Randomised controlled trial of the effects of increased energy intake on menstrual recovery in exercising women with menstrual disturbances: the ‘REFUEL’ study

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STUDY QUESTION: Does increased daily energy intake lead to menstrual recovery in exercising women with oligomenorrhoea (Oligo) or amenorrhoea (Amen)?

SUMMARY ANSWER: A modest increase in daily energy intake (330 ± 65 kcal/day; $18 \pm 4\%$) is sufficient to induce menstrual recovery in exercising women with Oligo/Amen.

WHAT IS KNOWN ALREADY: Optimal energy availability is critical for normal reproductive function, but the magnitude of increased energy intake necessary for menstrual recovery in exercising women, along with the associated metabolic changes, is not known.

STUDY DESIGN, SIZE, DURATION: The REFUEL study (trial # NCT00392873) is the first randomised controlled trial to assess the effectiveness of 12 months of increased energy intake on menstrual function in 76 exercising women with menstrual disturbances. Participants were randomised (block method) to increase energy intake 20–40% above baseline energy needs (Oligo/Amen + Cal, $n = 40$) or maintain energy intake (Oligo/Amen Control, $n = 36$). The study was performed from 2006 to 2014.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Participants were Amen and Oligo exercising women (age = 21.0 ± 0.3 years, BMI = 20.8 ± 0.2 kg/m², body fat = $24.7 \pm 0.6\%$) recruited from two universities. Detailed assessment of menstrual function was performed using logs and measures of daily urinary ovarian steroids. Body composition and metabolic outcomes were assessed every 3 months.

MAIN RESULTS AND THE ROLE OF CHANCE: Using an intent-to-treat analysis, the Oligo/Amen + Cal group was more likely to experience menses during the intervention than the Oligo/Amen Control group ($P = 0.002$; hazard ratio [CI] = 1.91 [1.27, 2.89]). In the intent-to-treat analysis, the Oligo/Amen + Cal group demonstrated a greater increase in energy intake, body weight, percent body fat and total triiodothyronine (TT₃) compared to the Oligo/Amen Control group ($P < 0.05$). In a subgroup analysis where $n = 22$ participants were excluded (ambiguous baseline menstrual cycle, insufficient time in intervention for menstrual recovery classification), 64% of the Oligo/Amen + Cal group exhibited improved menstrual function compared with 19% in the Oligo/Amen Control group (χ^2 , $P = 0.001$).

LIMITATIONS, REASONS FOR CAUTION: While we had a greater than expected dropout rate for the 12-month intervention, it was comparable to other shorter interventions of 3–6 months in duration. Menstrual recovery defined herein does not account for quality of recovery.

WIDER IMPLICATIONS OF THE FINDINGS: Expanding upon findings in shorter, non-randomised studies, a modest increase in daily energy intake (330 ± 65 kcal/day; $18 \pm 4\%$) is sufficient to induce menstrual recovery in exercising women with Oligo/Amen. Improved metabolism, as demonstrated by a modest increase in body weight (4.9%), percent body fat (13%) and TT_3 (16%), was associated with menstrual recovery.

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Introduction

During periods of energy deficiency in mammals, metabolic fuel is repartitioned away from the energetically costly processes of reproduction and growth in order to conserve fuel and maintain blood glucose for immediate survival (Wade et al., 1996). These adjustments signal a state of energy deficiency to the hypothalamus, ultimately leading to reproductive suppression associated with severe menstrual disorders, i.e. amenorrhoea (Amen) and oligomenorrhoea (Oligo) (Williams et al., 2001, 2015). In exercising women, the condition characterised by the inter-relationships among energy deficiency, menstrual dysfunction and low bone density is known as the Female Athlete Triad (Triad) (Nattiv et al., 2007; De Souza et al., 2014). The recommended treatment for the Triad is to 'refuel', i.e. increase energy intake to meet energy expenditure needs, including the energetic cost of exercise, and improve the underlying energetic status (De Souza et al., 2014).

Published reports on the efficacy of increasing food intake to reverse oligomenorrhoea and amenorrhoea in exercising women are limited to case studies (Dueck et al., 1996; Kopp-Woodroffe et al., 1999; Mallinson et al., 2013), a retrospective chart review (Arends et al., 2012) and uncontrolled interventions (Cialdella-Kam et al., 2014; Guebels et al., 2014; Lagowska et al., 2014a; Lagowska et al., 2014b). In a non-human primate model of exercise-associated amenorrhoea, increased food intake in exercising monkeys reversed amenorrhoea without any moderation in exercise energy expenditure (EEE) (Williams et al., 2001). While these studies provide evidence that increased energy intake is effective for recovery of menstrual function, the magnitude of energy required to restore menstrual function, the timeframe over which recovery occurs, and the associated metabolic changes have not been established. A randomised controlled trial (RCT), unbiased by self-selection, is necessary to properly address these gaps in the literature.

The purpose of this study was to determine whether a 12-month intervention of increased energy intake leads to menstrual recovery among women with severe exercise-associated menstrual disturbances. We hypothesised that exercising Oligo/Amen women who increased energy intake would demonstrate a greater frequency of menses and improved menstrual function, coincident with improvements in metabolic status, compared with an exercising Oligo/Amen Control group who maintained energy intake.

