Health Consequences of Low Energy Availability in Females and Their Underlying Physiological Mechanisms

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2023

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial
fulfillment of the requirements for graduation from the
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Abstract

Low energy availability (LEA) is a state in which energy intake is insufficient to support energy expenditure while maintaining energy balance, which has been shown to result in gastrointestinal distress, immunosuppression, reduced metabolic rate, menstrual dysfunction, diminished bone health, and impaired cardiovascular function in females. Alterations to the microbiome and mucosal lining may propagate gastrointestinal complaints and compromised immune function associated with LEA. The pathophysiology of LEA is otherwise largely driven by hormonal adaptations. Diminished production of thyroid hormones is known to contribute to reduced metabolic rate. Decreases in leptin and insulin and increases in ghrelin disrupt reproductive function through modulation of anorexigenic and orexigenic factors, and the resulting hypoestrogenism can impair bone health and cardiovascular function.

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In certain populations, caloric restriction is associated with health benefits such as increased lifespan, reduced oxidative damage, and improvements in biomarkers associated with age-related diseases such as diabetes, heart disease, and stroke (Kraus et al., 2019). However, severe caloric restriction, particularly in lean individuals, can result in negative health consequences, such as impaired reproductive function and diminished bone health (Mountjoy et al., 2018). Health detriments resulting from caloric restriction appear to arise when the degree of caloric restriction increases to the point at which there is insufficient energy to maintain homeostasis and maintain bodily functions.

Energy availability (EA) refers to the amount of dietary energy available to be utilized for physiological purposes after factoring out the energy expended during physical activity (Loucks et al., 1998). EA is calculated by obtaining the difference in total dietary energy intake and exercise energy expenditure in relation to an individual's lean body mass (LBM). LEA occurs when caloric consumption is insufficient to meet the energetic demands of both basal physiology and exercise due to decreased caloric intake, increased energy expenditure, or a combination of the two, resulting in significant disruption of whole-body homeostasis and dysregulation of multiple body systems. In healthy, young, menstruating women, EA < 30 kcal $kgLBM^{-1}$ day^{-1} has been demonstrated as a critical threshold which induces hormonal alterations and negative health consequences within 5 days, while an EA of 45 kcal $kgLBM^{-1}$ day^{-1} is generally considered to promote energy balance (Table 1) (Loucks & Thuma, 2003).

Table 1

Classification of Energy Availability Levels for Females

Energy Availability	Categorization	Example: **Female; 60 kg; 47 kg LBM** **1.5-2 hr moderate intensity training/day (EEE = 100 kcal/day)**
> 45 kcal kgLBM^{-1} day^{-1}	High EA: Used for periods of healthy weight gain	EI = 3,400 kcal/day EA = 3,400 – 1,000 = 2,400 kcal/day EA = 2,400 kcal day^{-1}/47 kgLBM = 51 kgLBM^{-1} day^{-1}
45 kcal kgLBM^{-1} day^{-1}	Optimal EA: Optimal intake to maintain weight while allowing optimal energy to maintain physiological function	EI = 3,100 kcal/day EA = 3,100 – 1,000 = 2,100 kcal/day EA = 2,100 kcal day^{-1}/47 kgLBM = 45 kgLBM^{-1} day^{-1}
30-45 kcal kgLBM^{-1} day^{-1}	Subclinical LEA: May be maintained for short periods of weight loss	EI = 2,600 kcal/day EA = 2,600 – 1,000 = 1,600 kcal/day EA = 1,600 kcal day^{-1}/47 kgLBM = 34 kgLBM^{-1} day^{-1}
< 30 kcal kgLBM^{-1} day^{-1}	Clinical LEA: Associated with negative health consequences resulting in damage to many body systems	EI = 2,200 kcal/day EA = 2,200 – 1,000 = 1,200 kcal/day EA = 1,200 kcal day^{-1}/47 kgLBM = 26 kgLBM^{-1} day^{-1}

Note. EA = energy availability; EEE = exercise energy expenditure; EI = energy intake; LEA = low energy availability; LBM = lean body mass. Adapted from "Energy availability in athletics: Health, performance, and physique" by A. K. Melin, I. A. Heikura, A. Tenforde, and M. Mountjoy, 2019, *International Journal of Sport Nutrition and Exercise Metabolism*, *29*(2), p. 153.

The concept of EA and its impact on human physiology was first noted in female athletes, where insufficient dietary intake to meet energy demand resulted in reduced luteinizing hormone (LH) pulsatility and triiodothyronine (T_3) levels (Loucks & Heath, 1994a; Loucks & Heath, 1994b). Alterations in LH pulsatility in response to LEA are associated with menstrual disturbances such as hypothalamic amenorrhea (HA) (Ackerman et al., 2012). Dysregulation of the menstrual cycle resulting from LEA has also been shown to negatively impact bone health in female athletes, a relationship described as the Female Athlete Triad (Triad) (Yeager et al. 1993). Recent studies have also suggested that LEA may negatively affect other physiological processes and can occur in males as well as females. Considering these findings, in 2014, the International Olympic Committee proposed the Relative Energy Deficiency in Sport (RED-S) model to describe the negative physiological consequences of LEA in athletes, including impairments in menstrual, gastrointestinal, metabolic, endocrine, and cardiovascular health (Mountjoy et. al, 2018). Although LEA and its health consequences have primarily been investigated in athletic populations, recent research suggests that females who exercise recreationally may also be at risk for LEA (Slater et al. 2016).

