Effects of low-carbohydrate time-restricted eating, with or without sprint interval training, on resting cardiac biomarkers in young adults: a sex-stratified randomized controlled trial

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Abstract

Purpose Low-carbohydrate time-restricted eating (LCTR) and sprint interval training (SIT) independently improve cardiometabolic health, but their combined effects on cardiac-specific biomarkers remain unknown. We investigated whether LCTR, with or without SIT, affects N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin T (cTnT) concentrations in young adults.

Methods Sixty-one young adults (31 males, 30 females) were randomized to a 4-week program of LCTR alone or LCTR+SIT. All participants consumed ≤10% of energy from carbohydrates within an 8-hour daily window. LCTR+SIT groups additionally performed 5 weekly sessions of 10×6-second sprints with 9-second recovery periods. Body composition, $\dot{V}O_{2max}$, and NT-proBNP and cTnT were assessed pre- and post-intervention. Data were analyzed using three-way mixed ANOVA with intervention (LCTR vs LCTR+SIT) and sex as between-subjects factors, and time as within-subjects factor.

Results Body fat decreased across all groups (range -1.2 to -2.6 kg, $P<0.05$), with no significant between-group differences. Significant intervention × time interaction was observed for $\dot{V}O_{2max}$ ($P=0.003$), whereas NT-proBNP showed a significant intervention × sex × time interaction ($P=0.016$). Post-hoc analyses revealed that only females in the LCTR+SIT group demonstrated a significant improvement in $\dot{V}O_{2max}$ (+17.1%, $P=0.014$) and a reduction in NT-proBNP (-48%, $P=0.0005$). Resting cTnT levels did not change in any group, with most values at or below detection limits.

Conclusion Brief LCTR, with or without SIT, reduced body fat in both sexes. Only females in the LCTR+SIT group demonstrated reduced resting NT-proBNP. Reasons for, and implications of, this rapid and sex-mediated adaptation in NT-proBNP are worthy of further study.

Keywords: cardiac biomarker; high-intensity interval training; cardiovascular risk factor; low-carbohydrate diet; time-restricted eating

Introduction

Low-carbohydrate (LC) diets effectively promote short-term weight loss and improve cardiovascular health markers in both clinical and non-clinical populations (Santos et al. 2012). Time-restricted eating (TR), a form of intermittent fasting with a limited daily eating window, can enhance these benefits when combined with LC diets (de Cabo and Mattson 2019; Alqusayer et al. 2025). Although LC diets effectively reduce adiposity and confer certain cardiovascular benefits, they are associated with potentially deleterious effects such as loss of lean body mass and compromised cardiorespiratory fitness. These reductions in muscle mass and aerobic capacity may contribute to adverse long-term health outcomes, including elevated cardiovascular risk (Church et al. 2005; Landry et al. 2021; Urbain et al. 2017). To mitigate these concerns, sprint interval training (SIT), characterized by brief bursts of maximal-intensity exercise interspersed with recovery periods, offers a time-efficient strategy to stimulate skeletal muscle protein synthesis and enhance cardiorespiratory fitness (Gibala and Little 2020). This training modality may therefore counteract the potentially negative effects of LC diets on body composition and aerobic capacity. Indeed, preliminary evidence indicates that combining LC with SIT improves body composition while simultaneously enhancing aerobic fitness (Sun et al. 2019). However, the impact of combining LC and/or TR with SIT on cardiovascular biomarkers remains unknown in young healthy adult males and females.

N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponin T (cTnT) represent well-established cardiac biomarkers traditionally employed in the diagnosis, management, and prognosis of cardiovascular diseases (Perrone et al. 2020). Recent advances in high-sensitivity immunoassay technologies have expanded their clinical utility, enabling the detection of subclinical cardiac stress and the assessment of long-term cardiovascular risk in currently asymptomatic populations (Daniels et al. 2008; Clerico et al. 2020; Farmakis et al. 2020). Even minimal elevations in baseline NT-proBNP and cTnT concentrations correlate with increased risk of future cardiac events, independent of traditional cardiovascular risk factors, with this association evident even in younger populations (Clerico et al. 2020; Farmakis et al. 2020). Notably, emerging evidence demonstrates sex-specific differences in both baseline levels and responses of these biomarkers to interventions. Females typically exhibit higher resting NT-proBNP concentrations compared to age-matched males (Welsh et al. 2022), while males demonstrate greater cTnT elevations in response to exercise stress

(Kong et al. 2017). These sex-related variations in cardiac biomarker profiles necessitate stratified analysis to accurately evaluate intervention effects. Importantly, emerging evidence suggests that these biomarkers demonstrate dynamic responses to lifestyle modifications, positioning them as surrogate endpoints for evaluating cardiovascular risk reduction strategies (Ciardullo et al. 2022; Farmakis et al. 2020). Despite preliminary data examining traditional cardiovascular risk factors during LC and TR interventions (Santos et al. 2012), the specific effects of these dietary approaches, particularly their integration with high-intensity exercise interventions, on NT-proBNP and cTnT concentrations in young adult males and females remain unexplored.

The primary aim of this study was to evaluate the effects of a low-carbohydrate time-restricted eating (LCTR), both independently and in combination with SIT, on resting concentrations of NT-proBNP and cTnT in young male and female adults. Given documented sex-specific metabolic and physiological responses to LC diets (Syed-Abdul et al. 2018), TR (Freire et al. 2020), and SIT (Skelly et al. 2021), a secondary aim was to determine whether biological sex mediates the effects of these interventions on cardiac biomarker concentrations.

Materials and Methods

Participants

One hundred twenty-eight volunteers (62 males, 66 females) responded to local advertisements for the study. Eighty-nine participants (45 males, 44 females) met the following inclusion criteria: (a) age 18-30 years; (b) stable body weight (±2 kg) for 3 months; (c) sedentary lifestyle without regular exercise training or variation in physical activity levels for 6 months, verified by the International Physical Activity Questionnaire (Ainsworth et al. 2006); (d) non-smoking status; (e) absence of any overt evidence of hormonal, orthopedic, or cardiovascular disease, diabetes, dyslipidemia, hypertension, or polycystic ovary syndrome (females only); f) for females, regular menstrual cycles (26-31 days) documented for the previous 6 months; and (g) no current medication use, including oral contraceptives. Thirteen eligible participants declined to participate for personal reasons, resulting in 85 enrolled participants (39 males, 46 females). Participants were stratified by sex and randomly assigned to either the low-carbohydrate time-restricted eating (LCTR) or LCTR combined with SIT. Initial group allocations were: LCTR males (LCTR_M, n=19), LCTR+SIT males (LCTR+SIT_M, n=20), LCTR