Materials and methods

Study design

Protocol information for The REFUEL study (Clinical Trial Number NCT00392873: 'Increased Calorie Intake to Reverse Energy Deficiency in Exercising Women: Impact on Bone and Menstrual Cyclicity') is available at <https://clinicaltrials.gov/ct2/show/NCT00392873>. The study was a 12-month parallel-design RCT designed to determine whether an increase in energy intake among young exercising women with amenorrhoea or oligomenorrhoea would improve the primary outcomes of menstrual recovery defined as (i) increased frequency of menses and (ii) improved menstrual function. The latter was defined as the resumption of menses in amenorrhoeic women and improved regularity of menses in oligomenorrhoeic women. An additional primary outcome included energy status as indicated by body weight and body composition. A secondary outcome was the metabolic hormone, total triiodothyronine (TT_3) (Williams et al., 2019). Research participants were randomised into study groups using the following methods: (i) a list of group assignments was generated and determined by coin toss, (ii) the block method was used for group assignment, and (iii) group assignment was placed in sealed envelopes. Envelopes were stored and distributed by the Clinical Research Center staff to the study coordinator at the time of randomisation. Lab technicians were blinded to group assignment by using study ID numbers that did not include group assignment information in the ID or on study materials. Women with Oligo/Amen were randomised into one of two groups, (i) a group who increased energy intake for the duration of the 12-month intervention (Oligo/Amen + Cal) or (ii) a control group who maintained habitual EEE and energy intake (Oligo/Amen Control). Both groups received calcium and vitamin D₃ supplements as the standard of care; the supplement dose was the amount necessary to consume an adequate intake of 1200 mg/day of calcium and 400 IU/day of Vitamin D₃. Energetic and reproductive status were repeatedly assessed throughout the study that included screening, a 4-week baseline period, a 12-month intervention and a post-study period (Williams et al., 2019). An abbreviated study design that includes the primary outcome measures assessed is provided in Fig. 1; detailed methods and figure of the overall study design have

been previously published (Williams et al., 2019). This study was conducted at two sites, the University of Toronto (UT) (2006 to 2008) and Penn State University (PSU) (2008 to 2014) with approval from the Research Ethics Board of UT and the Institutional Review Board of PSU, respectively. All subjects signed an approved informed consent prior to participation. Recruitment occurred from 2006 to 2013, and data collection was completed in 2014.

Eligibility criteria

Eligibility criteria for the study were: (i) age 18–35 years, (ii) BMI 16–25 kg/m², (iii) good health as determined by medical exam, (iv) no chronic illness, (v) ≥2 h/week of purposeful exercise, (vi) non-smoker, (vii) not currently dieting, (viii) no hormonal therapies for the past 6 months, (ix) no current clinical diagnosis of eating or psychiatric disorder, (x) not pregnant or lactating or planning a pregnancy, (xi) no medication use that would alter metabolic or reproductive hormone

concentrations, and (xii) no other contraindications to study participation. Women who reported no menses in the past 3 months or ≤6 cycles in the past 12 months were eligible.

To rule out pregnancy, a urine sample was collected to test for hCG. To ensure that the menstrual disturbances were not due to underlying endocrine or metabolic disease, a fasting blood sample was collected to measure complete blood count and an endocrine panel, which included thyroid-stimulating hormone (TSH), thyroxine (T₄), LH, FSH and prolactin. Women with primary amenorrhoea were not included.

Questionnaires

Participants were queried about health and medical background, basic demographics, medication and supplement use and history of eating disorders, menstrual cycles, physical activity, bone health and stress fractures (Williams et al., 2019).

Nutritional intervention

The nutritional intervention in the Oligo/Amen + Cal group consisted of prescribing an energy intake 20–40% above each participant's baseline energy requirements. The increase in energy intake was increased gradually to prevent any negative physiological or behavioural effects of a more abrupt increase, and to help with compliance. Baseline energy requirements were determined by using resting metabolic rate (RMR), daily energy expenditure and the thermic effect of food (TEF) (detailed methods published previously, summarised herein (Williams et al., 2019)). RMR was determined by indirect calorimetry using a ventilated hood system (SensorMedics Vmax). RMR measurements were performed in a fasted state for a 30–45 min period between 0630 and 1000 following 45 min of rest. Daily energy expenditure included exercise and non-exercise energy expenditure (NEAT, determined via 7-day accelerometry and physical activity logs). EEE was calculated using Polar heart rate monitoring (or Ainsworth et al. compendium (Ainsworth et al., 2011)) during purposeful exercise, corrected to subtract kcals associated with RMR. TEF was estimated as 10% of RMR + EEE + NEAT (Williams et al., 2019). Thus, total daily energy expenditure was determined to be RMR + EEE + NEAT + TEF. Using the calculation of total daily energy expenditure and calculating a caloric prescription 20–40% above that level, women in the Oligo/Amen + Cal group were counselled by a clinical dietitian to increase food intake. Participants were also supplied with energy bars (220–300 calories) and pre-measured servings of nuts, if desired. Women in the Oligo/Amen Control group were asked to maintain their usual diet and both groups were asked to maintain their baseline habitual exercise throughout the study (Williams et al., 2019).

