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Original Article

ORIGINAL ARTICLE

Effects of moderate sleep restriction during 8-week calorie restriction on lipoprotein particles and glucose metabolism

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Abstract

Study Objectives: This study examined how glucose, glucose regulatory hormones, insulin sensitivity, and lipoprotein subclass particle concentrations and sizes change with sleep restriction during weight loss elicited by calorie restriction.

Methods: Overweight or obese adults were randomized into an 8-week calorie restriction intervention alone (CR, n = 12; 75% female; body mass index = 31.4 ± 2.9 kg/m^2) or combined with sleep restriction (CR+SR, n = 16; 75% female; body mass index = 34.5 ± 3.1 kg/m^2). Participants in both groups were given the same instructions to reduce calorie intake. Those in the CR+SR group were instructed to reduce their habitual time-in-bed by 30–90 minutes 5 days each week with 2 ad libitum sleep days. Fasting venous blood samples were collected at pre- and post-intervention.

Results: Differential changes were found between the two groups (p = 0.028 for group × time interaction) in glucagon concentration, which decreased in the CR group (p = 0.016) but did not change in CR+SR group. Although changes in mean

Statement of Significance

This study adds important information to the literature regarding the effects of sleep restriction in the context of calorie restriction on health outcomes. The 8-week intervention used in this study is longer than many other controlled studies examining sleep restriction. Another feature of the study is that sleep restriction was on 5 days a week (≤90 minutes per day), and ad libitum sleep was on the other 2 days. Thus, the study design allows the results to have greater generalizability to populations in real life. Differential changes were found in serum glucagon concentrations between the groups undergoing calorie restriction with and without sleep restriction. Results also suggest differential changes in mean HDL particle size and visfatin concentration between the groups.

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HDL particle (HDL-P) size and visfatin concentration were not statistically different between groups (p = 0.066 and 0.066 for group×time interaction, respectively), mean HDL-P size decreased only in the CR+SR group (Cohen's d = 0.50, p = 0.022); visfatin concentrations did not change significantly in either group but appeared to decrease in the CR group (Cohen's d = 0.67, p = 0.170) but not in the CR+SR group (Cohen's d = 0.43, p = 0.225).

Conclusion: These results suggest that moderate sleep restriction, despite the presence of periodic ad libitum sleep, influences lipoprotein subclass particles and glucose regulation in individuals undergoing calorie restriction.

Clinical trial registration: ClinicalTrials.gov (NCT02413866, Weight Outlooks by Restriction of Diet and Sleep)

Key words: sleep restriction; catch-up sleep; weight loss; lipoprotein particles; glucagon; visfatin

Introduction

An estimated 71.6% of adults in the United States are classified as overweight or obese [1]. Excess adiposity is known to increase the risk for the development of impaired glucose metabolism, insulin sensitivity, and lipoprotein cholesterol profile [2, 3], and to increase the risk for diabetes and cardiovascular diseases [4, 5]. Weight loss, even a modest amount, improves glucose metabolism and insulin sensitivity [6]. Weight loss also improves traditional lipoprotein cholesterol profile, which includes total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) concentrations [7, 8]. However, lipoprotein particles are heterogeneous consisting of particles of a variety of sizes, with smaller particles carrying less cholesterol. LDL particle (LDL-P) concentration was a better indicator of atherosclerotic risk and peripheral artery disease than LDL-C in large-scale studies [9–11]. Low concentrations of HDL particles (HDL-P) were revealed to be superior to low HDL-C in terms of cardiovascular risk prediction [12]. Low mean HDL-P size was also associated with cardiovascular disease which may be secondary to the associations of HDL-P and HDL-C numbers to cardiovascular disease [12]. Reductions in small very-low-density lipoprotein particle (VLDL-P) concentration was found to be associated with reductions in atherosclerotic cardiovascular disease, independent of LDL-C [13]. As such, lipoprotein subclass particle concentrations and sizes are potentially better predictors of cardiovascular disease risks than traditional lipoprotein cholesterol profile. Few studies have examined the impact of weight loss on lipoprotein particle concentrations or sizes, and their results suggest that LDL-P size increased following weight loss [14, 15].

Insufficient sleep has also been found to influence glucose metabolism and insulin sensitivity [16–18]. Previously, glucose tolerance and insulin sensitivity were reduced after several nights of 4–5 hours of time-in-bed (TIB) per night compared to ≥8.5 hours TIB [19, 20]. More recent evidence showed that even an average of one-hour sleep restriction on three consecutive nights reduced insulin sensitivity compared to after three consecutive nights of ad libitum sleep [21]. Further, Depner et al. [22] reported that a weekend of recovery sleep did not prevent the reduced insulin sensitivity associated with recurrent insufficient sleep of two nights. These studies suggest that a short period of daily sleep restriction adversely affects glucose metabolism and insulin sensitivity. However, Zielinski et al. found no changes in glucose tolerance after 8 weeks of 90 minutes TIB restriction per night [23]. Thus, over a longer-period, the effects of sleep restriction on glucose metabolism and insulin sensitivity might be different from that of a short period.

Lipoprotein cholesterol profile has also been found to be associated with sleep duration. Shorter than 6 hours of sleep per night and longer than 8 hours of sleep per night are associated with higher total cholesterol, LDL-C, and TG concentrations, and associated with lower HDL-C concentration [24, 25]. In population cohorts, individuals reporting insufficient sleep had lower large HDL particle (HDL-P) concentrations than those reporting sufficient sleep [26]. Studies have also shown that a few days of sleep restriction influenced lipid metabolism [27, 28], and decreased small, medium, and large LDL-P, and small very-low-density lipoprotein particle (VLDL-P) concentrations [26].

The effects of sleep restriction during weight loss on glucose metabolism and insulin sensitivity is less studied, and we are not aware of published studies on changes in lipoprotein subclass. Nedeltcheva et al. examined the effects of a 14-day hypocaloric dietary weight loss program with two different sleep conditions, 5.5 hours of TIB and 8.5 hours of TIB [29]. Following the shortened sleep condition, fasting glucose and 24-hour average insulin concentration were significantly lower compared to the longer sleep condition. St-Onge et al. studied normal-weight adults following 4 hours of TIB and 9 hours of TIB over 5 consecutive nights while maintaining the same controlled diet [30]. Although the controlled diet was not designed to be hypocaloric, participants lost a small amount of weight of approximately 0.9 kg on average following both conditions. No differences in glucose or insulin concentrations were found between the sleep conditions. The results of these two studies [29, 30] seem to be different from those in the other abovementioned studies [17, 19–22], thus suggesting the effect of sleep restriction on glucose metabolism and insulin sensitivity may be influenced by weight loss. However, the durations of the sleep restriction intervention in these studies were short (14 and 5 days, respectively), and the effects of insufficient sleep on glucose metabolism and insulin sensitivity in a longer-term sleep restriction intervention and in the context of hypocaloric diet has not been studied.

