Food and Nutrition Sciences, 2026, 17(2), 180-194
https://www.scirp.org/journal/fns
ISSN Online: 2157-9458
ISSN Print: 2157-944X

Consumption of Two Eggs Daily Increases Serum Leptin in Amenorrheic Runners with Low Energy Availability without Changes in Lipid Profile

Lyra Clark[1], Michael Dellogono[1], Thomas A. Wilson[1,2*]

[1]Biomedical Engineering and Biotechnology Program, University of Massachusetts Lowell, Lowell, MA, USA
[2]Department of Biomedical and Nutritional Sciences, University of Massachusetts Lowell, Lowell, MA, USA
Email: *Thomas_Wilson@uml.edu

How to cite this paper: Clark, L., Dellogono, M. and Wilson, T.A. (2026) Consumption of Two Eggs Daily Increases Serum Leptin in Amenorrheic Runners with Low Energy Availability without Changes in Lipid Profile. *Food and Nutrition Sciences*, **17**, 180-194.
https://doi.org/10.4236/fns.2026.172014

Received: December 9, 2025
Accepted: February 6, 2026
Published: February 9, 2026

Abstract

Background/Objective: Low energy availability (EA) occurs in athletes due to inadequate dietary intake to meet the energy expenditure during exercise. A detrimental consequences of low EA in female athletes is suppression of menstrual function leading to chronic hypoestrogenism, which may reduce bone mass while elevating the risk of injury. The primary aim of this study was to investigate the effects of increased dietary cholesterol intake through the consumption of eggs on ovarian sex hormone and leptin levels in amenorrheic female endurance athletes. **Methods:** Division I female distance runners classified as amenorrheic (AMEN, n = 5) or eumenorrheic (EUMEN, n = 5) via self-report questionnaire participated in this study. Participants consumed two eggs per day for 12 weeks over the course of the outdoor track and field season. EA was calculated at baseline and post-intervention using 3-day dietary records, triaxial accelerometers, and heart rate (HR) monitors. Maximal oxygen uptake (VO_2max) was measured to calculate exercise energy expenditure with training HR data. Serum hormones, body composition, and blood lipids were measured at baseline and post-intervention. **Results:** There were no significant differences exhibited between the AMEN and EUMEN groups in body composition, EA, or sex hormones at baseline and after the dietary intervention. However, when examining differences within groups between baseline and post-intervention, serum leptin levels increased in the AMEN group by 70% ($p = 0.02$) compared to baseline following egg consumption, while no change in serum leptin was observed in the EUMEN group. Also, at baseline, EA was calculated to be below 30 kcal·kg^{-1}·FFM·d^{-1} threshold for only the AMEN group, while the EUMEN group was right at the threshold, whereas post-in-

tervention, EA was calculated to be below the threshold in both groups, but not significantly when compared to baseline. Importantly, no changes in serum lipids and lipoprotein cholesterol levels were observed in either treatment group post-intervention with the consumption of eggs compared to baseline. **Conclusions:** Twelve-week consumption of two eggs per day increased serum leptin in amenorrheic athletes with low EA, with no changes in ovarian sex hormones. The serum lipid profile was not adversely affected by increased dietary cholesterol in the form of eggs.

Keywords

Endurance Training, Running, Cholesterol, Estrogen, Leptin

1. Introduction

Low energy availability (EA) is a state that occurs in athletes due to inadequate dietary intake to meet the energy expenditure during exercise in addition to the requirements for optimal metabolic function [1]. Low EA is most commonly observed in endurance athletes with a high caloric expenditure or in activities that emphasize lean body composition for performance or aesthetics [2]. EA relative to physical activity is determined by the difference between energy intake (EI) and exercise energy expenditure (EEE) normalized for fat-free mass (FFM) [3]. After accounting for energy allocated to exercise training, EA is representative of the energy remaining for essential physiologic processes that include cellular maintenance, growth, reproduction, and thermoregulation [4]. As EA decreases due to either changes in energy intake or expenditure, the energy directed towards other metabolic processes decreases accordingly as a survival mechanism to conserve what valuable energy remains [5]. One of the detrimental consequences of low EA below the threshold of 30 $kcal \cdot kg^{-1} \cdot FFM \cdot day^{-1}$ [3] in female athletes is the suppression of menstrual function [6]. Relative energy deficiency in sport (REDs) is the condition that occurs when the energy deficit is severe enough to cause alterations to metabolic rate, menstrual function, immune function, bone health, protein synthesis, and cardiovascular function [7]. Exercise-associated menstrual dysfunction (EAMD) may range in severity from subclinical changes that include luteal phase defects (LPD) or anovulation to the clinical disturbances of oligomenorrhea and amenorrhea [8]. Functional hypothalamic amenorrhea is the most severe case that occurs due to suppression of the hypothalamic-pituitary-ovarian axis, reducing the secretion of gonadotropin releasing hormone (GnRH) and subsequently the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) [9] [10]. The resulting hypoestrogenic state has been linked to secondary adverse effects upon musculoskeletal health, beginning acutely with increased markers of bone resorption and decreased markers of formation [11]. Chronic hypoestrogenism impedes accrual of peak bone mass, thus reducing bone mineral density (BMD) [12]-[14] and elevating the risk of musculoskeletal injury [15]-[17]. Low EA, men-

strual dysfunction, and low BMD characterize the syndrome known as female athlete triad, which involves the presence of one or more of these components [18]. Adverse cardiovascular and metabolic effects have also been associated with FHA, including endothelial and lipid dysfunction [19] [20], reduction in circulating hormones including insulin, leptin, triiodothyronine (T3), and insulin-like growth factor-1 (IGF-1) [12] and reduced sympathetic response to exercise [21].