Energetic variables

Body weight was measured bi-weekly for the duration of the study on a digital scale to the nearest 1/100 kg.

Energy intake (kcal) was self-reported monthly using 3-day diet logs and analysed with Nutrition Data System for Research (NDSR 2008 Version). A daily EEE was estimated monthly by averaging EEE over a 7-day monitoring period.

Body composition was analysed by dual-energy X-ray absorptiometry (DXA). Participants were scanned on either a GE Lunar Prodigy or GE Lunar iDXA, and cross-calibration was performed consistent with International Society for Clinical Densitometry guidelines to remove system bias.

TT₃ was assessed monthly for the first 6 months and every 3 months for the second 6 months of the study. All blood sample collections occurred between 0700 and 1000 h; samples were processed and stored as published (Williams et al., 2019). Serum TT₃ was analysed using a competitive immunoassay (Siemens Healthcare Diagnostics, Inc, Tarrytown, NY, USA) on a chemiluminescence analyser (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). The sensitivity of the TT₃ assay was 0.54 nmol/l (35 ng/dl). The intra-assay and inter-assay coefficients of variation were 10.3 and 13.3%, respectively.

Reproductive variables and menstrual status

The determination of functional hypothalamic amenorrhoea/oligomenorrhoea was made using information from medical history, endocrine

measures (TSH, T₄, prolactin, estradiol (E₂), FSH, LH, LH/FSH ratio, total testosterone, sex hormone-binding globulin, free androgen index, hCG), physical exam (which included an evaluation of hirsutism and acne), diet and exercise history and presence/absence of current eating disorder and self-reported menstrual status corroborated by daily urinary hormone profiles of oestrogen (EIG, oestrone-1-glucuronide), progesterone (PdG, pregnanediol glucuronide) and LH for a 28-day monitoring period at baseline (Gordon et al., 2017). Criteria for assigning women as amenorrhoeic were the self-report of no menses for at least 3 months prior to the study and a suppressed hormonal profile with no evidence of menses during the baseline monitoring period. Criteria for assigning women as oligomenorrhoeic were the self-report of 1 or 2 menses in the past 3 months or <7 menses in the past 12 months, or a menstrual cycle 36–89 days in length during the baseline period (De Souza et al., 2010; Gordon et al., 2017). Additionally, a woman was also considered 'oligomenorrhoeic' if her self-reported menstrual history or baseline menstrual cycle indicated irregular menstrual cycles. If baseline menstrual status was not clearly discernable given the definitions provided, the menstrual status was considered 'ambiguous'. The occurrence of menses throughout the study was assessed using self-report and was corroborated by daily urinary reproductive hormone assessments and blinded determinations of menstrual function by two experts (De Souza et al., 2010). In the event of disagreement by the two experts, a third expert was consulted. Briefly, urinary concentrations of EIG, PdG, and LH were visually inspected for presence of hormonal fluctuations associated with eumenorrhoeic cycles or amenorrhoea. When menses was reported, the concentrations of EIG and PdG were aligned to the day of the self-reported menses. The presence or absence of ovulation was determined by a mid-cycle LH surge >25 mIU/ml which was preceded by an EIG peak >35 ng/ml. Luteal sufficiency was determined by a PdG concentration >5 µg/ml during the luteal phase of the cycle. In the event that no menses were reported, EIG and PdG measurements were visually inspected for chronically suppressed concentrations (Williams et al., 2019).

Statistical analysis

Data were screened for outliers and normality was assessed using the Shapiro–Wilk test. For normally distributed variables, independent *t*-tests were performed to compare baseline demographic variables between groups; for non-normally distributed variables, the Mann–Whitney U test was performed. Menstrual recovery was analysed two ways, (i) an intent-to-treat survival analysis that determined the effect of the intervention on menstrual frequency and (ii) a subgroup analysis that assessed the proportion of women who experienced improved menstrual function in each group (chi-square). The subgroup (*n* = 28 Oligo/Amen + Cal and *n* = 26 Oligo/Amen Control) did not include randomised participants who received an ambiguous cycle determination at baseline (*n* = 5) nor those who were in the study insufficient time for intervention data to be collected (*n* = 7) or recovery criteria to be applied (*n* = 10).

Based on a chi-square analysis for resumption of menstrual function, a sample size of 17 women per group will provide 80% power to demonstrate a statistically significant difference between groups for the primary outcome of improved menstrual function, as described previously (Williams et al., 2019). The sample size for the overall REFUEL

study, with consideration of multiple primary outcomes, is provided in our study design publication (Williams *et al.*, 2019). To assess data missingness, Little's MCAR test indicated that data were missing completely at random (Little, 1988). R Statistical Computing Platform (version 3.4.1) or IBM SPSS Statistics for Windows (Version 25.0. Armonk, NY, USA: IBM Corp.) were used for analyses. Data were reported as mean $\pm$ SEM and the significance level was $\alpha = 0.05$.

