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Review article

A summary of the influence of exogenous estrogen administration across the lifespan on the GH/IGF-1 axis and implications for bone health

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ABSTRACT

Bone growth, development, and remodeling are modulated by numerous circulating hormones. Throughout the lifespan, the extent to which each of the hormones impacts bone differs. Understanding the independent and combined impact of these hormones on controlling bone remodeling allows for the development of more informed decision making regarding pharmacology, specifically the use of hormonal medication, at all ages. Endocrine control of bone health in women is largely dictated by the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis and the hypothalamic-pituitary-ovarian (HPO) axis. Growth hormone, secreted from the pituitary gland, stimulates cells in almost every tissue to secrete IGF-1, although the majority of circulating IGF-1 is produced hepatically. Indeed, systemic IGF-1 concentrations have been found to be correlated with bone mineral density (BMD) in both pre- and post-menopausal women and is often used as a marker of bone formation. Sex steroids produced by the ovaries, namely estradiol, mediate bone resorption through binding to estrogen receptors on osteoclasts and osteoblasts. Specifically, by increasing osteoclast apoptosis and decreasing osteoblast apoptosis, adequate estrogen levels prevent excessive bone resorption, which helps to explain the rapid decline in bone mass that occurs with the menopausal decrease in estrogen production. Though there are documented correlations between endogenous estrogen concentrations and GH/IGF-1 dynamics, this relationship changes across the lifespan as sex-steroid dynamics fluctuate and, possibly, as tissue responsiveness to GH stimulation decreases. Aside from the known role of endogenous sex steroids on bone health, the impact of exogenous estrogen administration is of interest, as exogenous formulations further modulate GH and IGF-1 production. However, the effect and extent of GH and IGF-1 modulation seems to be largely dependent on age at administration and route of administration. Specifically, premenopausal women using combined oral contraceptive therapy (COC), post-menopausal women taking oral hormone therapy (HT), and both pre- and post-menopausal women using a transdermal form of estrogen therapy (COC or HT) demonstrate disparate GH/IGF-1 responses to exogenous estrogen. This review serves to summarize what is currently known regarding the influence of exogenous estrogen administration across the lifespan on the GH/IGF-1 axis and implications for bone health.

1. Introduction

In women of all ages, bone health is largely influenced by circulating estrogen, a hormone that fluctuates throughout the course of the menstrual cycle, decreases precipitously after menopause, and is known to prevent bone resorption [1]. Bone turnover is further influenced by the GH/IGF-1 axis, in which growth hormone releasing hormone (GHRH) is released by the hypothalamus and stimulates the pituitary to secrete growth hormone (GH), which stimulates peripheral production of insulin-like growth factors (IGFs) [2–4]. The IGFs are single-chain polypeptides that bind to IGF receptors to elicit a response [5]. IGF-1, the majority of which is produced in the liver, has a profound bone trophic effect through binding to the IGF-1 receptor on osteoblasts, activating second messenger systems, and resulting in osteoblast differentiation and resultant bone formation [5,6]. Circulating estrogen, whether of endogenous or exogenous origin, modulates the GH/IGF-1 axis and can therefore influence bone turnover through mechanisms independent of its direct anti-resorptive effects [7]. Thus, the use of exogenous estrogen throughout a woman's lifespan, either through premenopausal hormonal contraception or postmenopausal estrogen therapy, its specific impact on the GH/IGF-1 axis, and potential downstream effects on bone will be explored in both premenopausal and postmenopausal women. (See Tables 1 and 2.)

Eighteen million premenopausal women use combined oral contraceptives (COCs), and use of hormonal contraception is particularly common during adolescence and young adulthood [8–10], a time when peak bone mass acquisition is ongoing [11–15]. Despite the increasing use of COC therapy in younger women, investigators have failed to

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Table 1
Summary of the effects of oral vs. transdermal estrogen therapy on the GH/IGF-1 axis and bone health in postmenopausal women. Progestins are oral unless otherwise specified. EE = ethinyl estradiol; CEE = conjugated equine estrogen; 17β = 17β-estradiol; DYDR = dydrogesterone; NETA = norethisterone acetate; MPA = medroxyprogesterone acetate; CMA = chlormadinone acetate; CA = cyproterone acetate; NS = not significant; L Spine = lumbar spine; Tot Hip = total hip; Prox Femur = proximal femur.