Therefore, the aim of this exploratory study was to examine the effects of moderate sleep restriction of less than or equal to (≤) 90 minutes of nightly TIB during an 8-week calorie restriction program on glucose concentrations, insulin sensitivity, hormones associated with glucose metabolism, and lipoprotein subclass particle sizes and concentrations in overweight or obese adults. We hypothesized that sleep restriction during calorie restriction would negatively impact glucose metabolism compared to calorie restriction alone in overweight or obese adults.

Methods

Data were obtained from the Weight Outlooks by Restriction of Diet and Sleep (WORDS) study (ClinicalTrials.gov identifier: NCT02413866). This study utilized a randomized controlled

design to examine the influence of sleep restriction on outcomes associated with 8-week calorie restriction. Primary results on body composition have been previously described [31]. The study protocol was approved by the University of South Carolina Institutional Review Board and all participants signed an informed consent form prior to participation. Figure 1 shows the sample sizes from the parent study to the present study, which included 28 participants who had lipoprotein subclass data at pre- and post-intervention. Of these participants, 23 also had glucose regulatory hormone data available.

Participants and intervention groups

Participants were 35–55 years of age, overweight or obese [25 ≤ body mass index (BMI) ≤ 40 kg/m^2], weight stable (≤3% body weight change) during the previous 3 months and did not smoke during the past year. No female participant was pregnant or lactating. None of the participants reported having been diagnosed with diabetes; however, we did not purposely screen for diabetes. Participants who were taking medications had been taking those in a stable dose for >6 months prior to participating in the study. Specifically, 4 participants reported taking lipid or glucose-controlling medications. No participant was a shift worker, nor had work that required long-distance driving or operating heavy equipment. All participants reported current 24-hour sleep duration was 6.5–8 hours, including naps of <90 min/day, and no use of medications, devices, or hypnotics to help sleep. Further, participants were assessed for sleep apnea utilizing a WatchPat monitoring device (Itamar Medical, Israel) at home for one night, and no one had signs of severe sleep apnea (i.e. apnea-hypopnea index ≥ 30). All participants were informed of potential safety issues associated with moderate sleep restriction during an individual orientation prior to participation in the study.

Participants were randomized into one of two 8-week interventions, calorie restriction alone or combined with sleep restriction (CR and CR+SR, respectively), following pre-intervention measurements. The present study included 12 participants in the CR group and 16 in the CR+SR group (Figure 1). During the intervention, all participants were asked to maintain normal daily activities and to not participate in other interventional research studies.

Calorie restriction

All participants were instructed to self-report all calorie-containing items consumed, including any beverages or snacks, for one week prior to the intervention, throughout the intervention, and 1 week after completion of the intervention. Participants were instructed on using the MyFitnessPal application (MyFitnessPal, Inc.) that is available on smartphones or web-based devices. If a smartphone or computer was unavailable, participants were provided with a self-report paper form to record any calorie-containing food or drink consumed, including portion size, calories, and macronutrient breakdown (carbohydrate, protein, and fat) utilizing The Calorie King Calorie Counter.

Each individual's resting metabolic rate was measured, and 95% of this value was calculated and used as a daily calorie intake goal for each respective participant [31]. Prepackaged meals, based on each participant's calorie intake goals, were provided for lunch and dinner 4 days each week during the intervention. These meals also served as samples that meet the calorie intake goal for participants. Dietary instruction was provided to each participant for breakfast and snacks throughout the day, as well as during the 3 days of ad libitum food consumption when participants were able to eat freely. Each week research staff collected the dietary records and reviewed with participants.

Sleep restriction

Participants wore an actigraphy monitor (ActiGraph GT3X+, ActiGraph, Pensacola, FL) on their non-dominant wrist for a week prior to the intervention, throughout the 8-week intervention, and for a week after the intervention to evaluate sleep. Participants were also instructed to maintain a sleep diary during these days recording time getting into bed, lights turned off and trying to sleep, waking up, and getting out of bed, and any naps taken throughout the day. Sleep diaries and actigraphy recordings were reviewed by staff and any issue was discussed with the participant each week.

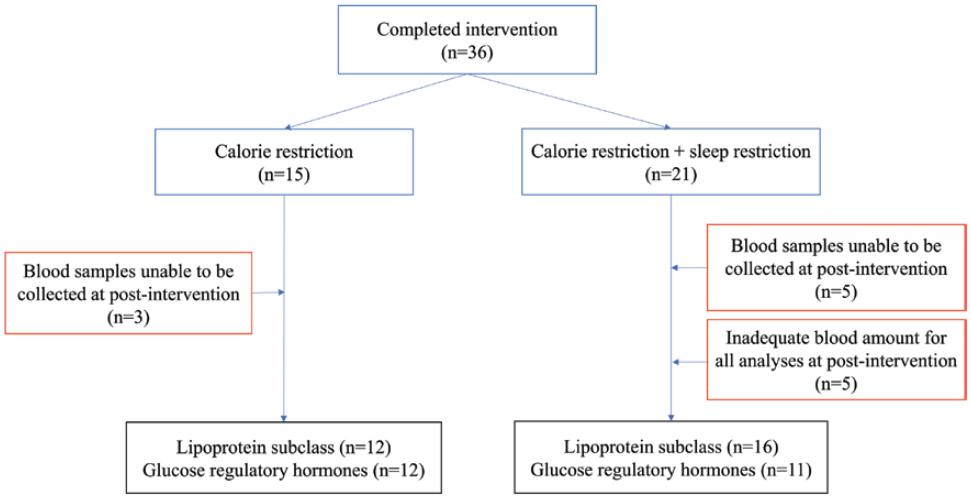

Figure 1. Participant flow diagram for the analytic sample.

