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# Effects of transdermal estrogen on body composition in adolescent female athletes

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BOSTON UNIVERSITY  
SCHOOL OF MEDICINE

Thesis

**EFFECTS OF TRANSDERMAL ESTROGEN ON BODY COMPOSITION IN  
ADOLESCENT FEMALE ATHLETES**

by

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Submitted in partial fulfillment of the  
requirements for the degree of  
Master of Arts

2013

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## **ACKNOWLEDGEMENTS**

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ADOLESCENT FEMALE ATHLETES**

**MARY SIMS**

Boston University School of Medicine, 2013

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**ABSTRACT**

**OBJECTIVE.** The effect of transdermal estrogen on body composition in adolescent female athletes with oligo-/amenorrhea has yet to be examined. This is important because female amenorrheic athletes often express a reluctance to take replacement estrogen given concerns that this will cause weight gain and accumulation of body fat. In this study we performed a randomized, placebo-controlled study to examine the effects of transdermal estrogen on body composition parameters, specifically fat mass and lean mass, in this specific population. We hypothesized that body composition does not change in adolescent athletes with oligo-/amenorrhea receiving transdermal estrogen when compared to no estrogen.

**METHODS.** In a cross-sectional study, we examined baseline characteristics of 51 athletes with oligo-/amenorrhea, 24 athletes with eumenorrhea, and 23 non-

athlete control subjects. Of the 51 athletes with oligo-/amenorrhea, 11 were randomized to no estrogen and 8 were randomized to receive transdermal estrogen for a period of 6 months. Changes in body composition parameters were assessed. Subjects were 14 to 21 years of age.

RESULTS. Athletes with oligo-/amenorrhea had lower weight, BMI, fat mass, lean mass, trunk fat, and % body fat when compared with athletes with eumenorrhea and non-athlete controls at baseline. Athletes with oligo-/amenorrhea randomized to transdermal estrogen (OAM E+) did not differ from athletes randomized to placebo (OAM E-) after 6 months for changes in weight, BMI, fat mass, or lean mass.

CONCLUSIONS. Our results support our hypothesis that transdermal estrogen does not change body composition parameters in adolescent athletes with oligo-/amenorrhea. After assessing our data we believe further studies are necessary to determine the effects transdermal estrogen in this subset of athletes.

## TABLE OF CONTENTS

Title	i
Reader's Approval Page	ii
Acknowledgements	iii
Abstract	iv
Table of Contents	v
List of Tables	vi
List of Figures	vii
List of Abbreviations	viii
Introduction	1
The Female Athlete Triad	1
Hormone Replacement Therapy	7
Bone Mineral Density	7
Body Composition	9
Methods	12
Subject Selection	12
Study Treatment	14
Experimental Protocol	15
Body-Composition Measurements	16
Statistical Analysis	16
Results	18

Baseline Characteristics	18
Changes in Body Composition Parameters	21
Discussion	26
Baseline Findings	26
Comparison of Oral and Transdermal Estrogen Treatment	30
Limitations	32
Future Studies	33
Conclusion	35
References	36
Vita	40

## LIST OF TABLES

Table	Title	Page
1	BMD Definitions	7
2	Baseline Characteristics of Oligo-/amenorrheic Athletes (OAM), Eumenorrheic Athletes (EA), and non-athlete controls (HC)	19
3	Changes in Body Composition Parameters in OAM E+ versus OAM E-	22
4	Multivariate Analysis Results	24
5	Within Group Analysis of OAM E+ and OAM E- Groups Body Composition Parameters After 6 Months of Treatment.	24

## LIST OF FIGURES

Figure	Title	Page
1	The Spectrum Proposed for the Female Athlete Triad by the American College of Sports Medicine	2
2	Regulation of the Reproductive Axis by the Hypothalamus	5
3	Baseline Characteristics with 3-group Comparison Results	20,21
4	Percent Changes in Body Composition Parameters in OAM E+ versus OAM E-	22
5	Associations Between Changes in Weight and Changes in Fat Mass	23
6	Mean Differences from Within Group Analysis of Body Composition Parameters	25
7	The Hormonal Mechanism of Leptin Secretion and Female Reproductive Consequences	29

## ABBREVIATIONS

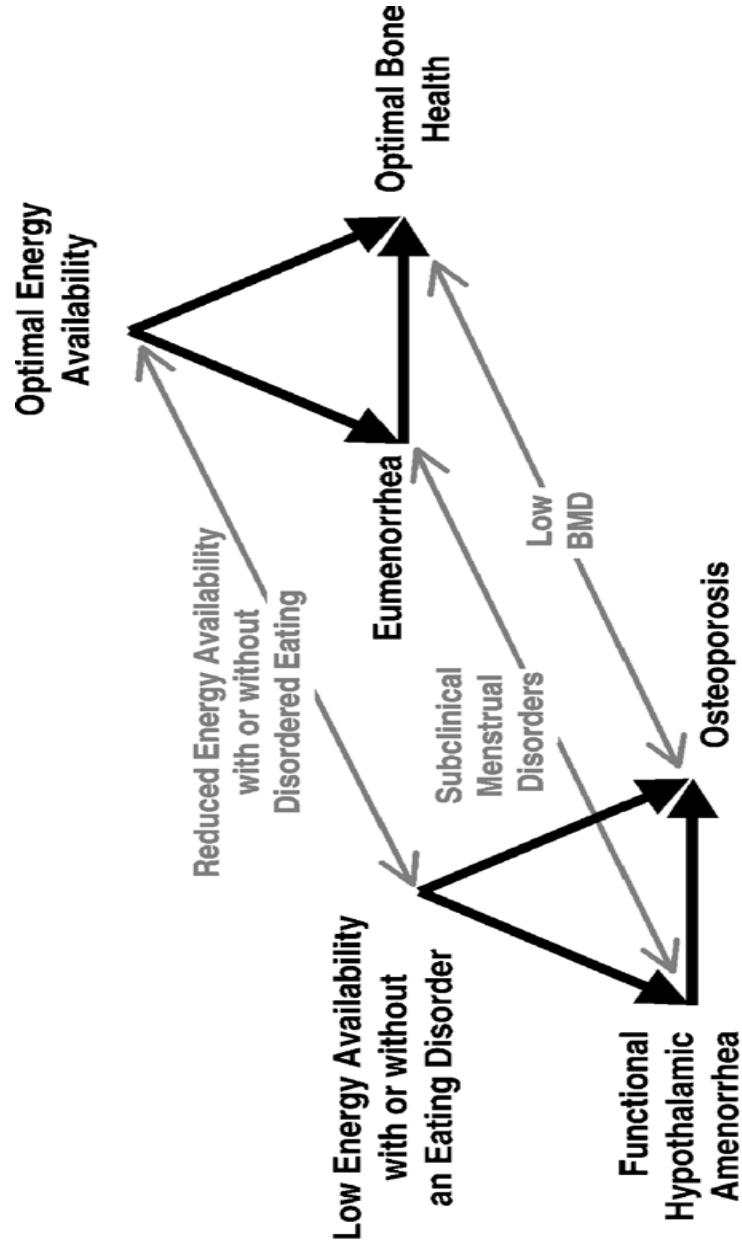
AA	oligo-/amenorrheic athletes
ACSM	American College of Sports Medicine
ALT	alanine amino transferase
AST	aspartateaminotransferase
BMD	bone mineral density
BMI	body mass index
BN	bulimia nervosa
CRH	corticotropin-releasing hormone
DXA	dual X-ray absorptiometry
EA	eumenorrheic athletes
FHA	functional hypothalamic amenorrhea
FSH	follicle-stimulating hormone
GH	growth hormone
GnRH	Gonadotropin releasing hormone
HC	non-athlete healthy controls
IGF-1	Insulin-like growth factor-
K	potassium
LH	Luteinizing hormone
MGH	Massachusetts General Hospital
OAM	Oligo-/amenorrheic
OCP	oral contraceptive pills