Although the health complications of LEA have been well documented in clinical studies, the mechanisms underlying the physiological complications of LEA remain poorly understood. In the Triad model, menstrual dysfunction and LEA act synergistically to compromise bone health, but it is unclear if the hormonal alterations associated with LEA also interact to cause the other impairments in physiological function described by the RED-S model. The aim of this review is to explore the underlying mechanisms of the physiological consequences which result from LEA due to a combination of inadequate energy intake and excess energy demands.

Endocrine

In a state of energy deficiency, numerous hormonal adaptations occur to maintain energy balance within the body. These hormonal alterations are primarily responsible for many of the symptomologies associated with LEA, such as menstrual dysregulation, diminished bone health, and reduced metabolism. Changes in certain key hormones which have been documented in athletes with LEA are summarize in Table 2. Most of these hormones regulate a multitude of physiological functions across multiple body systems. The respective effects of these hormonal changes will be discussed in the context of each body system affected by LEA in subsequent sections.

Table 2

Summary of Endocrine Changes During Low Energy Availability

Hormone	Response (Females)	Comments
Appetite and Metabolism		
Leptin	↓ Hilton & Loucks (2000)	Leptin is suppressed at an EA threshold below 30 kcal $kgLBM^{-1}$ day^{-1}, but reaches asymptotic limit below 20 kcal $kgLBM^{-1}$ day^{-1}.
Ghrelin	↑ Scheid et al. (2011)	Increases in ghrelin are significantly magnified in individuals with amenorrhea.
Insulin	↓ Loucks et al. (1998)	Insulin decreases linearly with reductions in LEA.
HPG Axis		
Estradiol	↓ Loucks & Thuma (2003)	Reduced estradiol levels are only observed when EA was restricted to 10

		kcal kgLBM^{-1} day^{-1}, and not at an EA of 20 kcal kgLBM^{-1} day^{-1} or above
HPA axis		
Cortisol	↓ Loucks & Thuma (2003)	Cortisol response is restricted when energy availability falls below threshold of 20 kcal kgLBM^{-1} day^{-1}, rather than decreasing linearly
HPT axis		
Total T_3	↓ Loucks & Heath (2004)	An EA threshold of 25 kcal kgLBM^{-1} day^{-1} is necessary to see significant reductions in T_3
GH and IGF-1 axis		
GH	↓ Loucks & Thuma (2003)	Increases in GH reach an asymptotic below 20 kcal kgLBM^{-1} day^{-1}.
IGF-1	↓ Loucks & Thuma (2003)	IGF-1 was disrupted at an EA < 30 kcal kgLBM^{-1} day^{-1}, but these effects were not magnified when EA was reduced to 10 kcal kgLBM^{-1} day^{-1}

Note. ↓ indicates a decrease in response to LEA. ↑ indicates an increase in response to LEA. LEA = low energy availability; EA = energy availability; HPG = hypothalamic-pituitary-adrenal; HPA = hypothalamic-pituitary-adrenal; HPT = hypothalamic-pituitary-thyroid; T_3 = triiodothyronine; GH = growth hormone; IGF-1 = insulin-like growth hormone. Adapted from "Endocrine effects of relative energy deficiency in sport" by K. J. Elliott-Sale, A. S. Tenforde, A. L Parziale, B. Holtzman, and K. E. Ackerman, 2018, *International Journal of Sport Nutrition and Exercise Metabolism*, *28*(4), pp. 336-337.

Reproductive

Of the body systems which are affected by LEA, the reproductive system is particularly sensitive to changes in metabolic and nutrition status. Negative energy balance can result in ovulatory disorders, irregular menses, amenorrhea, and disruptions in sexual maturation (Gordon et al., 2017). LEA is associated with menstrual cycle irregularities and impaired reproductive function. Menstrual irregularities can range from mild to severe, and include both subclinical abnormalities such as luteal phase defects (LPDs) and anovulatory cycles and clinically diagnosed disturbances such as oligomenorrhea and amenorrhea (De Souza & Williams, 2004). An estimated 50% of female athletes experience subclinical menstrual irregularities and 33% suffer from amenorrhea (De Souza et al., 1998). The presence or absence of menses is commonly used to assess reproductive function and health in females with LEA, given that post-menarche and pre-menopausal individuals are often most affected by conditions of LEA. As such, this section will focus on the changes in reproductive hormones in response to LEA and its subsequent effects on the menstrual cycle.