females (LCTR_F, n=23), and LCTR+SIT females (LCTR+SIT_F, n=23). During the intervention, 15 participants withdrew due to scheduling conflicts or non-adherence (LCTR_M: 4; LCTR+SIT_M: 4; LCTR_F: 5; LCTR+SIT_F: 2). Non-adherence was defined as failure to maintain nutritional ketosis (absence of urinary ketones for >3 consecutive days), completion of <80% of prescribed training sessions (LCTR+SIT groups), or failure to maintain the 8-hour feeding window on >3 occasions per week. Additionally, 9 female participants who completed the intervention were excluded from analysis due to mistiming of assessments relative to their menstrual cycle phase. Thus, the final analysis included 61 participants (LCTR_M: 15; LCTR+SIT_M: 16; LCTR_F: 13; LCTR+SIT_F: 17), with baseline characteristics comparable between intervention groups within each sex. Males averaged 23.0 ± 4.3 years with BMI 26.2 ± 4.3 $kg \cdot m^{-2}$, while females averaged 22.0 ± 3.6 years with BMI 24.4 ± 3.8 $kg \cdot m^{-2}$. **Figure 1** illustrates the participant flow, including recruitment, randomization, and retention. All participants provided written informed consent after receiving a detailed study briefing. The study was conducted in accordance with the Declaration of Helsinki and approved by the regional ethics committee for research involving human subjects.

Insert Figure 1 here

Experimental design

Participants received standardized nutritional counseling for the LCTR protocol and were familiarized with all testing and training procedures, including a practice maximal oxygen uptake ($\dot{V}O_{2max}$) test session to ensure valid baseline measurements. They maintained habitual physical activity and dietary patterns while completing a 2-week baseline food record before intervention. Pre-intervention assessments occurred following 48-h abstention from moderate-to-vigorous physical activity. For female participants, all baseline and post-intervention assessments were scheduled 3-7 days after the onset of menses (i.e., early follicular phase) to minimize hormonal influences on outcome measures. The phases of the menstrual cycle were determined by measuring morning basal body temperature and urine luteinizing hormone concentration using home ovulation prediction kits (Runbio Biotech Co., Ltd., Shantou, China). Baseline measurements included venous blood sampling, body composition assessments, and $\dot{V}O_{2max}$ testing on separate days. The 4-week intervention commenced 5 days after baseline testing, with all participants initiating the LCTR protocol and

continuing dietary recording. Participants assigned to LCTR+SIT groups simultaneously began their exercise intervention. During the 4-week intervention, participants were instructed to refrain from additional physical activity beyond their habitual activity levels. Daily physical activity was monitored using a pedometer plugin (WeRun) installed on participants' phones through the WeChat app (Xu et al. 2021), with step counts recorded throughout the 2-week baseline and 4-week intervention periods. Post-intervention assessments occurred 48 h after the final training session, with participants maintaining the LCTR protocol until blood sampling to capture cumulative intervention effects. Assessment timing was based on evidence that acute exercise-induced elevations in NT-proBNP and cTnT normalize within 48 h (Gresslien and Agewall 2016). All laboratory assessments were standardized to 11:00 h in a climate-controlled environment (20°C, 50% relative humidity).

Dietary Intervention Protocol

Before intervention, participants completed a standardized nutrition education program led by a registered dietitian, encompassing portion estimation, macronutrient identification, and LCTR-specific meal planning. Participants received comprehensive educational materials including food lists, sample menus, and adherence guidelines. The LCTR protocol required a macronutrient distribution of 65% fat, 25% protein, and 10% carbohydrate (Goss et al. 2020), with daily energy intake confined to an 8-hour feeding window (10:00-18:00 h) followed by 16-hour fasting (de Cabo and Mattson 2019). Participants were provided with detailed meal plans and food exchange lists to facilitate adherence to macronutrient targets while allowing flexibility in food choices.

Dietary adherence was assessed using 3-day food records (two weekdays, one weekend day) collected during a 2-week baseline period and throughout the 4-week intervention. Records were analyzed weekly using validated software (Sports Nutrition Centre, National Research Institute of Sports Medicine, version 3.1, China). Participants attended weekly consultations for dietary monitoring and protocol adjustments. Nutritional ketosis was confirmed via daily morning urinary ketone testing (UROPAPER reagent strips, Suzhou First Pharmaceutical Co. Ltd., China). Participants recorded urinary ketone results daily throughout the 4-week intervention. Ketone levels ≥0.5 mmol/L were classified as positive, indicating nutritional ketosis. The overall positive rate was calculated as the percentage of all daily measurements across all participants that showed positive ketone results.

Sprint Interval Training

Participants assigned to the LCTR+SIT groups performed sprint interval training 5 days per week for 4 weeks. Each session consisted of 10 × 6-second maximal sprints interspersed with 9-second passive recovery periods on a mechanically braked cycle ergometer (Monark 894E, Vansbro, Sweden). Initial resistance was set at 1.0 kg for females and 2.0 kg for males. Resistance remained constant throughout all 10 sprints within each session. Progression decisions were evaluated every third training session (sessions 3, 6, 9, 12, 15, and 18). If participants successfully maintained the target pedal cadence (≥90 rpm) throughout all 10 sprints during the evaluation session, resistance was increased by 0.5 kg for the subsequent training session. If cadence dropped below 90 rpm during any sprint within the evaluation session, resistance remained unchanged. Each sprint commenced from a stationary position with rapid acceleration to 100 rpm before automatic resistance application, followed by maximal effort for 6 seconds. Sessions included a 3-minute warm-up and 3-minute cool-down of unloaded cycling at self-selected cadence. All training was supervised, with monitoring of power output, heart rate (HR) and ratings of perceived exertion (RPE, Borg 6-20 scale). Training sessions were scheduled between 15:00-18:00 h to minimize circadian variation in performance.

Outcome Variable Assessments

Body composition

Standing height was measured to the nearest 0.1 cm using a stadiometer. Body mass and composition including total body water were determined via multi-frequency bioelectrical impedance analysis (InBody 770, Biospace Co., Seoul, Korea) following overnight fasting and bladder voiding. This device has demonstrated excellent agreement with dual-energy X-ray absorptiometry for measuring fat-free mass (concordance correlation coefficient = 0.98) and body fat percentage (concordance correlation coefficient = 0.97) across diverse BMI categories (Hurt et al. 2021).