OAM E+	Olig-/amenorrheic with estrogen
OAM E-	olig-/amenorrheic without estrogen
PBM	peak bone mass
PCOS	Polycystic Ovarian Syndrome
TSH	thyroid stimulation hormone

## **INTRODUCTION**

### **The Female Athlete Triad**

The female athlete triad refers to a syndrome common among female athletes due to the interrelation of three clinical problems. The components of the triad include low energy availability, menstrual dysfunction, and decreased bone mineral density (BMD) (Nazem & Ackerman, 2012). A recent publication from the American College of Sports Medicine (ACSM) on the state of knowledge of the triad presented each component as the pathological end of a spectrum ranging from normal to frankly abnormal. The other end of the spectrum, referred to as the healthy end, comprises optimal energy availability, eumenorrhea, and optimal bone health (Figure 1). An athlete's location on the spectrum depends on her diet and exercise habits (Nattiv et al., 2007). Low energy availability directly leads to the development of menstrual disorders and subsequently to low BMD (Souza & Toombs, 2010).



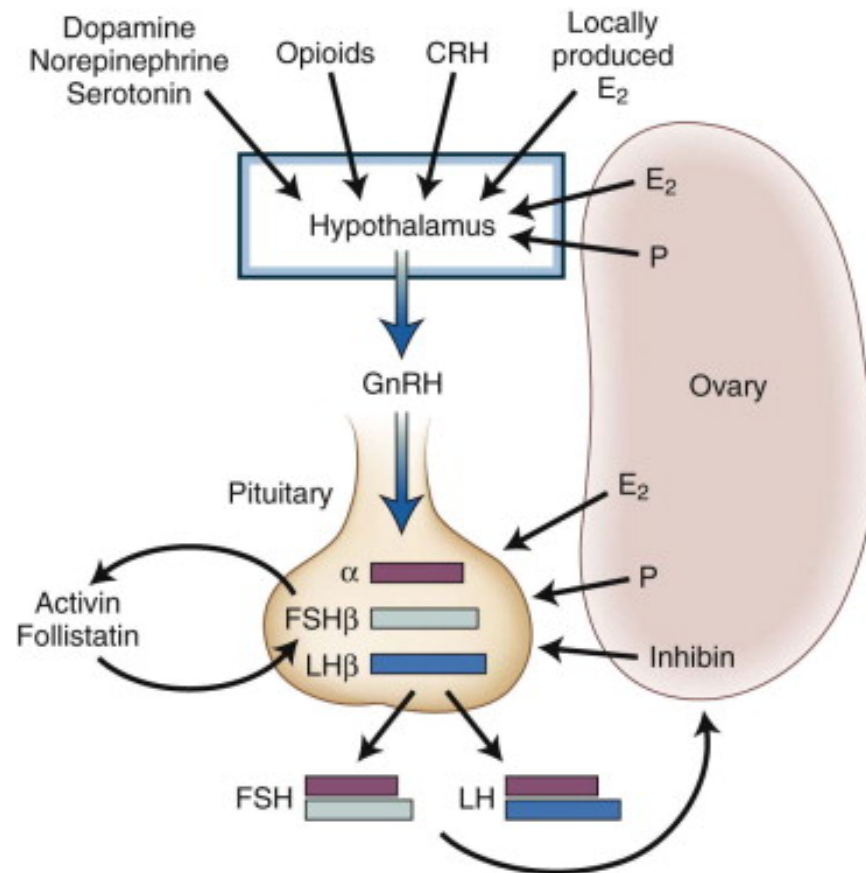
**Figure 1. The Spectrum Proposed for the Female Athlete Triad by the American College of Sports Medicine.** The lower left triangle reflects the most pathologic conditions related to the triad. The upper right triangle coincides with profile of a healthy athlete. Figure taken from Nattiv et al, 2007.

A female athlete's energy availability plays a valuable role in the progression of Triad-related conditions. Energy availability, defined as dietary energy intake minus exercise energy expenditure, is responsible for maintaining body functions (Nattiv et al., 2007). In the case of the Triad, the energy deficit results from some form of dietary restriction, with or without a more severe clinical mental disorder such as anorexia nervosa (AN) or bulimia nervosa (BN). Smolak, Murnen, and Ruble (2000) reported some form of disordered eating behavior in as many as 62% of athletes involved in lean build sports such as gymnastics, ballet, and cross-country running. When energy availability is suboptimal, the body is forced to compensate and reduce the amount of energy available for growth and reproduction (Liu & Lebrun, 2006). Body weight, however, is not directly correlated with energy status and we therefore can not assume that low body weight signifies an energy deficit state or that normal body weight represents energy stability.

Menstrual dysfunction related to the Triad occurs secondary to energy deficiency because of the dependence of the reproductive system on the net state of energy balance. When energy balance becomes too low, the hypothalamic-pituitary-ovarian axis is suppressed in order to preserve energy for vital functions, resulting in functional hypothalamic amenorrhea (FHA) (Gordon, 2010; Nazem & Ackerman, 2012). Amenorrhea is the most pathological condition related to the Triad. Primary amenorrhea is defined as absence of menarche at the age of 16, whereas secondary amenorrhea is classified as the absence of

menstrual cycles for greater than three months after menarche (Nattiv et al., 2007). Oligomenorrhea reflects menstrual cycles lasting longer than 35 days and eumenorrhea refers to menstrual cycles near the median interval of 28 days for young women (21-35 days) (Nattiv et al., 2007; Souza & Toombs, 2010). The most important consequence of menstrual irregularities in young women is low bone mineral density (Christo et al., 2008; Cobb et al., 2003).

Functional hypothalamic amenorrhea as a result of a negative energy balance stems from a change in gonadotropin releasing hormone (GnRH) pulse secretion. GnRH pulsatile secretion from the medial basal hypothalamus is decreased in frequency and amplitude, leading to suppression of the reproductive axis and consequently conservation of energy (Melmed, Polonsky, Larsen, & Kronenberg, 2011). The alteration in GnRH secretion causes the downhill effect of decreasing luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion from the pituitary and ultimately results in understimulated ovarian follicles responsible for producing estrogen. This progression to estrogen deficiency from a lack of GnRH is shown in Figure 2. Functional hypothalamic amenorrhea is reversible and contingent upon adequate energy balance to restore proper GnRH secretion (Couzinet et al., 1999; Deligeoroglou et al., 2010).



**Figure 2. Regulation of the Reproductive Axis by the Hypothalamus.** GnRH is released into portal circulation from the hypothalamus depending on stimulation from regulating hormones. GnRH and other circulating hormones and steroids regulate the formation and release of FSH and LH from the pituitary. FSH and LH ultimately act on the ovary to induce the formation of estradiol (E<sub>2</sub>) and progesterone (P). Corticotropin-releasing hormone (CRH). Figure taken from Melmed et al., 2011.