For the CR+SR group, participants were directed to reduce their total TIB by a minimum of 30 minutes and up to 90 minutes on 5 nights per week, and to sleep freely on the other 2 nights each week. To aid in retention and adherence to the CR+SR group, each participant chose their own sleep restricted and ad libitum sleep days, and allowed a range of TIB reduction between 30 and 90 minutes. Participants were instructed to achieve this reduction by going to bed later, getting up earlier, or a combination of both, and maintaining their nap habits. Participants were also asked to maintain their sleep schedule throughout the 8-week intervention and to not shift nightly sleep schedules. Participants in the CR only group were instructed to maintain their daily sleep and nap habits throughout the study.

Output from the monitors was analyzed using the ActiLife 6.11 software program provided by the manufacturer. Minute-by-minute asleep/awake status was determined using the Cole-Kripke Algorithm [32]. Self-reported times from the sleep diary were entered into the ActiLife software program to help quantify total sleep time (TST) and TIB for each participant. TST was defined as the total number of minutes considered asleep by the actigraphy device, while TIB was total time from getting into bed with lights off trying to sleep until getting out of bed plus any naps recorded by the participant throughout the day. The difference in midpoint of nightly TIB during sleep restricted days and ad libitum days for participants in the CR+SR group was calculated. The difference in midpoint of TIB on workdays and free days for those in the CR group was also calculated.

To monitor adverse changes due to the intervention, all participants were asked to complete the Center for Epidemiological Studies Depression Scale (CES-D) [33], Epworth Sleepiness Scale (ESS) [34], Functional Outcomes of Sleep Questionnaire (FOSQ) [35], Pittsburg Sleep Quality Index (PSQI) [36], the 36-Item Short Form (SF-36) [37], and Psychomotor Vigilance Test (PVT) [38, 39] at pre-intervention and every 2 weeks during the intervention. Indications for exclusion from the study were set at clinical depression (CES-D > 16), excessive sleepiness (ESS > 12), a change >10% in FOSQ, or PVT response time >500 ms. No participant experienced changes based on these criteria that would be considered exclusionary from the study.

Measurements

Height, body weight, and physical activity

Height was measured using a stadiometer at pre- and post-intervention. Bodyweight, with participants wearing standard scrubs, was measured at pre-intervention, weekly during intervention, and post-intervention using an electronic scale that was calibrated annually (CC Vaughan & Sons, Incorporated, Columbia, SC).

Previous evidence suggests that both hypocaloric diet and sleep restriction could affect physical activity [40, 41], which may subsequently affect glucose control and lipoprotein profile. Thus, physical activity at pre- and post-intervention were quantified by the actigraphy monitor (ActiGraph GT3X+, ActiGraph, Pensacola, FL) using manufactured provided software (Actilife version 6.11). This monitor quantifies accelerations resulting from physical activity-associated bodily motion at a fixed point of the body to determine physical activity intensities. Physical activity counts per minute per day were determined by combining the magnitude of sampled acceleration from all three axes measured by the monitor as an integrated measure of physical activity. Previously, physical activity counts measured by the Actigraph GT3X+ worn on the wrist and on the waist were found to be strongly correlated as a non-intensity specific measure of physical activity [42] and was therefore included as a measure of total physical activity.

Serum sample collection

Venous blood samples were collected in a BD serum Vacutainer following a minimum of a 12-hour fast (not including water), between 06:00 and 09:00 hours, at pre- and post-intervention. Following approximately 30 minutes allowing the blood to clot, the whole blood was centrifuged at 3000 rpm at 4°C for 20 minutes. Serum separated after centrifugation was aliquoted and stored at –80°C until all participant samples were ready for analysis.

Lipoprotein subclass particle concentrations and sizes, and NMR-calculated values

Serum samples were analyzed by LipoScience, Inc. (Raleigh, NC) for determination of lipoprotein subclass particle concentrations and sizes by nuclear magnetic resonance (NMR) spectroscopy. The LipoProfile-3 algorithm [43] was used to determine concentrations of small, medium, and large (including chylomicron) very-low-density lipoprotein particle (VLDL-P) and HDL-P, small and large LDL-P and intermediate-density lipoprotein particle (IDL-P). Weighted-average VLDL-P, LDL-P, and HDL-P diameter was calculated as the sum of the lipoprotein subclass diameters multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal. Apolipoprotein B (ApoB)-containing lipoprotein particles, including VLDL-P, IDL-P, and LDL-P, possess atherogenic properties [44]; thus, ApoB-containing lipoprotein concentration was calculated as the sum of the three. Total TG and HDL-C concentrations were calculated by NMR. In addition, a lipoprotein insulin resistance score (LP-IR), an index for insulin sensitivity [45], was also calculated.

Glucose, glucose regulatory hormones and indices of insulin sensitivity

Serum glucose concentrations were analyzed in duplicate samples using YSI 2300 STAT Plus (YSI Life Sciences, Yellow Springs, OH), which was calibrated according to manufacturer instruction. A single Bio-Plex Pro Human Diabetes Panel 10-Plex assay (BIO-RAD Laboratories, Hercules, CA) was used to analyze serum concentrations in duplicate samples of insulin, glucagon, gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), visfatin and resistin using a MAGPIX system (Luminex Corporation, Austin, TX) following manufacturer instruction. Insulin and glucagon are hormones that regulate glucose concentrations. GIP and GLP-1 are incretins that stimulate insulin secretion [46–48]. Visfatin mimics insulin [49] and resistin promotes insulin resistance [50].

The updated homeostasis model assessment of insulin resistance (HOMA2-IR) was calculated using the HOMA Calculator program v2.2.3, which simulates the physiological processes

that influence circulating glucose and insulin levels in order to provide model-derived estimates of insulin resistance [51, 52]. The quantitative insulin-sensitivity check index (QUICKI) was calculated as 1/[log(I)+log(G)], where I and G were concentrations of fasting insulin and glucose, respectively [53].

Statistical analysis

Statistical analysis was performed using SAS version 9.4 (Cary, NC). The Shapiro-Wilk and Kolmogorov-Smirnov tests were performed to test whether the data were normally distributed. Non-normally distributed data were transformed using log(10) to be used in statistical models. Pre-intervention values were compared between CR and CR+SR using independent *t*-test or Chi-square test as appropriate. A general linear model with repeated measures, including a group×time interaction, was utilized to determine if any variables changed differently between the two groups following their respective interventions. Pre-intervention body weight or change in body weight was adjusted in the general linear models to account for potential influences of these variables. Paired sample *t*-tests were performed within each group to compare pre- and post-intervention values and effect sizes (Cohen's d and partial Eta Squared) were also calculated. Change values were calculated by subtracting pre-intervention values from post-intervention values. A p value of < 0.05 was considered statistically significant.