Cholesterol is the biological precursor in the synthesis of the ovarian sex hormones estrogen and progesterone. In healthy individuals, the bulk of cholesterol is synthesized endogenously while the remaining 30% is obtained through the diet [22]. Cholesterol biosynthesis is energetically demanding and is tightly regulated by the enzyme HMG-CoA reductase (HMGR) in response to metabolic changes [22]. Low EA inhibits HMGR activity, subsequently reducing cholesterol biosynthesis [22]. There is evidence in non-energy-restricted sedentary [23] [24] and low EA exercise-trained [25] animal models that increased dietary cholesterol consumption raises sex hormones, which is hypothesized to occur due to increased amounts of the precursor available for biosynthesis. In female endurance athletes with low EA, it is proposed that supplemental dietary cholesterol may be effective in increasing estrogen and progesterone levels to attenuate some of the adverse effects of FHA.

Hypoleptinemia that is observed in athletes with FHA in comparison to eumenorrheic controls occurs due to the biological action of leptin as a mediator between nutritional status and reproductive function [26] [27]. Leptin levels circulate relative to adipose tissue stores and fluctuate in response to changes in acute energy balance [28]. Elevation in energy intake or lipid stores signals an energy surplus that causes appetite suppression and increased energy expenditure [29]. Conditions of low EA causes suppression in circulating leptin and an absence of diurnal leptin variation, stimulating energy conservation through reduction of thyroid hormones, metabolic rate, and reproductive function [27] [30] [31]. Due to the influence upon reproduction, leptin replacement therapy has been examined as a pharmacological treatment to restore menstruation. Recombinant human leptin administration was successful in the recovery of menstruation and metabolic hormone abnormalities, including unfavorable changes in bone markers without differences in caloric intake [32] [33]. Though the advantages of leptin administration to resume menses in FHA athletes include the avoidance of modifications to exercise training or weight gain, it requires twice-daily injection and dose adjustments to maintain stable weight, which may be invasive or inconvenient for participants. A potential alternative is increased dietary cholesterol intake, given that evidence in animal models demonstrates an associated increase in serum leptin when additional cholesterol is supplemented through the diet [34] [35]. This association has been reported to occur in sedentary, non-energy-restricted animal experiments and has not yet been investigated in exercise-trained human populations.

The aims of this study are to assess the effects of increased dietary cholesterol

through the consumption of eggs upon ovarian sex hormones and leptin in amenorrheic female athletes with and without active controls. The objective is to evaluate the potential for egg consumption as a novel dietary intervention to improve metabolic health in athletes with low EA to prevent any permanent impacts on bone health and fertility.

2. Materials and Methods

2.1. Subjects

Ten Female Division I Student-Athletes from The Women's Cross Country/Track and Field Team (age 20.2 ± 1.0 yrs.) participated in this study (refer to **Table 1** for baseline characteristics). The inclusion criterion for the study was current participation in outdoor track and field in endurance events, defined as 800-meter to 10-kilometer races. Exclusion criteria included chronic illness, use of oral contraceptives, pregnancy, or the inability to participate in exercise training. This study was approved by The University of Massachusetts Lowell Institutional Review Board (15 - 140-WIL-EPD). All subjects signed an informed consent prior to participation in the study. Subjects recruited for participation in the study were a subset of participants who were eligible based on previous cross-sectional survey data [25]. Individuals eligible for the study based on inclusion/exclusion criteria (n = 10) were recruited for participation in the 12-week dietary intervention.

Table 1. Comparison of baseline data in amenorrheic (AMEN) versus eumenorrheic (EUMEN) subjects. Data is displayed as mean ± SD or median [interquartile range].

Variable	AMEN (n = 5)	EUMEN (n = 5)	p Value
BMI ($kg \cdot m^{-2}$)	20.7 ± 1.7	21.1 ± 1.6	0.72
FFM (kg)	47.0 ± 1.9	45.1 ± 2.9	0.25
Body Fat (%)	19.7 ± 2.8	19.7 ± 2.5	0.98
EA ($kcal \cdot kg^{-1} \cdot FFM \cdot d^{-1}$)	28.4 ± 13.2	30.2 ± 11.7	0.82
Estradiol ($pg \cdot mL^{-1}$)	32.8 ± 14.9	40.4 ± 16.0	0.46
Progesterone ($ng \cdot mL^{-1}$)	1.9 [0.7 - 5.4]	2.8 [0.7 - 6.2]	1.00
Leptin ($ng \cdot mL^{-1}$)	4.3 ± 1.7	5.2 ± 2.8	0.56
TC ($mg \cdot dL^{-1}$)	165.2 ± 8.0	169.0 ± 22.5	0.73
LDL-C ($mg \cdot dL^{-1}$)	85.4 ± 7.5	88.8 ± 17.3	0.70
HDL-C ($mg \cdot dL^{-1}$)	64.4 ± 7.5	64.8 ± 9.6	0.94
Triglycerides ($mg \cdot dL^{-1}$)	55.2 ± 23.6	63.2 ± 29.0	0.65

Abbreviations: BMI = body mass index; FFM = fat-free mass; EA = energy availability; TC = total cholesterol, p value represents difference via independent-samples *t* test or Mann-Whitney U-test, *Significant difference ($p < 0.05$) between groups.

2.2. Metabolic Assessment

Metabolic data collection was scheduled over two assessment sessions on separate days. Assessment session I consisted of anthropometric measurement with a plat-

form scale and stadiometer (QuickMedical, Issaquah, WA), fasting venous blood sample collection, body composition analysis with BC-418 Segmental Body Composition Analyzer (Tanita, Arlington Heights, IL), and resting metabolic rate (RMR) measurement using Parvo Medics TrueOne 2400 metabolic cart (Parvo Medics, Sandy, UT). Assessment session I was conducted in the morning hours (700 - 1000) following a 12-hour fast and absence of intense exercise training within the prior 24 hrs. Eumenorrheic female athletes were assessed during the early follicular phase (days 2 - 6 immediately following the onset of menses via self-report) to avoid fluctuations in hormone levels and RMR due to variation in menstrual cycle phase [36]. Assessment session II was conducted in the afternoon (1300 - 1600) and included the measurement of maximal oxygen consumption (VO_2 max) using the standard Bruce Protocol treadmill test using Parvo Medics TrueOne 2400 metabolic cart.

