ketogenic or very-low-carbohydrate ketogenic diets may reduce triglycerides level[32,33]. Moreover, blood total cholesterol, LDL-C, and HDL-C levels are more variable and may depend on baseline phenotype, diet composition, duration, and weight loss[33]. By comparison, liver enzyme levels showed a clearer sex-dependent temporal pattern, with ALT and AST decreasing mainly in men, who also had higher baseline values. Similar improvement outcomes have been reported in studies of very-low-calorie ketogenic diets in individuals with overweight or obesity, including reductions in ALT and AST, and improvements in other hepatic steatosis markers[34,35]. We suggest that the lipid profile changes did not follow a single consistent pattern during KD, whereas liver enzyme changes may suggest partial normalization in groups with higher baseline enzyme levels.

Appetite score and appetite-related hormones were analyzed because weight loss usually activates compensatory mechanisms that promote hunger and weight regain. Ketosis has been suggested to attenuate this response[14,17,36]. In the present study, appetite scores differed primarily by metabolic phenotype, indicating that subjective appetite responses were not uniform across participants. Although no significant uniform time-dependent effect was observed, appetite scores tended to increase during KD in participants with lean and overweight phenotypes. This indicating lower perceived hunger and greater fullness over time. In contrast, participants with obesity showed lower and less consistent appetite scores, particularly men. These suggesting stronger perceived hunger or lower fullness during KD treatment.

Among the appetite-related hormones, leptin showed a clearer pattern of KD-related changes than ghrelin. This is consistent with previous evidence that KDs may reduce leptin levels, while their effect on ghrelin is less consistent[37]. The stronger leptin response is also biologically plausible, as circulating leptin is closely associated with adiposity[38]. Leptin levels also differ between women and men, partly reflecting sex-related differences in body fat amount and distribution[39]. In our study, the observed significant association between percentage change in fat mass and change in leptin levels from baseline to day 60 further supports the role of adiposity change in shaping the leptin response.

Perceived stress and stress-related biomarkers were assessed because adaptation to a KD involves not only metabolic changes, but also behavioral and physiological demands related to dietary restriction, and changes in eating patterns. In the present study, PSS-10 scores increased after diet initiation in women with lean and obese phenotypes, men with overweight and obesity. In contrast, women with overweight showed no significant change, while lean men showed a decrease by day 60. Overall, these results suggest that KD treatment was not associated with a consistent increase in perceived stress across all metabolic phenotype–sex groups. Cortisol showed a temporal response, with changes most evident during the early phase of the KD treatment. This is consistent with evidence that VLCKDs may influence HPA-axis-related outcomes, particularly during early adaptation or weight loss[23,40,41]. Noradrenaline did not change significantly. Overall, these findings indicate that KD treatment was accompanied by a heterogeneous perceived stress response, which was only partly reflected in circulating stress-related biomarkers.

Body composition was assessed to determine whether the metabolic and hormonal responses to the ketogenic diet were accompanied by changes in body mass and adiposity-related compartments. In the present study, body mass and fat mass decreased following KD treatment across all groups, while the magnitude of fat mass reduction showed a sex-dependent pattern and was often more pronounced in men. Similar reductions in body weight and fat mass have been reported in previous low-carbohydrate or KD studies, including interventions in individuals with obesity[19,20]. VAT and SAT also decreased, although their response patterns were not identical, indicating that regional adipose tissue compartments may adapt differently during ketogenic weight loss. Lean mass changed over time. Early changes in lean or muscle mass during carbohydrate restriction may partly reflect shifts in glycogen-associated water and hydration status, rather than only structural tissue loss[42,43]. These body composition changes provide an important context for the hormonal and glucose–insulin responses observed in the present study. We showed that the reduction in fat mass may be consistent with the observed changes in leptin. Reductions in body mass and adiposity-related compartments may also partly explain the decreases in insulin and HOMA-IR, particularly in participants with overweight and obesity.

This study has several limitations. The final sample size was relatively small, and participant dropout occurred during the KD treatment, particularly in the early phase. The absence of a non-ketogenic control group limits the ability to separate the effects of nutritional ketosis from those of weight loss or energy

restriction. Because the study was conducted under free-living conditions, dietary intake could not be fully controlled despite daily BHB and glucose monitoring.