Results

Participant characteristics

Participant characteristics prior to the intervention are shown in Table 1. No differences between the CR and CR+SR groups for age, sex or race composition, height, blood pressure, proportion of participants having signs of mild or moderate sleep apnea, calorie intake, or total physical activity were found. However, body weight and BMI were significantly greater in the CR+SR group compared to the CR group. This sub-sample was comparable in these characteristics to the full sample.

The CR and CR+SR groups had similar degree of calorie restriction compared to pre-intervention throughout the 8 weeks (14.0 ± 9.2% and 17.8 ± 7.9%, $p = 0.264$). After the intervention, body weight significantly decreased in both groups with no significant difference found in the amount of absolute or percent weight loss between groups ($p = 0.974$ and 0.818, respectively). The amount of weight loss in this sub-sample was also comparable to the full sample.

Total physical activity did not change differently between the two groups from pre- to post-intervention ($p = 0.454$ for group × time interaction). There was no significant change found in physical activity within either the CR or CR+SR group ($p = 0.132$ and 0.500, respectively) or in the overall sample ($p = 0.119$).

Sleep duration, midpoint sleep time, and self-reported questionnaires and assessment

No differences in TIB, TST, or midpoint sleep time between CR and CR+SR groups prior to intervention were found (Table 2). For the CR+SR group, TIB and TST were shorter on the 5 days when sleep was restricted, by 49 and 66 minutes on average, respectively, and longer on the 2 ad libitum days, by 75 and 57 minutes on average, respectively, during the 8-week intervention compared to pre-intervention values. For the CR group, both TIB and TST remained stable throughout the intervention. The difference between midpoint sleep time on sleep-restricted days and ad libitum sleep days for the CR+SR group was not different from the difference in midpoint sleep on workdays and free days for the CR group ($p = 0.412$).

Presented in Supplementary Table 1 are the scores of self-administered questionnaires and assessments at pre-intervention and every 2 weeks throughout intervention. No participant in either the CR or CR+SR group had changes that met the pre-determined criteria for exclusion during the intervention.

Lipoprotein subclass particle concentrations and sizes

Fasting serum lipoprotein subclass particle concentrations and sizes are found in Table 3. Prior to intervention, no significant differences in any of the lipoprotein subclass particle concentrations or sizes were found between the CR and CR+SR groups ($p \geq 0.187$ for all), which persisted after adjusting for pre-intervention body weight ($p \geq 0.141$ for all).

Although the differences between the CR and CR+SR groups in the changes in mean HDL-P size and TG concentration (NMR-calculated) were not statistically significant (p for group × time interaction = 0.066 and 0.077, respectively), we further examined the changes within each group given the small sample size and exploratory nature of the study. Mean HDL-P size decreased in the CR+SR group with medium effect size (Cohen's $d = 0.50$, $p = 0.022$), but did not change in the CR group (Cohen's $d = 0.00$, $p = 0.790$) (Figure 2). TG concentration did not change significantly in either group and the effect sizes were small (CR: Cohen's $d = 0.16$, $p = 0.078$; CR+SR: Cohen's $d = 0.47$, $p = 0.269$; using transformed data).

All other NMR-determined concentrations and sizes did not change differently from pre- to post-intervention between the CR and CR+SR groups. After adjustment for pre-intervention weight or weight change from pre- to post-intervention, the changes in CR and CR+SR groups were not different for all lipoprotein subclass particle concentrations and sizes, and NMR-calculated variables (all p values for group × time interaction ≥ 0.109).

Glucose, glucose regulatory hormones, and insulin sensitivity indices

Fasting serum concentrations of glucose, insulin, glucagon, GLP-1, GIP, visfatin, and resistin, and indices of insulin sensitivity are presented in Table 4. Prior to the intervention, CR+SR had a significantly higher insulin concentration and lower QUICKI compared to the CR group ($p = 0.029$, and 0.027, respectively). Glucose and the other glucose regulatory hormone concentrations, as well as HOMA2-IR index were not different ($p > 0.059$ for all). After adjustment for pre-intervention body weight, there were no differences at pre-intervention between CR and CR+SR groups for any of these concentrations or indices of insulin sensitivity ($p \geq 0.051$ for all).

Differential changes from pre- to post-intervention in glucagon concentrations were found between the CR and CR+SR

groups. It significantly decreased in the CR group with a large effect size of change (Cohen's d = 0.93, p = 0.016), but did not change in the CR+SR group with a small effect size of change (Cohen's d = 0.21, p = 0.701) (Figure 3). Glucagon concentration changes were still different following adjustment for body weight change from pre- to post-intervention (p for group × time interaction = 0.035). In each group, glucagon changes were similar to without adjustment (CR: a large effect size reflected by partial Eta Squared = 0.324, p = 0.054; CR+SR: a small effect size reflected by partial Eta Squared = 0.010, p = 0.765). Glucagon concentration changes were no longer different between the CR and CR+SR groups after adjustment for pre-intervention body weight (p for group × time interaction = 0.262).

We also examined changes in visfatin concentration within each group, considering the small sample size and a near significant group × time interaction (p = 0.066). Visfatin concentration did not change significantly in either group (p = 0.170 and 0.225, for CR and CR+SR, respectively, using transformed data). However, it decreased with a medium effect size in the CR group (Cohen's d = 0.67) but increased with a small effect size in the CR+SR group (Cohen's d = 0.43) (Figure 4). After adjustment for pre-intervention body weight or weight change from pre- to post-intervention, visfatin concentrations were still near statistically different between the CR and CR+SR groups in the changes after interventions (p for group × time interaction =0.063 and 0.069, respectively).

The CR and CR+SR groups did not change differently from pre- to post-intervention in concentrations of glucose, insulin, GLP-1, GIP, or resistin, or any index of insulin sensitivity (all p values for group × time interaction >0.123). Adjustment for pre-intervention body weight or weight change from pre- to post-intervention did not influence these findings (all p values for group×time interaction ≥0.140).