2.3. Assessment of Energy Availability

Dietary energy intake and energy expenditure were measured over 3 consecutive days to calculate energy availability (EA) at baseline and at the end of the 12-week dietary intervention. Subjects completed a 3-day dietary record following education by the researchers and were instructed to maintain their habitual eating patterns and exercise training during the data collection period. ActiGraph GT9X Link (ActiGraph, Pensacola, FL) tri-axial accelerometers were worn by subjects on their non-dominant wrist for the 3-day period to measure non-exercise physical activity thermogenesis (NEAT). Subjects were instructed to wear Polar H7 Heart Rate (HR) Monitors (Polar, Stockholm, Sweden) during all exercise sessions to determine exercise energy expenditure (EEE).

Subject energy intake (EI) was calculated using 3-day dietary record data with the 2013-2014 Version of the Food and Nutrient Database for Dietary Studies (FNDDS; Food Surveys Research Group, Beltsville, MD) [37]. NEAT was calculated using ActiLife Software, version 6.13.3 (ActiGraph). EEE was calculated from the HR data measured during training sessions using corresponding energy expenditure data recorded during VO_2 max testing. EA was calculated by subtraction of EEE from EI normalized for fat-free mass (FFM), or EA = (EI – EEE)/kg FFM expressed in $kcal \cdot kg^{-1} \cdot FFM \cdot d^{-1}$.

2.4. Serum Hormone and Lipid Measurement

Fasting venous blood samples were centrifuged for 15 minutes at 2500 RPM and serum was collected and immediately stored at −80˚C until analysis. Serum estradiol, progesterone, and leptin were measured using enzyme-linked immunoassay (ELISA) kits (ALPCO, Salem, NH) on a microplate reader (Infinite 200 PRO series, Tecan Trading AG, Switzerland). Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) were measured by use of a clinical chemistry auto-analyzer (Medica EasyRA, Bedford, MA).

2.5. Dietary Intervention

Following baseline data collection, subjects were instructed to consume two eggs per day in the form of their choice for a 12-week time period that took place over the course of the outdoor track and field season. Subjects were instructed to otherwise maintain normal eating habits during this intervention period. Egg consumption provided between 328 - 372 mg of supplemental dietary cholesterol daily based on the content supplied by two medium-to-large eggs in the FNDDS [36].

2.6. Statistical Analysis

Statistical analysis was performed using SPSS version 24.0 (IBM, Armonk, NY, USA). Study data is presented as mean ± SD for normal distribution as determined by the Shapiro-Wilk test. Data is alternatively presented as median (interquartile range) for non-normal distribution. The subjects were divided into two groups based on menstrual status for analysis. Subjects were classified in the amenorrheic group (AMEN, n = 5) if self-report revealed the absence of the menstrual cycle for >90 days prior to recruitment into the study or Eumenorrheic (EUMEN, n = 5) if self-report indicated consistent menstrual cycles occurring at 26 to 32-day intervals.

Independent-samples *t* test was used to compare normal data between the AMEN and EUMEN groups at baseline and post-dietary intervention. Mann-Whitney U-test was used to compare non-normal data between groups. Paired-samples *t* test was used to compare data at baseline to data post-dietary intervention within the AMEN and EUMEN groups. Related-samples Wilcoxon Signed Rank Test was used to compare non-normal data within AMEN and EUMEN groups. Statistical significance for all analyses was set at an alpha level of 0.05.

3. Results

At baseline, there were no significant differences between treatment groups in measurements of body composition, energy availability, serum hormones, or serum lipids (**Table 1**). Mean EA in AMEN group was calculated to be 28.4 ± 13.2 kcal·kg^{-1}·FFM·d^{-1}, falling below the threshold of 30 kcal·kg^{-1}·FFM·d^{-1}. Mean EA was slightly at this threshold in EUMEN group at 30.2 ± 11.7, though this difference in comparison to AMEN (+6.1%) was not significant (p = 0.82). Mean serum estradiol, progesterone and leptin levels were lower in AMEN versus EUMEN (−19%, −32%, and −17%, respectively), but these differences were not statistically significant. There was no difference between AMEN and EUMEN groups in serum lipid levels.

There were also no significant differences between AMEN and EUMEN groups in body composition data, energy availability, serum hormones, or serum lipids after the 12-week dietary intervention (**Table 2**). Although both the AMEN and EUMEN groups now had mean EA below the threshold of 30 kcal·kg^{-1}·FFM·d^{-1} after week 12 (25.2 ± 14.3 kcal·kg^{-1}·FFM·d^{-1} and 27.0 ± 4.7 kcal·kg^{-1}·FFM·d^{-1}, re-

spectively). Also, the AMEN group still had lower mean serum estradiol and progesterone levels than the EUMEN group (−24% and −64%, respectively), though these differences were not statistically significant. However, after the 12-week intervention, the AMEN group had slightly higher mean serum leptin levels than the EUMEN group (24%).

Table 2. Comparison of post-intervention data in amenorrheic (AMEN) versus eumenorrheic (EUMEN) subjects. Data is displayed as mean ± SD or median [interquartile range].

Variable	AMEN (n = 5)	EUMEN (n = 5)	p Value
BMI ($kg \cdot m^{-2}$)	20.4 ± 1.4	20.9 ± 1.7	0.75
FFM (kg)	47.0 ± 1.7	46.0 ± 2.9	0.53
Body Fat (%)	20.1 ± 3.6	18.8 ± 6.0	0.69
EA ($kcal \cdot kg^{-1} \cdot FFM \cdot d^{-1}$)	25.2 ± 14.3	27.0 ± 4.7	0.79
Estradiol ($pg \cdot mL^{-1}$)	29.8 ± 11.5	39.4 ± 32.9	0.56
Progesterone ($ng \cdot mL^{-1}$)	2.1 [0.4 - 3.9]	5.8 [2.8 - 13.7]	0.22
Leptin ($ng \cdot mL^{-1}$)	7.3 ± 1.7	5.9 ± 3.5	0.44
TC ($mg \cdot dL^{-1}$)	173.4 ± 18.6	165.4 ± 16.5	0.49
LDL-C ($mg \cdot dL^{-1}$)	94.0 ± 17.7	84.8 ± 9.1	0.33
HDL-C ($mg \cdot dL^{-1}$)	67.0 ± 7.8	69.4 ± 11.3	0.71
Triglycerides ($mg \cdot dL^{-1}$)	59.8 ± 29.6	55.0 ± 10.8	0.74

Abbreviations: BMI = body mass index; FFM = fat-free mass; EA = energy availability; TC = total cholesterol, p value represents difference via independent-samples *t* test or Mann-Whitney U-test, *Significant difference ($p < 0.05$) between groups.