Regulation of the Menstrual Cycle

The menstrual cycle is a collection of regular cyclic changes which occur in the reproductive system of healthy females to prepare the female body for possible pregnancy (Acevedo-Rodriguez et al., 2018). Proper functioning of the menstrual cycle is often used as an indicator of normal reproductive physiology. Reproductive function is primarily regulated by the hypothalamic-pituitary-gonadal (HPG) axis, which is largely controlled by hypothalamic gonadotropin-releasing hormone (GnRH) secretions. At the onset of puberty, GnRH is secreted from the hypothalamus in a pulsatile fashion to trigger the release of follicle stimulating hormone

(FSH) and luteinizing hormone (LH) from the anterior pituitary. The release of FSH and LH regulates the production of estrogens, progesterone, and inhibin from the ovaries throughout the menstrual cycle, which then act in a series of feedback loops to alter FSH and LH release throughout the course of the menstrual cycle and ovulation.

LEA Mediates Alterations in GnRH and LH Pulsatility

Decreases in GnRH pulsatility are commonly detected by using the pulsatile secretion of LH, since GnRH secretion from hypothalamic neurons is difficult to measure directly. In a study by Loucks and Heath (1994a), females who were fed a dietary intake of 10 kcal $kgLBM^{-1}$ day^{-1} (a state of LEA) for five days had a 23% reduction in LH pulse frequency and a 40% increase in LH pulse amplitude compared to participants consuming 45 kcal $kgLBM^{-1}$ day^{-1} (a state of balanced EA). When a treadmill exercise regime with an exercise energy expenditure of 30 kcal $kgLBM^{-1}$ day^{-1} per day was added to both groups of participants, similar alterations in LH pulse frequency and amplitude were observed between the balanced EA and LEA conditions (Loucks et al., 1998). The similarity in LH pulse alterations with or without the addition of an exercise regimen suggests that LEA, rather than exercise stress, is the main cause of impairments to the reproductive cycle. While previous research suggests that LH pulsatility was disrupted when EA fell below a certain critical threshold (Loucks & Thuma, 2003), more recent research indicates that the extent of reproductive impairments is dependent on the magnitude of the energy deficiency, with menstrual disturbances increasing as EA decreases (Lieberman et al., 2018). Furthermore, restoration of energy balance in previously amenorrheic athletes resulted in the restoration of menses, providing further evidence that menstrual cycle dysregulation is dependent on EA (Dueck et al., 1996).

LEA Reduces GnRH Secretions to Suppress Reproductive Activity

The brain is largely responsible for monitoring nutrition and energy status and appears to temporarily suppress reproductive function to conserve energy for more vital physiological processes during times of LEA. In a state of negative energy balance, the brain suppresses HPG activity by inhibiting GnRH. Alterations in GnRH release has been identified as the main cause of reproductive dysfunction due to metabolic and nutrient deficiencies (Reame et al., 1985). Reduced GnRH secretion from the hypothalamus diminishes LH and FSH release from the pituitary, preventing follicular development and resulting in decreased estradiol (E_2) and progesterone levels. These changes manifest as HA, a form of secondary amenorrhea which presents with severe estrogen deficiency. Females with HA have markedly lower LH pulse frequency than healthy controls. Pulsatile GnRH treatment restored ovulation in previously HA patients, providing further evidence that reduced pulsatile secretion is the main cause of reproductive dysfunction induced by negative energy balance (Santoro & Elzahr, 1993).

Orexigenic and Anorexigenic Factors Regulate GnRH Secretions During LEA

Although the mechanism by which LEA reduces GnRH secretion is not fully understood, several factors involved in the regulation of appetite and metabolism may alter GnRH secretion depending on energy status. Leptin, insulin, and ghrelin are particularly important hormones which alter GnRH secretions by regulating the production of anorexigenic and orexigenic factors from neurons in the hypothalamus (Cone, 2005). When produced by these neurons, anorexigenic factors suppress the appetite, while orexigenic factors signal an increase in feeding behaviors. In a state of negative energy balance, decreases in insulin and leptin and increases in ghrelin result in both a decrease in anorexigenic factors and an increase in orexigenic factors (Celik et al.,

2015). Alterations in anorexigenic and orexigenic factors in response to insulin, leptin, and ghrelin is a primary cause of reproductive dysfunction through suppression of GnRH release.

Leptin

Leptin plays a role in sexual development and reproduction, and has been shown to affect the hypothalamic-pituitary-gonadal axis by stimulating GnRH and gonadotropin secretion (Barash et al., 1996). Reduced leptin is associated with low gonadotropin levels, and leptin treatment was able to restore gonadotropin and restore puberty in leptin-deficient mice. Although GnRH neurons themselves do not have leptin receptors, leptin appears to indirectly influence GnRH-secreting cells by acting through leptin-responsive afferent neurons. In murine studies, deletion of leptin receptors from forebrain neurons prevented puberty and caused infertility, indicating that other forebrain neurons mediate the effects of leptin on GnRH to cause alterations in menstrual function in response to energy status (Quennell et al., 2009).