		Estrogen	Dose	Route of administration	Intermittent progestin	Dose	Population studied	GH/IGF-1 findings	Bone findings
1986	Dawson-Hughes et al.	EE	20 μg/day, 15 days	Oral	–	–	Age 50–73 n = 12	↑GH AUC ↓IGF-1	–
1988	Frohlander and von Schoultz	EE	10 μg/day, 3 months	Oral	–	–	Age 44–62 n = 14	↑GH AUC ↓IGF-1	–
		EE + Tamoxifen	10 μg/day 20 mg/day 3 months					GH and IGF-1 unchanged	
1991	Bellantoni et al.	17β	0, 50, 100, & 150 μg/day 8 weeks/dose	Transdermal	MPA	10 mg/day	Age 45–72 n = 28	↓GH peak response to GHRH injection ↔IGF-1	–
1991 + 992	Weissberger et al. + Ho and Weissberger	EE	20 μg/day, 2 months	Oral	Norethisterone	5 mg/day	Age 53–77 n = 7	↑GH mean ↑GH pulse amplitude ↓IGF-1	↓ osteocalcin
		17β	100 μg/day, 2 months	Transdermal			n = 7	↑IGF-1	↑ osteocalcin ↑ procollagen I ↑ procollagen III
1992	Slowinska-Srzednicka et al.	17β	100 μg/day, 6 months	Transdermal	CMA	2 mg/day	Age 44–59 N = 12	↑IGF-1	–
1993	Campagnoli et al.	CEE	0.625 mg/day, 6 months	Oral	DYDR	20 mg/d	Age 43–58 n = 16	↓IGF-1	–
		17β	50 μg/day, 6 months	Transdermal			n = 14	↑IGF-1	
1993	Kelly et al.	EE CEE EV	20 μg/day, 1 month 1.25 mg/day, 1 month 2 mg/day, 1 month	Oral	MPA	10 mg/day	Age 54–71 n = 6	↑GH mean ↓IGF-1	–
1994	Campagnoli et al.	CEE	0.625 mg/day, 9 months	Oral	DYDR	10 mg/day, 6 months	Age 40–56 n = 6	↓IGF-1	–
					NETA	5 mg/day, 3 months		↔IGF-1	
		17β	50 μg/day, 9 months	Transdermal	DYDR	10 mg/day, 6 months	n = 6	↔IGF-1	
					NETA	5 mg/day, 3 months		↑IGF-1	
1994	Dall'Anglio et al.	17β	50 μg/day, 6 months	Transdermal	MPA	10 mg/day	n = 7	↔GH peak response to GHRH injection ↔IGF-1	–
1994	Hillard et al.	CEE	0.625 mg/day, 3 years	Oral	dl-norgestrel	0.15 mg/day	Age 52 ± 4 n = 33	–	↑L. Spine BMD ↑Prox Femur BMD
		17β	50 μg/day, 3 years	Transdermal	NETA	0.25 mg/day (transdermal)	n = 33		
1994	Lieberman et al.	Various	Various	Oral	Various	Various	Age 60–69 n = 13	↓IGF-1	–
1996	Bellantoni et al.	CEE	1.25 mg/day, 6 weeks	Oral	–	–	Age 49–75 n = 16	↑GH AUC ↓IGF-1	–
		17β	100 μg/day, 6 weeks	Transdermal				↔GH AUC ↔IGF-1 (↓IGF-1 > 62 yr)	
1996	Friend et al.	17β	2 mg/day, 15 days 200 μg/day, 15 days	Oral Transdermal	–	–	Age 52–80 n = 8	↑GH AUC ↑GH mean ↑GH pulse amplitude ↓IGF-1	–
1996	Helle et al.	17β	2 mg/day, 6 months	Oral	NETA	1 mg/day	Age 43–63 n = 14	↓IGF-1	–
		17β	50 μg/day, 6 months	Transdermal	NETA	250 μg/day (transdermal)		↔IGF-1	
1998	Campagnoli et al.	17β	50 μg/day, 6 months	Transdermal	DYDR	20 mg/day	Age 42–58 n = 39	Low baseline IGF-1: ↑ IGF-1 High baseline IGF-1: ↓ IGF-1	–
1998	Moe et al.	Various	Various	Oral	Various	Various	Age 57 + n = 24	↑GH mean ↓IGF-1	–
2000	Heald et al.	CEE	0.625 mg, 12 months	Oral	–	–	Age 49 ± 1.9 n = 10	↓↓↓↓IGF-1	–
					MPA	10 mg/day, 3 months		↓↓↓ IGF-1	
					Desogestrel	75 μg/day, 3 months		↓↓ IGF-1	
					Norethindrone	1.05 mg/day, 3 months		↓ IGF-1	