In conclusion, a 60-day KD treatment induced nutritional ketosis across all metabolic phenotype–sex groups. However, the accompanying changes in lipid substrate availability, glucose–insulin homeostasis, appetite-related hormones, perceived stress and body composition were heterogeneous. These findings indicate that adaptation to nutritional ketosis should be interpreted in relation to baseline metabolic phenotype and biological sex rather than as a uniform dietary response. This may be particularly important when KDs are applied in metabolically diverse populations. From an applied perspective, these findings may suggest the necessity of development personalized nutritional strategies in which ketogenic diets are applied and monitored according to individual metabolic phenotype and biological sex.

METHODS

Study design

The present manuscript reports a prospective, longitudinal, single-arm analysis of participants undergoing the KD intervention. Although the broader registered DIETMICRO protocol includes dietary intervention arms, the present report focuses exclusively on the ketogenic diet component and does not present between-arm randomized comparisons.

All participants followed the same KD treatment for 60 days. Assessments were performed at four time points: baseline (day 0), day 14, day 30, and day 60, with an accepted measurement window of ± 1 day. Participants were classified by baseline metabolic phenotype and sex into six analytical groups. The lean group was used as a reference metabolic phenotype rather than as a non-intervention control.

The primary focus of the study was the time course of nutritional ketosis, assessed using plasma BHB concentrations. Secondary analyses included changes in glucose–insulin homeostasis, lipid metabolism, appetite-related hormones, subjective hunger and satiety, perceived stress, stress-related biomarkers, and body composition parameters. Hormonal and biochemical markers were analyzed as plasma concentrations measured at each study time point, whereas body composition variables were analyzed as percentage changes from baseline to days 14, 30, and 60.

Participants and eligibility criteria

Participants were recruited during routine visits to a clinical dietitian between September 30, 2024, and September 30, 2025. Individuals who met the eligibility criteria were invited to participate in the study and were enrolled after providing written informed consent. Participants were adults aged at 25–59 years at enrollment.

A total of 48 participants were initially recruited, with 8 planned for each of the 6 analytical groups: LEAN_F, LEAN_M, OVERWEIGHT_F, OVERWEIGHT_M, OBESE_F, and OBESE_M (F – female, M – male).

After the first two weeks of the KD treatment, 14 participants discontinued the study: 4 from LEAN_M, 2 from LEAN_F, 3 from OVERWEIGHT_M, 4 from OVERWEIGHT_F, 0 from OBESE_M, and 1 from OBESE_F. Thus, 34 participants remained in the study through day 30: 6 LEAN_F, 4 LEAN_M, 4 OVERWEIGHT_F, 5 OVERWEIGHT_M, 7 OBESE_F, and 8 OBESE_M. Twenty-eight participants completed the full 60-day KD treatment: 5 LEAN_F, 4 LEAN_M, 3 OVERWEIGHT_F, 4 OVERWEIGHT_M, 7 OBESE_F, and 5 OBESE_M (Fig. 1). The main reasons for early discontinuation were non-adherence to dietary recommendations and failure to achieve nutritional ketosis, accounting for 64.3% of early dropouts. Other reasons included loss of contact with the study team (14.2%), family- or work-related reasons reported by participants (14.2%), and dissatisfaction with results or worsening well-being during the diet (7.3%).

Participants were classified into metabolic phenotype groups at baseline according to sex-specific clinical criteria based on BMI, total fat percentage, and MFR. The MFR was calculated as muscle mass divided by fat mass on day 0. Assignment to a given metabolic phenotype required meeting at least two of the three predefined criteria.