Insulin

Insulin is a hormone produced and released from pancreatic beta cells which regulates energy homeostasis and plays a role in regulating the neuroendocrine reproductive axis. Disruption of the insulin receptor (*IR*) gene from specific neurons in mice caused a reduction in LH levels and reproductive hormones (Brüning et al., 2000). Insulin has been shown to stimulate the expression and secretion of GnRH in vitro, but ablation of the *IR* gene from GnRH neurons resulted in normal puberty and fertility, suggesting that insulin indirectly targets GnRH neurons to affect reproductive function (Burcelin et al., 2003). In vivo studies supported these findings, as administration of insulin significantly increased the LH pulse frequency to a greater extent than could be generated at the level of the pituitary gland alone, indicating that insulin modulates

neurons involved in GnRH secretion (Moret et al., 2008). Direct targets which may mediate the effects of insulin on GnRH include anorexigenic factors such as pro-opiomelanocortin (POMC), orexigenic factors such as neuropeptide Y (NPY), IGF-1, and GABA neurons (Gamba & Pralong, 2006). More recent evidence suggests that insulin may also directly affect hypothalamic GnRH neurons. In vitro, expression of the insulin receptor at the protein and mRNA levels was found in a clonal GnRH neuronal cell line. These cells were also shown to display characteristics similar to those of other insulin-sensitive tissues, although these findings have yet to be confirmed in vivo (Salvi et al., 2006).

Ghrelin

Ghrelin is an orexigenic factor which migrates to the hypothalamus from endocrine cells in the gastric submucosa, in addition to being produced directly by cells in the hypothalamic arcuate nucleus (ARC) (Lu et al., 2002). During energy restriction, increases in ghrelin stimulate GH secretion to stimulate appetite and promote feeding (Nakazato et al., 2001). In a state of LEA, increased ghrelin levels are associated with reductions in GnRH secretion and LH levels, as well as hypogonadism (Kluge et al., 2007). Ghrelin has been shown to influence GnRH levels through both indirect mechanisms, via anorexigenic and orexigenic factors, and via direct mechanisms involving the growth hormone secretagogue receptor (GHS-R) (Ogata et al., 2009; Farkas et al., 2013).

Anorexigenic Factors

Leptin, insulin, and ghrelin exert their effects by altering the release of anorexigenic factors, such as POMC and its derivatives like alpha-melanocyte stimulating hormone (αMSH)

(Cone, 2005). Insulin and leptin promote the expression of anorexigenic factors while ghrelin reduces their expression (Cowley et al., 2003).

POMC. POMC is a peptide precursor which undergoes posttranslational modifications to produce a variety of smaller, biologically active peptides. The peptides processed from POMC depend on the processing enzymes present in the tissue in which the *POMC* gene is expressed. In the hypothalamus, expression of POMC leads to the production of α-, β-, and γMSH (Coll et al., 2004). POMC-expressing neurons in the ARC are important anorexigenic neurons which mediate the effects of insulin and leptin (Qiu et al., 2014). POMC neurons make synaptic connections with GnRH neurons in the region of the brain where GnRH neurons are concentrated, suggesting that POMC-derived peptides act directly on GnRH secretion (Leranth et al, 1988).

αMSH. αMSH acts as an anorexigenic neuropeptide and is a cleavage product released by POMC upon stimulation by insulin and leptin (Benoit et al., 2002). αMSH binding to the melanocortin4 receptor (MC4R), expressed on GnRH neurons, stimulates GnRH production and increases serum LH levels (Celis, 1985). Mice deficient in either the MC4R or that lack both LepR and IR have reproductive deficiencies, suggesting that melanocortin signaling mediates the effects of insulin and leptin in the neuroendocrine axis. Ghrelin boutons also make connections with POMC neurons in the ARC, and ghrelin reduces POMC neuropeptide expression (Cowley et al., 2003). Accordingly, decreases in insulin and leptin in response to LEA inhibit the release of αMSH from POMC neurons while increases in ghrelin reduces POMC neuronal activity, resulting in reduced GnRH secretion.

***β*-Endorphin**. *β*-Endorphin is another POMC-derived peptide which has been shown to inhibit GnRH and gonadotropin secretion by interacting with opioid receptors (Ciechanowska et al., 2007). Administration of ghrelin in mice reduced LH concentration and pulse frequency, while co-administration of ghrelin with naloxone, an opioid antagonist, restored LH concentrations and frequency, suggesting that the effects of ghrelin on LH secretion was mediated by *β*-Endorphins (Ogata et al., 2009). Although it is unclear if *β*-Endorphins act directly on GnRH neurons or through indirect mechanisms, they are another potential mediator of ghrelin reduction in response to energy insufficiency and may suppress reproductive function through alterations in GnRH secretion.