Graded exercise test

Participants performed a graded exercise test on a cycle ergometer (Monark 839E, Vansbro, Sweden) beginning at 25 W, with 15-W increments every 3 min while maintaining a cadence of 60 rpm. Oxygen uptake was measured continuously using a calibrated metabolic analysis system (MetaMax 3B, Cortex Biophysik GmbH, Leipzig, Germany). The test was terminated when participants reached

volitional exhaustion, defined as inability to maintain the required cadence despite verbal encouragement. $\dot{V}O_{2max}$ was determined as the highest 30-s average value achieved during the test. $\dot{V}O_{2max}$ was expressed in absolute terms ($L \cdot min^{-1}$), relative to body mass ($ml \cdot kg^{-1} \cdot min^{-1}$), and relative to fat-free mass ($ml \cdot kgFFM^{-1} \cdot min^{-1}$) to account for body composition changes.

Cardiac Biomarker Analysis

Pre- and post-intervention, venous blood samples (3 mL) were collected from the antecubital vein with participants seated. After clotting at room temperature, samples were centrifuged at 3500 × g for 20 min. Serum was separated and stored at −20°C until analysis. NT-proBNP and cardiac troponin T concentrations were measured using electrochemiluminescence immunoassay (Cobas e411, Roche Diagnostics Ltd., Rotkreuz, Switzerland). The fifth-generation high-sensitivity assay demonstrated intra-assay coefficients of variation of 2.4% at 126 $ng \cdot l^{-1}$ for NT-proBNP and 3.1% at 12.2 $ng \cdot l^{-1}$ for cTnT. Detection limits were 5 $ng \cdot l^{-1}$ and 3 $ng \cdot l^{-1}$ for NT-proBNP and cTnT, respectively. All analyses followed manufacturer specifications.

Statistical analysis

Data distribution was evaluated using the Kolmogorov-Smirnov test. To address non-normality in cardiac biomarkers while maintaining analytical consistency across all outcome variables, NT-proBNP and cTnT values underwent logarithmic transformation. All primary outcome variables (body composition, $\dot{V}O_{2max}$, and log-transformed cardiac biomarkers) were analyzed using three-way mixed ANOVA with two between-subjects factors (intervention: LCTR vs LCTR+SIT; sex: male vs female) and one within-subjects factor (time: pre vs post). When significant interactions were detected, simple main effects were examined using Bonferroni-adjusted pairwise comparisons. Dietary intake variables and daily step counts were similarly analyzed to assess protocol fidelity. Training characteristics (peak power output, heart rate, RPE) were analyzed using a two-way mixed ANOVA with sex as a between-subjects factor and time (training weeks) as a within-subjects factor. Statistical significance was set at $p < 0.05$. Data are expressed as mean ± SD for normally distributed variables and median (range) for non-normally distributed variables. All analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). In addition, Individual NT-proBNP responses were analyzed both as absolute concentrations and percentage changes from baseline to comprehensively assess response

heterogeneity and clinical relevance. Due to detection limit constraints, individual analysis was not performed for cTnT.

Results

Dietary Changes

The overall positive rate of urinary ketones was 97%, with no significant differences among the four groups. Energy intake decreased significantly from baseline to intervention in all groups ($P < 0.05$, Figure 2). Macronutrient composition shifted significantly across all groups (Table 1, Figure 2). Fat intake increased from baseline values of 36-38% (~72-90 g) to intervention values of 58-61% (~87-118 g) of total energy ($P < 0.05$). Protein intake increased from 17-20% (~70-106 g) to 25-29% (~86-125 g) of total energy ($P < 0.05$). Carbohydrate intake decreased from 42-47% (~202-235 g) to 10-17% (~43-59 g) of total energy ($P < 0.05$). No significant two-way or three-way interactions were observed for energy intake or any macronutrient variable (all $P > 0.05$).

Insert Figure 2 and Table 1 here

Training Data

Overall, training adherence was 96% in the LCTR+SIT groups, with no significant differences between males and females. Peak power output during SIT sessions was significantly higher in males than females across all weeks (main effect of sex: $P < 0.05$; **Table 2**). Peak exercise heart rate (150-157 beats·min^{-1}) and RPE (11-12) remained stable across the intervention period, with no significant main effects of time or sex and no sex × time interactions (all $P > 0.05$).

Insert Table 2 here

Physical Activity Monitoring

Daily step counts remained consistent throughout the study, with no significant differences between the 2-week baseline (7452±3176 steps/day) and 4-week intervention (8031±2816 steps/day) periods (main effect of time: $P > 0.05$). Furthermore, no significant intervention × sex × time interaction or main effects of intervention or sex were observed (all $P > 0.05$).

Anthropometric Changes

All intervention groups demonstrated significant and similar relative body weight reductions across the intervention period ($P < 0.05$, **Table 3**). Males in LCTR and LCTR+SIT groups decreased body weight by 4.2 kg (80.5 ± 11.1 to 76.3 ± 10.4 kg) and 3.1 kg (80.3 ± 15.8 to 77.2 ± 14.9 kg), respectively. Female participants showed similar patterns, with LCTR and LCTR+SIT groups reducing body weight by 2.6 kg (68.1 ± 11.6 to 65.5 ± 11.2 kg) and 2.0 kg (63.7 ± 9.0 to 61.7 ± 9.4 kg), respectively. These changes corresponded to BMI reductions of 1.0-1.3 $kg \cdot m^{-2}$ across all groups ($P < 0.05$).

Insert Table 3 here

Body Composition Changes

All intervention groups demonstrated significant changes in body composition (**Table 3**), with fat mass decreasing by 1.2-2.6 kg across groups ($P < 0.05$), consequently body fat percentage decreased by 1.5-2.2% across all groups ($P < 0.05$). There was a significant main effect of intervention for fat-free mass data. This main effect indicated that the addition of SIT attenuated fat-free mass loss compared to LCTR alone. A significant drop in fat-free mass occurred in LCTR males (1.2 kg) and LCTR females (0.9 kg; both $P < 0.05$), whereas the addition of exercise (LCTR+SIT) reduced fat-free mass loss (males: 0.6 kg decrease, females: 0.4 kg decrease; both $P > 0.05$). Total body water showed no significant changes from pre- to post-intervention in any group (all $P > 0.05$).