Women were classified as lean when BMI was 18.5–24.9 kg/m², total fat percentage was 21–33%, and the MFR was > 1.0–1.4; as overweight when BMI was 25.0–29.9 kg/m², total fat percentage was 34–39%, and the MFR was 0.8–1.0; and as obese when BMI was ≥ 30.0 kg/m², total fat percentage was ≥ 40%, and the muscle mass-to-fat mass ratio was < 0.8. Men were classified as lean when BMI was 18.5–24.9 kg/m², total fat percentage was 8–20%, and the muscle mass-to-fat mass ratio was > 1.6–2.0; as overweight when BMI was 25.0–29.9 kg/m², total fat percentage was 21–25%, and the muscle mass-to-fat mass ratio was 1.2–1.6; and as obese when BMI was ≥ 30.0 kg/m², total fat percentage was ≥ 26%, and the muscle mass-to-fat mass ratio was < 1.2.

The inclusion criteria were: age approximately 25–59 years at enrollment, signed informed consent, willingness to follow the KD protocol, stable body mass during the three months preceding enrollment, no major dietary changes before the start of the study, and either good general health or stable metabolic status appropriate for the assigned metabolic phenotype group.

The exclusion criteria were: pregnancy or breastfeeding; diagnosed type 1 diabetes, uncontrolled type 2 diabetes or severe metabolic disorders; history of eating disorders; severe kidney, liver, cardiovascular or pancreatic disease; active cancer or severe chronic inflammatory disease; use of medications affecting appetite, body mass, glucose or lipid metabolism, or ketone body metabolism; use of a KD before enrollment; alcohol or psychoactive substance abuse; participation in another clinical study during the previous three months; and inability to comply with the dietary intervention or study procedures.

Ethical approval

and informed consent

The study protocol was approved by the Bioethics Committee of the Jerzy Kukuczka Academy of Physical Education in Katowice, Poland (Polish name: Uczelniana Komisja Bioetyczna Akademii

Wychowania Fizycznego im. Jerzego Kukuczki w Katowicach; approval no. 2/2021). The study was retrospectively registered at ClinicalTrials.gov (NCT07672691; first posted on 2 July 2026). All methods were performed in accordance with the relevant institutional, national, and international guidelines and regulations and with the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. Before providing consent, participants were informed about the study procedures, potential risks and benefits, and their right to withdraw from the study at any time without providing a reason.

Ketogenic diet treatment

The dietary intervention consisted of a KD designed to induce and maintain nutritional ketosis by restricting carbohydrates, increasing fat intake, and maintaining moderate protein intake. Participants were instructed to follow a classical ketogenic dietary pattern, with carbohydrate intake generally limited to < 50 g/day or approximately ≤ 10% of total daily energy intake. Fat was prescribed as the predominant energy source, typically accounting for approximately 65–75% of total daily energy intake, whereas protein intake was maintained at a moderate level, usually corresponding to approximately 20–25% of total daily energy intake. Total energy intake was individualized based on body mass, baseline metabolic phenotype, dietary tolerance, and the clinical goal of body mass reduction or metabolic improvement.

Day 0 was defined as the baseline visit immediately preceding the initiation of the KD. During this visit, participants attended the first group meeting, received dietary recommendations, and were instructed on the practical implementation of the diet and self-monitoring procedures. The dietary plan consisted of two main meals per day, lunch and dinner, consumed within a 5–10 h eating window. Participants received a set of examples 25 lunch recipes and 25 dinner recipes, which could be modified according to individual preferences, tolerance, and product availability, provided that the ketogenic macronutrient assumptions were maintained.

Meals were based on high-quality protein sources, including meat, fish, eggs, seafood, and organ meats; fat sources such as clarified butter, lard, coconut oil, and olive oil; and low-carbohydrate vegetables, including leafy greens, cruciferous vegetables, radish, and fermented cucumbers. Fermented foods and selected natural ingredients, such as cinnamon, turmeric, and apple cider vinegar, were recommended as supportive dietary components. The diet excluded cereal products, added sugars, high-sugar fruits, highly processed foods, and alcohol. After the initial two-week adaptation phase, selected ketogenic-compatible foods and additions, such as mayonnaise, guacamole, and pumpkin seeds, could be introduced depending on individual tolerance and dietary adherence.

To reduce the risk of symptoms commonly associated with early ketogenic adaptation, participants were advised to maintain adequate fluid and electrolyte intake. The dietary protocol included daily intake of electrolyte-rich broth and supplementation with selected vitamins and minerals, including magnesium, potassium, B vitamins, vitamin D3, vitamin K2 MK-7, and omega-3 fatty acids, according to individual needs and tolerance.