Orexigenic Factors

In contrast to anorexigenic factors, which are decreased during LEA, orexigenic factors are upregulated in a state of negative energy balance, and have been shown to suppress LH secretion. Leptin and insulin inhibit orexigenic factor activity while ghrelin promotes their expression.

NPY. NPY is a hypothalamic orexigenic factor which may mediate the effects of leptin, insulin, and ghrelin on reproduction. NPY neurons are found in close contact with GnRH neurons, and may input signals directly into GnRH cell bodies and nerve terminals through NPY Y1 receptors (Li et al, 1999). Activity and gene expression of NPY increases during energy restriction. NPY is associated with reduced LH secretion and exogenous administration of NPY has been shown to reduce gonadotropin levels in mice (Catzeflis et al., 1993). In NPY-deficient mice, gonadotropin levels were unaffected by fasting (Hill & Levine, 2003). In addition, although leptin-deficient mice are commonly obese and sterile, these consequences were not

observed in NPY-deficient mice, suggesting that leptin may act through an NPY-dependent mechanism (Erickson et al., 1996). Indeed, expression of both leptin and insulin reduced expression of *NPY* gene expression in rats, offering a potential mechanism through which LEA-induced leptin and insulin deficiencies may allow for greater NPY activity and subsequent reductions in GnRH and LH secretion (Schwartz et al., 1992).

AgRP. Agouti-related peptide (AgRP) is another hypothalamic orexigenic factor that is co-expressed with NPY in the ARC and inhibits LH secretion (Vulliémoz et al., 2005). Deletion of neurons expressing AgRP restored fertility in leptin-deficient mice, suggesting that AgRP also mediates the effects of leptin deficiency on reproductive function (Wu et al., 2012). NPY and AgRP may also mediate the effects of ghrelin on GnRH. Administration of ghrelin has been shown to increase mRNA levels of both AgRP and NPY in the ARC, primarily through the GHS-R (Kamegai et al., 2001).

Orexin. Orexin is an orexigenic hormone involved in appetite control. It is secreted by hypothalamic neurons which project into the ARC where GnRH neurons are concentrated (Peyron et al., 1998). In mice, 80% of GnRH neurons express orexin receptors, and orexin has been shown to decrease GnRH neuron activity and pulse frequency through β-Endorphin and corticotropin-releasing hormone receptors (Campbell et al., 2003; Gaskins & Moenter, 2012). Leptin inhibits orexin-expressing neurons (Yamanaka et al., 2003), suggesting that orexin may indirectly suppress GnRH hormones in response to decreases in leptin concentration observed during LEA.

Kisspeptin Regulates GnRH Secretions During LEA

Kisspeptin is a peptide found in the hypothalamus which positively regulates GnRH synthesis and release through its receptor Kiss1r (de Roux et al., 2003). The kisspeptin receptor is coupled to the GPR54 protein, which regulates reproduction. Kisspeptin and GPR54 stimulate the HPG axis and directly stimulate the secretion of GnRH from the hypothalamus (Seminara et al., 2003). Kisspeptin has also been shown to integrate the effects of estrogen on GnRH neurons. Kisspeptin neurons in the anteroventricular periventricular nucleus mediate positive feedback signaling of estrogen on GnRH and LH release, while kisspeptin neurons in the ARC mediate negative feedback signaling of estrogen (Adachi et al., 2007).

Kisspeptin appears to act as a nutrition sensor which mediates the effects of LEA on reproductive function. In murine models, negative energy balance reduced *Kiss1* gene expression and gonadotropin levels, resulting in a delayed onset of puberty, while administration of exogenous kisspeptin restored gonadotropin secretion and normalized puberty onset (Castellano et al., 2005). Leptin, AgRP, and NPY have been proposed as effectors which mediate the effects of low energy status on kisspeptin. Kisspeptin neurons in the ARC contain leptin receptors, which GnRH neurons themselves lack (Sanchez-Garrido & Tena-Sempere, 2013). Downregulation of leptin in response to LEA reduced hypothalamic *Kiss1* mRNA expression and kisspeptin action, while administration of leptin restored *Kiss1* mRNA expression in leptin-deficient rats (Castellano et al., 2006; Quennell et al., 2011). Thus, reduced leptin levels in response to LEA likely suppresses kisspeptin action, resulting in reduced GnRH secretion. AgRP and NPY may also play a role in kisspeptin action. AgRP and kisspeptin neurons share inhibitory synaptic connections, and kisspeptin neurons express NPY receptors (Padilla et al., 2017). In a

state of LEA, AgRP and NPY expression is upregulated, which may result in reduced GnRH secretion through inhibition of kisspeptin action.

Bone Health

Another major health consequence linked to LEA is diminished bone health. Bone health is often evaluated by using bone mineral density (BMD) testing through a dual-energy x-ray absorptiometry scan. Various other bone metabolic markers can be used to indicate rates of bone formation and resorption. LEA is associated with poor bone health outcomes in exercising women, and may be exasperated by reproductive dysfunction (Nattiv et al., 2007).