Cardiorespiratory Fitness Responses

Three-way mixed ANOVA revealed significant main effects of intervention ($P = 0.025$) and sex ($P = 0.0001$), and intervention × time interaction ($P = 0.003$) for $\dot{V}O_{2max}$. Post-hoc analyses showed differential responses across groups (Table 3). When expressed relative to body mass, only LCTR+SIT females demonstrated significant improvement in $\dot{V}O_{2max}$ (25.2±3.2 to 29.5±6.7 $ml \cdot kg^{-1} \cdot min^{-1}$, +17.1%, $P = 0.014$). All other groups remained unchanged (all $P > 0.05$). When normalized to fat-free mass to account for body composition changes, LCTR males showed a 15% reduction (42.8±8.4 to 36.4±9.7 $ml \cdot kgFFM^{-1} \cdot min^{-1}$, $P = 0.015$), while LCTR+SIT females demonstrated a 14.1% increase (37.6 ± 4.2 to 42.9 ± 8.7 $ml \cdot kgFFM^{-1} \cdot min^{-1}$, $P = 0.029$).

Cardiac Biomarker Responses

For NT-proBNP, three-way mixed ANOVA on log-transformed values revealed a significant intervention × sex × time interaction ($P = 0.016$). Post-hoc analyses identified that only LCTR+SIT

females demonstrated a significant reduction from baseline (median: 26.0 ng·l^{-1}, range: 5.0-91.7) to post-intervention (median: 13.6 ng·l^{-1}, range: 5.0-30.8; P = 0.0005). All other groups showed no significant changes (all P > 0.05; **Table 3**). **Figure 3** illustrates individual response patterns, showing both absolute concentrations (Panel A) and percentage changes from baseline (Panel B).

Insert Figure 3 here

For cTnT, the majority of values remained at or below the 3 ng·l^{-1} detection limit across all time points. Three-way mixed ANOVA on log-transformed values showed no significant main effects or interactions (all P > 0.05). Only sporadic detectable values occurred, with no systematic pattern across groups or time points (**Table 3**).

Discussion

The current randomized controlled trial examined the effects of a 4-week LCTR intervention, with or without SIT, on resting cardiac biomarkers and other traditional health outcomes in sedentary young male and female adults. Our primary finding was a sex-specific response in resting NT-proBNP, with only females in the LCTR+SIT group demonstrating a significant (48%) reduction despite identical intervention protocols across sexes. While all groups achieved comparable fat loss, the addition of SIT to LCTR provided additional advantages: it attenuated the loss of fat-free mass observed with LCTR alone and, in females specifically, enhanced cardiorespiratory fitness. These differential responses highlight the importance of sex-stratified analyses in exercise intervention studies and suggest that even ultra-brief high-intensity exercise (12 minutes weekly) can produce meaningful cardiovascular adaptations when combined with metabolic interventions.

Cardiac Biomarkers: NT-proBNP

While population-based dietary surveys suggest associations between reduced carbohydrate intake and lower NT-proBNP levels (Yang et al. 2023), direct experimental evidence examining LC and/or TR dietary interventions on cardiac-specific biomarkers is lacking. In support of our study design, high-intensity exercise has emerged as a potent modulator of NT-proBNP, with moderate-to-vigorous physical activity associated with 37% lower levels per 10-minute increment (Parsons et al. 2018), and high-intensity interval training showing superior efficacy over moderate-intensity exercise (Zare Karizak et al. 2023). The present study revealed that a brief period of LCTR alone does not significantly

alter resting NT-proBNP concentrations in young adults, with no sex-specific differences observed. Although this contrasts with Zheng et al. (Zheng et al. 2024), who reported decreased levels of traditional myocardial enzymes (lactate dehydrogenase, creatine kinase, and related isoforms) in metabolic syndrome patients following LC diets, regardless of time-restriction status, the choice of biomarkers and young healthy adults may explain some of the difference in outcome. We employed cardiac-specific biomarkers that more accurately reflect long-term cardiovascular risk compared to traditional myocardial enzymes (Farmakis et al. 2020; Ahmad et al. 2024), with NT-proBNP demonstrating superior prognostic value (Kvisvik et al. 2017; Ahmad et al. 2024). Unlike traditional enzymes that lack tissue specificity and can be elevated by skeletal muscle damage from various sources, NT-proBNP specifically reflects cardiac wall stress and neurohormonal activation. In the present study, despite significant weight loss and reduced caloric intake, NT-proBNP remained unchanged, challenging the previous hypothesis that weight loss *per se* mediates natriuretic peptide changes (Minami et al. 2000). This finding is particularly relevant given the inconsistent results from previous LC intervention studies, which have variously reported decreased (Minami et al. 2000), unchanged (Hollstein et al. 2021), or increased (Kistorp et al. 2014) NT-proBNP levels following dietary weight reduction. Notably, these contrasting findings emerged from studies in diverse populations: Minami (2000) studied obese patients, Hollstein (2021) examined overweight adults during severe caloric restriction, while Kistorp (2014) investigated heart failure patients with elevated baseline NT-proBNP. These divergent outcomes suggest that modulation of resting NT-proBNP may require more than simple caloric restriction, especially in young healthy participants.

In the LCTR+SIT group, female participants demonstrated a significant reduction in resting NT-proBNP (median change: -48%), with no change in the male group. This sex-specific response to high-intensity exercise extends previous observational findings (Parsons et al. 2018) by demonstrating, in a controlled intervention setting, that vigorous physical activity can modulate NT-proBNP levels. Despite an identical intervention protocol, the sex-based difference in response may reflect the higher pre-intervention data in females and/or other mechanistic processes that remain speculative. The observed sex-specific response is, however, important given the relatively small exercise "dose". Previous research, including work from our group (Sun et al. 2022) and others (Boullosa et al. 2022), has established that this brief intervention can rapidly enhance cardiorespiratory fitness in sedentary

populations. When combined with other similar studies, these results suggest that exercise of high relative and absolute intensity may be more important than a large exercise dose based on prolonged exercise duration. Further exploration of a potential sex-specific response to LCTR+SIT is warranted, potentially in different (e.g., clinical) groups.

The mechanistic underpinning of this sex-specific NT-proBNP response warrants exploration. Whether young adult women are inherently more "trainable" has been proposed based on the fact that young women demonstrate superior baseline endothelial function compared to men (Stanhewicz et al. 2018). Whether this reflects a pre-disposition to amplified training-responsiveness or is a consequence of inherent differences in the female cardiovascular system remains unknown. Exercise stimulates endothelial nitric oxide production, an effect potentially enhanced by estrogen's vasculoprotective actions (Moreau et al. 2013). This synergy could reduce cardiac afterload and wall stress, the primary stimulus for NT-proBNP release, more effectively in females. Animal studies provide biological precedent, demonstrating inherent sex differences in cardiac adaptation with females exhibiting superior adaptive capacity (Konhilas et al. 2004). While our 4-week intervention revealed some sex-specific responses, the mechanisms remain speculative without direct measurements. Future studies incorporating endothelial function assessment, cardiac imaging, and hormonal profiling could help elucidate these pathways and identify factors determining individual responsiveness to combined metabolic-exercise interventions.