Dietary adherence was monitored throughout the KD treatment using several complementary methods. Participants maintained food diaries and recorded daily home measurements of capillary blood glucose and BHB. Measurements were performed by participants under home conditions using a FreeStyle Optium Neo blood glucose and ketone monitoring system (Abbott Diabetes Care Inc., USA). Participants remained in regular contact with the supervising dietitian. The dietary recommendations, recipes, and practical implementation of the KD were discussed during four in-person meetings held over the entire dietary cycle, both in group format and individually when required.

Nutritional ketosis was defined as capillary blood BHB > 0.7 mmol/L during home monitoring. Participants who did not achieve or maintain nutritional ketosis, or who did not adhere to the dietary recommendations, were classified as non-adherent and were excluded from subsequent longitudinal analyses.

Measurement schedule

All study measurements were performed at four time points: baseline (day 0), day 14, day 30, and day 60. At each time point, blood sampling, questionnaire assessment, dual-energy X-ray absorptiometry (DXA) examination, were performed in the morning between 7:00 and 9:00 a.m. Blood samples were collected after an overnight fast. Hormonal and biochemical markers were assessed as plasma levels at each study time point. These included BHB, glucose, insulin, HOMA-IR, FFA, leptin, ghrelin, glucagon, cortisol, noradrenaline, total cholesterol,, LDL-C, HDL-C, ALT, and AST. Appetite score, and perceived stress score were assessed at each time point using questionnaire-based methods. Body composition variables were assessed at each study time point using DXA analysis. For the longitudinal analyses, body composition outcomes were expressed as percentage changes from baseline to days 14, 30, and 60. The analyzed variables included body mass, fat mass, lean mass, VAT, and SAT.

Anthropometric and body composition assessment

DXA measurements were performed using a GE Healthcare Lunar iDXA densitometer (GE Healthcare, Madison, WI, USA) and analyzed with enCORE software (GE Healthcare, Madison, WI, USA). Participants were scanned in light clothing, without metal objects, in the supine position according to the manufacturer’s procedures. The muscle mass-to-fat mass ratio used for baseline metabolic phenotype classification was calculated as DXA-derived muscle mass divided by DXA-derived fat mass on day 0 (baseline). DXA quality control and calibration procedures were performed according to the manufacturer’s guidelines.

Blood sampling and biochemical/hormonal analyses

Venous blood was collected into EDTA tubes and centrifuged at 1000 × g for 15 min at 4°C. Plasma samples were separated and stored at − 80°C until analysis. Plasma levels of hormonal and metabolic markers were measured using commercially available ELISA kits according to the manufacturers’ instructions: DRG® Insulin ELISA kit (EIA-2935; DRG Instruments GmbH), AFFIGEN AffiELISA β-hydroxybutyrate kit (AFG-BTL-025028), Human COR ELISA kit (EH0641; FineTest), Human Noradrenaline

ELISA kit (EU2565; FineTest), Free Fatty Acids ELISA kit (EU3592; FineTest), Human GC ELISA kit (EH1944; FineTest), Human Leptin ELISA kit (RD191001100; BioVendor Group), and Human Ghrelin ELISA kit (orb439772; Biorbyt Ltd.).

The plasma lipid panel and biochemical markers were determined using an Epoll 200 analyzer (Alpha Diagnostics, Poland), according to the manufacturer's instructions, with the following reagents: cholesterol (C6608-100), triglycerides (T6630-100), HDLc-D Direct (MI1001096), LDLc-D Direct (MI41023), glucose (G6620-100), ALT (A6424-125), AST (A6461-125). HOMA-IR was calculated as fasting insulin [μIU/mL] × fasting glucose [mg/dL] / 405.