Table 3. Comparison of pre-intervention to post-intervention data within amenorrheic (AMEN) and eumenorrheic (EUMEN) groups.

Variable	Group	Baseline	Week 12	p Value
BMI ($kg \cdot m^{-2}$)	AMEN	20.7 ± 1.7	20.4 ± 1.4	0.84
	EUMEN	21.1 ± 1.6	20.9 ± 1.7	0.79
FFM (kg)	AMEN	47.0 ± 1.9	47.0 ± 1.7	0.97
	EUMEN	45.1 ± 2.9	46.0 ± 2.9	0.46
Body Fat (%)	AMEN	19.7 ± 2.8	20.1 ± 3.6	0.71
	EUMEN	19.7 ± 2.5	18.8 ± 6.0	0.64
EA ($kcal \cdot kg^{-1} \cdot FFM \cdot d^{-1}$)	AMEN	28.4 ± 13.2	25.2 ± 14.3	0.62
	EUMEN	30.2 ± 11.7	27.0 ± 4.7	0.50
Cholesterol Intake ($mg \cdot d^{-1}$)	AMEN	195.8 ± 144.7	388.6 ± 131.7	0.14
	EUMEN	303.9 ± 263.2	343.1 ± 271.9	0.40
Estradiol ($pg \cdot mL^{-1}$)	AMEN	32.8 ± 14.9	29.8 ± 11.5	0.72
	EUMEN	40.4 ± 16.0	39.4 ± 32.9	0.91
Progesterone ($ng \cdot mL^{-1}$)	AMEN	1.9 [0.7 - 5.4]	2.1 [0.4 - 3.9]	0.89
	EUMEN	2.8 [0.7 - 6.2]	5.8 [2.8 - 13.7]	0.23

Continued

Leptin (ng·mL^{-1})	AMEN	4.3 ± 1.7	7.3 ± 1.7	0.02*
	EUMEN	5.2 ± 2.8	6.2 ± 4.0	0.37
TC (mg·dL^{-1})	AMEN	165.2 ± 8.0	173.4 ± 18.6	0.28
	EUMEN	169.0 ± 22.5	165.4 ± 16.5	0.63
LDL-C (mg·dL^{-1})	AMEN	85.4 ± 7.5	94.0 ± 17.7	0.17
	EUMEN	88.8 ± 17.3	84.8 ± 9.1	0.59
HDL-C (mg·dL^{-1})	AMEN	64.4 ± 7.5	67.0 ± 7.8	0.20
	EUMEN	64.8 ± 9.5	69.4 ± 11.3	0.19
Triglycerides (mg·dL^{-1})	AMEN	55.2 ± 23.6	59.8 ± 29.6	0.79
	EUMEN	63.2 ± 29.0	55.0 ± 10.8	0.49

Abbreviations: BMI = body mass index; FFM = fat-free mass; EA = energy availability; TC = total cholesterol, p value represents difference via paired-samples *t* test or related-samples Wilcoxon Signed Rank Test, *Significant difference ($p < 0.05$) between groups.

When comparing the pre-intervention and post-intervention data within groups there were still no significant differences in body composition, serum estradiol and progesterone levels, or blood lipids, however, there was a significant increase in serum leptin levels in the AMEN but not with the EUMEN group (Table 3). This difference in serum leptin level in the AMEN group, increased from 4.3 ± 1.7 ng·dL to 7.3 ± 1.7 ng·dL at week 12 of the dietary intervention (+70%, $p = 0.02$). Whereas, there was no significant change in serum leptin in EUMEN subjects (19%). EA decreased similarly within both AMEN and EUMEN groups (−11%) over the 12 weeks. As far as the dietary intervention of consuming 2 whole eggs per day for 12 weeks, the mean daily cholesterol intake increased greatly in the AMEN group (+98%) but only slightly in the EUMEN group (13%), but not statistically significant ($p = 0.14$ and $p = 0.40$, respectively). In a follow-up interview regarding menstrual status, it was revealed that one of five amenorrheic athletes regained menses by the end of the 12 week dietary intervention period.

4. Discussion

This is the first known study to assess the effects of supplemental dietary cholesterol in amenorrheic female athletes upon ovarian sex hormones and leptin. The present study was conducted prospectively in free-living conditions with no alterations to exercise training in participants. Though increased exogenous cholesterol intake did not increase serum estrogen or progesterone, an increase in serum leptin occurred in amenorrheic athletes following the 12-week dietary intervention. No adverse effects of the increased cholesterol intake upon lipid profile were observed.

Supplementary dietary cholesterol was consumed by subjects in the form of eggs, which in addition to supplying numerous nutrients are inexpensive and easily obtained and prepared. Eggs are an ideal food to be used to increase exogenous

cholesterol intake as they are high in dietary cholesterol without excessive amounts of saturated fat, as in other animal products. The high bioavailability of egg protein [38] provides essential amino acids (EAAs) that aid muscle protein synthesis, which may preserve lean body mass and facilitate recovery from intense training [39]-[41]. Eggs provide several B-vitamins required by energy production through cellular respiration in the body, including thiamin, riboflavin, folate, B_6, and B_{12} [42]. In addition, eggs contain high amounts of leucine, an EAA that mediates skeletal muscle glucose uptake in tandem with the insulin signaling pathway [43]. Increased levels of intracellular leucine enable glucose-sparing and blood sugar maintenance during exercise and energy restriction [42], which may enhance endurance performance in female athletes with FHA [44].