Table 1. Participant characteristics by intervention group

	CR (n = 12)	CR+SR (n = 16)*	p value
Age (years)	44.8 ± 6.4	44.2 ± 5.5	0.789
Sex (M/F)	3/9	4/12	1.000
Race (C/AA/H)	5/7	7/8/1	0.655
Height (m)	1.69 ± 0.05	1.71 ± 0.07	0.349
Body mass index (kg/m^2)	31.4 ± 2.9	34.5 ± 3.1	**0.010**
Blood pressure, systolic (mmHg)	122.6 ± 8.5	125.3 ± 6.3	0.335
Blood pressure, diastolic (mmHg)	79.5 ± 4.1	80.3 ± 5.6	0.699
Apnea-Hypopnea Index† <5 / ≥5 and <15 / ≥15 and <30 events per hour (n)	6/3/3	8/5/3	0.840
Calorie Intake (kcal/day)			
Pre-Intervention	1642 ± 361	1926 ± 545	0.144
During intervention	1395 ± 257	1562 ± 379	0.216
Bodyweight (kg)			
Pre-Intervention	89.2 ± 9.2	100.8 ± 9.3	**0.003**
Post-Intervention	86.4 ± 8.8‡	98.0 ± 9.9‡	
Physical activity (counts/minute/day)			
Pre-Intervention	1633 ± 371	1558 ± 301	0.556
Post-Intervention	1761 ± 414	1582 ± 413	

Data presented as Mean ± SD. CR: calorie restriction; CR+SR: calorie restriction and sleep restriction. C: Caucasian; AA: African American; H: Hispanic. Listed p values are for comparisons between CR and CR+SR at pre-intervention. The p values in bold are < 0.05.

*The subset of participants who also had glucose regulatory hormones data (n = 11) had similar values to these reported here.

†Assessed by WatchPat (Itamar Medical, Israel) home testing.

‡p < 0.05 significant difference from pre- to post-intervention within the specific group.

Discussion

To our knowledge, this study is the first to examine the effects of chronic moderate sleep restriction of ≤90 minutes TIB with periodic ad libitum sleep during a calorie restriction weight loss intervention in overweight or obese adults. The primary findings are that there were differential changes in serum glucagon concentration between the CR and CR+SR groups, which decreased in the CR group only. Additionally, mean HDL-P size decreased only in the CR+SR group, and visfatin concentrations had a medium effect size of reduction in the CR group but only a small effect size of change in the CR+SR group. The changes in mean HDL-P size and visfatin were not statistically different between the CR and CR+SR groups, likely due to the small sample size. These data suggest that allowing ad libitum sleep on 2 nights per week did not prevent the differential changes in mean HDL-P size, and glucagon and visfatin concentrations between the CR and CR+SR groups. These findings are in line with recent literature suggesting that ad libitum sleep on the weekend did not prevent decreases in insulin sensitivity induced by sleep restriction [22].

Table 2. Time in bed (TIB) and total sleep time (TST) at pre-intervention and during intervention, and midpoint sleep time by intervention group

	CR (n = 12)		CR+SR (n = 16)	
	Pre-intervention	During intervention	Pre-intervention	During intervention
Average TIB (min/day)	421.6 ± 67.2	422.3 ± 26.4	423.6 ± 75.0	408.9 ± 52.7
Sleep restricted days				379.9 ± 7.0§
Ad libitum sleep days				504.0 ± 18.0§
Average TST (min/day)	372.9 ± 69.6	374.0 ± 49.3	367.9 ± 68.4	353.8 ± 47.4
Sleep restricted days				314.1 ± 16.7*
Ad libitum days				437.2 ± 18.6*
Midpoint sleep time (HH:MM)	03:07 ± 00:51	03:13 ± 00:30	02:48 ± 01:23	03:16 ± 01:24
Sleep restricted or workdays		03:01 ± 00:35		03:05 ± 01:29
Ad libitum or free days		03:24 ± 00:32		03:27 ± 01:25

Data presented as Mean ± SD. CR: calorie restriction; CR+SR: calorie restriction and sleep restriction.

*p < 0.01 compared to pre-intervention values; §p < 0.001 compared to pre-intervention values.

Table 3. Lipoprotein subclass particle concentrations and sizes, and NMR-calculated values at pre- and post-intervention by intervention group

	CR (n = 12)		CR+SR (n = 16)		Group×Time interaction
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	p value
CHY-P and VLDL-P Concentration (nmol/L)					
Total CHY-P and VLDL-P	47.2 ± 19.1	43.2 ± 22.6	41.8 ± 16.7	48.8 ± 20.8	0.104
Large CHY-P and VLDL-P	2.6 (0.5–11.2)	4.3 ± 3.5	3.6 ± 2.4	3.8 ± 1.7	
	0.45 ± 0.45	0.47 ± 0.42	0.44 ± 0.37	0.53 ± 0.23	0.507†
Medium VLDL-P	10.6 ± 7.5	6.9 (0.6–26.9)	7.6 (1.6–21.9)	6.5 (0.2–29.8)	
	0.92 ± 0.33	0.75 ± 0.44	0.76 ± 0.33	0.72 ± 0.51	0.450†
Small VLDL-P	32.3 ± 18.9	30.8 ± 18.9	30.8 ± 13.2	36.8 ± 17.8	0.224
LDL-P and IDL-P Concentration (nmol/L)					
Total LDL-P	1008 ± 249	861 (685–1389)	882 ± 240	925 ± 261	
	2.99 ± 0.11	2.97 ± 0.12	2.93 ± 0.12	2.95 ± 0.14	0.301†
IDL-P	217 ± 114	184 ± 130	202 ± 85	184 ± 103	0.662
Large LDL-P	324 ± 241	361 ± 257	184 (0.0–770)	253 (0.0–758)	
	2.36 ± 0.54	2.54 ± 0.36	2.39 ± 0.51	2.52 ± 0.38	0.289†
Small LDL-P	467 ± 276	431 ± 280	399 ± 165	453 ± 217	0.229
Apo-B Containing Lipoprotein-P	1055 ± 255	928 (730–1425)	923 ± 238	974 ± 272	
Concentration (nmol/L)	3.01 ± 0.11	2.99 ± 0.12	2.95 ± 0.11	2.97 ± 0.13	0.251†
HDL-P Concentration (µmol/L)					
Total HDL-P	28.5 ± 7.5	27.1 ± 8.1	27.4 ± 11.5	26.5 ± 10.9	0.883
Large HDL-P	6.7 (3.3–13.6)	6.8 ± 3.9	7.4 ± 3.2	6.4 ± 3.0	
	0.83 ± 0.22	0.76 ± 0.28	0.82 ± 0.22	0.76 ± 0.21	0.927†
Medium HDL-P	7.1 ± 4.6	8.1 ± 5.3	7.3 (3.8–19.0)	9.1 ± 4.4	
	0.84 ± 0.23	0.87 ± 0.31	0.91 ± 0.21	0.91 ± 0.22	0.917†
Small HDL-P	13.8 ± 8.6	12.2 ± 8.5	10.8 ± 7.9	11.0 ± 9.0	0.552
Mean Particle Size (nm)					
VLDL-P	50.7 ± 7.0	49.5 ± 6.5	49.8 ± 5.9	50.4 ± 7.2	0.506
LDL-P	20.8 ± 0.8	20.9 (19.7–21.5)	20.7 ± 0.8	21.0 (19.6–22.1)	
	1.32 ± 0.02	1.31 ± 0.01	1.32 ± 0.02	1.32 ± 0.02	0.491†
HDL-P	9.7 ± 0.8	9.7 ± 0.7	9.9 ± 0.6	9.6 ± 0.6	0.066
NMR-calculated values (mg/dL)					
TG	104.4 ± 56.0	91.4 ± 55.1	65.0 (24.0–158.0)	91.8 ± 40.4	
	1.96 ± 0.24	1.88 ± 0.27	1.85 ± 0.23	1.92 ± 0.20	0.077†
HDL-C	49.6 ± 18.6	45.9 ± 19.4	46.5 ± 22.3	43.2 ± 21.4	0.941
LP-IR Score	44.8 ± 23.6	43.3 ± 24.1	40.4 ± 15.5	46.1 ± 17.0	0.202