Appetite, satiety and perceived stress questionnaires

The appetite score were assessed using the Polish version of the numeric hunger–fullness scale. Participants rated their current perceived hunger or fullness on a 1–10 scale, where lower values indicated greater hunger, middle values indicated a neutral state, and higher values indicated greater fullness. Perceived stress was assessed at each study time point using the Polish version of the PSS-10 score. The questionnaire evaluates the frequency of stress-related thoughts and feelings experienced during the previous month. Each item was scored on a 5-point Likert-type scale (0–4), and the total PSS-10 score was calculated according to the standard scoring procedure, with higher scores indicating greater perceived stress.

Statistical analysis

Statistical analyses were performed using R software, version 4.6.0. For participants characteristic at baseline level, before KD treatment, Kruskal-Wallis test following Shapiro-Wilk test for normality distribution was applied. The corresponding Kruskal–Wallis test statistics and p-values are reported in Supplementary Table A.

Plasma biochemical and hormonal variables, questionnaire scores and metabolic indices were analyzed across four time points: baseline, day 14, day 30 and day 60. Body composition outcomes were analyzed as percentage changes from baseline to day 14, day 30 and day 60. The main analytical model included time, metabolic phenotype and sex as fixed factors, together with all two-way and three-way interactions. For repeated-measures analyses, participant ID was included as a random effect to account for within-subject correlations over time. Thus, the primary model structure was defined as time × metabolic phenotype × sex. For body composition outcomes, time represented the interval of percentage change from baseline. Model assumptions were assessed before analysis by inspection of residual distributions and evaluation of variance homogeneity. When required, variables were transformed before modeling to improve compliance with model assumptions. Specifically, glucagon, leptin, ghrelin, and AST were analyzed after $\log_2$ transformation. Descriptive values are presented on the original scale.

A linear mixed-effects model was fitted and 3-way ANOVA was used to test main effects and interactions (lme4 package)[44]. When significant main effects of at least three-level-factor (time, metabolic phenotype) or interactions (time × metabolic phenotype × sex, time × metabolic phenotype,

metabolic phenotype × sex, time × sex) were revealed, post hoc comparisons were performed using estimated marginal means with adjustment for multiple comparisons using Tukey's method (emmeans package)[45]. For appetite score, an ordinal cumulative linear mixed model was applied, and post hoc comparisons were adjusted using Holm's method. For the presentation of inferential results in the main tables, only the highest-order significant interaction and post-hoc multiple comparison were reported. Full post hoc comparisons corresponding to the significant effects reported in the manuscript are provided in the supplementary tables B - F.

Correlation analyses were performed using Pearson's correlation coefficient to assess selected associations between changes in metabolic, hormonal, questionnaire and body composition variables. For these analyses, changes were calculated from baseline to day 14 and from baseline to day 60, depending on the analyzed response window, and provided in supplementary table G. All data are presented as mean ± standard deviation unless otherwise stated. Statistical significance was set at $p < 0.05$.

Abbreviations

ALT
alanine aminotransferase
AST
aspartate aminotransferase
BHB
β-hydroxybutyrate
DXA
dual-energy X-ray absorptiometry
FFA
free fatty acids
HDL
C-high-density lipoprotein cholesterol
HOMA
IR-homeostatic model assessment of insulin resistance
KD
ketogenic diet
LDL
C-low-density lipoprotein cholesterol
MFR
muscle-to-fat mass ratio
PSS
10-10-item Perceived Stress Scale
SAT

subcutaneous adipose tissue
VAT
visceral adipose tissue
VLCKD
very-low-carbohydrate ketogenic diet.

Declarations

Author contributions

M.G. and K.G. performed the experiments, contributed to the methodology, prepared the tables, and wrote the original draft of the manuscript. M.G. conceptualized the study, acquired funding for the study. M.M. performed the experiments, supervised the study. G.Z. and G.K. and M.P. contributed to the methodology, performed the experiments. A.M. reviewed and edited the manuscript, supervised the study, acquired funding for the study. M.N.Ch. reviewed and edited the manuscript, supervised the study. All authors reviewed and approved the final manuscript.

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Data availability

The data supporting the findings of this study are included in the Supplementary Materials. Additional data generated or analyzed during the current study are available from the corresponding author upon reasonable request.

Additional information’s

Competing interests

The authors declare that they have no competing interests.