Hypoestrogenism as a result of functional hypothalamic amenorrhea leads to an inability to achieve peak bone mass and low BMD, because estradiol is a key regulator of bone metabolism, particularly during the teenage and young adult years (Meczekalski, Podfigurna-Stopa, & Genazzani, 2010a). Estradiol inhibits osteoclast function by increasing osteoprotegerin (Riggs, 2000), and in early puberty, it increases levels of the important bone anabolic hormones, growth hormone (GH) and Insulin-like growth factor-1 (IGF-1)(Wennink et al.,

1991). Adolescents generally experience the largest gain in bone density from the rise in estrogen levels that occurs during puberty (Ott, 1991). Girls generally achieve up to 90% of their peak bone mass (PBM) by 18 years of age, with 40% of bone accrual occurring during puberty (Meczekalski, Podfigurna-Stopa, & Genazzani, 2010b). The Z-score, a subject specific specialized score, compares the subject's BMD obtained by DXA against values from age, race, and sex-matched peers. The score reflects standard deviations above and below the mean for age, gender and race. Low BMD in athletes has been defined by the ACSM as a Z-score between -1.0 and -2.0, while osteoporosis, a more pathological condition is defined as a Z-score of less than -2.0 with other clinical risk factors (Nazem & Ackerman, 2012; Nattiv et al., 2007). Table 1 highlights the various BMD definitions based on organization and population. Reporting DXA results as BMD Z-Scores is important for our subject selection because the values are compared to matched peers, whereas T-Scores compare DXA values to peak bone mass, typically achieved only in the early to mid-20s (Nazem & Ackerman, 2012). With reports citing BMD values 2-6% lower at the spine, hip, and total body among amenorrheic athletes compared with controls (Souza & Toombs, 2010) it is important to optimize energy balance to restore menses in these athletes and hence increase bone accrual, a process predominantly mediated by estrogen in adolescence.

**Table 1. BMD Definitions.** BMD definitions are represented by organization and vary based on population. The World Health Organization (WHO) bases the criteria on T-Scores, BMD results of postmenopausal women compared to young adult premenopausal women, whereas the International Society for Clinical Densitometry (ISCD) and ACSM use Z-Scores, DXA results compared to age-matched females, for diagnostic criteria. Table taken from Nazem & Ackerman, 2012.

	World Health Organization		International Society for Clinical Densitometry		American College of Sports Medicine	
Population	Postmenopausal women		Premenopausal women		Premenopausal female athletes	
Terminology	Osteopenia	Osteoporosis	BMD within expected range for age	BMD below expected range for age	Low BMD	Osteoporosis
Criteria	T-Score: -1 to -2.5 (1 to 2.5 SD below the average value for young healthy women)	T-Score: $\leq$ -2.5 (2.5 SD or more below the average value for young healthy women)	Z-Score: $>$ -2 ( $<$ 2 SD below the average value for age-, sex-, and race-matched controls)	Z-Score: $\leq$ -2 (2 SD or more below the average value for age-, sex-, and race-matched controls)	Z-Score: -1 to -2 with secondary clinical risk factors for fracture (eg, chronic malnutrition, eating disorders, hypogonadism, glucocorticoid exposure, previous fractures)	Z-Score: $\leq$ -2 with secondary clinical risk factors for fracture

## **Hormone Replacement Therapy**

### ***Bone Mineral Density***

With the primary goal of treatment being to restore regular menstrual cycling and enhancement of BMD, 92% of physicians support estrogen replacement therapy, routinely prescribing oral contraceptive pills (OCPs) (Cumming DC, 1996; Haberland, Seddick, Marcus, & Bachrach, 1995). However, the effects of hormone replacement therapy on BMD remain unclear. Vescovi, Jamal, and De Souza (2008) conducted a systematic literature review of 36 journal articles between 1960 to January 2007 of women with hypothalamic amenorrhea receiving hormonal therapeutic strategies. Of the reports pertaining to effectiveness of oral contraceptives in restoring BMD, eight studies reported a positive effect and nine reported no significant effect after 8-12 months of treatment. The inability to significantly enhance BMD in adults with hypothalamic amenorrhea by oral estrogen has been accredited to the reduction in IGF-1 following oral estrogen administration (Ho & Weissberger, 1992). Further suppression of already low levels of IGF-1 in hypothalamic amenorrhea by oral estrogen (Couzinet et al., 1999) has led to investigation of other forms of estrogen replacement therapy.

The metabolic effects of estrogen are dependent on its route of administration (O'Sullivan, Crampton, Freund, & Ho, 1998). Oral estrogen suppresses IGF-1 by a hepatic first-pass mechanism, which is avoided with transdermal estrogen (Burkman, 2007). Because transdermal estrogen does not

suppress IGF-1, it may exert a greater impact on BMD than oral estrogen. In a study of 417 postmenopausal women in an estrogen deficient state, BMD increased with ultra low-dose transdermal estradiol (Ettinger et al., 2004). Although these results cannot be extrapolated to adolescent amenorrheic athletes, the same rationale applies. The anti-suppressive IGF-1 effects of transdermal estrogen are under investigation as a treatment to restore BMD in young women with exercise-related amenorrhea.

### ***Body Composition***

A key concern for athletes is a potential change in body weight and body composition with estrogen replacement, and the potential impact of estrogen replacement and body composition changes on athletic performance. Functional hypothalamic amenorrhea is not only associated with hypoestrogenism, but with changes in GH and IGF-1, strong determinants of body composition and resting energy expenditure (Dos Reis, De Melo, Meirelles, Vezozzo, & Halpern, 2003). Rickenlund and colleagues examined oligo-/amenorrheic athletes between the ages of 16-35 after 10 months of oral contraceptive treatment. Results showed an increase in weight and fat mass in the oligo-/amenorrheic (OAM) athletes compared to eumenorrheic athletes and controls with oral estrogen administration (Rickenlund et al., 2004). In a study of postmenopausal women, oral estrogen administration was associated with an increase in fat mass and decrease in lean mass. The findings were attributed to the decrease in IGF-1

observed with oral estrogen given the known anabolic effects of IGF-1 on lean mass (O'Sullivan et al., 1998).

To date, no studies have examined changes in body composition in oligo-/amenorrheic athletes following administration of transdermal estrogen. A study in postmenopausal women reported that oral estrogen increased fat mass and decreased lean mass, whereas transdermal estrogen caused an increase in lean mass, with no changes in fat mass (O'Sullivan et al., 1998). Importantly, the ability of transdermal estrogen to prevent chronic IGF-1 suppression may have beneficial effects on BMD of adolescent oligo-amenorrheic athletes (OAM) while also avoiding the subsequent changes in body composition.

### **Specific Aims**

The effect of transdermal estrogen on body composition in adolescents with olig-/amenorrhea has not yet been examined. In this study we performed a randomized, placebo-controlled study to examine the impact of physiologic estrogen replacement on body composition in adolescent athletes with olig-/amenorrhea. We hypothesized that body composition, specifically fat mass and lean mass, does not change in adolescent athletes with oligo/amenorrhea receiving transdermal estrogen when compared to controls not receiving estrogen. Our goal was to determine if transdermal estrogen is a viable treatment option for female adolescent athletes concerned about the possible effects on their body composition, with the common perception that oral estrogen increases weight and fat mass.

Assessing body composition changes is especially important in this subset of adolescent females because the perceived changes due to estrogen replacement may lead to an athlete's refusal of estrogen supplementation rather than choosing the treatment needed for optimal bone and overall health. The high prevalence of reproductive dysfunction in adolescents, with a corresponding deficit in peak bone mass, makes it essential to identify the effects of all possible therapeutic interventions.

## **METHODS**

In a cross-sectional analysis at baseline, characteristics of oligo-/amenorrheic athletes, eumenorrheic athletes, and non-athletic healthy controls will be assessed and compared to one another. A 6 month longitudinal analysis will determine the effects of transdermal estrogen use on body composition in a group of oligo-/amenorrheic athletes.