Cardiac Biomarkers: cTnT

Resting cTnT concentrations were not affected by either LCTR or LCTR+SIT in both sexes. Although we employed a high-sensitivity assay, most values remained at or below the assay detection limit before and after the interventions, albeit with some individual variability. Across all sample points, detectable cTnT values (>3 $ng.l^{-1}$) were observed in 61% of males and but only 3% of females. While community-based population studies have reported resting cTnT detection rates of 69-83% in males and 42-54% in females (Kimenai et al. 2021; Schneider et al. 2014), these predominantly middle-aged and older cohorts demonstrate age-dependent trends with lower detection rates observed in younger participants—a pattern consistent with our findings. The remarkably low detection rate in our young females likely reflects their robust cardiovascular health status and the consistently lower baseline

cTnT concentrations documented in premenopausal women compared to age-matched men (Kimenai et al. 2021), possibly reflecting enhanced cardioprotection during premenopausal years.

Given these low baseline values, our ability to detect intervention-induced changes was limited. Studies demonstrating exercise training-induced reductions in resting cTnT have predominantly involved older adults or clinical populations with elevated baseline values, such as the 12-week exercise training intervention by Koppen et al. (2021) in heart failure patients (mean age 61 years) where significant cTnT reductions correlated with $\dot{V}O_{2max}$ improvements. Thus, the inability to detect changes in resting cTnT due to diet and/or exercise interventions appears to be influenced by participants' age, baseline health status, and current limitations in assay sensitivity for young, healthy populations. Therefore, cTnT may have limited utility as a biomarker for monitoring cardiovascular adaptations in this demographic.

Body Composition and Training Adaptations

While LC diets effectively reduce adiposity, they frequently result in concurrent losses of fat-free mass and compromised cardiorespiratory fitness (Urbain et al. 2017). Consistent with these concerns, participants in our LCTR-only groups experienced significant reductions in fat-free mass (males: 1.2 kg; females: 0.9 kg), with males additionally demonstrating a 15% decline in $\dot{V}O_{2max}$ relative to fat-free mass. However, the addition of SIT partially mitigated these adverse effects, with attenuated fat-free mass losses observed in both LCTR+SIT groups. This relative preservation of lean tissue aligns with findings from Sartor et al. (Sartor et al. 2010), who reported similar protective effects when combining LC diets with high-intensity interval training. Remarkably, female participants in the LCTR+SIT group demonstrated improvements in $\dot{V}O_{2max}$ (17.1% relative to body mass, 14.1% relative to fat-free mass), an adaptation not observed in their male counterparts. These sex-specific $\dot{V}O_{2max}$ improvements are consistent with established female advantages in cardiac adaptive capacity (Konhilas et al. 2004), though underlying mechanisms require further exploration. Collectively, our findings extend previous LC diet research by demonstrating that incorporating both time-restriction and high-intensity exercise not only protects against the catabolic effects of dietary restriction but also reveals sex-specific enhancements in cardiorespiratory fitness that warrant further investigation.

Implications

The combination of resting NT-proBNP improvements, partial preservation of fat-free mass, and enhanced aerobic capacity, observed with LCTR+SIT in young healthy females, supports the addition of SIT and highlights the critical limitations of dietary restriction alone. These physiological adaptations could translate to improved functional capacity and metabolic health—factors directly linked to long-term cardiovascular outcomes. The current trial highlights the particular importance for female health, though young adult males may need a stronger exercise stimulus over a longer period to achieve similar positive effects.

The clinical significance of our NT-proBNP findings warrants emphasis. NT-proBNP serves as both a marker of cardiac wall stress and a robust predictor of cardiovascular events. In clinical populations, including diabetic patients, each standard deviation increase corresponds to a 59% higher cardiovascular risk (Ahmad et al. 2024). Furthermore, even minimal elevations within the normal range predict adverse outcomes in community populations (Wang et al. 2004). The 48% reduction in NT-proBNP observed among female participants represents a substantial improvement in cardiovascular risk profile, despite their low baseline values (median 26 $ng.l^{-1}$) compared to age-matched norms of 49-53 $ng.l^{-1}$ (Welsh et al. 2022). These findings suggest that early interventions even in young healthy adults could yield meaningful cardiovascular benefits before traditional risk factors emerge.

From a translational perspective, our intervention achieved these benefits, predominantly in females, through a remarkably time-efficient approach—just 12 minutes of exercise weekly. The rapid onset of benefits, with significant changes within 4 weeks, adds to the practical appeal of this intervention strategy. Moreover, the 96% adherence rate indicates that this ultra-brief protocol is feasible, acceptable, and reduces time-related barriers to exercise. Collectively, these findings suggest that minimal-volume, high-intensity exercise combined with dietary modification may offer a pragmatic strategy for cardiovascular risk reduction in young healthy females.

Strengths and limitations

This study has several methodological strengths. The randomized controlled design and rigorous experimental controls—including standardized nutritional education, daily ketone monitoring, and supervised training—ensure high internal validity. The prospective sex-specific analysis, combined with

comprehensive assessments spanning body composition, cardiorespiratory fitness, and cardiac biomarkers, provides a multi-dimensional view of intervention effects. Additionally, the brief exercise protocol (12 minutes weekly) offers exceptional feasibility for real-world application.

Several limitations warrant consideration. While the sample size (n=61) is comparable to other controlled laboratory intervention studies (Moro et al. 2021) and yielded significant effects (48% NT-proBNP reduction in females receiving LCTR+SIT), the relatively small size of sex-stratified subgroups may limit the generalizability of findings, the detection of smaller effects, and the exploration of moderating factors. Longer studies incorporating multiple timepoints and follow-up assessments are needed to evaluate the sustainability of effects and characterize temporal dynamics of biomarker changes, especially in young adult males. Additionally, blood volume status was not directly assessed through gold-standard methods such as indicator dilution techniques or dual-isotope labeling. While total body water showed no significant change between pre- and post-intervention measurements, providing indirect evidence of maintained hydration status, future studies should consider direct measurement of plasma volume to definitively exclude volume-mediated effects on cardiac biomarkers.