Primary reproductive outcome: intent-to-treat analysis

We conducted a survival analysis (conditional recurrent events Cox Proportional Hazards survival model (Prentice *et al.*, 1981)) to determine whether the intervention increased the frequency of menses in the Oligo/Amen + Cal group compared to the Oligo/Amen Control group. Women who did not experience menses by the end of their time participating in the intervention were right-censored; their last week of study participation was noted, and their data were used in the analysis up to the time of censorship (Kleinbaum and Klein, 2012). The resulting model thus estimated whether the study's intervention affected the likelihood (hazard) of experiencing a menses. We controlled for baseline fat mass and menstrual status in the model. Baseline menstrual status was categorised as either amenorrhoeic (1) or not amenorrhoeic (0) (i.e. oligomenorrhoeic or ambiguous), as explained above. The proportional hazard assumption for the model was satisfied ($P > 0.05$).

Primary reproductive outcome: subgroup analysis

A Pearson's chi-square analysis was conducted to compare the proportion of subjects who improved menstrual function between groups. For women with amenorrhoea at baseline, improved menstrual function was defined as experiencing at least one menses during the intervention. For women with oligomenorrhoea at baseline, improved menstrual function was an improvement in menstrual regularity, defined as at least 1, 3, 5 or 7 menstrual cycles < 36 days in length during 3, 6, 9 or 12 months of the intervention, respectively. This analysis was performed in the subgroup of women who were in the study for adequate time for these definitions to be applied and who had a clear baseline menstrual status of amenorrhoea or oligomenorrhoea. For some women ($n=5$), it was not possible to categorise a woman's menstrual status due to missing baseline urine samples or a baseline menstrual status that could not be discerned (Williams *et al.*, 2019), thus menstrual status was considered 'ambiguous', and these subjects were not included in this sub-analysis.

Primary and secondary energetic outcomes: intent-to-treat analysis

To compare the effects of the intervention on the changes in the primary outcomes of body weight and body composition and in the secondary outcome of energy status and TT_3 concentrations between the study groups, we used a generalised linear fixed effects model on the raw values at five time points during the study with time, study group, study group* time interaction, and baseline body weight and fat mass as fixed effects. This model was also used for descriptive variables such as energy intake and EEE. Notably, we initially ran a generalised linear mixed effects model with subjects as a random effect; however, it produced a zero estimated variance for the random effect, indicating an overparametrised specification. Therefore, the variable of subject ID was removed from the model. When the marginal effect of either baseline body weight or fat mass was not significant, we opted

for the most parsimonious model by removing insignificant terms. For variables with a significant interaction (time*group), simple contrasts using sequential Bonferroni correction were performed. The measurements at five time points were used in the models, including baseline, month 3, month 6, month 9 and post-study (month 13). Absolute and percent change from baseline were also calculated using the data collected at months 3, 6 and 9 and post-study (month 13).

Results

Of 233 women who consented and began the screening period as either ovulatory (reference control group, not described in this article) or with menstrual disturbances (oligomenorrhoea or amenorrhoea), 142 met initial eligibility criteria and entered the baseline monitoring period. Of these women, 116 (82%) completed baseline monitoring, and this included 76 women with menstrual disturbances. Of the 76 Oligo/Amen women, 40 were randomised to the Oligo/Amen + Cal group and 36 were randomised to the Oligo/Amen Control group (Fig. 2). With an average dropout/early termination rate of 57% during the entire 12-month intervention period (40% at 6 months), 17 Oligo/Amen + Cal and 16 Oligo/Amen Control completed all 12 months of the intervention. Reasons for withdrawal or termination after randomisation are provided in Fig. 2. The dropout rate (including both voluntary withdrawal and early termination) was similar between groups (chi-square, $P = 1.000$; Oligo/Amen Control, 56%; Oligo/Amen + Cal, 58%). There were no significant baseline differences in descriptive characteristics, body composition, energy intake and expenditure, and menstrual status between women who completed the study and those who dropped out. TT_3 was significantly greater among non-completers compared with completers (Supplementary Table S1).

Table 1 provides baseline descriptive information. Screening endocrine data are consistent with a diagnosis of functional hypothalamic amenorrhoea (FHA) in both groups. The proportion of amenorrhoeic compared with oligomenorrhoeic women in the two Oligo/Amen study groups was not significantly different ($\chi^2 = 4.1$, $P = 0.059$). In the Oligo/Amen + Cal group, 55% (22/40) of women were amenorrhoeic, 35% (14/40) were oligomenorrhoeic, and the remaining 10% (4/40) had an ambiguous baseline menstrual status. In the Oligo/Amen Control group, 36% (13/36) were amenorrhoeic, 61% (22/36) were oligomenorrhoeic, and 3% (1/36) were ambiguous. Furthermore, there was no significant difference in the self-reported history of menses (in the past 12 months) between the Oligo/Amen groups at baseline. The majority of women (79%) were recreational exercisers; whereas, 21% were competitive athletes. Most women (71%) primarily participated in endurance exercise, such as distance running, cycling, swimming and aerobic exercise at fitness centres, while 12% and 9% of the women primarily participated in ball sports and aesthetic sports (gymnastics, dance), respectively, and the remaining 8% participated in weight-class and power sports or multiple exercise modalities.