### **Subject Selection**

A total of 51 adolescent athletes who met the criteria for diagnosis of oligo-/amenorrhea, 24 athletes with eumenorrhea, and 23 healthy control subjects were enrolled in the study. The study was performed at the Clinical Research Center of Massachusetts General Hospital (MGH), Boston, MA, USA. All subjects were 14 to 21 years of age, and all athletes were endurance athletes. Endurance training was defined as at least 4 hours of aerobic weight-bearing training of the legs or specific endurance training weekly, or at least 20 miles of running weekly for a period of at least 6 months in the previous year. Cyclists and swimmers were excluded because their training does not include true weight-wearing activities. Rowers and gymnasts were also excluded given that these activities have been associated with a preservation of bone density even when associated with amenorrhea, likely consequent to differences in the nature of weight bearing and impact.

Athletes with amenorrhea were required to have had an absence of menses for at least three months, or absence of menarche at  $\geq 16$  years, whereas athletes with oligoamenorrhea were required to have a cycle length greater than six weeks for at least six months. Eumenorrheic athletes (EA) were defined as those who had at least nine menses (cycle length 21-35 days) in the preceding year. Healthy non-athletes were required to not participate in organized team sports or engage in weight bearing exercise activity for more than two hours a week.

Subjects were excluded from the study if there was a condition other than endurance training that may have caused amenorrhea; including hypothyroidism, hyperprolactinemia, premature ovarian failure and PCOS (Polycystic Ovarian Syndrome). The use of medications affecting bone metabolism, such as estrogen, progesterone, anabolic steroids, glucocorticoids (except local application of glucocorticoid creams), phenytoin, and phenobarbitone also excluded subjects from enrollment. For the oligo-/amenorrhic athletes to be randomized to one of the treatment groups, other exclusion criteria included a past medical history of conditions that may increase the risk of thromboembolism, a current history of smoking, migraines or peanut allergies, and any undiagnosed or abnormal genital bleeding.

Subjects were recruited through mailings to pediatricians, nutritionists, therapists, sports medicine specialists, high schools and colleges in the New

England area, and through advertisements in local newspapers and magazines. Prior to performing any testing, we obtained assent from the subject or consent from the parents following a briefing of study procedures in the presence of a study investigator.

All subjects were counseled regarding the risk of pregnancy, options for birth control measures, and possible side effects from estrogen treatment at the time of consent and also at follow-up visits. Minor side effects of estrogen include nausea, dizziness, breast tenderness and fullness, headache and bloating. More serious side effects include liver and gall bladder disorders, thromboembolism, and abnormal vaginal bleeding (protocol 136-138). Subjects were advised that transdermal estrogen patches do not provide contraceptive protection or protection against sexually transmitted diseases.

Athletes with OAM were randomized to receive transdermal or no estrogen for 12 months. Girls receiving OAM transdermal estrogen also received cyclical progesterone for 12 days of every month. All subjects took 1200 mg of elemental calcium and 800 IU of vitamin D daily.

### **Study Treatment**

Of the 51 athletes with oligo-/amenorrhea, data are available for 11 who were randomized to no estrogen and 8 who were randomized to receive transdermal estrogen for a period of 6 months. All subjects received 1200 mg elemental calcium and 800 IU vitamin D daily. The athletes randomized to the

transdermal estrogen patch (releasing 100 mcg/day of 17 $\beta$ -estradiol) applied the patches biweekly (Sundays/Wednesdays) and received cyclical progesterone (micronized progesterone 200 mg) for the first 12 days of the month. The doses of estrogen were based on recommended replacement doses for this age group.

### **Experimental Protocol**

At the screening visit a history, physical examination, and screening labs were obtained, including a complete blood count and levels of potassium, ALT (alanine aminotransferase), AST (aspartateaminotransferase) TSH, and FSH. Bone density and body composition, specifically fat and lean mass, were assessed by dual energy X-ray absorptiometry (DXA) at the spine, hip, and total body. Spine BMD Z-scores were used to stratify the OAM subjects during randomization. A Bouchard 3-day activity record confirmed inclusion criteria for endurance training.

Subjects then completed a baseline visit within eight weeks of the screening including a history and physical examination with anthropometric measurements and a DXA scan to assess body composition. The same testing was performed at a 6 month follow up visit.

## **Body-Composition Measurements**

Body composition was assessed using DXA (HologicInc., QDR-4500, Waltham, MA). Measurements were taken at the lumbar spine, hip, and whole body for bone density, and of the whole body for body composition. Body composition measures included fat mass, lean mass, percent body fat and trunk fat. DXA measures were repeated at the 6-month follow-up visit.

## **Statistical Analysis**

We used JMP (version 10, SAS Institute, Inc., Cary, NC) for all analyses and set a p value of less than 0.05 on a two-tailed test to indicate significance. Three-group comparisons of baseline characteristics of OAM, EA and HC were performed by an overall ANOVA, followed by a Tukey-Kramer analysis to assess between-group differences while controlling for multiple comparisons with normally distributed parametric data. A Kruskal-Wallis test followed by the Steel Dwaas All Pairs analysis was using for nonparametric data sets. Weight, body mass index (BMI), and lean mass measures required log transformations to fit a normal distribution.

For our primary longitudinal analysis we compared changes in weight, BMI, fat mass, and lean mass in oligo-/amenorrheic athletes receiving transdermal estrogen (OAM E+) versus oligo-/amenorrheic athletes not receiving estrogen (OAM E-) using a Student t-test. We controlled for chronological age, height, and changes in weight in our analysis using

multivariate methods for further analysis. We confirmed that changes in weight were predictive of subsequent changes in the fat mass, but not lean mass using simple linear (Pearson's) correlation. For our secondary analysis, a paired *t* test was used to compare within group changes in body composition measures over 6 month.

## RESULTS

### Baseline Characteristics

Bone age, height, and trunk fat did not differ between groups. Athletes with oligo-/amenorrhea had significantly lower weight ( $p=0.03$ ), BMI ( $p=0.003$ ), fat mass ( $p=0.04$ ), and lean mass ( $p=0.047$ ) compared with eumenorrheic athletes, and lower fat mass ( $p=0.01$ ) and percent body fat ( $p<0.0001$ ) when compared with the non-athlete control subjects (Table 1, Figure 3). Athletes with eumenorrhea had significantly higher lean mass ( $p=0.004$ ) (Figure 3F) and significantly lower percent body fat ( $p=0.049$ ) (Figure 3G) when compared with non-athletes.