(continued on next page)

Table 1 (*continued*)

		Estrogen	Dose	Route of administration	Intermittent progestin	Dose	Population studied	GH/IGF-1 findings	Bone findings
2002	Cetinkaya et al.	CEE	Not Specified, 2 year	Oral	MPA	10 mg/day	Age 41–56 n = 17	–	↓L Spine BMD (NS)
					–	–	n = 18		
		17β	Not Specified, 2 year	Transdermal	–	–	n = 15		
2003	Nugent et al.	CEE (n = 9)	1.25 mg/day, 4 months	Oral	CA DYDR MPA DYDR	5 mg/day 20 mg/day 10 mg/day 2.5 mg/day	Age 57 ± 3 n = 9	↓ IGF-1 ↓ IGF-1 ↓ IGF-1 ↔IGF-1	–
		17β (n = 10)	100 μg/day, 4 month	Transdermal	CA DYDR MPA NETA	5 mg/day 20 mg/day 10mg/day 2.5 mg/day	n = 10	↔IGF-1 ↔IGF-1 ↑IGF-1 ↑IGF-1	
2011	Veldhuis et al.	17β	2 mg/day, 21 days	Oral	MPA	5 mg/day	Age 52–74 n = 11	↑GH ↓IGF-1	–
2014	Kim et al.	CEE	0.625 mg/day, 2 years	Oral	– Not Specified	– Not Specified	Age 54 ± 6 n = 16 n = 30	–	↑L Spine BMD ↑Tot Hip BMD
		17β	1.5 mg/day, 2 years	Transdermal	– Not Specified	– Not Specified	n = 15 n = 39		

definitively answer the basic question, i.e. are COCs helpful or harmful to bone? COCs suppress bone turnover markers, including those of bone formation [16–20], and some studies suggest a deleterious impact of COCs on bone mineral density (BMD) in younger compared to older women [10,21]. Interpreting reports of effects of COCs on bone is complicated by varied study designs and populations studied, lack of

Table 2
Summary of the effects of oral vs. transdermal contraceptive therapy on the GH/IGF-1 axis and bone health in premenopausal women. Progestins are oral unless otherwise specified. EE = ethinyl estradiol; L. spine = lumbar spine; Tot hip = total hip; WB = whole body; NA = not applicable.

		Estrogen	Dose		Route of administration	Progestin	Dose	Population studied	GH/IGF-1 findings	Bone findings
1990	Karlsson et al.	EE	30 μg/day, 2–3 months	Monophasic	Oral	desogestrel or levonorgestrel	150 μg/day	Age 22–31 n = 9	↓GH pulse amplitude ↑GH pulse frequency	–
1993	Massa et al.	EE	20–35 μg/day	Not specified	Oral	Various	Various	Age 30 ± 7.5 n = 10	↑GHBP ↔GH ↔IGF-1	–
1994	Jernstrom and Olsson	EE	Various	Monophasic (n = 21) triphasic (n = 12)	Oral	Various		Age 19–25 n = 17 (present OC) n = 16 (former OC)	↓IGF-1 (present users only)	–
2000	Balogh et al.	EE	30 μg/day	Monophasic	Oral	Dienogest	2 mg/day	Age 24 ± 3 n = 9	↔GH ↓IGF-1	–
						Levonorgestrel	125 μg/day	n = 9	↑GH AUC ↓IGF-1	
2001	Jernstrom et al.	EE	Various	Various	Oral	Various	Various	Age 17–35 n = 155	↓IGF-1 ↑IGFBP-3	–
2002	Grinspoon et al.	EE + rhIGF-1	35 μg/day, 30 μg 2x/day, 9 months	Monophasic	Oral	Norethindrone	0.4 mg/day	Age 18–38 n = 14	↑IGF-1	↑L. spine BMD
		rhIGF-1	30 μg 2x/day, 9 months	NA	NA	NA	NA	n = 16	↑IGF-1	
		EE + rhIGF-1 placebo	35 μg/day, 9 months	Monophasic	Oral	Norethindrone	0.4 mg/day	n = 15		
2010	Harel et al.	EE	20 μg/day, 1 year	NA	Transdermal	Norelgestromin (transdermal)	150 μg/day	Age 12–21 n = 5	↔IGF-1	↔BMD (↑L. spine/Tot hip in control group)
2011	Blackmore et al.	Not specified	Not specified	Not specified	Oral	Various	Various	Age 18–21 n = 80 Age 31–40 n = 112	↓IGF-1 ↔IGF-1	–
2015	Elkazaz and Salama	Not specified	Not specified	Not specified	Oral	Various	Various	Age 20–40 n = 43 (present)	↓IGF-1	↓spine T-score ↓femur T-score ↓forearm T-score
								n = 41 (past)	↔IGF-1	↔T-scores