An increase in serum leptin was observed in amenorrheic female athletes despite persistence of energy deficiency as determined by EA below 30 $kcal \cdot kg^{-1} \cdot FFM \cdot d^{-1}$ both at baseline and post-intervention. Leptin levels are responsive to both changes in acute energy balance and adipose tissue stores [28], both of which remained stable in study participants over 12 weeks. The clinical relevance of this elevation in leptin has been established by previous literature in amenorrheic female athletes, where it has been proposed as a treatment to restore menstruation and improve bone health [32] [33]. The first prospective interventional study administering twice-daily intravenous recombinant leptin treatment reported efficacy in stimulating follicular growth and ovulation, increasing mean LH and LH pulse frequency, estradiol, IGF-1, thyroid hormone, and markers of bone formation with no change in markers of resorption [32]. A second interventional study also reported stimulation of menses, an increase in estradiol and progesterone, and favorable changes in bone markers corresponding to increases in serum leptin [33]. These studies show how serum leptin levels change the hormones involved in the Activin-Follistatin-Inhibin Axis (AFI axis), where females with HA demonstrate lower circulating levels of Activins and FSTL3, and higher levels of FST compared to healthy women [45]. Although the exact mechanism of action has not been observed, Activins and Inhibins belong to the transforming growth factor beta (TGF-β) family, and are highly expressed in the pituitary gland, gonads, placenta, and corpus luteum [45] [46]. Activins enhance pituitary follicle-stimulating hormone (FSH) secretion, whereas Inhibins have an opposite action on FSH secretion [45] [46]. Hence, Activins and Inhibins both play a role in the regulation of the menstrual cycle. At the same time, they have paracrine effects associated with ovarian follicular development and steroidogenesis, which are important processes of reproductive function [45] [46]. Further prospective research should be conducted to assess the benefit of elevations in serum leptin due to dietary cholesterol consumption upon bone markers in female athletes with FHA.

Serum estradiol and progesterone did not change in response to the 12-week dietary cholesterol intervention. This data contrasts with literature examining increased dietary cholesterol in animal models that produced elevations in ovarian steroid hormones as early as 4 weeks in carp (24) and 9 weeks in ovariectomized

mice [23]. It should be noted that these studies were conducted in sedentary animal populations fed ad libitum without significant physical energy expenditure. Data in exercise-trained animal populations with supplemental dietary cholesterol is also limited, though a 12-week prospective study showed an increase in serum estradiol in female exercise-trained rats receiving a high-cholesterol diet [25]. Though it was hypothesized that an increase in serum leptin would increase ovarian hormones accordingly, the duration of the intervention may not have been adequate to allow neuroendocrine adaptations in response to energy homeostasis to occur. Previous literature regarding intravenous leptin treatment measured substantial elevations in serum leptin after 12 weeks; treatment did not elicit changes in estradiol or progesterone until assessment after 24 weeks of treatment [33]. Dietary cholesterol supplementation extended over a longer period should be conducted to determine if leptin increases will effectively induce other favorable metabolic and hormonal changes accordingly.

There were no unfavorable changes observed in blood lipid levels of amenorrheic or eumenorrheic participants in response to the increased dietary cholesterol intervention involving the consumption of two eggs per day. This is the first known study assessing the impact of egg consumption on lipid profile in amenorrheic female athletes. There has been much controversy over the past several decades regarding the impact of dietary cholesterol upon blood lipids, though the regulation of cholesterol homeostasis is now better understood. An increase in dietary cholesterol intake reduces endogenous biosynthesis due to activation of the negative feedback loop that suppresses HMGR activity [47]. The most recent version of Dietary Guidelines for Americans has been updated to remove the previous recommendations to limit daily cholesterol intake [48]. Literature examining the effect of egg consumption on cardiovascular health report absence of correlation with the risk for coronary artery disease and stroke in non-diabetic individuals [49] [50]. Investigation of the impact upon genes involved with regulation of cholesterol homeostasis indicated that consumption of three eggs per day did not impact total cholesterol or genes involving cholesterol uptake and transport in healthy individuals [51]. In addition, research conducted in overweight males consuming three eggs per day on a carbohydrate-restricted diet had increases in HDL-C without a change in LDL-C [52]. Though cholesterol absorption varies greatly among individuals [53], previous studies have shown a mean increase of 2.2 $mg \cdot dL^{-1}$ in plasma total cholesterol per every 100 $mg \cdot day^{-1}$ increase [54]. Hyper responders, comprising approximately 25% of the population, experience increases in both LDL-C and HDL-C; however, the LDL-C/HDL-C ratio remains constant [55] [56].

Several limitations of the current study should be noted. Menstrual status of the participants was determined through self-report, and no other markers of fertility or hypothalamic-pituitary-ovarian axis function were assessed for confirmation. The dietary intervention was a 12-week prospective study that may not have been of adequate duration to measure changes in ovarian sex hormones in response to the elevation in serum leptin. The group sizes in the current study were limited

due to the exclusion of a high number of subjects due to oral contraceptive use, and therefore may not have been adequate to provide statistical power to detect differences in sex hormones. Due to the small number of available eligible study participants, no amenorrheic or eumenorrheic control groups without egg consumption were included. However, it is of note that one of five amenorrheic athletes recovered menses at the end of the dietary intervention, which warrants further investigation. Future prospective studies involving egg consumption in amenorrheic athletes with group sizes of adequate statistical power to determine further impacts upon sex hormones and/or specific changes in menstrual status for a longer time period than 12 weeks should be conducted. In addition, the impact of increases in serum leptin on bone markers and other markers of metabolic status due to egg consumption should also be assessed.

In conclusion, twelve-week consumption of two eggs per day increased serum leptin in energy-deficient amenorrheic athletes despite the presence of low EA. Egg consumption had no effect on estradiol or progesterone regardless of changes in leptin. Serum lipids were not adversely affected by the increase in dietary cholesterol. Further study to determine the effects of increased dietary cholesterol upon ovarian sex hormones over a longer duration is warranted, as this would be a potential nutritional treatment to improve metabolic health for amenorrheic athletes.