Overview of Bone Metabolism

Bone microarchitecture is constantly being remodeled through bone formation and resorption. When these processes become imbalanced, a higher relative rate of bone resorption to formation can alter bone structure and reduce bone mass. Markers of bone metabolism can be used to identify rates of bone resorption and formation and serve as indicators of bone health.

Bone Formation

Markers of osteoblast activity, including bone-specific alkaline phosphatase (BAP), carboxy-terminal propeptides of type 1 procollagen (P1CP), and osteocalcin, are used to measure rates of bone formation (Vasikaran et al., 2010). Osteocalcin is a type of collagen protein in the bone matrix which is synthesized by osteoblasts during bone formation. P1CP are peptides which are cleaved by procollagen molecules synthesized by osteoblasts, and circulating concentrations are representative of the rate of collagen type 1 production. BAP is an enzyme specific to bone metabolism and represents the rate of bone mineralization by osteoblasts.

Bone Resorption

Markers of osteoclast activity, β-C-terminal telopeptide (β-CTx), N-terminal telopeptide (NTX), pyridinoline (PYD), and deoxypyridinoline (DPD), give an indication of bone resorption (Kuo & Chen, 2017). During bone resorption, pyridinium crosslinks in the bone are released in urine and can be detected by measuring PYD and DPD, which are components of the crosslinks. Hydroxyproline is also released into the bloodstream during bone resorption and can give an indication of the rate of bone resorption, although these are not specific to bone metabolism.

Alterations in Bone Microarchitecture and Bone Mineral Density Resulting from LEA

In a study by Ihle and Loucks (2004), markers of bone metabolism were assessed after five days of a diet of either 10, 20, 30, or 45 kcal $kgLBM^{-1}$ $day^{-1}$of EA in healthy, young, exercising, pre-menopausal women. Markers of bone formation, P1CP and total osteocalcin, were both reduced at EA levels of 30 kcal $kgLBM^{-1}$ day^{-1} or less. However, while decreases in P1CP varied linearly with reductions in EA, osteocalcin only significantly decreased when EA was between 20-30 kcal $kgLBM^{-1}$ day^{-1}. Bone resorption, as measured by NTX concentrations, was only increased when EA was reduced to 10 kcal $kgLBM^{-1}$ day^{-1}. These results were supported in another study where LEA (15 kcal $kgLBM^{-1}$ day^{-1}) resulted in higher β-CTx and lower P1NP when compared with controls consuming a balanced EA of 45 kcal $kgLBM^{-1}$ day^{-1} (Papageorgiou et al., 2018). These alterations in bone microarchitecture were associated with other hormonal alterations, suggesting that these effects are mediated by hormone changes in response to states of energy deficiency.

Direct Mechanisms of LEA on Bone Health

Insulin and leptin decrease in response to LEA, and may play a role in the poor bone outcomes associated with LEA (Ihle & Loucks, 2004). Osteoblasts and osteoclasts both contain insulin receptors, and insulin has been shown to increase bone formation and decrease bone resorption in vivo (Thomas et al., 1998). In exercising women, reductions in P1CP in response to LEA were linearly related to changes in insulin (Ihle & Loucks, 2004). Leptin may alter bone metabolism both directly, via osteoblasts and chondrocytes, and indirectly, via other hormones, including estrogen, cortisol, and IGF-1 (Upadhyay et al., 2015). Reduced bone mineral density is also associated with decreased levels of T_3 and IGF-1 which are correlated with decreases in osteocalcin, P1CP, and BAP (Ihle & Loucks, 2004). Alterations in these hormones in response to LEA might also mediate the disruption of bone health induced directly by LEA.

Effects of LEA-Induced Reproductive Dysfunction on Bone Health

Although LEA has direct effects on bone health, it also indirectly influences bone integrity through alterations in estrogen levels and reproductive dysfunction (De Souza et al., 2008). As described in the Triad, menstrual function appears to be closely linked to bone health, with early studies demonstrating that amenorrheic athletes had lower BMD in the lumbar spine than eumenorrheic athletes (Drinkwater et al., 1984). The negative consequences of LEA on bone health appears to be exacerbated by estrogen deficiency (De Souza et al., 2008). Women who were deficient in both energy and estrogen concentrations had the lowest P1NP and T_3 concentrations and the highest β-CTX and ghrelin concentrations (Ihle & Loucks, 2004). Restoration of menstrual function has been shown to improve bone health, with a 6.3% increase in BMD in previously amenorrheic athletes who resumed menses as opposed to a 3.4% decrease

in BMD in athletes who remained amenorrheic (Drinkwater et al., 1986). Although it can be improved by restoration of menses, BMD still remains lower in athletes with a history of amenorrhea than eumenorrheic athletes with no history of menstrual irregularities, and the extent of BMD is often associated with the length of menstrual disturbances.