randomized, controlled trials (RCTs), and the rapidly-evolving nature of contraceptive therapies [10,22]. Further, some prospective studies have reported a higher risk of fracture in *young* COC users compared to older COC users [23,24], though data are not definitive. Given the economic and health care burden associated with poor bone health and fractures, it is critical to determine whether non-oral routes of hormonal contraception afford advantages over COCs in young women.

A potential physiologic mechanism for the observed harmful effect of COCs on bone in young women is the hepatic "first-pass effect," which describes how metabolism of oral estrogen suppresses the potential for liver production of IGF-1 and reduces IGF-1 bioavailability by altering hepatic IGF binding proteins (IGFBPs) [25,26]. Further, the type progestin used in the COC formulation may modify the impact on the GH/IGF-1 axis. However, COC effects on the GH/IGF-1 axis may be obviated by alternate routes of administration such as the transdermal route.

Menopause and aging in and of itself result in alterations in the GH/IGF-1 axis, with overall decreased GH and IGF-1 concentrations exhibited in older premenopausal versus younger premenopausal women [27]. The profound decline in circulating estrogen concentrations that occurs with menopause further impinges upon the GH/IGF-1 axis. Left untreated, lower estrogen and IGF-1 concentrations create an environment in which bone resorption is uninhibited and bone formation is suppressed, creating a net loss of bone that can result in osteoporosis and an increased fracture risk [28]. In postmenopausal women, estrogen therapy (ET) is widely prescribed to manage menopausal symptoms, such as vasomotor instability, and is also beneficial for bone health [29]. Indeed, several studies indicate the effectiveness of ET in maintaining bone mass in postmenopausal women [1,30–35]. However, ET also influences the GH/IGF-1 axis through mechanisms that have not yet been fully elucidated, but it is clear that the response is dependent upon the estrogen dose, the use of a progestin with estrogen, and the route of administration of exogenous estrogen. By understanding how endogenous and exogenous estrogen interact in postmenopausal women to affect the GF/IGF-1 axis, we may be able to capitalize upon these mechanisms and develop alternative therapies to prevent postmenopausal bone loss.

The purpose of this review is to summarize what is currently known regarding the influence of exogenous estrogen administration in both premenopausal and postmenopausal women on the GH/IGF-1 axis and implications for bone health.

2. Postmenopausal women

2.1. Changes in the GH/IGF-1 axis with oral estrogen therapy

The response of the GH/IGF-1 axis in postmenopausal women using ET has been thoroughly investigated over the past three decades in women using a variety of exogenous estrogens with and without various classes of progestins. Both the direction and magnitude of changes in GH and IGF-1 that occur with ET are dependent on the type of therapy, dose, duration of therapy, and route of administration. We begin with a summary of the effects of three common oral estrogen preparations: ethinyl estradiol (EE), conjugated equine estrogens (CEE), and 17β-estradiol.

2.1.1. Section 1. Oral ethinyl estradiol (EE)

Investigators have consistently reported increases in GH and decreases in IGF-1 as a result of oral EE therapy in postmenopausal women [36–38]. One of the earliest studies conducted was an investigation of the GH and IGF-1 responses to oral EE in normal weight postmenopausal women (n = 12, age 50–73 years) who underwent 24 h blood sampling before and after 15 days of oral EE (20 μg/day). A 53% increase in mean GH concentration ($p = 0.039$) and a 42% decrease in mean IGF-1 concentration ($p = 0.006$) were observed after EE therapy. In addition, participants underwent a GHRH stimulation test (bolus injection: 1.5 μg GHRH/kg body weight) both before and after EE therapy and the mean peak GH response to the GHRH stimulation was significantly greater after oral EE therapy than before therapy (23.0 ± 3.7 vs. 15.3 ± 3.1 ng/mL). The GH response pattern was attributed to increased pituitary sensitivity to GHRH with increasing serum estradiol and a loss of negative feedback due to decreased IGF-1 [36].