Primary reproductive outcome: intent-to-treat analysis

Figure 3 depicts the results of the survival analysis, demonstrating that increased energy intake had a significant positive effect on the

Figure 2. Flow chart of subject enrollment during the study for this sample and the reasons for dropout during the study. OCs, oral contraceptives.

likelihood (hazard) of experiencing a menses ($P=0.002$) after controlling for baseline fat mass and menstrual status. Women in the Oligo/Amen + Cal group were almost twice as likely (91% increase; hazard ratio [CI] = 1.91 [1.27, 2.89]) to experience a menses during the study than those in the Oligo/Amen Control group. Baseline fat mass significantly influenced the model (hazard ratio [CI] = 1.08 [1.04, 1.13]) such that the higher a participant's fat mass at baseline, the greater the likelihood (hazard) of experiencing a menses. Specifically, a one unit (kg) increase in fat mass at baseline increased the likelihood (hazard) of experiencing menses by 8%. Baseline menstrual status did not influence the model (hazard ratio [CI] = 0.77 [0.57, 1.04]).

Figure 4 illustrates the occurrence of menses during the intervention for each participant in the Oligo/Amen + Cal (Fig. 4a) and Oligo/Amen Control (Fig. 4b) groups. These descriptive figures visually demonstrate that women in the Oligo/Amen + Cal group experienced a greater frequency of menses compared with women in the Oligo/Amen Control group over 12 months.

Primary reproductive outcome: subgroup analysis:

In the subgroup of women who had a clear baseline menstrual status and completed adequate time in the study for the definitions of improved menstrual function to apply ($n=28$ Oligo/Amen + Cal and $n=26$ Oligo/Amen Control), 64% of women in the Oligo/Amen + Cal group exhibited improved menstrual function compared with 19% in the Oligo/Amen Control group ($\chi^2 = 11.2$, $P=0.001$; Table II). The odds of women in the Oligo/Amen + Cal group improving menstrual function were seven times greater than that of the Oligo/Amen Control group (odds ratio (OR) [95% CI]: 7.6 [2.2, 26.2] and relative risk [95% CI]: 3.3 [1.5, 7.7]). The absolute difference for improved menstrual function between the Oligo/Amen + Cal and Oligo/Amen Control groups was 45% [95% CI: 22%, 68%]. Within the menstrual groups, 65% of amenorrhoeic women and 63% of oligomenorrhoeic women in the Oligo/Amen + Cal group met the criteria for improved menstrual function compared with 18% of amenorrhoeic women ($\chi^2 = 6.2$, $P=0.023$; OR [95% CI]: 8.4 [1.4, 49.9]) and 20% of

Table 1 Baseline characteristics of the study groups.

	Oligo/Amen + Cal (n = 40)	Oligo/Amen Control (n = 36)
Demographics		
Age (years)	21.3 ± 0.5	20.7 ± 0.5
Age of Menarche (years)	13.5 ± 0.2	13.7 ± 0.3
Gynecological age (years)	7.8 ± 0.5	7.0 ± 0.5
Height (cm)	164.2 ± 1.0	166.6 ± 1.1
Weight (kg)	55.0 ± 1.0	59.1 ± 1.3
BMI (kg/m ²)	20.4 ± 0.3	21.3 ± 0.4
% body fat	23.8 ± 0.7	25.7 ± 1.0
Fat mass (kg)	13.1 ± 0.5	15.3 ± 0.8
Lean mass (kg)	39.7 ± 0.7	41.1 ± 0.8
Energy intake (kcal/day) ^a	1,907 ± 85	1,910 ± 157
Exercise energy expenditure (kcal/day) ^{*b}	309 ± 43	366 ± 47
Exercise volume (min/week) ^{**}	303 ± 33	342 ± 45
History of exercise—past 6 months (min/week)	466 ± 50	548 ± 68
Hormones		
Total triiodothyronine (ng/dl) ^{***}	78.2 ± 3.5	85.8 ± 3.5
Thyroid-stimulating hormone (mIU/l) ^{****}	1.8 ± 0.1	1.7 ± 0.1
LH (IU/l)	6.9 ± 1.0	7.8 ± 1.4
FSH (IU/l)	5.2 ± 0.3	5.4 ± 0.9
LH/FSH ratio	1.4 ± 0.2	1.4 ± 0.2
Prolactin (µg/l)	8.2 ± 0.8	9.5 ± 0.9
Total testosterone (ng/dl)	34.4 ± 4.0	32.2 ± 2.4
Free androgen index	2.1 ± 0.3	2.5 ± 0.3
Racial category		
Caucasian	33/40 (83%)	31/36 (86%)
Asian	5/40 (13%)	2/36 (6%)
African American	0/40 (0%)	2/36 (6%)
Other	2/40 (5%)	1/36 (3%)

Data are mean ± SEM.

*n = 37 Oligo/Amen + Cal, n = 35 Oligo/Amen Control.

**n = 34 Oligo/Amen + Cal, n = 30 Oligo/Amen Control.

***n = 39 Oligo/Amen + Cal.

****n = 34 Oligo/Amen Control.

^a3-day mean.^b7-day mean.

oligomenorrhoeic women (Fisher's exact test, $P = 0.071$; OR [95% CI]: 6.7 [1.0, 45.0]) in the Oligo/Amen Control group. Of the women who recovered menstrual function in the Oligo/Amen + Cal group, 67% (12/18) did so in the first 3 months of the intervention, 78% (14/18) recovered in the first 6 months of the intervention, and the remaining 22% (4/18) initially recovered in the last 6 months of the intervention (between months 7 and 12).