The association between negative bone health and menstrual dysfunction observed in amenorrheic athletes is likely mediated by a state of hypoestrogenism associated with HA. In exercising females with LEA, reduced E_2 is associated with increased osteocalcin (Ihle & Loucks, 2004). Osteoblasts and osteocytes both express estrogen receptors. Estrogen inhibits bone resorption by suppressing the formation of osteoclasts while stimulating osteoblast activity via estrogen receptor α. Estrogen also regulates skeletal health by influencing calcium absorption and increasing GH secretion (Riggs et al., 2002). Reduced GnRH secretion and LH pulsatility in response to LEA results in a hypoestrogenic condition, which alters bone metabolism in favor of bone demineralization, which compounds to progressively diminish bone mineral density as the length of menstrual disturbance increases.

Metabolism

Resting metabolic rate (RMR) is the amount of energy the body uses to maintain homeostasis and vital physiological processes at rest. RMR and non-resting energy expenditure, which includes basal activity thermogenesis, the thermic effect of food, and exercise activity thermogenesis, make up the total daily energy expenditure (Maclean et al., 2011). LEA is associated with reductions in total RMR, which is likely an adaptive mechanism to conserve energy when EA is low (Melin et al., 2014).

Effects of Leptin on Metabolic Adaptations during LEA

Leptin concentrations have repeatedly been demonstrated to decrease in response to negative energy balance and may in part mediate the metabolic adaptations associated with LEA. Leptin interacts with skeletal muscle proteins to influence metabolic adaptations, with decreases in leptin increasing expression of the myosin heavy chain (MHC I) isoform, an efficient muscle fiber with low energy cost (Baldwin et al., 2011). Restoration of leptin levels stimulated the MHC IIx isoform, which is more energetically costly than MHC I. These results suggest that decreases in leptin allow for the skeletal muscle adaptations which increase energy efficiency by lowering the energy cost of aerobic contractions, contributing to overall reductions in RMR.

Impact of LEA-Induced Alterations of Hypothalamic-Pituitary-Thyroid Axis on Metabolism

The hypothalamic-pituitary-thyroid axis is largely mediated by thyroid hormones and is an important regulator of energy expenditure as it adapts the metabolic rate in response to energy intake (Kim, 2008). Specifically, the reductions in T_3 associated with LEA is associated with reduced thermogenesis and metabolic rate. Thyroid hormones are known to regulate metabolic processes, such as lipolysis, glycogenesis, and protein synthesis (Yavuz et al., 2019). Reductions in thyroid hormones may therefore be responsible for the reductions in RMR seen in athletes with LEA.

Cardiovascular

LEA is associated with endothelial dysfunction and aberrant lipid metabolism, leading to chronic heart issues. Amenorrheic athletes with LEA are at particular risk for cardiovascular

complications, and often present with bradycardia, hypotension, and impaired ability to perform orthostatic challenges due to alterations in the renin-angiotensin system (Mountjoy et al., 2018).

Mechanisms Underlying Effects of LEA on Cardiovascular Function

In addition to its negative effects on bone health, hypoestrogenism in amenorrheic athletes with LEA can result in cardiovascular impairments. E_2 regulates blood vessel function via estrogen receptors located in the coronary and peripheral vessels. Estrogens stimulate nitric oxide (NO) synthesis and production, which enhances vasodilation (Murphy, 2011). E_2 also affects the endothelium, blood vessels, heart muscle, and metabolic parameters to provide a cardioprotective effect (Reckelhoff, 2005). Estrogen decreases low-density lipoprotein (LDL) oxidation and accumulation of excess oxidized LDL, providing a protective effect against atherosclerosis (Cid et al., 2002). Hypoestrogenism can impair NO activity, cause endothelial dysfunction, alter autonomic function, and activate the renin-angiotensin system (Schunkert et al., 1997). Decreased estrogen is also associated with unfavorable changes in the lipid profile, such as increased concentrations of total cholesterol, triglycerides, and LDL (Ouyang et al., 2006). Premenopausal women on calorie restricted diets have elevated lipid levels, and amenorrheic athletes have been shown to have elevated LDL compared with eumenorrheic athletes (Friday et al., 1993). Women with HA also have impairments in the dilation of the brachial artery, which proceeds endothelial dysfunction of the coronary artery (Gordon et al., 2017). Taken together, these results suggest that estrogen concentration reduction is a primary cause of cardiovascular dysfunction in women with LEA.

Gastrointestinal

Gastrointestinal complaints are common in athletes with LEA, and include nausea, vomiting, diarrhea, abdominal pain, bloating, and loss of appetite (Rogers et al., 2021). Studies have also reported constipation, delayed gastric emptying, increased intestinal transit time, and sphincter dysfunction (Mountjoy et al., 2018).