Clinically, ET often persists for several months or years, making the translation of studies reporting on short-term EE somewhat limited. Nevertheless, investigations of the impact of longer-term oral EE therapy on the GH/IGF-1 axis have yielded similar results. For example, in postmenopausal women aged 53–77 years. (n = 12), 24 h serial blood samples collected every 10 min before and after 2 cycles of EE therapy (20 μg EE/day for 28 days + 5 mg norethisterone/d on days 15–21 of each cycle) resulted in a 250% increase in mean GH concentration, 107% increase in GH pulse amplitude, and a 273% increase in basal GH concentration with a 33% decrease in IGF-1 concentration [38]. As such, these data support the notion that EE stimulates GH production while suppressing IGF-1 production, which diminishes the negative inhibition of GH production.

In an effort to further understand the mechanism by which oral EE is impacting the GH/IGF-1 axis, an investigation was conducted in which low dose EE therapy (10 μg EE for 3 cycles of 3 weeks on treatment + 1 week off treatment) was administered with and without the addition of tamoxifen (an antiestrogen, 10 mg 2×/day) in postmenopausal women (n = 14, age 44–62). Low dose oral EE therapy alone resulted in significant increases in GH concentrations and significant decreases in IGF-1 concentrations. However, the addition of tamoxifen to low dose oral EE caused GH and IGF-1 concentrations to return to baseline values. Because tamoxifen acts to block estrogen receptors, this finding indicates that estrogen must bind to its receptor in order to modulate the GH/IGF-1 axis in postmenopausal women [37].

2.1.2. Section 2. Oral conjugated equine estrogens (CEE)

Similar to oral EE therapy, oral CEE therapy results in consistent increases in GH and decreases in IGF-1 concentrations in postmenopausal women. One of the first studies to describe this effect included postmenopausal women (n = 30, age 43–58 years) treated with 0.625 mg/day of oral CEE for 24 days/month for 6 months and oral dydrogesterone (10 mg 2×/day) during the last 12 days of each treatment cycle in the non-hysterectomized women. Significant increases in GH concentrations and an average 28% decrease in IGF-1 concentrations after EE therapy were observed [39]. These findings were expanded upon in a RCT of postmenopausal women (n = 10) before and after 15 months of CEE therapy alone (0.625 mg CEE) and in combination with three trials of different progestins (medroxyprogesterone acetate (MPA), 10 mg; desogestrel, 75 μg; or norethindrone, 1.05 mg) added during the last 14 days of each 28-day cycle for 3 months. With CEE treatment alone, a 37% decrease in IGF-1 concentration was observed ($p = 0.0001$). However, this reduction was reversed as the progestin administered increased in androgenicity such that IGF-1 was significantly greater when norethindrone (the most androgenic progestin given) was administered compared to CEE with no progestin ($p = 0.0015$) [40]. It is clear that giving a progestin in combination with estrogen therapy, as it done to protect against endometrial hyperplasia in non-hysterectomized women, may modulate the GH/IGF-1 response to exogenous estrogens in concert with the androgenicity profile of the specific progestin utilized.

Of further interest is the impact of longer-term oral CEE use. A larger cross-sectional study was conducted in postmenopausal who had been taking CEE (0.625 mg/day, 5–7daya/week) for at least 3 years (n = 24) and women who were not taking any ET (n = 24). Serial blood samples were collected every 20 min for 24 h and demonstrated that oral CEE was associated with greater mean 24 h GH concentration (1.21 ± 0 0.11 vs. 0.92 ± 0.10 μg/L, $p = 0.041$), more GH peaks (9.5 ± 0.4 vs. 7.3 ± 0.3, $p < 0.001$), and a shorter interpeak interval (2.66 ± 0.11 vs. 3.43 ± 0.17 min, $p = 0.006$) compared to non-users. Further, IGF-1 concentrations in women taking CEE were only 73% of those in women not

taking ET ($p < 0.001$) [31], demonstrating that IGF-1 production is suppressed as a result of long-term CEE therapy.