Conclusion

This randomized controlled trial demonstrated that combining short-term LCTR diet with SIT produced a sex-specific response in resting NT-proBNP. While LCTR alone did not significantly alter cardiac biomarkers and compromised traditional training adaptations, the addition of just 12 minutes weekly of SIT not only mitigated fat-free mass loss with LCTR in both sexes but also significantly reduced resting NT-proBNP levels in females by 48%. These findings contribute to emerging evidence that extremely time-efficient high-intensity exercise interventions, when strategically combined with metabolic interventions, offer potentially broad health benefits. Future investigations should elucidate the mechanistic basis for these sex-specific responses and evaluate whether such brief interventions can produce sustained cardiovascular benefits across diverse populations.

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Author contributions ZK, OKL and JN designed the experiment. OKL, YZ, and XS performed the data collection. YZ, XS, HZ, KG, JN, QS, OKL and ZK analyzed the data and YZ wrote the first draft of the manuscript. All authors have read and approved the manuscript.

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 1. Daily macronutrient intake (grams) at baseline and during the intervention period

	LCTR_M (n=15)		**LCTR+SIT_M** (n=16)		**LCTR_F** (n=13)		**LCTR+SIT_F** (n=17)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Fat (g)	89.7±39.4	117.5±42.1*	87.9±37.5	116.6±41.1*	71.0±21.5	89.3±26.7*	74.2±21.0	87.1±28.3*
Protein (g)	106.3±45.9	121.3±49.2*	101.6±41.6	124.8±47.5*	75.4±29.3	86.6±32.8*	70.3±28.2	86.3±35.8*
Carbohydrate (g)	223.1±97.9	47.7±30.1*	235.3±104.5	43.0±33.1*	208.4±63.7	58.9±37.8*	202.2±64.3	49.8±27.0*

LCTR_M, low-carbohydrate time-restricted eating, males; **LCTR+SIT_M**, low-carbohydrate time-restricted eating combined with sprint interval training, males; **LCTR_F**, low-carbohydrate time-restricted eating, females; **LCTR+SIT_F**, low-carbohydrate time-restricted eating combined with sprint interval training, females. * Significantly different from corresponding PRE values, $P < 0.05$

Table 2. Training characteristics during 4-week sprint interval training in LCTR+SIT groups

	Week 1 (sessions 1-5)		Week 2 (sessions 6-10)		Week 3 (sessions 11-15)		Week 4 (sessions 16-20)	
	Males	Females	Males	Females	Males	Females	Males	Females
Peak power (W)	511±93	328±78*	514±93	329±49*	491±86	322±52*	493±92	342±61*
HR (beats.min^{-1})	157±11	151±11	157±9	152±9	156±14	151±9	154±10	150±10
RPE	12±1	11±1	12±1	12±1	12±1	12±1	12±2	12±1

Values are averaged across all training sessions within each week; LCTR+SIT, low-carbohydrate time-restricted eating combined with sprint interval training; HR, heart rate; RPE, rating of perceived exertion (Borg 6-20 scale). * Significantly different from corresponding males' values, $P < 0.05$

Table 3. Anthropometric characteristics, body composition, cardiorespiratory fitness, and cardiac markers pre- (PRE) and post-intervention (POST) in male and female participants assigned to low-carbohydrate time-restricted eating (LCTR) or LCTR combined with sprint interval training (SIT)

	LCTR_M (n=15)		**LCTR+SIT_M** (n=16)		**LCTR_F** (n=13)		**LCTR+SIT_F** (n=17)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Anthropometric data								
Age (yr)	23.5±5.5	-	22.5±3.1	-	21.8±3.6	-	22.2±3.6	
Height (cm)	175.5±5.4	-	175.0±5.1	-	163.4±5.0	-	164.8±7.4	
Body weight (kg)	80.5±11.1	76.3±10.4*	80.3±15.8	77.2±14.9*	68.1±11.6	65.5±11.2*	63.7±9.0	61.7±9.4*
BMI ($kg.m^{-2}$)	26.1±3.3	24.8±2.9*	26.2±5.2	25.2±4.9*	25.5±4.1	24.5±3.9*	23.4±3.1	22.7±3.2*
Body composition								
Body fat (%)	23.9±8.4	22.4±8.2*	26.2±8.8	24.0±8.4*	38.3±5.3	37.0±5.4*	32.4±5.5	30.6±6.0*
Fat mass (kg)	19.8±8.5	17.6±7.8*	22.2±11.2	19.6±10.4*	26.6±8.1	25.1±8.5*	20.8±5.9	19.6±5.6*
FFM (kg)	59.9±6.7	58.7±6.6*	58.2±5.8	57.6±5.8	41.3±3.6	40.4±3.3*	42.5±5.1	42.1±5.5
TBW (L)	43.1±4.2	43.0±3.9	43.3±4.3	43.1±4.1	30.7±3.3	30.5±3.1	30.5±3.1	30.5±4.0
Cardiorespiratory fitness								
$\dot{V}O_{2max}$ ($ml.kg^{-1}.min^{-1}$)	31.7±5.3	28.4±8.6	30.9±6.0	33.3±9.5	23.4±5.5	22.2±5.0	25.2±3.2	29.5±6.7*
$\dot{V}O_{2max}$ ($ml.kg_{FFM}^{-1}.min^{-1}$)	42.8±8.4	36.4±9.7*	41.8±6.8	43.6±10.2	38.3±9.2	35.7±8.2	37.6±4.2	42.9±8.7*
Cardiac markers ($ng.l^{-1}$)								
NT-pro-BNP								
Median	12.1	11.9	5.0	5.0	14.5	19.0	26.0	13.6
Range	5.0-63.7	5.0-34.6	5.0-23.9	5.0-21.4	5.0-46.5	5.0-59.6	5.0-91.7	5.0-30.8*
cardiac troponin T								
Median	3.0	3.7	3.0	3.4	3.0	3.0	3.0	3.0
Range	3.0-12.1	3.0-14.3	3.0-9.5	3.0-9.0	3.0-3.0	3.0-3.0	3.0-14.1	3.0-3.0

LCTR_M, low-carbohydrate time-restricted eating, males; **LCTR+SIT_M**, low-carbohydrate time-restricted eating combined with sprint interval training, males; **LCTR_F**, low-carbohydrate time-restricted eating, females; **LCTR+SIT_F**, low-carbohydrate time-restricted eating combined with sprint interval training, females; FFM, fat-free mass; TBW, total body water. * Significantly different from corresponding PRE values, $P < 0.05$