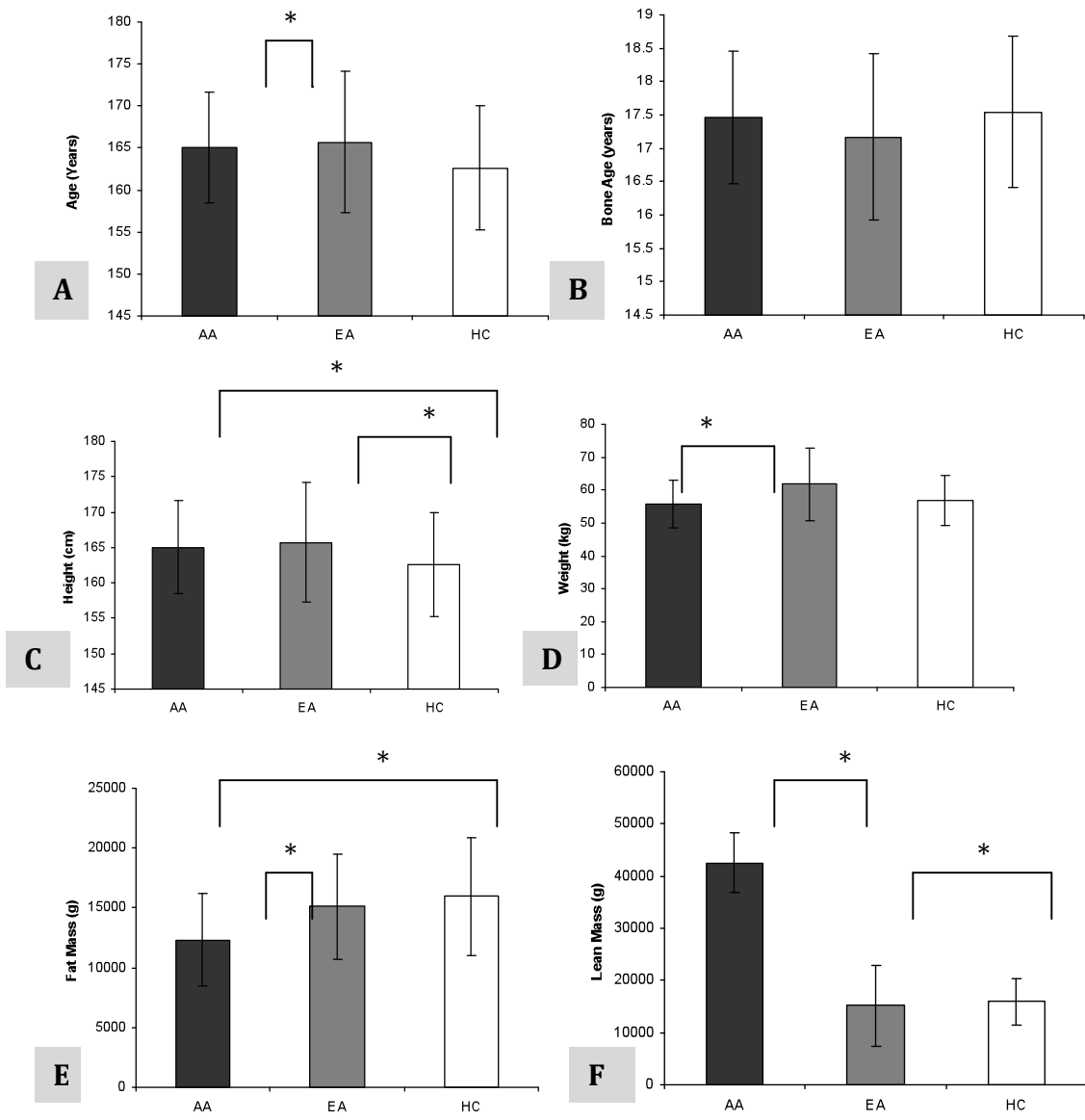
**Table 2. Baseline Characteristics of Oligo-/amenorrheic Athletes (AA), Eumenorrheic Athletes (EA), and non-athlete controls (HC).** Data are shown as mean  $\pm$  standard deviation. Abbreviations: n = number of subjects, BMI = body mass index. Significance at  $p \leq 0.05$  ANOVA between groups.

	Oligo- /Amenorrheic athletes n=51	Eumenorrheic Athletes n=24	Non- Athletes n=23	<i>p</i> ANOVA	<i>p</i> OAM vs EA	<i>p</i> OAM vs HC	<i>p</i> EA vs HC
Chronological age (years)	19.3 $\pm$ 2.1	19.9 $\pm$ 2.1	19.1 $\pm$ 1.7	0.03 <sup>a</sup>	0.03		
Bone age (years)	17.47 $\pm$ 0.99	17.17 $\pm$ 1.25	17.54 $\pm$ 1.14	0.2			
Weight (kg)	55.68 $\pm$ 7.26	61.80 $\pm$ 11.15	56.83 $\pm$ 7.59	0.02 <sup>a</sup>	0.015		
Height (cm)	165.01 $\pm$ 6.54	165.72 $\pm$ 8.42	162.60 $\pm$ 7.38	0.3			
BMI (kg/cm <sup>2</sup> )	20.42 $\pm$ 2.33	22.35 $\pm$ 2.31	21.50 $\pm$ 2.52	0.003 <sup>a</sup>	0.003		
Total fat mass (g)	12332.2 $\pm$ 3862.94	15141.1 $\pm$ 4392.22	15962.8 $\pm$ 4916.66	0.005 <sup>a,b</sup>	0.04	0.01	
Total lean mass (g)	42564.1 $\pm$ 5700.32	46304.4 $\pm$ 7688.43	40456.0 $\pm$ 4438.85	0.006 <sup>a,c</sup>	0.05		0.004
Trunk fat (g)	4636.63 $\pm$ 1849.44	5614.45 $\pm$ 2156.6	5701.83 $\pm$ 2051.87	0.04			
% body fat	21.46 $\pm$ 0.70	23.5 $\pm$ 1.02	26.98 $\pm$ 5.23	0.0001 <sup>a,c</sup>		<0.0001	0.05

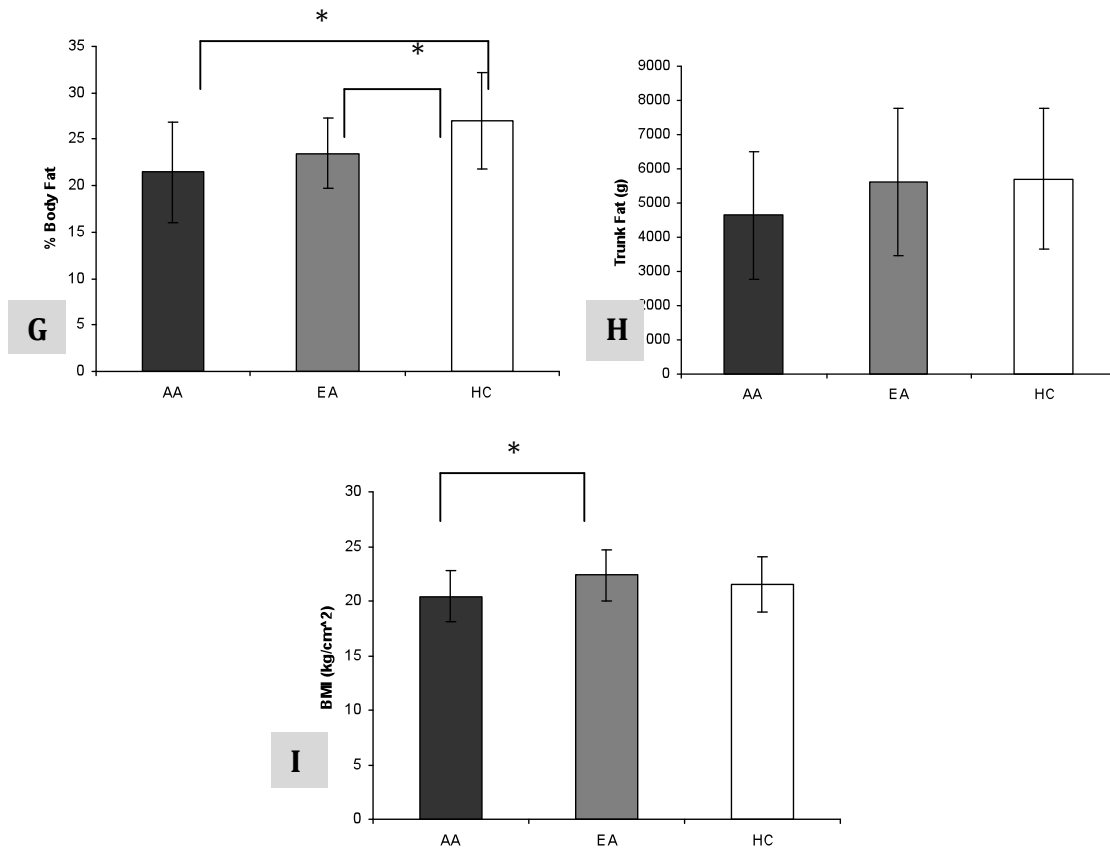
<sup>a</sup>Oligo-/Amenorrheic athletes vs. Eumenorrheic athletes  $p < 0.05$