2.1.3. Section 3. Oral 17β-estradiol

Postmenopausal women using oral 17β-estradiol experience similar changes in GH and IGF-1 as other exogenous oral estrogens. In one investigation, postmenopausal women ($n = 10$, mean age 58 years) were monitored for a control period during which no ET was given and for a 21 day cycle wherein oral 17β-estradiol (1.0 mg oral micronized E_2, 2×/day) was administered. A 152% increase in GH concentration and an 18% decrease in IGF-1 concentration were observed after oral 17β-estradiol therapy [41], effects that are similar to that previously reported in response to other exogenous estrogens, such as EE and CEE.

2.2. Differential effects of oral estrogen preparations

While it is apparent that oral estrogen therapies (EE, CEE, 17β-estradiol) alter the GH/IGF-1 axis in a similar manner, the magnitude of the increase in GH and decrease in IGF-1 is not comparable across therapies. In order to distinguish the degree to which different oral preparations impact the GH/IGF-1 axis, a crossover design study was utilized. Three different oral estrogen therapies were compared in postmenopausal women ($n = 6$, age 54–71 years) randomly assigned to receive 20 μg EE, 1.25 mg CEE, and 2 mg estradiol valerate (EV) for 4-week crossover cycles, adding 10 mg MPA daily for the last 12 days of each cycle. Hourly blood samples were collected for 24 h prior to therapy and during the third week of each treatment cycle for a total of 4 24 h blood collections. Significant increases in GH concentrations and decreases in IGF-1 concentrations were observed with all forms of oral estrogen therapy. The most marked changes were observed with EE therapy, though the differences between oral therapy types were not significant. However, while GHBP was significantly elevated with all three oral estrogen therapy types, GHBP was significantly greater with EE therapy than EV therapy, suggesting a more marked impact of oral EE on the liver, as GHBP is derived from proteolytic cleavage of hepatic GH receptors [2]. Further, there was a significant correlation between the percentage increase in GH concentrations and the percentage reduction in IGF-1 concentrations ($r = 0.5$, $p = 0.04$), pointing to a reduction in negative IGF-1 feedback driving increased GH production from the pituitary [42]. These results highlight the indisputable impact of oral estrogens on the GH/IGF-1 axis regardless of the oral estrogen formulation.

The mechanism by which exogenous oral estrogen results in the aforementioned changes is likely an increased sensitivity of the pituitary to GHRH stimulation, but a reduced peripheral responsiveness to GH, as measured through IGF-1 production [43]. Reduced peripheral responsiveness has been measured using an IGF-1 Generation Test, in which a bolus injection of GH is administered and serial blood samples are collected following the injection to observe the hepatic IGF-1 response. In an investigation comparing postmenopausal women ($n = 13$, age 60–69) after a GH injection (0.1 mg/kg body weight), those who reported taking oral estrogen therapy (various preparations) had significantly lower basal IGF-1 concentrations ($p < 0.005$) and a reduced rise in IGF-1 in response to the GH injection compared to the women not taking oral estrogen (111 ± 21 vs. 268 ± 27 μ/L increase in IGF-1, $p < 0.01$) [44]. Thus, it can be concluded that despite an increase in bioavailable GH, as occurs in postmenopausal women as a result of exogenous estrogen therapy, there is a decreased hepatic responsiveness to produce IGF-1. It is widely believed that this is due to the first pass effect, in that metabolizing the oral estrogen results in decreased protein synthesis capabilities of the liver [45,46].