Mechanisms Underlying Gastrointestinal Dysfunction Associated with LEA

Limited research has been done on the gastrointestinal effects of LEA in athletes, and most evidence for the underlying mechanisms of gastrointestinal dysfunction resulting from insufficient energy balance come from the extreme LEA state of anorexia nervosa (AN). However, alterations to the gut microbiota may contribute to some of the gastrointestinal complaints associated with LEA. Profound disturbances in the gut microbiota are associated with severe energy deficits, which may also alter gastrointestinal function. For instance, AN patients have increased levels of *Enterobacteriacee* and *Methanobrevibacter smithii* (Morita et al., 2015). This shift in microbiota abundance appears to be an energy conserving mechanism to obtain more nutrients, as these species are able to extract more calories from food by transforming hydrogen into methane (Million et al., 2013). Increased production of methane is also associated with reduced intestinal transit time and constipation, offering a potential mechanism for the reported gastrointestinal complications during LEA (Gottleib et al., 2016).

Immunological

Although it is plausible that there is an association between LEA and impaired immunity given that the body diverts energy away from certain physiological systems during periods of insufficient energy intake, there is limited evidence regarding this interaction. However, it is

hypothesized that this may be due to the immune system being the last system to shut down in response to LEA. Even so, LEA is associated with increased incidence of respiratory and gastrointestinal illnesses and symptoms such as body aches and headaches (Drew et al., 2018). Some studies have also reported that athletes with amenorrhea exhibit more upper respiratory symptoms and reduced immunoglobulin A (IgA) secretion compared with eumenorrheic athletes (Shimizu et al., 2012). Similarly, military recruits with LEA had reduced cell-mediated and humoral immune function. Restoration of energy balance resulted in an increase in IgA compared with controls (O'Leary et al., 2020).

HSC Aging

In a study in physique athletes undergoing physical training and concurrent energy restriction, athletes with LEA had increased levels of neutrophils and rates of hematopoietic stem cell (HSC) proliferation (Sarin et al., 2019). The fact that excessive cell proliferation promotes aging is consistent with the observation that phenotypic characteristics of an aging HSC population were observed in the energy-restricted athletes. HSC aging results in the loss of self-renewal and regenerative potential and thus may be responsible for impaired immune function in athletes with LEA (Nikolich-Žugich, 2018).

Leptin and Immunosuppression

Alterations in leptin levels are associated with changes in both T and B lymphocytes. Decreased levels of leptin caused by LEA are associated with reduced T-cell maturation and proliferation, an imbalanced ratio of $CD4^+/CD8^+$ T-lymphocytes, atrophy of the thymus, and a dominant Th2 helper response (Lord et al., 1998; Howard et al., 1999). Leptin administration was shown to reverse these alterations and inhibit their immunosuppressive effects, suggesting

that LEA induces immunosuppression partially through reductions in leptin levels. Leptin also mediates the suppression of B-lymphocyte proliferation and function (Tanaka et al., 2011). LEA is associated with reduced proliferation of B-lymphocytes, an expansion of the geminal center containing memory B-lymphocytes, reduction of the pool of naïve B-cells, and reduced IgG production (Sarin et al., 2019). Similar changes have been observed in aging immune systems, suggesting that these alterations may contribute to immunosuppression in athletes with LEA (Sellami et al., 2018).

Altered Cytokine Profile

Alterations in cytokines which favor anti-inflammatory cytokines, in addition to an enhanced $CD4^+$ Th2 response, is associated with dominance of the regulatory/wound-healing macrophage (M2/M3) (Jolly, 2004). These macrophages suppress the immune response by limiting inflammation, which is consistent with the downregulation of systemic inflammation and immunometabolism observed in athletes with LEA (Sarin et al., 2019).

Mucosal Disturbances

Severe states of LEA, such as AN, have been associated with an increased risk for autoimmunity (Raevuori et al., 2014). Athletes in a state of energy deficiency had increased pro-inflammatory IgG activity, reduced IgG antibody production, reduced affinity of IgGs with specific B-cell receptors, impaired IgE signaling, increased eotaxin, and a dominant Th2 response (Sarin et al., 2019). These changes are associated with autoimmune diseases, particularly of the lungs and intestine, suggesting a possible mechanism for the increased susceptibility to autoimmune conditions seen in individuals with LEA (Hogan et al., 2001).

Conclusion

Negative energy balance, resulting from a combination of insufficient energy intake and excess energy expenditure, results in numerous hormonal adaptations which take place in order to conserve energy for basic physiological processes, but at the expense of numerous body systems. In female athletes, LEA has been shown to have detrimental consequences on reproductive, bone, metabolic, cardiovascular, gastrointestinal, and immunological health. The mechanisms by which LEA induces these effects on various body systems have not been fully elucidated, but appear to involve complex interactions between hormones such as leptin, estrogen, and ghrelin, with the HPG axis, which affects a number of body systems, playing a particularly important role in regulating menstrual function. Although many of the physiological complications of LEA are reversible by restoring EA to normal levels, certain consequences, such as bone loss, may be irreversible. As such, it is particularly vital to monitor for this condition and rapidly restore energy balance in female athletes to restore homeostasis and avoid long term complications related to LEA.

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