Normally distributed data are presented as Mean ± SD; non-normally distributed data are presented as median (lower quartile-upper quartile) and log(10) transformed (mean ± SD) underneath. NMR: nuclear magnetic resonance; CR: calorie restriction; CR+SR: calorie restriction and sleep restriction; CHY-P/C: chylomicron particle/cholesterol; VLDL-P/C: very-low-density lipoprotein particle/cholesterol; LDL-P/C: low-density lipoprotein particle/cholesterol; IDL-P: intermediate-density lipoprotein particle; TG: triglyceride; Apo-B: apolipoprotein-B; HDL-P/C: high-density lipoprotein particle/cholesterol; LP-IR: lipoprotein insulin resistance
†p value for test using transformed data.

Lipoprotein subclass particle concentrations and sizes

Due to the small number of studies completed, how sleep restriction may affect the lipoprotein profile remain inconclusive. Reynolds et al. found that TG concentration was lower following five nights of 4-hour TIB compared to two nights of 10-hour TIB in healthy weight adults [54]. However, O'Keeffe et al. found no changes in total cholesterol, HDL-C, or TG concentrations following five nights of 4-hour TIB compared to 9-hour TIB in a randomized cross-over trial of young normal weight adults [55]. In healthy adults undergoing five nights of 4-hour TIB compared to controls achieving five nights of >7-hour TIB, small, medium, and large LDL-P, small VLDL-P, and ApoB concentrations decreased with no changes in small, medium, or large HDL-P concentrations [26].

To date, we are not aware of any study that has examined the influence of prolonged period of sleep restriction on lipoprotein profile. Aho at el. examined the putative effects of prolonged insufficient sleep, assessed by self-report, using two independent large sample epidemiologic cohorts [26]. They found that large and extra-large HDL-P concentrations were lower among individuals who reported subjective sleep insufficiency compared to individuals without subjective sleep insufficiency. Our study found mean HDL-P size decreased in the CR+SR group but not the CR group. Given that larger HDL-P size is associated with lower cardiovascular disease risk [12], these results suggest that lipoprotein profile was negatively influenced by the addition of sleep restriction to calorie restriction in the CR+SR group when compared to CR weight loss alone in overweight or obese adults.

Glucose, glucose regulatory hormones, and insulin sensitivity indices

In our study, glucose and insulin concentrations did not have differential changes between the CR and CR+SR groups; however,

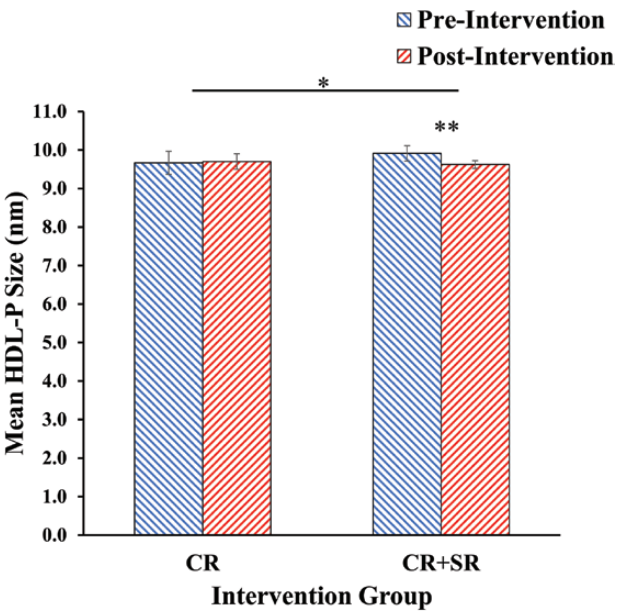

Figure 2. Mean high-density lipoprotein particle (HDL-P) size at pre- and post-intervention by intervention group. Error bars represent standard error of the mean. CR: calorie restriction; CR+SR: calorie restriction and sleep restriction. *p = 0.066 for group×time interaction. **p = 0.022 for pre- to post-intervention comparison within CR+SR group.

glucagon concentration decreased in the CR group but did not change in CR+SR group. The two previous studies involving sleep restriction during calorie restriction did not measure glucagon [29, 30]. St-Onge et al. did not find differences in fasting or average glucose and insulin concentrations in the morning, afternoon, or at night between two different sleep opportunities, 4-hour and 9-hour TIB, in normal weight men and women [30]. Nedeltcheva et al. noted that during a 14-day combined exposure to hypocaloric diet and short sleep periods, lower levels of fasting and 24-hour insulin concentrations following 5.5-hours of sleep opportunity versus 8.5-hours were found in overweight individuals (BMI=27.4±2.0 kg/m^2) [29]. These changes are consistent with a state of reduced carbohydrate availability potentially induced by calorie restriction. The weight loss in their study was similar with our study, ~3–4% weight loss. These two studies did not suggest sleep restriction during a short period of calorie deficit had adverse effects on glucose metabolism. In contrast, our results suggest adverse effects of sleep restriction during a longer calorie restriction intervention period in participants who had a higher BMI than participants in the two previous studies. These results are important given that calorie restriction for a few weeks may be used more by obese individuals than overweight and normal weight individuals in order to obtain health benefits.