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Tables

Tables are available in the Supplementary Files section.

Figures

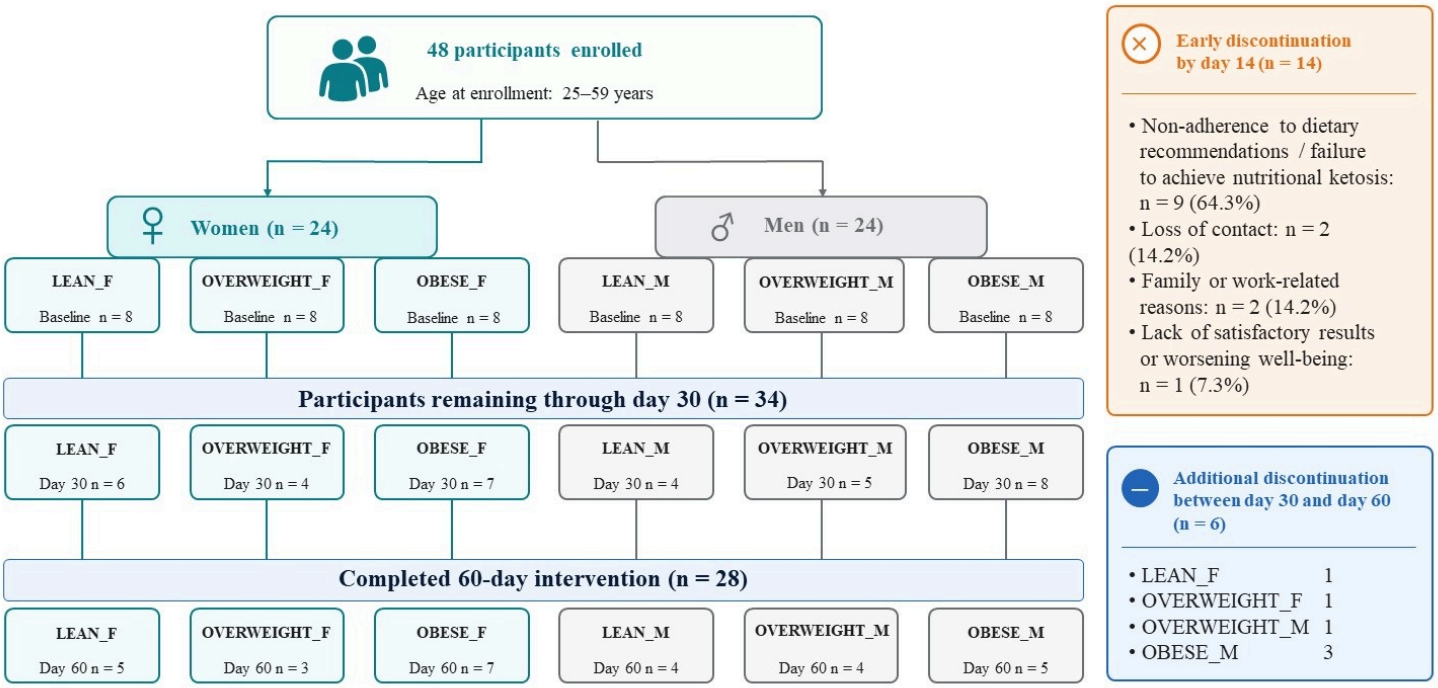


Figure 1

Participant flow diagram. Participant enrollment, sex-based allocation, metabolic phenotype classification and retention during the 60-day ketogenic diet treatment. Forty-eight participants were enrolled, 34 remained through day 30 and 28 completed the ketogenic diet treatment. Metabolic phenotype classification was based on sex-specific criteria including BMI, total fat percentage and muscle-to-fat mass ratio.