2.3. Transdermal estrogen therapy (ET)

Exogenous estrogen impacts the GF/IGF-1 axis differently if estrogens are administered transdermally rather than orally, as the transdermal administration avoids the first-pass effect that oral ET has on the liver. The first investigator to report the impact of transdermal ET on the GH/IGF-1 axis studied postmenopausal women ($n = 28$, age 45–72 years) who underwent 4 8-week cycles of transdermal ET at differing doses of 17β-estradiol (0, 50, 100, 150 μg/day). The women were randomized to receive the doses in an increasing or decreasing order and oral MPA (10 mg/d) was administered during weeks 3, 4, 7, and 8 of each patch phase. Blood samples were collected before transdermal ET and during weeks 6 and 8 of each dose of the patch. After each basal blood collection, a bolus iv injection of GHRH (1 μg/kg body weight) was administered and blood samples were collected at 30, 60, 90, and 120 min. A reduced responsiveness of GH to the bolus GHRH injection was observed with increasing transdermal 17β-estradiol dose, indicated by significantly lower peak GH and GH area under the curve (AUC) ($p < 0.01$). There were also an increased numbers of non-responders to the GHRH bolus injection as the transdermal 17β-estradiol dose increased ($p < 0.01$). On the other hand, IGF-1 concentrations did not differ in response to any dose of transdermal 17β-estradiol use in postmenopausal women [47]. In a group of postmenopausal women ($n = 7$) given a moderate dose of transdermal 17β-estradiol (50 μg/d) for 6 months, there were no differences in IGF-1 concentration before and after transdermal ET. However, the GH response to GHRH injection was also unchanged after transdermal ET in that sample of women [48]. Another investigation of transdermal ET revealed slightly different results. Postmenopausal women ($n = 12$, age 44–59 years) were observed over 6 28-day cycles of transdermal ET. Women were administered 17β-estradiol patches (100 μg/day) from days 1–21 of the cycle and were administered oral chlormadinone acetate (2 mg/day) on days 15–21 of each cycle. Blood samples were collected before and after the third and sixth therapy cycle. Concomitant with increases in plasma estradiol, the women experienced a 67% increase in IGF-1 concentration after 3 months of transdermal ET and a further 11% increase in IGF-1 from month 3–6 ($p < 0.02$). In addition, there was a positive correlation noted between plasma estradiol and IGF-1 concentration ($r = 0.439$, $p < 0.01$) during transdermal ET [49]. This study again highlights the notion that transdermal ET does not suppress IGF-1 release from the liver to the same extent as oral ET.

However, a dichotomous relationship has been observed as a result of transdermal ET dependent on baseline IGF-1 concentrations. For example, postmenopausal women ($n = 39$, age 42–58) were administered transdermal 17β-estradiol patches (50 μg/day) for 24 days/month. Non-hysterectomized women further received dydrogesterone (10 mg 2×/day) during the last 12 days of each 28-day treatment cycle. When examining the group as a whole, there was a significant increase in GH concentration after 6 months of transdermal 17β-estradiol therapy (2.72 ± 4.15 vs 5.08 ± 7.7 ng/mL) and no significant change in IGF-1 concentration (169 ± 48.39 vs. 156 ± 42.22 ng/mL). However, when the women were subdivided into those with higher vs. lower baseline IGF-1 concentrations, the women with low baseline IGF-1 concentrations had a non-significant increase in IGF-1 after 6 months of transdermal 17β-estradiol therapy (133.5 ± 19.8 vs. 156.7 ± 42.2 ng/mL), but the women with higher baseline IGF-1 concentration experienced a significant *decrease* in IGF-1 after 6 months of transdermal 17β-estradiol therapy (206.5 ± 40.5 vs. 165.2 ± 49.1 ng/mL). This is the first study to suggest a bimodal response of transdermal exogenous estrogen dependent on an baseline IGF-1 levels [50].

2.4. Oral vs. transdermal estrogen therapy

From the plethora of studies that have been conducted to compare the impact of oral vs. transdermal estrogen administration of GH and IGF-1 concentrations in postmenopausal women, it has become apparent that the transdermal route of estrogen administration impacts the GH/IGF-1 axis differently than the oral route, though the results of these studies have been varied in terms of the magnitude of the effect, especially on IGF-1 concentration, which has been reported to be increased, decreased, or unchanged in response to various transdermal

ET regimens [37–40,44,48,51–53]. It is also important to note that the radio-immunoassays available to measure IGF-1 pose limitations, as erroneous measurements can be made based on the concentration of IGFBPs is the laborious process of separating these through gel chromatography [54]. Thus, results should be cautiously interpreted.