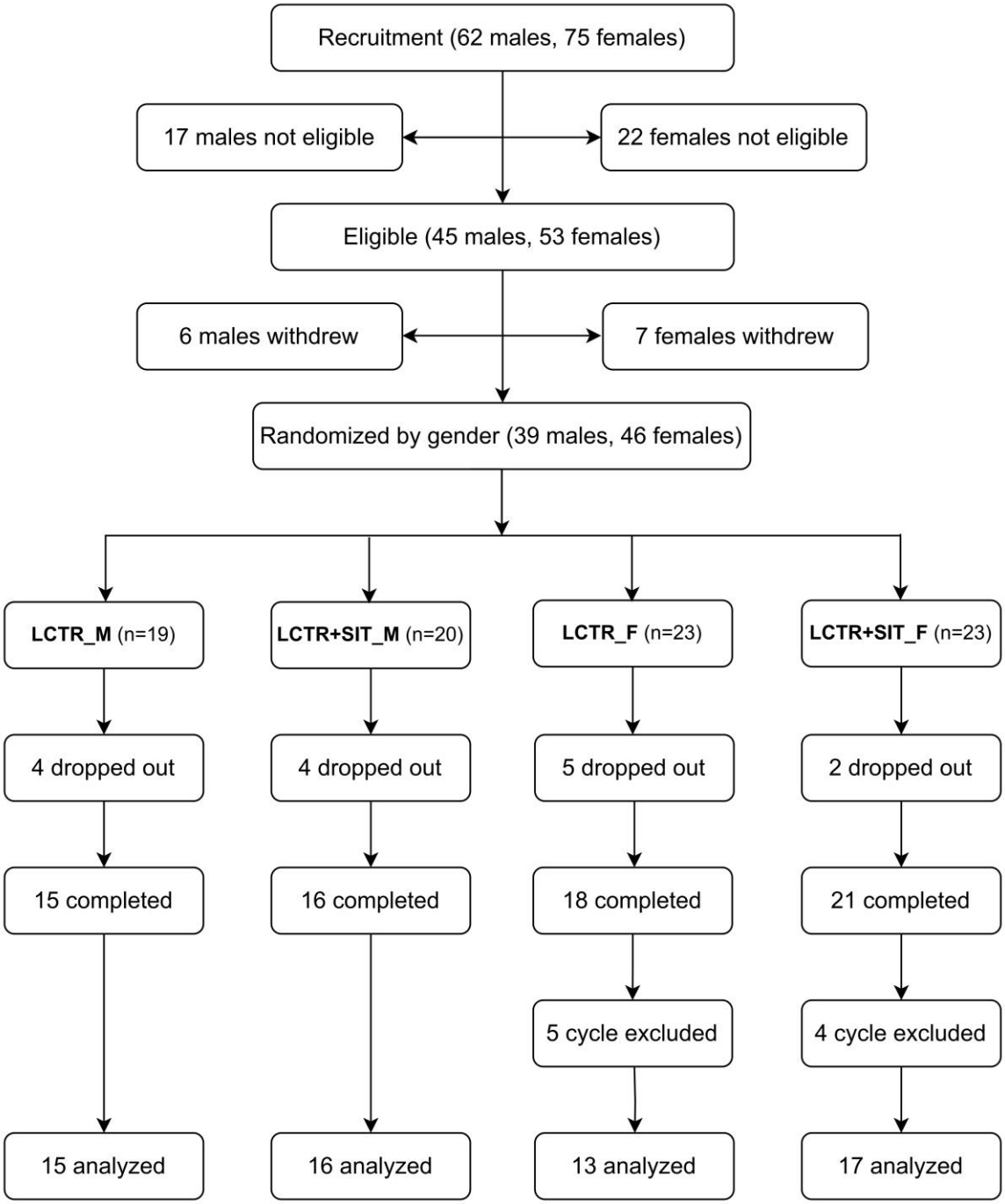

Figure 1. Participant flow diagram showing recruitment, randomization, and retention throughout the intervention trial. Participants were stratified by sex and randomly allocated to dietary intervention alone or combined dietary and exercise intervention. **LCTR_M**, low-carbohydrate time-restricted eating, males; **LCTR+SIT_M**, low-carbohydrate time-restricted eating combined with sprint interval training, males; **LCTR_F**, low-carbohydrate time-restricted eating, females; **LCTR+SIT_F**, low-carbohydrate time-restricted eating combined with sprint interval training, females.