<sup>b</sup>Oligo-/Amenorrheic athletes vs. non-athletes  $p < 0.05$

<sup>c</sup>Eumenorrheic athletes vs. non-athletes  $p < 0.05$



**Figure 3. Baseline Characteristics with 3-group Comparison Results.** Oligo-/amenorrheic athletes (AA), eumenorrheic athletes (EA), and non-athlete controls (HC). A: age, B: bone age, C: height, D: weight, E: fat mass, F: lean mass. Significance at  $p \leq 0.05$  ANOVA between groups.



**Figure 3 continued. Baseline characteristics with 3-group comparison results.** Oligo-/amenorrheic athletes (AA), eumenorrheic athletes (EA), and non-athlete controls (HC). G: % body fat, H: trunk fat, I: BMI. Significance at  $p \leq 0.05$  ANOVA between groups.

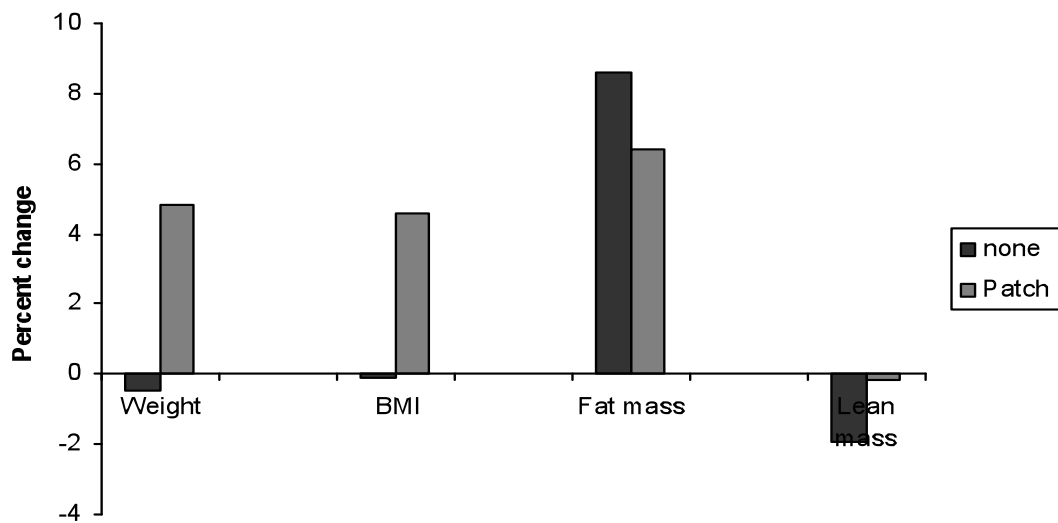
### Changes in Body Composition Parameters

In our primary analysis, OAM E+ girls did not differ from OAM E- girls over 6 months for changes in weight ( $p=0.09$ ), BMI ( $p=0.15$ ), fat mass ( $p=0.81$ ), and lean mass ( $p=0.30$ ) (Table 3 and Figure 4). OAM E+ females experienced a 4.79% and 4.59% increase for weight and BMI, whereas the OAM E- females experienced a 0.49% and 0.08% decrease in weight and BMI. Both OAM E+ and OAM E- females demonstrated increases in percent change in fat mass (6.43%, 8.61%) and decreases in their percent changes in lean mass (-0.16%,

-1.94%), although the OAM E+ group had a smaller percent increase in fat mass and smaller percent decrease in lean mass than the OAM E- group, and the increase in weight in OAM E+ was mostly from an increase in lean mass.

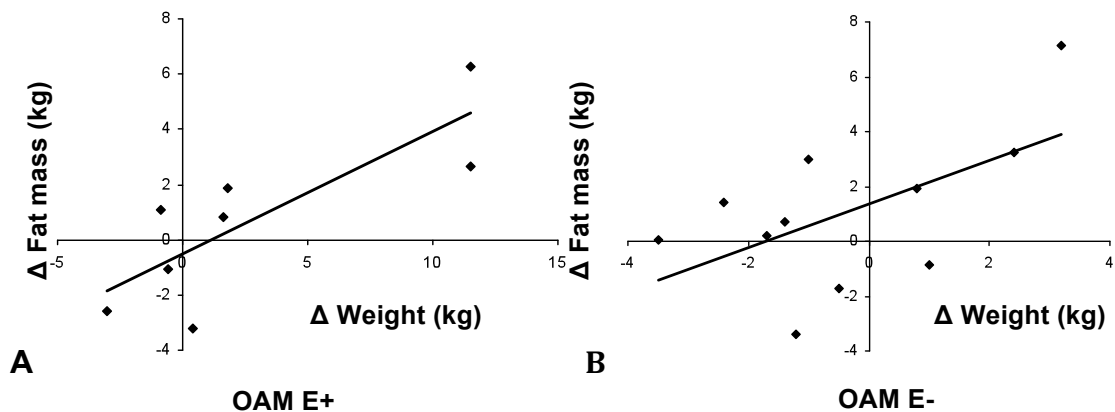
**Table 3. Changes in Body Composition Parameters in OAM E+ versus OAM E-.**  
 $\Delta$ =difference in, 6 months compared with baseline. Significance at  $p \leq 0.05$  ANOVA between groups.

	AN E+	AN E-	<i>p</i> values
$\Delta$ Weight (kg)	2.79 $\pm$ 5.59	-0.39 $\pm$ 2.04	0.09
$\Delta$ BMI (kg/cm <sup>2</sup> )	0.89 $\pm$ 1.84	-0.05 $\pm$ 0.8	0.15
$\Delta$ Fat mass (g)	730.6 $\pm$ 3066.1	1062.9 $\pm$ 2819.5	0.81
$\Delta$ Lean mass (g)	-92.6 $\pm$ 1262.6	-916.28 $\pm$ 1910.52	0.3



**Figure 4. Percent Changes in Body Composition Parameters in OAM E+ versus OAM E-.**  
 Percent change in body composition measures in oligo-/amenorrheic females randomized to no estrogen (black bars) and females randomized to estrogen (grey bars).

We also examined differences in OAM E- girls versus OAM E+ girls after controlling for (1) baseline age, height, and weight changes, and for (2) baseline age and height. There were no correlations of baseline age, height, and weight changes with changes in lean mass. Correlation analysis did show an association between changes in weight and absolute and percent changes in fat mass ( $r=0.59$   $p=0.0075$ ;  $r=0.55$   $p=0.014$ ). Differences between OAM E- girls vs. OAM E+ girls for changes in fat mass over 6 months became significant in the positive direction after controlling for changes in weight over this period (Table 4). Weight changes significantly predicted changes in fat mass in OAM E+ girls, but not OAM E- (Figure 5). Multivariate analysis did not reveal any other significant confounding variables.



**Figure 5: Associations Between Changes in Weight and Changes in Fat Mass.** A) OAM E+ girls change in fat mass correlated positively with changes in weight ( $r=0.81$ ,  $p=0.014$ ), whereas B) OAM E- girls did not have a significant correlation between change in fat mass and change in weight ( $r=0.57$ ,  $p=0.067$ ).