Visfatin is an adipocytokine known to possess insulin-mimetic properties, functions to activate insulin receptors, and acts as a determinant of insulin resistance in obesity [49]. A previous study in middle-aged, obese females who slept less than 6 hours found higher circulating visfatin concentration and lower insulin sensitivity compared to women who achieved adequate sleep [56]. A two-month intervention was implemented in these women to increase the amount of sleep achieved each night and found that visfatin concentration decreased and insulin sensitivity increased. In our study, visfatin concentration decreased in the CR group with medium effect size, suggesting the non-significant finding in this group was likely due to the small sample size. These results are in line with previous study findings that sleep restriction potentially increases visfatin concentrations. However, no differential changes were found between CR and CR+SR groups in insulin sensitivity from pre- to post-intervention, as indicated by their similar changes in HOMA2-IR, QUICKI, and LP-IR.

A few explanations exist for the lack of differential changes in insulin sensitivity from pre- to post-intervention between the CR and CR+SR groups. One reason is the influence of recent nights' sleep. Previously, insulin sensitivity is influenced by only 2–3 nights of restricted or extended sleep [21, 57]. We did not control the last 2–3 nights' sleep duration before blood sampling in our study, as the CR+SR group chose their restricted and ad libitum sleep days. Therefore, we were not able to determine if any chronic effect due to the 8-week intervention that occurred was influenced by recent nights' sleep. These data suggest that any influence of chronic moderate sleep restriction with periodic ad libitum sleep on insulin sensitivity, compared to the effect of weight loss, was not substantial. Second, similar to the study performed by Zielinski et al. [23], the possibility remains that the duration of the intervention may have allowed for chronic adaptations to occur due to sleep restriction. Lastly, the degree of sleep restriction in our study may not be large enough to influence insulin sensitivity.

The way in which accessory glucose regulatory hormones, such as GIP, GLP-1, and resistin, relate to sleep restriction during calorie restriction has yet to be elucidated. GIP is an incretin which stimulates insulin secretion but does not inhibit glucagon secretion and is known to be elevated in individuals with impaired glucose metabolism [47]. GLP-1 is another incretin that stimulates glucose-dependent insulin secretion when plasma glucose concentration is high, but not when plasma glucose concentration falls below a normal range [46, 48]. Lastly, resistin, an adipocytokine, is altered in obese adults [50]. The study by St-Onge et al. found no differences in fasting GLP-1 following 4-hour vs. 9-hour TIB with a controlled diet [30]. Thus, our findings are in line with previous results suggesting that fasting GLP-1 concentration may not be directly influenced by modest weight loss in the presence or absence of moderate sleep restriction.

Strengths and limitations

This study adds important information to the literature regarding the effects of sleep restriction in the context of calorie restriction on cardiometabolic health outcomes. Very few studies have examined outcomes in this nature, and, to our knowledge, this study has been the only study completed examining a sleep restriction intervention lasting several weeks. This 8-week study duration is considerably longer than other controlled studies examining sleep restriction. Our study was unique in that it involves modest sleep restriction, an average of less than 90 minutes per day shorter TIB on 5 days a week, and ad libitum sleep on the other 2 days of the week. By allowing participants to choose restricted sleep days make the results more generalizable to the larger populations where catch-up sleep occurs throughout the week. In addition, the average difference in midpoint sleep on sleep restricted days and ad libitum days in

Table 4. Glucose, its regulatory hormones, and indices of insulin sensitivity at pre- and post-intervention by intervention group

	CR (n = 12)		CR+SR (n = 11)		Group×time interaction
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention	p value
Glucose (mg/dL)	76.3 ± 16.6	74.4 ± 17.1	91.6 ± 20.1	82.5 ± 17.4	0.123
Insulin (uU/mL)	6.6 (0.8–31.7)	5.3 (1.8–24.4)	15.6 ± 8.4ǂ	13.9 (3.3–44.3)	
	0.77 ± 0.43	0.78 ± 0.32	1.13 ± 0.26	1.05 ± 0.34	0.491†
Glucagon (þg/mL)	1776 (1428–2631)	1823 ± 317	1789 ± 243	1771 ± 278	
	3.26 ± 0.07	3.18 ± 0.07	3.25 ± 0.06	3.24 ± 0.07	**0.028**†
GLP-1 (þg/mL)	231 ± 95	185 ± 70	186 ± 41	154 (87–525)	
	2.34 ± 0.16	2.16 ± 0.46	2.26 ± 0.11	2.25 ± 0.22	0.166†
GIP (þg/mL)	367 (260–819)	322 ± 70	382 ± 135	349 (149–2223)	
	2.61 ± 0.17	2.50 ± 0.10	2.56 ± 0.15	2.57 ± 0.31	0.169†
Visfatin (þg/mL)	5513 ± 2462	4161 ± 1405	3883 ± 1659	3992 (1377–15772)	
	3.71 ± 0.18	3.59 ± 0.16	3.55 ± 0.20	3.66 ± 0.27	0.066†
Resistin (þg/mL)	5677 (2845–22092)	5515 (3496–13999)	5802 ± 1478	5718 (3312–23666)	
	3.82 ± 0.25	3.81 ± 0.21	3.75 ± 0.12	3.83 ± 0.26	0.240†
HOMA2-IR	1.25 (0.42–4.15)	0.73 (0.52–3.27)	1.89 (0.49–4.17)	1.72 (0.38–5.59)	
	0.05 ± 0.32	0.05 ± 0.26	0.23 ± 0.27	0.14 ± 0.34	0.969†
QUICKI	0.39 ± 0.08	0.39 ± 0.05	0.32 ± 0.04†	0.34 ± 0.05	0.316

Normally-distributed data are presented as Mean±SD; non-normally distributed data are presented as median (lower quartile-upper quartile) and log(10) transformed (mean ± SD) underneath. CR: calorie restriction; CR+SR: calorie restriction and sleep restriction; GLP-1: glucagon-like peptide-1; GIP: gastric inhibitory peptide; HOMA2-IR: updated homeostasis model assessment of insulin resistance; QUICKI: quantitative insulin-sensitivity check index. The p values in bold are < 0.05.
ǂp < 0.05 significant difference at pre-intervention between groups; †p value for test using transformed data.