Acknowledgements

This study was supported by the Egg Nutrition Center Graduate Fellowship Research Grant.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Loucks, A.B., Kiens, B. and Wright, H.H. (2011) Energy Availability in Athletes. *Journal of Sports Sciences*, **29**, S7-S15. https://doi.org/10.1080/02640414.2011.588958

[2] Fahrenholtz, I.L., Melin, A.K., Wasserfurth, P., Stenling, A., Logue, D., Garthe, I., *et al.* (2022) Risk of Low Energy Availability, Disordered Eating, Exercise Addiction, and Food Intolerances in Female Endurance Athletes. *Frontiers in Sports and Active Living*, **4**, Article ID: 869594. https://doi.org/10.3389/fspor.2022.869594

[3] Loucks, A.B. and Thuma, J.R. (2003) Luteinizing Hormone Pulsatility Is Disrupted at a Threshold of Energy Availability in Regularly Menstruating Women. *The Journal of Clinical Endocrinology & Metabolism*, **88**, 297-311. https://doi.org/10.1210/jc.2002-020369

[4] Wade, G.N. and Schneider, J.E. (1992) Metabolic Fuels and Reproduction in Female Mammals. *Neuroscience & Biobehavioral Reviews*, **16**, 235-272. https://doi.org/10.1016/s0149-7634(05)80183-6

[5] Wade, G.N., Schneider, J.E. and Li, H.Y. (1996) Control of Fertility by Metabolic Cues. *American Journal of Physiology-Endocrinology and Metabolism*, **270**, E1-E19.

https://doi.org/10.1152/ajpendo.1996.270.1.e1

[6] Williams, N.I., Helmreich, D.L., Parfitt, D.B., Caston-Balderrama, A. and Cameron, J.L. (2001) Evidence for a Causal Role of Low Energy Availability in the Induction of Menstrual Cycle Disturbances during Strenuous Exercise Training. *The Journal of Clinical Endocrinology & Metabolism*, **86**, 5184-5193. https://doi.org/10.1210/jcem.86.11.8024

[7] Gallant, T.L., Ong, L.F., Wong, L., Sparks, M., Wilson, E., Puglisi, J.L., *et al.* (2025) Low Energy Availability and Relative Energy Deficiency in Sport: A Systematic Review and Meta-Analysis. *Sports Medicine*, **55**, 325-339. https://doi.org/10.1007/s40279-024-02130-0

[8] De Souza, M.J., Toombs, R.J., Scheid, J.L., O'Donnell, E., West, S.L. and Williams, N.I. (2010) High Prevalence of Subtle and Severe Menstrual Disturbances in Exercising Women: Confirmation Using Daily Hormone Measures. *Human Reproduction*, **25**, 491-503. https://doi.org/10.1093/humrep/dep411

[9] Berga, S.L., Mortola, J.F., Girton, L., Suh, B., Laughlin, G., Pham, P., *et al.* (1989) Neuroendocrine Aberrations in Women with Functional Hypothalamic Amenorrhea. *The Journal of Clinical Endocrinology & Metabolism*, **68**, 301-308. https://doi.org/10.1210/jcem-68-2-301

[10] Meczekalski, B., Podfigurna-Stopa, A., Warenik-Szymankiewicz, A. and Genazzani, A.R. (2008) Functional Hypothalamic Amenorrhea: Current View on Neuroendocrine Aberrations. *Gynecological Endocrinology*, **24**, 4-11. https://doi.org/10.1080/09513590701807381

[11] Ihle, R. and Loucks, A.B. (2004) Dose-Response Relationships between Energy Availability and Bone Turnover in Young Exercising Women. *Journal of Bone and Mineral Research*, **19**, 1231-1240. https://doi.org/10.1359/jbmr.040410

[12] De Souza, M.J. and Williams, N.I. (2005) Beyond Hypoestrogenism in Amenorrheic Athletes: Energy Deficiency as a Contributing Factor for Bone Loss. *Current Sports Medicine Reports*, **4**, 38-44. https://doi.org/10.1097/01.csmr.0000306070.67390.cb

[13] De Souza, M.J., West, S.L., Jamal, S.A., Hawker, G.A., Gundberg, C.M. and Williams, N.I. (2008) The Presence of Both an Energy Deficiency and Estrogen Deficiency Exacerbate Alterations of Bone Metabolism in Exercising Women. *Bone*, **43**, 140-148. https://doi.org/10.1016/j.bone.2008.03.013

[14] Mallinson, R.J., Williams, N.I., Hill, B.R. and De Souza, M.J. (2013) Body Composition and Reproductive Function Exert Unique Influences on Indices of Bone Health in Exercising Women. *Bone*, **56**, 91-100. https://doi.org/10.1016/j.bone.2013.05.008

[15] Kelsey, J.L., Bachrach, L.K., Procter-Gray, E., Nieves, J., Greendale, G.A., Sowers, M., *et al.* (2007) Risk Factors for Stress Fracture among Young Female Cross-Country Runners. *Medicine & Science in Sports & Exercise*, **39**, 1457-1463. https://doi.org/10.1249/mss.0b013e318074e54b

[16] Barrack, M.T., Gibbs, J.C., De Souza, M.J., Williams, N.I., Nichols, J.F., Rauh, M.J., *et al.* (2014) Higher Incidence of Bone Stress Injuries with Increasing Female Athlete Triad-Related Risk Factors. *The American Journal of Sports Medicine*, **42**, 949-958. https://doi.org/10.1177/0363546513520295

[17] Nattiv, A., Kennedy, G., Barrack, M.T., Abdelkerim, A., Goolsby, M.A., Arends, J.C., *et al.* (2013) Correlation of MRI Grading of Bone Stress Injuries with Clinical Risk Factors and Return to Play: A 5-Year Prospective Study in Collegiate Track and Field Athletes. *The American Journal of Sports Medicine*, **41**, 1930-1941. https://doi.org/10.1177/0363546513490645

[18] Nattiv, A., Loucks, A.B., Manore, M.M., Sanborn, C.F., Sundgot-Borgen, J. and War-

ren, M.P. (2007) American College of Sports Medicine Position Stand. The Female Athlete Triad. *Medicine & Science in Sports & Exercise*, **39**, 1867-1882.