**Table 4: Multivariate Analysis Results.** Changes in body composition parameters in OAM E- versus OAM E+ girls after controlling for covariates. Significance at  $p \leq 0.05$  ANOVA between groups.

Body composition changes	$p$ OAM E- versus OAM E+	$p^a$	$p^b$
$\Delta$ Fat mass (g)	0.81	0.035*	0.9
$\Delta$ Lean mass (g)	0.3	0.58	0.76

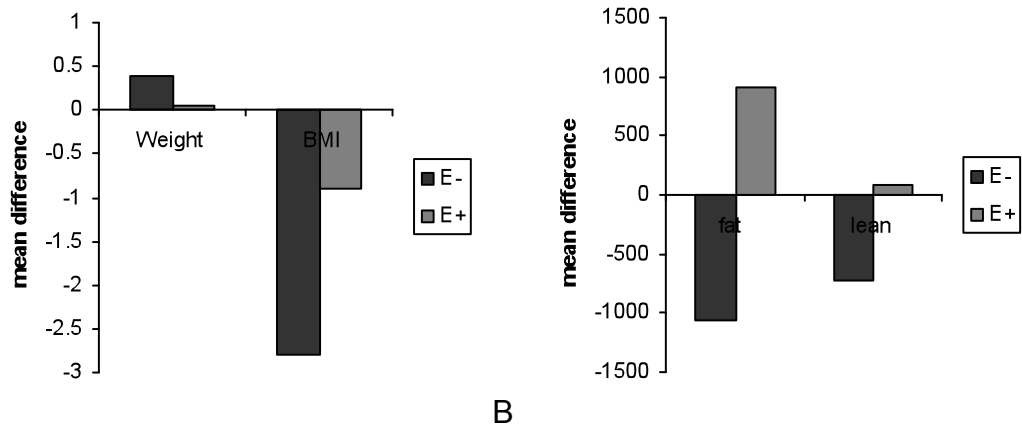
$p^a$ Value after controlling for baseline age, height, and changes in weight

$p^b$ Value after controlling for baseline age and height

Additionally, on secondary analysis using a paired t-test, we found no significant changes over 6 months within OAM E+ girls and within OAM E- girls for weight, BMI, fat mass, and lean mass (Table 5 and Figure 6).

**Table 5. Within Group Analysis of OAM E+ and OAM E- Groups Body Composition Parameters After 6 Months of Treatment.** A paired t test compared differences from BL to 6M within a group for weight, BMI, fat mass, and lean mass. Significance at  $p \leq 0.05$ .

	BL	6M	Mean Difference	P-Value
Weight E-	57.3909	57	0.39091	0.5388
Weight E+	53.0625	55.85	-2.7875	0.2010
BMI E -	21.246	21.1923	0.05373	0.8290
BMI E +	19.5536	20.4454	-0.8918	0.2129
Fat Mass E -	13193.9	14256.8	-1062.9	0.2397
Fat Mass E +	10921.1	11651.8	-730.64	0.5219
Lean Mass E -	43200.5	42284.2	916.282	0.1428
Lean Mass E +	43911.5	43818.9	92.6375	0.8415



**Figure 6. Mean Differences from Within Group Analysis of Body Composition Parameters.** (A) weight and BMI and (B) fat and lean mass mean differences. Results are reported as mean differences between BL and 6M.

## DISCUSSION

This is the first study to examine the effects of transdermal estrogen on body composition in adolescents with oligo-/amenorrhea. We demonstrate that transdermal estrogen does not significantly change fat or lean mass over the 6 month period of treatment in oligo-/amenorrheic athletes compared to other oligo-/amenorrheic athletes who do not receive estrogen therapy. Our findings are especially relevant to this subset of adolescent athletes because of their stage of bone development and the possible deleterious effects of long term amenorrhea. Adequate estrogen levels are essential to restore bone accrual and achieve an optimal BMD. These data suggest transdermal estrogen may serve as a viable treatment option for adolescent oligo-/amenorrheic athletes without significantly affecting body composition.

### **Baseline Findings**

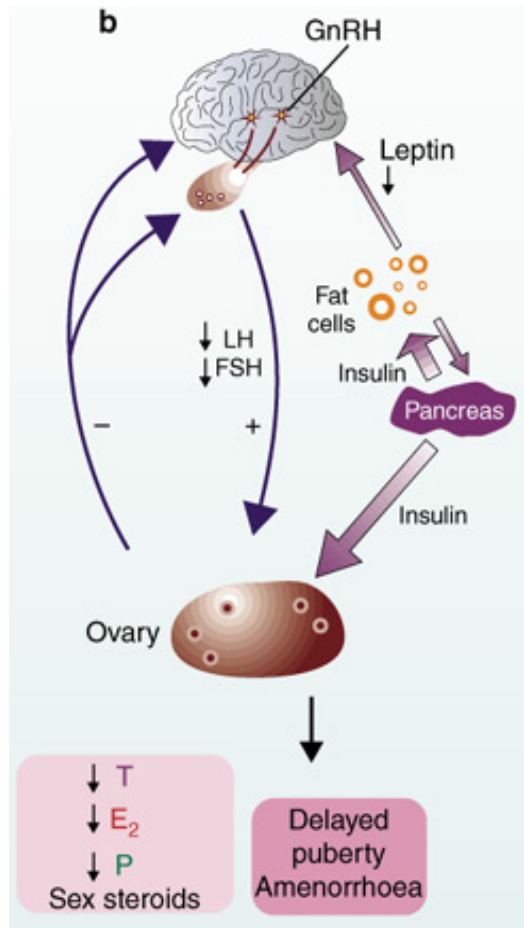
Our analysis included a cross-sectional examination of baseline characteristics in oligo-/amenorrheic athletes versus eumenorrheic athletes and non-athlete controls. It is important to identify individuals with Triad-related dysfunction and determine specifically what differentiates the different groups. One way is to recognize the major contributors to the components of the triad. Cristo and colleagues (2008) found that lean mass and BMI were the strongest predictors of bone density in athletes. Our results also indicate differences in body composition between the three subject groups that would predispose them

to variable BMD measures. Importantly, oligo-/amenorrheic athletes had significantly lower BMI and lean mass than eumenorrheic athletes (Table 2 and Figure 3).

Baseline body composition measures are also important to understand and differentiate because of their relation to energy availability. Low energy availability begins the progression towards menstrual dysfunction and low estrogen levels, ultimately leading to low BMD. Fat mass reflects energy stores, and thus the chronic state of energy availability in any individual. Low fat mass indicates low energy availability, and is a predictor of menstrual dysfunction (Misra et al., 2006). Consistent with this, the OAM athletes had significantly lower fat mass than EA and non-athletes..

Fat mass is also an important baseline characteristic to compare between groups because fat related hormones possess the ability to alter reproductive function. Low fat mass not only indicates a disruption in energy balance, but could explain the mechanism leading to amenorrhea, namely from changes in hormones that are secreted by fat (such as leptin) or are regulated by fat (such as ghrelin and peptide YY) and then impact GnRH pulsatility. Leptin, a hormone secreted by fat mass, has previously been explored as an influence on the hypothalamic-pituitary-ovarian axis. Lower leptin levels as a result of low fat mass have been linked to altered LH pulsatility. A recent study on adolescent athletes demonstrated significantly lower fat mass and leptin

levels in amenorrheic subjects compared to eumenorrheic subjects and non-athletic controls (Ackerman et al., 2012). Figure 6 highlights the progression from a decrease in fat mass leading to lower leptin levels and eventually amenorrhea. The orexigenic hormone ghrelin, secreted by gastric oxyntic cells, is inversely related to fat mass and also linked to the hypothalamic-pituitary-ovarian axis. In the same study by Ackerman and colleagues (2012), adolescent athletes demonstrated an independent association between higher ghrelin levels and lower fat mass, and an inverse association with altered LH pulsatility. The associations between fat related hormones and changes to LH pulsatility reflect an endocrine link between a low energy state, low fat mass and altered GnRH or LH pulsatility in adolescent athletes.