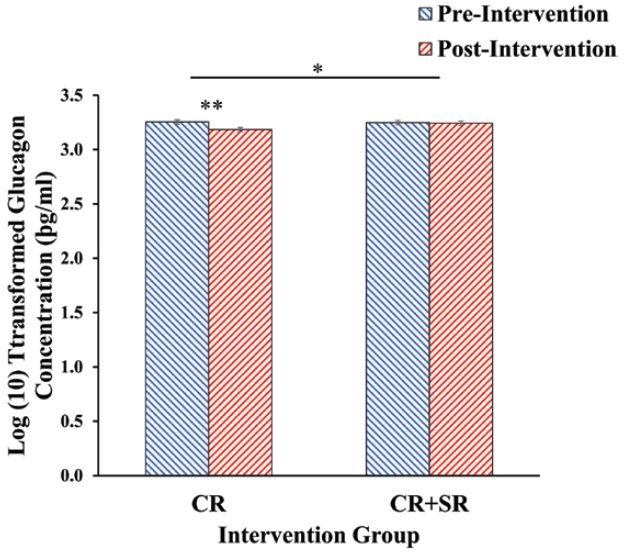

Figure 3. Serum glucagon concentration [log(10) transformed] at pre- and post-intervention by intervention group. Error bars represent standard error of the mean. CR: calorie restriction; CR+SR: calorie restriction and sleep restriction. *p = 0.028 for group×time interaction. **p = 0.016 for pre- to post-intervention comparison within CR group.

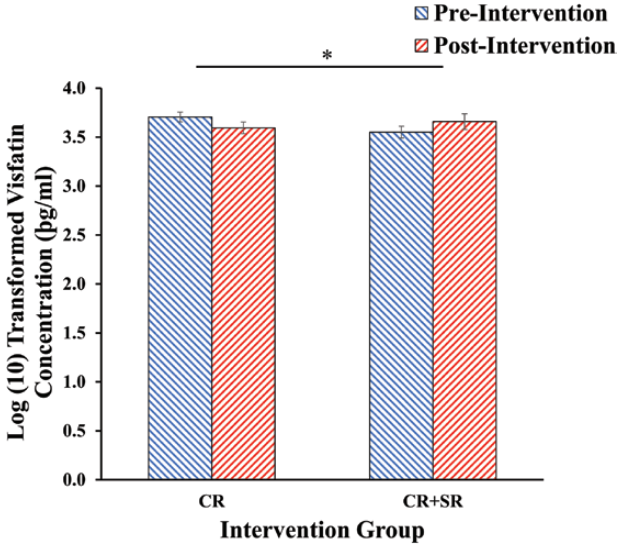

Figure 4. Serum visfatin concentration [log(10) transformed] at pre- and post-intervention by intervention group. Error bars represent standard error of the mean. CR: calorie restriction; CR+SR: calorie restriction and sleep restriction. *p = 0.066 for group × time interaction.

the CR+SR group was less than one hour and not different compared to the workday and free day difference in the CR group. Social Jetlag of greater than 1 hour compared to less than 1 hour, specifically, the difference in sleep schedule on weekdays and weekend days, was previously found to be associated with increased metabolic syndrome risk and diabetes/prediabetes [58]. Therefore, the sleep timing differences were unlikely to contribute to our findings regarding group differences.

A few limitations also exist. First, only a single fasting blood sample was collected at pre- and post-intervention. Therefore, variables of interest, specifically glucose and its regulatory hormones, were not measured after meals or throughout 24-hour period. We did not control the previous few nights' sleep, which may influence the results as we discussed above. We did not use more advanced techniques, such as the insulin clamp, which is more sensitive to changes in glucose metabolism. Second, this study is from a clinical trial with different primary outcomes where blood samples were only available from a subset of participants. As such, statistical power was not determined *a priori*. As a result, some findings were of medium effect sizes for the degree of change from pre- to post-intervention, but not statistically significant.

The small sample size also did not allow us to examine sex or racial differences within or between groups. In both groups, 75% of participants were women; if there were sex differences in any outcomes, the results may be more influenced by women than men participants. We also did not

control menstrual cycle phase of the blood sample collection date. The sample characteristics and small sample size limit the generalizability of the results. Although the statistical methods of repeated measures analysis accounted for the pre-intervention values, differences between CR and CR+SR in pre-intervention body weight, insulin and QUICKI, could impact the findings that cannot be completely addressed by statistical analysis. Specifically, greater body weight, higher insulin concentration, and lower QUICKI in the CR+SR group suggest this group may be less metabolically healthy than the CR group at baseline. This may contribute to different changes in response to interventions even though the primary differences found between the two groups were not these three outcomes per se.

Conclusion

In this study, 8 weeks of calorie restriction with and without sleep restriction of less than 90 minutes per day on 5 days with 2 days of ad libitum sleep per week, resulted in similar degrees of weight loss. However, differential changes in serum glucagon concentration were found between the CR and CR+SR groups. Our findings also suggest differential changes in mean HDL-P size and visfatin concentrations between the CR and CR+SR groups. These findings suggest this moderate amount of sleep restriction, despite periodic catch-up sleep, influenced glucose regulation and lipoprotein metabolism. Thus, individuals who are pursuing weight loss should be encouraged to avoid short sleep on some days to prevent potential undesirable influence on glucose and lipoprotein metabolism in order to obtain better weight loss outcomes.

Supplementary material

Supplementary material is available at *SLEEP Advances* online.

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J.R.S. acquired data, analyzed and interpreted data, and drafted the manuscript. R.R.P. acquired data and reviewed the manuscript. S.D.Y. assisted with obtaining funding, provided consultation to the study, and reviewed the manuscript. K.P.B. acquired data, assisted with data analysis, and reviewed the manuscript. J.L.D. critically reviewed the manuscript. X.W. designed the study, obtained funding, supervised the study, and critically reviewed the manuscript. All authors approved the final version.

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Disclosure Statement

None declared.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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