[19] Friday, K.E., Drinkwater, B.L., Bruemmer, B., Chesnut, C. and Chait, A. (1993) Elevated Plasma Low-Density Lipoprotein and High-Density Lipoprotein Cholesterol Levels in Amenorrheic Athletes: Effects of Endogenous Hormone Status and Nutrient Intake. *The Journal of Clinical Endocrinology & Metabolism*, **77**, 1605-1609. https://doi.org/10.1210/jcem.77.6.8263148

[20] Rickenlund, A., Eriksson, M.J., Schenck-Gustafsson, K. and Hirschberg, A.L. (2005) Amenorrhea in Female Athletes Is Associated with Endothelial Dysfunction and Unfavorable Lipid Profile. *The Journal of Clinical Endocrinology & Metabolism*, **90**, 1354-1359. https://doi.org/10.1210/jc.2004-1286

[21] Schaal, K., van Loan, M.D. and Casazza, G.A. (2011) Reduced Catecholamine Response to Exercise in Amenorrheic Athletes. *Medicine & Science in Sports & Exercise*, **43**, 34-43. https://doi.org/10.1249/mss.0b013e3181e91ece

[22] Lieberman, M. and Marks, A.D. (2012) Cholesterol Synthesis. In: Peet, A, Ed., *Marks' Basic Medical Biochemistry*, 4th Edition, Wolters Kluwer, 630-632.

[23] Li, L., Xiao, N., Yang, X., Gao, J., Ding, J., Wang, T., *et al.* (2012) A High Cholesterol Diet Ameliorates Hippocampus-Related Cognitive and Pathological Deficits in Ovariectomized Mice. *Behavioural Brain Research*, **230**, 251-258. https://doi.org/10.1016/j.bbr.2012.02.024

[24] Peyghan, R., Gooraninejad, S., Shahriari, A. and Jamshidi, Z. (2012) Feeding Effect of Cholesterol in the Diet on Sex Hormones Concentrations and the Gonads' Growth of Yearling Common Carp. *Iranian Journal of Veterinary Medicine*, **6**, 23-28.

[25] Clark, L.R., Dellogono, M.J., Mangano, K.M. and Wilson, T.A. (2018) Clinical Menstrual Dysfunction Is Associated with Low Energy Availability but Not Dyslipidemia in Division I Female Endurance Runners. *Journal of Exercise Physiology Online*, **21**, 265-276.

[26] Londraville, R.L., Macotela, Y., Duff, R.J., Easterling, M.R., Liu, Q. and Crespi, E.J. (2014) Comparative Endocrinology of Leptin: Assessing Function in a Phylogenetic Context. *General and Comparative Endocrinology*, **203**, 146-157. https://doi.org/10.1016/j.ygcen.2014.02.002

[27] Kyriakidis, M., Caetano, L., Anastasiadou, N., Karasu, T. and Lashen, H. (2016) Functional Hypothalamic Amenorrhoea: Leptin Treatment, Dietary Intervention and Counselling as Alternatives to Traditional Practice—Systematic Review. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, **198**, 131-137. https://doi.org/10.1016/j.ejogrb.2016.01.018

[28] Hill, J.W., Elmquist, J.K. and Elias, C.F. (2008) Hypothalamic Pathways Linking Energy Balance and Reproduction. *American Journal of Physiology-Endocrinology and Metabolism*, **294**, E827-E832. https://doi.org/10.1152/ajpendo.00670.2007

[29] Dardeno, T.A., Chou, S.H., Moon, H., Chamberland, J.P., Fiorenza, C.G. and Mantzoros, C.S. (2010) Leptin in Human Physiology and Therapeutics. *Frontiers in Neuroendocrinology*, **31**, 377-393. https://doi.org/10.1016/j.yfrne.2010.06.002

[30] Laughlin, G.A. and Yen, S.S.C. (1997) Hypoleptinemia in Women Athletes: Absence of a Diurnal Rhythm with Amenorrhea. *The Journal of Clinical Endocrinology & Metabolism*, **82**, 318-321. https://doi.org/10.1210/jcem.82.1.3840

[31] Chan, J.L., Heist, K., DePaoli, A.M., Veldhuis, J.D. and Mantzoros, C.S. (2003) The Role of Falling Leptin Levels in the Neuroendocrine and Metabolic Adaptation to Short-Term Starvation in Healthy Men. *Journal of Clinical Investigation*, **111**, 1409-

1421. https://doi.org/10.1172/jci200317490

[32] Welt, C.K., Chan, J.L., Bullen, J., Murphy, R., Smith, P., DePaoli, A.M., *et al.* (2004) Recombinant Human Leptin in Women with Hypothalamic Amenorrhea. *New England Journal of Medicine*, **351**, 987-997. https://doi.org/10.1056/nejmoa040388

[33] Chou, S.H., Chamberland, J.P., Liu, X., Matarese, G., Gao, C., Stefanakis, R., *et al.* (2011) Leptin Is an Effective Treatment for Hypothalamic Amenorrhea. *Proceedings of the National Academy of Sciences*, **108**, 6585-6590. https://doi.org/10.1073/pnas.1015674108

[34] Zhao, S. and Wu, Z. (2005) Atorvastatin Reduces Serum Leptin Concentration in Hypercholesterolemic Rabbits. *Clinica Chimica Acta*, **360**, 133-140. https://doi.org/10.1016/j.cccn.2005.04.021

[35] Huang, Q., He, B., Yang, F., Zeng, H. and Zhao, Q. (2012) Effect of High-Cholesterol Diet on Serum Leptin and Blood Lipid in Rabbits. *Journal of Animal and Veterinary Advances*, **11**, 1719-1721. https://doi.org/10.3923/javaa.2012.1719.1721

[36] Day, D.S., Gozansky, W.S., Van Pelt, R.E., Schwartz, R.S. and Kohrt, W.M. (2005) Sex Hormone Suppression Reduces Resting Energy Expenditure and β-Adrenergic Support of Resting Energy Expenditure. *The Journal of Clinical Endocrinology & Metabolism*, **90**, 3312-3317. https://doi.org/10.1210/jc.2004-1344