**Figure 7. The Hormonal Mechanism of Leptin Secretion and Female Reproductive Consequences.** While in a state of negative energy balance and low fat mass leptin is secreted from fat cells. The reproductive system is subsequently shut down because of reduced GnRH production, therefore reducing LH and FSH. This progression leads to hypothalamic amenorrhoea. Image taken from Sharpe and Franks, 2002.

These data indicate that adolescent oligo-/amenorrhoeic athletes can be differentiated from adolescent eumenorrhoeic athletes by weight, BMI, fat mass, and lean mass.

## **Comparison of Oral and Transdermal Estrogen Treatment**

Oral estrogen is being used as a treatment option for adolescents with oligo-/amenorrhea, however, studies have reported changes in body composition following treatment with estrogen. In a subset of oligo/amenorrheic endurance athletes between the ages of 16-35, who received oral estrogen for 10 months, there was a significant increase in weight and fat mass with estrogen administration (Rickenlund et al., 2004). Body composition changes due to estrogen have previously been attributed to the relationship between estrogen and GH. Oral estrogen exerts effects in the liver by inhibiting the metabolic effects of GH by decreasing IGF-1 production and fat oxidation. The clinical consequences in postmenopausal women include a decrease in lean mass and increase in whole body fat mass (Leung, Johannsson, Leong, & Ho, 2004). The IGF-1 mediated effects of GH are impacted by oral estrogen and may cause changes in body composition from oral estrogen administration.

Transdermal estrogen, which does not suppress IGF-1, should be explored as a treatment option in this subset of individuals to restore bone health without the possible 'negative' effects on body composition. Based on this rationale, we hypothesized that administration of transdermal estrogen to oligo-/amenorrheic athletes between the ages of 14-21, compared to no estrogen therapy, should not significantly alter body composition parameters. Our results support our hypothesis, and we show that transdermal estrogen

does not change weight, BMI, fat mass, or lean mass between groups. Additional multivariate analysis revealed that changes in fat mass across groups were associated with changes in weight, and adjusting for weight changes led to a significant difference between groups for an increase in fat mass.

It is possible that our study was underpowered and did not completely reveal the interaction between transdermal estrogen usage and body composition changes in oligo-/amenorrheic girls. We found no significant differences between OAM E+ versus OAM E- for weight, BMI, fat mass, and lean mass changes. However, the data did suggest a trend towards differences between the treatment groups (Table 3). Adequate sample size may reflect changes in body composition measures, and further assist our understanding of how different forms of estrogen specifically impact fat mass and lean mass in athletes.

We found no significances for our within group analysis for changes in weight, BMI, fat mass, and lean mass in OAM E+ girls (Table 5), indicating that transdermal estrogen therapy does not cause weight or body composition changes. This may help promote transdermal estrogen as a form of hormone replacement therapy for adolescent oligo-/amenorrheic athletes concerned about an increase in weight or an impact on performance. Additionally, if

anything, our study showed a non-significant decrease in fat mass in the OAM E+ group, as well as an increase in lean mass.

## **Limitations**

Data from this study does further support transdermal estrogen as a possible treatment option for adolescent athletes, but the results should be considered as a starting point for further investigation. Although outcomes presented were statistically viable, small sample size limited the power of the study. There were only eight athletes receiving the transdermal patch and eleven athletes with no treatment. Additionally, the 6-month treatment time frame limited the longitudinal analysis of body composition changes. Subjects should be followed over a longer duration to determine whether the observed lack of changes in fat and lean mass with treatment persists over time (Souza & Toombs, 2010).

The number of treatment groups also limits the findings from the study. Although we hypothesized that transdermal estrogen would not change body composition parameters compared to no treatment, the addition of an oral estrogen treatment group would provide more insight to the mechanisms whereby these changes may have occurred. Previous studies on postmenopausal women have compared oral estrogen to transdermal estrogen and found significant differences between the two groups for body composition

changes (Osullivan). There were not enough subjects receiving oral estrogen treatment to compare within our cohort at this time.

### **Future Studies**

The results of our study, as well as its limitations, indicate the need to include other parameters in future investigations. Hormone levels, specifically IGF-1, should be monitored in order to more accurately describe the mechanisms whereby estrogen replacement therapy affects body composition, particularly with respect to route specific actions. BMD measures should also be included in the analysis when assessing transdermal estrogen as a treatment to improve BMD without subsequent changes in body composition. As mentioned earlier, more subjects should be assessed over a longer period of time to increase the power of the study, and should also be compared to an oral estrogen treatment group.

Although our findings are consistent with findings from previous studies examining the effects of transdermal estrogen on body composition, our data support the need to examine these effects among specific groups of subjects. The profiles of adolescent oligo-/amenorrheic adolescent athletes and postmenopausal women both reflect a state of estrogen deficiency, but confer different changes on the body based on weight. Sullivan and colleagues (1998) proposed that body composition changes observed in women receiving oral estrogen may be attributable to increases in fat mass and decreases in lean

mass from aging. Transdermal estrogen has also been proposed as a strategy to increase BMD in adolescents with AN, and may cause changes in body composition over time that differ from those in athletes given that AN subjects having even lower IGF-1 levels at baseline than athletes (Misra et al., 2011). Findings related to body composition changes with estrogen treatment cannot be assumed to be the same for all conditions and thus further studies are necessary to study the effects of transdermal estrogen in different subject cohorts.

Our subset of athletes also requires specific attention to the time of year during which measurements are recorded. Any athlete's body composition has the capacity to change depending on whether they are in or out of season. The activities of our subjects are mostly seasonal. Therefore, body composition parameters are likely to vary through out the year. It is important to demonstrate the effects of transdermal estrogen on body composition while controlling for these factors.

After identifying transdermal estrogen as a beneficial treatment option to restore BMD without effecting body composition or performance, it is important to assess the effects of different estrogen doses on body composition and performance. Subjects in this study, OAM female athletes between the ages of 15 and 21, received 100 mcg/day of 17B-estradiol transdermally, similar to a study of postmenopausal women in which the subjects also received 100

mcg/day 17 $\beta$ -estradiol transdermally (O'Sullivan et al., 1998). However, in a group of 24-34 year old amenorrheic adults, the transdermal patches used delivered only 50 mcg estradiol/day (Cumming DC, 1996). Variances in the amount and type of estrogen used in various studies demonstrate the need to systematically compare and explore the effects of various doses and types of transdermal estrogen on body composition.

## **Conclusion**

We have demonstrated that transdermal estrogen does not significantly change lean mass or fat mass over a 6-month period compared to no treatment in a group of adolescent female athletes. Although our findings support the use of transdermal estrogen as a potential treatment option for adolescent athletes with amenorrhea, changes in diet and exercise habits should be the primary treatment for athletes with triad related symptoms (Souza & Toombs, 2010). The origin of bone and menstrual disturbances can be attributed to changes in energy balance that can be preserved by improving nutritional status. Adolescent athletes should be encouraged to engage in the proper balance of physical activity and eating as a way to ensure optimal bone development.

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