Primary and secondary energetic outcomes: intent-to-treat analysis

Figure 5 depicts the results of the generalised linear fixed effects model and estimated marginal means for the energetic variables. There was a significant interaction between study group and time for energy intake,

body weight, percent body fat and TT_3 concentrations ($P < 0.05$), indicating that the Oligo/Amen + Cal group had a greater energy intake, gained more body weight, and had a greater increase in percent body fat and TT_3 concentrations during the study compared to the Oligo/Amen Control group. There was no effect of time, group, nor a time*group interaction for EEE and lean mass. Energy intake increased 330 ± 65 kcal/day (18 ± 4%) in the Oligo/Amen + Cal group compared with a change of -66 ± 68 kcal/day (1 ± 4%) in the Oligo/Amen Control group. Among those who completed the study, the change in energy intake at the end of the study compared with baseline was 434 ± 148 kcal/day (23 ± 8%) in the Oligo/Amen + Cal group and -64 ± 126 kcal/day (−1.4 ± 7%) in the Control group. The Oligo/Amen + Cal group experienced an average increase in body weight of 0.9 ± 0.2 kg (1.6 ± 0.4%) at 3 months, which further

Figure 3. Results of recurrent event cox proportional hazard survival model for the intervention. Hazard ratios are depicted by closed black circles; 95% confidence intervals depicted by horizontal lines. Women in the Oligo/Amen + Cal group were 1.9 times more likely to experience menses compared with the Oligo/Amen Control group. Baseline fat mass significantly influenced the model; the higher a participant's fat mass at baseline, the greater her hazard of experiencing menses. A one unit (kg) increase in fat mass at baseline increased the hazard of experiencing menses by 8%.

increased to 2.6 ± 0.4 kg ($4.9 \pm 0.8\%$) after 12 months. Women in the Oligo/Amen + Cal group increased body weight 1.6 ± 0.3 kg ($3.0 \pm 0.6\%$) by the end of study participation (including dropouts and completers); in contrast, the average change in the Oligo/Amen Control group was 0.7 ± 0.4 kg ($1.2 \pm 0.6\%$). Among study completers in the Oligo/Amen + Cal group, fat mass and percent body fat increased by 2.0 ± 0.3 kg ($18 \pm 3\%$) and $2.7 \pm 0.4\%$ ($13 \pm 2\%$), respectively, and TT_3 increased by 9 ± 4 ng/dl ($16 \pm 6\%$) at the post-study measurement. Minimal body composition changes were observed in the Oligo/Amen Control group (0.6 ± 0.4 kg for fat mass and $0.7 \pm 0.5\%$ for percent body fat), while TT_3 concentration decreased by -8 ± 5 ng/dl (8%) post-study.

Discussion

REFUEL is the first RCT to quantify the effects of increasing energy intake on the recovery of menses in previously oligomenorrhoeic and amenorrhoeic exercising women. Our results demonstrated that menstrual recovery was achieved with a modest increase of 330 kcal/day

(18% above baseline energy intake) with no change in EEE. The likelihood of experiencing menses was greater in the Oligo/Amen + Cal group compared with the Oligo/Amen Control group, secondary to the increase in energy intake along with a modest increase in body weight ($+2.6$ kg, $+4.9\%$), fat mass ($+18\%$), percent body fat ($+13\%$), and TT_3 ($+16\%$). In addition to demonstrating menstrual recovery by examining the overall frequency of menses throughout the intervention, we showed that increased energy intake improved menstrual function in both amenorrhoeic and oligomenorrhoeic participants as defined by the initial resumption of menses in amenorrhoeic women and the development of more regular, normal length cycles in oligomenorrhoeic women. Of interest, in the women who recovered in the Oligo/Amen + Cal group, a large majority recovered menses by month 6 of the intervention.

These findings suggest that the intervention effects on menstrual recovery were due to the increase in energy intake and improved energetic status (i.e. body weight, percent body fat and metabolic hormone TT_3) rather than any change in exercise habits or volume, since EEE remained unchanged. Interestingly, the time course of change for body composition was different than that observed for TT_3 . Body weight and percent body fat increased gradually throughout the study; whereas, TT_3 did not demonstrate an increase until the end of the study, suggesting that there may have been a delay in the metabolic and hormonal response to increased energy intake. We surmise that a greater volume of prescribed energy intake may have resulted in an earlier hormonal response. Notably, TT_3 concentrations decreased by nearly 10% in the Oligo/Amen control group, suggesting that without improved energy intake metabolism may be further suppressed and likely contributed to the significant group differences at the end of the intervention. The sample size for the overall REFUEL study, with consideration of multiple primary outcomes, is provided in our study design publication (Williams et al., 2019).