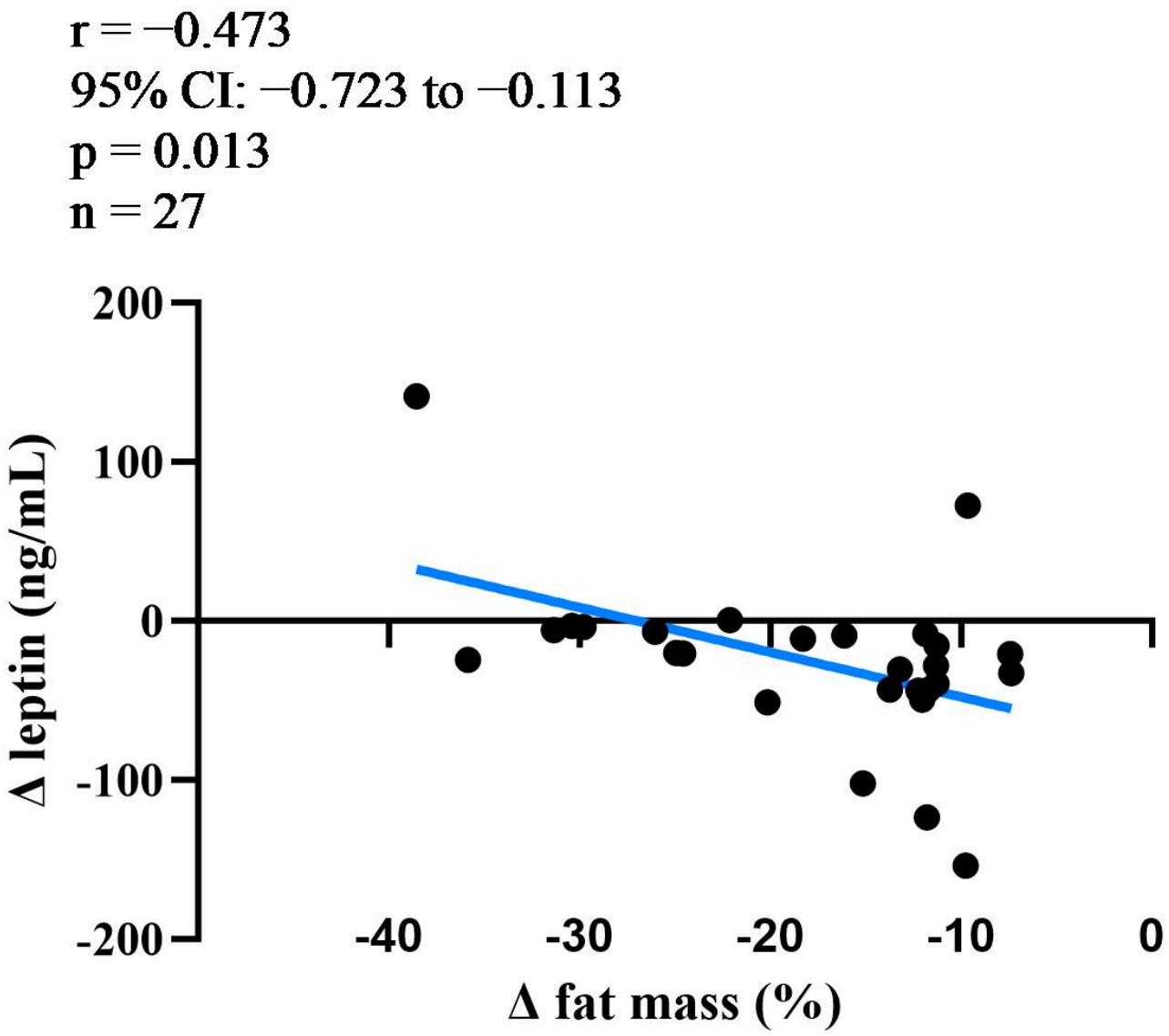


Figure 2

Association between percentage change in fat mass and change in leptin levels from baseline to day 60. Each point represents one participant. The line indicates the simple linear regression fit. Pearson correlation analysis showed a significant moderate negative association between Δfat mass (%) and Δleptin (r = −0.473, 95% CI: −0.723 to −0.113, R^2 = 0.223, p = 0.013, n = 27). Δ values were calculated as day 60 minus baseline, with fat mass expressed as percentage change from baseline.

Supplementary Files

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- Supplementarymatherials03.07.2026.xlsx
- Tables.docx