[37] Montville, J.B., Ahuja, J.K.C., Martin, C.L., Heendeniya, K.Y., Omolewa-Tomobi, G., Steinfeldt, L.C., *et al.* (2013) USDA Food and Nutrient Database for Dietary Studies (FNDDS), 5.0. *Procedia Food Science*, **2**, 99-112. https://doi.org/10.1016/j.profoo.2013.04.016

[38] Schaafsma, G. (2000) The Protein Digestibility-Corrected Amino Acid Score. *The Journal of Nutrition*, **130**, 1865S-1867S. https://doi.org/10.1093/jn/130.7.1865s

[39] Wolfe, R.R. (2006) The Underappreciated Role of Muscle in Health and Disease. *The American Journal of Clinical Nutrition*, **84**, 475-482. https://doi.org/10.1093/ajcn/84.3.475

[40] Norton, L.E. and Layman, D.K. (2006) Leucine Regulates Translation Initiation of Protein Synthesis in Skeletal Muscle after Exercise. *The Journal of Nutrition*, **136**, 533S-537S. https://doi.org/10.1093/jn/136.2.533s

[41] Rodriguez, N.R., Vislocky, L.M. and Gaine, P.C. (2007) Dietary Protein, Endurance Exercise, and Human Skeletal-Muscle Protein Turnover. *Current Opinion in Clinical Nutrition and Metabolic Care*, **10**, 40-45. https://doi.org/10.1097/mco.0b013e3280115e3b

[42] Layman, D.K. and Rodriguez, N.R. (2009) Egg Protein as a Source of Power, Strength, and Energy. *Nutrition Today*, **44**, 43-48. https://doi.org/10.1097/nt.0b013e3181959cb2

[43] Layman, D.K. and Baum, J.I. (2004) Dietary Protein Impact on Glycemic Control during Weight Loss. *The Journal of Nutrition*, **134**, 968S-973S. https://doi.org/10.1093/jn/134.4.968s

[44] Layman, D.K. and Walker, D.A. (2006) Potential Importance of Leucine in Treatment of Obesity and the Metabolic Syndrome. *The Journal of Nutrition*, **136**, 319S-323S. https://doi.org/10.1093/jn/136.1.319s

[45] Bouzoni, E., Perakakis, N. and Mantzoros, C.S. (2020) Circulating Profile of Activin-Follistatin-Inhibin Axis in Women with Hypothalamic Amenorrhea in Response to Leptin Treatment. *Metabolism*, **113**, Article 154392. https://doi.org/10.1016/j.metabol.2020.154392

[46] Hall, J.E. (2019) Neuroendocrine Control of the Menstrual Cycle. In: Strauss, III, J.F.

and Barbieri, R.L., Eds., *Yen and Jaffe's Reproductive Endocrinology*, Elsevier, 149-166.e5. https://doi.org/10.1016/b978-0-323-47912-7.00007-x

[47] Lee, A. and Griffin, B. (2006) Dietary Cholesterol, Eggs and Coronary Heart Disease Risk in Perspective. *Nutrition Bulletin*, **31**, 21-27. https://doi.org/10.1111/j.1467-3010.2006.00543.x

[48] US Department of Health and Human Services and US Department of Agriculture (2015) 2015-2020 Dietary Guidelines for Americans (8th Edition).

[49] Kritchevsky, S.B. (2004) A Review of Scientific Research and Recommendations Regarding Eggs. *Journal of the American College of Nutrition*, **23**, 596S-600S. https://doi.org/10.1080/07315724.2004.10719429

[50] Qureshi, A.I., Suri, F.K., Ahmed, S., Nasar, A., Divani, A.A. and Kirmani, J.F. (2007) Regular Egg Consumption Does Not Increase the Risk of Stroke and Cardiovascular Diseases. *Medical Science Monitor*, **13**, CR1-8.

[51] DiMarco, D.M., Missimer, A., Murillo, A.G., Lemos, B.S., Malysheva, O.V., Caudill, M.A., *et al.* (2017) Intake of up to 3 Eggs/Day Increases HDL Cholesterol and Plasma Choline While Plasma Trimethylamine-*N*-Oxide Is Unchanged in a Healthy Population. *Lipids*, **52**, 255-263. https://doi.org/10.1007/s11745-017-4230-9

[52] Mutungi, G., Ratliff, J., Puglisi, M., Torres-Gonzalez, M., Vaishnav, U., Leite, J.O., *et al.* (2008) Dietary Cholesterol from Eggs Increases Plasma HDL Cholesterol in Overweight Men Consuming a Carbohydrate-Restricted Diet. *The Journal of Nutrition*, **138**, 272-276. https://doi.org/10.1093/jn/138.2.272

[53] Grundy, S.M. (1983) Absorption and Metabolism of Dietary Cholesterol. *Annual Review of Nutrition*, **3**, 71-96. https://doi.org/10.1146/annurev.nu.03.070183.000443

[54] McNamara, D. (2000) Dietary Cholesterol and Atherosclerosis. *Biochimica et Biophysica Acta* (*BBA*)*-Molecular and Cell Biology of Lipids*, **1529**, 310-320. https://doi.org/10.1016/s1388-1981(00)00156-6

[55] Herron, K.L., Vega-Lopez, S., Conde, K., Ramjiganesh, T., Roy, S., Shachter, N.S., *et al.* (2002) Pre-Menopausal Women, Classified as Hypo- or Hyper-Responders, Do Not Alter Their LDL/HDL Ratio Following a High Dietary Cholesterol Challenge. *Journal of the American College of Nutrition*, **21**, 250-258. https://doi.org/10.1080/07315724.2002.10719218

[56] Herron, K.L., Vega-Lopez, S., Ramjiganesh, T., Fernandez, M.L., Conde, K. and Shachter, N.S. (2003) Men Classified as Hypo- or Hyperresponders to Dietary Cholesterol Feeding Exhibit Differences in Lipoprotein Metabolism. *The Journal of Nutrition*, **133**, 1036-1042. https://doi.org/10.1093/jn/133.4.1036