Similar improvements in body weight and body fat have been documented in shorter nutritional interventions in exercising women (Dueck et al., 1996; Kopp-Woodroffe et al., 1999; Guebels et al., 2014), while increased body weight and TT_3 have been documented in men refed after caloric restriction (Friedl et al., 2000) and in a non-human primate model of menstrual recovery with increased energy intake (Williams et al., 2001). Because female athletes may be hesitant to gain weight, as demonstrated by a tendency for a high drive for thinness (De Souza et al., 2007; Gibbs et al., 2011), it is important to emphasise that the improvements in menstrual function we observed can occur with relatively small gains in body weight and is in line with the average weight gain of 1.0 kg to 5.3 kg reported by others who tracked the occurrence of menses among oligo/amenorrhoeic athletes during interventions of increased energy intake (Kopp-Woodroffe et al., 1999; Arends et al., 2012; Cialdella-Kam et al. 2014; Guebels et al. 2014; Lagowska et al., 2014a), and in a recent case study which demonstrated that ~ 5 kg increase in body weight was associated with menstrual recovery (Areta, 2020). The success of our intervention, however, appears to be dependent on baseline energetic status, such that the higher the fat mass at baseline, the greater the likelihood of experiencing menses. Similarly, Falsetti et al. (2002) demonstrated that for each one unit (1 kg/m^2) increase in BMI, the probability of recovery of menses in anorexic women with FHA increased by 25%, while a one unit (1 kg/m^2) decrease in BMI doubled the odds of persistent amenorrhoea in anorexic women (Dempfle et al., 2013).

A previous observational study demonstrated that increasing energy intake is associated with the recovery of menstrual function in exercising women with severe menstrual disturbances (Arends *et al.*, 2012), and several published interventions have also demonstrated that increasing energy intake by at least 360 kcal/day leads to recovery of menses in previously oligo/amenorrhoeic exercising women (Dueck *et al.*, 1996; Kopp-Woodroffe *et al.*, 1999; Cialdella-Kam *et al.*, 2014; Guebels *et al.*, 2014; Lagowska *et al.*, 2014a). However, such interventions lacked a control group and had small sample sizes, therefore limiting the generalisability of the findings. In contrast, our participants were randomised into either an intervention or control group which allows for the control of 'spontaneous' recovery of menstrual function due to factors that are not measured or not yet understood. RCTs also control for 'self-selection', such that women who were willing to consume more food, or conversely, less willing to consume more food, were equally distributed among the two groups. An important consideration in randomised trials is the retention of participants. Our drop-out rate did not differ between groups, and there were no significant differences in the key characteristics of age, body weight and BMI when dropouts in the two study groups were compared and likely not different when compared to studies of a shorter duration.

Our intervention was designed to test a modest increase in energy intake, thereby, inducing modest weight gain. We did provide individualised guidance from a nutritionist and a clinical psychologist to support participants' adherence to the intervention. Importantly, future studies that implement strategies to provide more personalised dietary interventions accounting for food preferences (Reed *et al.*, 2011), dietary patterns across the day (Reed *et al.*, 2014), timing of food intake and macronutrient composition may have the potential to be even more effective. Overall, the exercising women in our study, who were primarily recreational athletes, were receptive to the increase in intake and small increases in body weight in order to improve menstrual health.

Because this investigation included both amenorrhoeic and oligomenorrhoeic exercising women, we also investigated the effect of baseline menstrual cycle status on menstrual recovery. Notably, baseline menstrual status did not significantly influence the success of the intervention in our recurrent events survival analyses, demonstrating that the intervention can be generalised to exercising women with a range of exercise-associated menstrual disturbances.

At present, there is a lack of established definitions of 'menstrual recovery'. Investigators have used varying definitions (Pape *et al.*, 2020), including the occurrence of a single menses (Kopp-Woodroffe *et al.*, 1999; Cialdella-Kam *et al.*, 2014), positive

Table II Subgroup analysis of the proportion of amenorrhoeic and oligomenorrhoeic women who recovered menstrual function.

	Recovered	Did NOT recover	P-value
All women			
Oligo/Amen + Cal	64% (18/28)	36% (10/28)	0.001
Oligo/Amen Control	19% (5/26)	81% (21/26)	
Amenorrhoeic women			
Oligo/Amen + Cal	65% (13/20)	35% (7/20)	0.023
Oligo/Amen Control	18% (2/11)	82% (9/11)	
Oligomenorrhoeic women			
Oligo/Amen + Cal	63% (5/8)	37% (3/8)	0.071
Oligo/Amen Control	20% (3/15)	80% (12/15)	

The subgroup analysis excludes women who did not have a clear baseline menstrual status ($n = 5$) and those who dropped out immediately after starting the intervention and before any intervention data were collected, $n = 7$. An additional 10 oligomenorrhoeic women (4 Oligo/Amen + Cal and 6 Control) could not be included due to inadequate time in the study to determine whether recovery criteria were met or not (oligomenorrhoeic women had to be in the study for at least 3 months in order to determine if the criteria for improved menstrual function were met).

ovulation test and two consecutive cycles (Guebels et al., 2014), menses followed by two menstrual cycles <36 days in length (Lagowska et al., 2014), menstrual cycles <~36 days for at least 3 months (Arends et al., 2012), and at least three menses in a 6-month time period (Misra et al., 2008). In non-exercising women with FHA, recovery has been defined as occurrence of spontaneous ovulatory menstrual cycle with/without pregnancy (Falsetti et al., 2002), or menses and evidence of ovulation with $E_2 > 100$ pg/ml and progesterone > 5 ng/ml (Berga et al., 2003), whereas partial recovery has been defined as $E_2 > 60$ pg/ml and $P_4 < 5$ ng/ml (Berga et al., 2003). It is currently unknown which specific definitions are adequate to indicate optimised menstrual recovery. Furthermore, due to the different hormonal patterns associated with amenorrhoea and oligomenorrhoea, it is not clear whether the same definitions of menstrual recovery can be applied to both types of menstrual disturbances. More research is needed, and we will attempt to address this issue in subsequent analyses and publications.