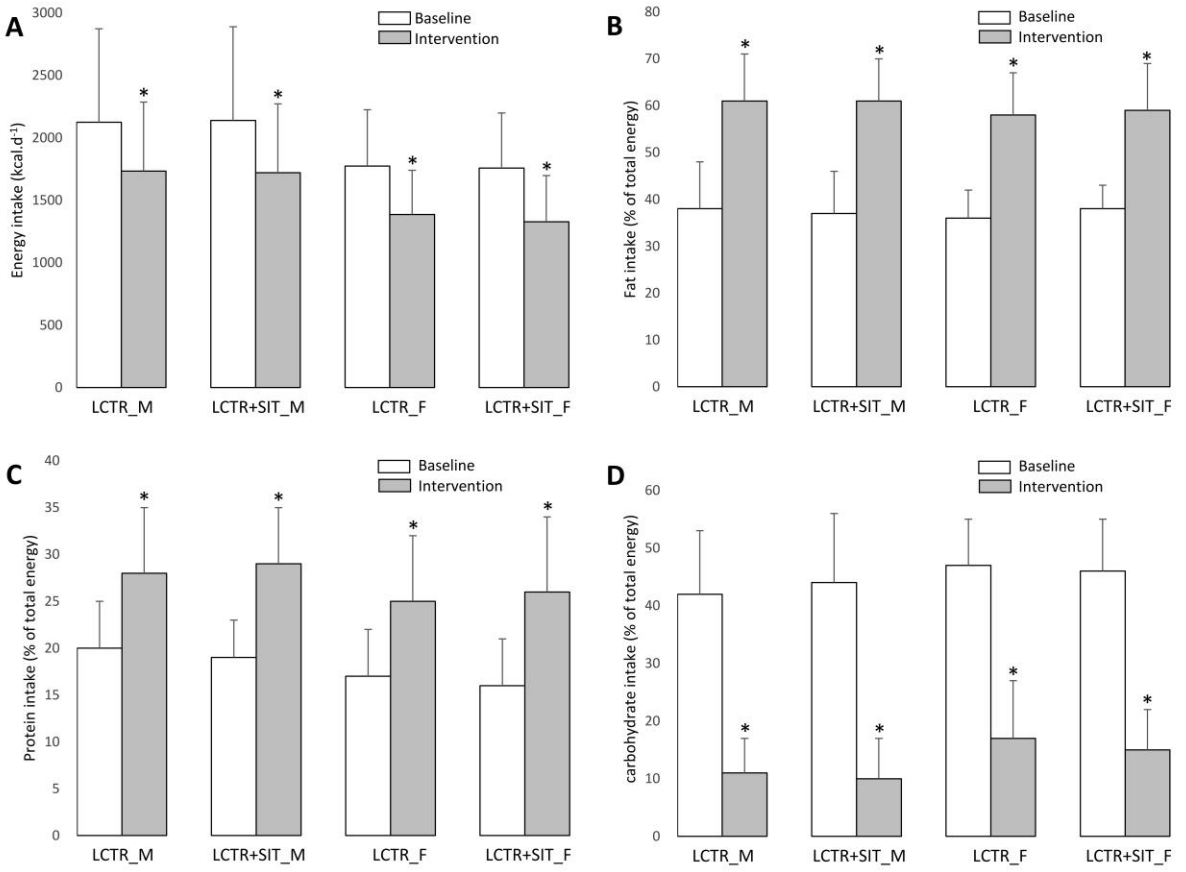

Figure 2. Dietary intake and macronutrient composition during baseline and intervention periods. Data are presented as mean ± standard deviation. Changes in (A) daily energy intake, (B) fat intake as percentage of total energy, (C) protein intake as percentage of total energy, and (D) carbohydrate intake as percentage of total energy across four intervention groups. LCTR_M, low-carbohydrate time-restricted eating in males; LCTR+SIT_M, LCTR combined with sprint interval training in males; LCTR_F, LCTR in females (n=13); LCTR+SIT_F, LCTR combined with sprint interval training in females. *P < 0.05 compared to baseline values

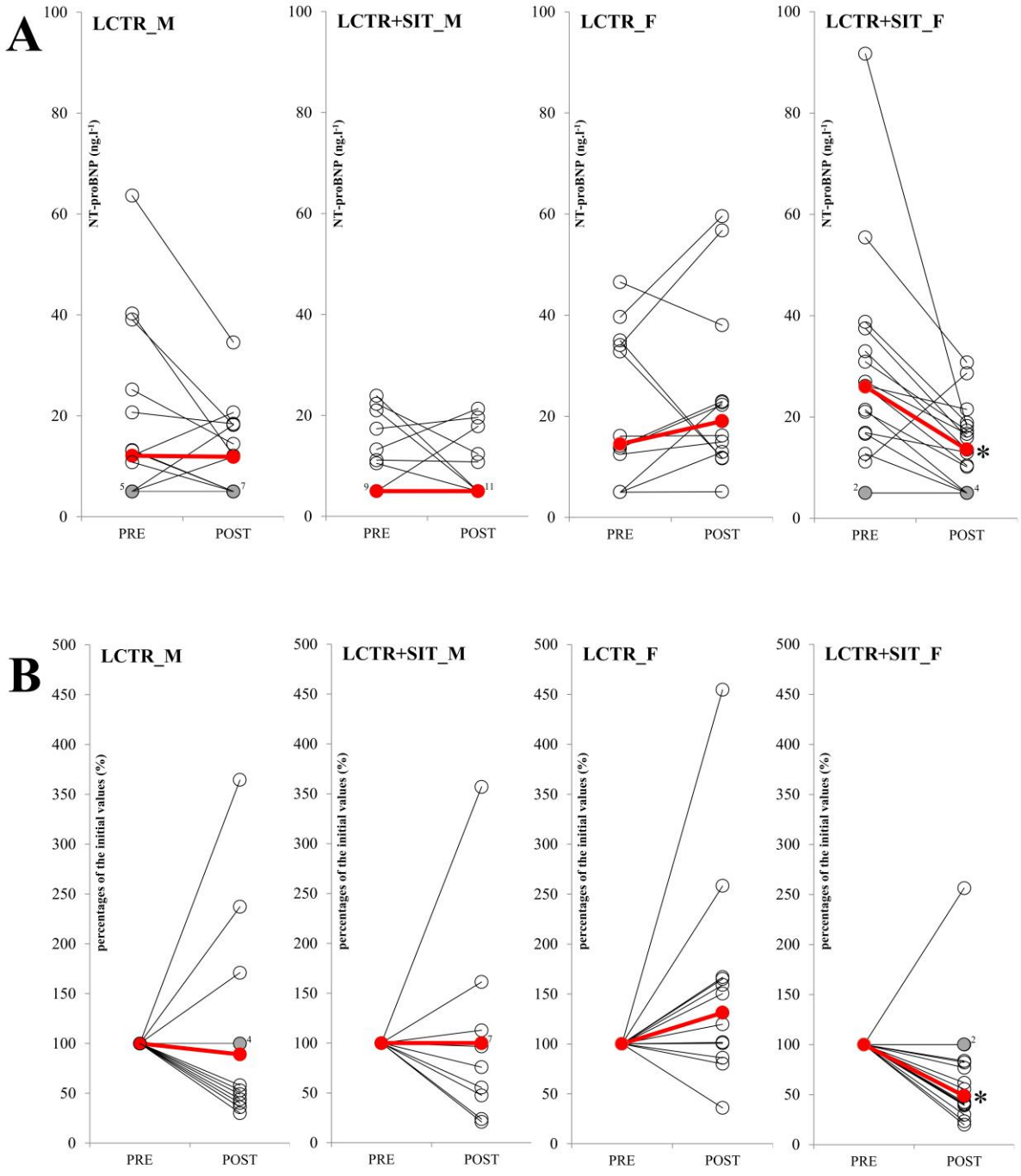

Figure 3. Individual N-terminal pro-brain natriuretic peptide (NT-pro-BNP) values before (PRE) and after (POST) dietary and exercise interventions. (**A**) Absolute NT-proBNP concentrations before and after intervention, with individual participants shown as connected circles and group medians indicated by thick red lines. (**B**) Data are expressed as a percentage of baseline values, with individual participants shown as connected circles and group medians indicated by thick red lines. Sample sizes are denoted where multiple subjects overlap. **LCTR_M**, low-carbohydrate time-restricted eating, males (n=15); **LCTR+SIT_M**, low-carbohydrate time-restricted eating combined with sprint interval training, males (n=16); **LCTR_F**, low-carbohydrate time-restricted eating, females (n=13); **LCTR+SIT_F**, low-carbohydrate time-restricted eating combined with sprint interval training, females (n=17). *P < 0.05 versus PRE.