The current study has limitations. Due to the rigorous nature of the REFUEL RCT, there was a relatively high dropout rate. We acknowledge that this dropout rate may affect the generalisability of the results, although the randomised nature of the intervention and the similar dropout rate between groups may have prevented bias. Notably, the dropout rate by month 6 of our intervention (40%) was comparable to that reported by other investigators, (~30%) for an uncontrolled intervention (Guebels et al., 2014); if other investigators had extended their intervention for a full year, it is plausible to speculate that a similarly high dropout rate may have resulted. To account for participant attrition, mixed modelling and recurrent event survival analysis were utilised. Because we observed a statistically significant difference between groups for our primary outcomes in both our intent-to-treat and sub-analyses, it is likely that the study was adequately powered. We acknowledge, however, that the confidence intervals for the ORs are wide, indicating some uncertainty due to the small sample size in the sub-analyses. Furthermore, we acknowledge that the definitions of menstrual recovery and improved menstrual function used herein do not account for the quality of recovery, i.e. change in the

reproductive hormones, which will be explored in future analyses. Lastly, we did not perform ovarian ultrasounds or imaging of the hypothalamic-pituitary region, and we acknowledge there was a lack of racial diversity in our study sample.

Strengths of the REFUEL study is the comprehensive assessment of 'objective' measures over the 12-month RCT intervention, such as measurements of body composition and metabolic hormones that help to confirm metabolic status and daily urinary assessments of ovarian steroids throughout the 12-month study. Importantly, REFUEL is the first and longest dietary intervention and only RCT designed to improve energy status, menstrual cyclicity and bone health in exercising women with exercise-associated menstrual disturbances. A clear highlight of this study is the detailed and prospective assessments of menstrual function with daily urinary reproductive hormones and repeated assessments of anthropometric, energetic and metabolic status.

In summary, our study provides novel and important information regarding the success of a dietary intervention to promote menstrual recovery in exercising amenorrhoeic and oligomenorrhoeic women. We demonstrated that only a modest increase in daily energy intake, equivalent to about 300–350 kcal/day was sufficient to induce menstrual recovery and was associated with a modest degree of weight and fat gain and improved metabolism, as evidenced by an increase in TT_3 , indicative of the key role that energetic recovery plays in menstrual recovery. It is notable that our prescription of increasing dietary energy intake was independent of changes in exercise training. Lastly, in a high proportion of subjects, menstrual recovery occurred within the first 6 months of the intervention. The results of this study will directly inform recommendations for treatment and return-to-play for exercising women with the Female Athlete Triad. Future REFUEL data analyses will inform our understanding of the degree to which recovery of 'menses' corresponds with the 'hormonal quality' of menstrual cyclicity and whether the quality of menstrual recovery depends on the degree of metabolic recovery.

Figure 5. Estimated marginal means and results of the generalised linear fixed effects model for the two study groups. Results are for five time points during the study (Baseline (BL), Month 3, Month 6, Month 9, Post-study) for a) energy intake, b) exercise energy expenditure, c) body weight, d) percent body fat, e) lean mass, f) fat mass, and g) TT_3 . The Oligo/Amen + Cal group is depicted in blue squares; the Oligo/Amen Control group is depicted by red circles. P -values of the fixed effects in the model and study group*time interaction are displayed for each model. *Indicates significant change from baseline ($P < 0.05$). ^aIndicates significant differences between groups at that time point ($P < 0.05$). TT_3 , total triiodothyronine; BW, body weight; FM, fat mass.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

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

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Authors' roles

M.J.D.S. and N.I.W. conceptualised and designed the study, acquired the funding, supervised and executed the study, provided the resources for study completion, supervised data management and data analyses, and supervised and worked on writing and editing the manuscript. R.J.M. was involved in coordination of the project, data collection, data analysis and interpretation, creation of the figures, verification of the data/results and in writing and editing the manuscript. N.C.A.S. was involved in data analysis, creation of the figures, verification of the data/results and in writing

and editing the manuscript. K.J.K. helped with data analysis and writing and editing the manuscript. M.P.O. helped to conceptualise and design the study, was involved with study execution, and edited the manuscript. J.L.S. and H.C.A. were involved in coordination of the project, data collection, data analysis and editing of the manuscript. E.A.R. helped with data analysis and editing of the manuscript. D.J.M. was involved in statistical analysis and creation of the figures. P.K.D. was consulted for statistical analysis and editing of the statistical writing in the manuscript.

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Conflict of interest

All authors report no conflict of interest.

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