We previously described the findings of a study in which oral EE was administered for 2 cycles (20 μg EE/day for 28 days + 5 mg norethisterone/d on days 15–21 of each cycle) and significant increases in GH concentrations and decreases in IGF-1 concentrations were observed [38]. An additional group of women in that study (n = 7) were administered transdermal 17β-estradiol (100 μg/day) in order to compare the two routes of administration. While the serum estradiol concentration achieved with the oral and transdermal preparations did not differ, the women using transdermal ET experienced no change in GH concentrations or GH dynamics. Further, in contrast to the 33% decrease in IGF-1 concentrations observed in the oral EE group, the women using transdermal ET experienced a 28% *increase* in IGF-1 concentrations ($p < 0.005$) [38]. Concomitant with these changes in IGF-1, changes in markers of bone turnover were discordant after oral vs. transdermal ET. The women taking oral EE experienced a 63% decrease in osteocalcin, while those administered transdermal ET experienced a 92% increase in osteocalcin in addition to significant increases in procollagen I and procollagen III ($p < 0.05$). These changes reflect fibroblast and osteoblast function and were significantly related to changes in IGF-1, demonstrating that increases in IGF-1 concentration during transdermal ET are indicative of important downstream effects on bone formation [55].

Comparisons have also been made between transdermal ET and oral CEE therapy. As described above, use of oral CEE (0.625 mg/day) resulted in significant increases in GH and decreases in IGF-1 concentrations after 6 months [39]. In this same study, an additional group of women were administered transdermal 17β-estradiol (50 μg/day) (n = 14) and experienced no change in GH concentrations after 6 months of transdermal ET. On the other hand, 2 women experienced a decrease in IGF-1, 6 women experienced no change within 20% of baseline IGF-1, and 6 women experienced an increase in IGF-1 concentrations. The group as a whole experienced, on average, an 11.7% increase in IGF-1, and this was significantly greater than the IGF-1 concentration observed after 6 months of oral EE [39]. In a similar study, investigators administered higher doses of oral CEE and transdermal ET to postmenopausal women (n = 23, mean age 57 years). This study utilized a crossover design in which women were randomized to receive oral CEE (1.25 mg/day) and transdermal 17β-estradiol (100 μg/day) for 6 months each. Oral CEE resulted in a 250% increase in GH concentration and a 29% decrease in IGF-1 concentration ($p < 0.05$) at month 6, whereas transdermal ET did not produce significant changes in GH or IGF-1 concentrations compared to baseline [53], further demonstrating the divergent effects of oral compared to transdermal ET.

Age may be an additional factor modulating the GH/IGF-1 response to ET. To better elucidate the effect of age, a cross-over study was designed in which postmenopausal women (n = 16) representing a wide age range (49–75 years) were administered oral CEE (1.25 mg/day) and transdermal 17β-estradiol (100 μg/day) for 6 weeks each, separated by an 8-week washout period. Blood samples were collected before and after ET and analyses were conducted on the group as a whole and in two subgroups of women: ≤62 years and >62 years in age. Overall, GH AUC was significantly increased after oral CEE ($p < 0.003$) but did not change after transdermal ET. Similarly, IGF-1 concentrations were significantly decreased after oral CEE ($p = 0.002$) but did not change after transdermal ET. However, when the women were subdivided into older and younger age groups, a 13% decrease in IGF-1 was observed in the older subgroup after transdermal ET ($p = 0.02$). This finding of decreased IGF-1 after transdermal ET differs from the aforementioned reports, in which the majority of transdermal ET users experienced increases or no change in IGF-1 concentrations after transdermal ET. In order to explore differences in GH responsiveness to GHRH after oral CEE vs. transdermal ET, a bolus injection of GHRH (1 μg/kg body weight) was administered and serial blood samples were collected for two hours thereafter at baseline and after each ET period. At baseline, the older postmenopausal women experienced a significantly lower GH peak amplitude and GH AUC after GRHR administration compared to the younger postmenopausal women. While this age-dependent difference in GHRH response persisted following ET, there were no differences in GH dynamics following oral ET compared to transdermal ET. These findings suggest that independent, age-related changes in the GH/IGF-1 axis likely modulate the response to transdermal exogenous estrogen. [52].

One speculation as to the why oral EE and CEE impact GH and IGF-1 differently than transdermal ET is the different *type* of estrogen delivered via the two routes of administration. In order to examine if this was the case, investigators conducted crossover studies comparing the effects of *oral* 17β-estradiol to *transdermal* 17β-estradiol on the GH/IGF-1 axis. In one study, postmenopausal women (n = 8, age 52–80 years) received oral 17β-estradiol (1 mg 2×/day) and transdermal 17β-estradiol (100 μg/day) for 15 days each in a random order and 24 h blood samples were collected before ET and after each cycle of ET. Oral ET resulted in significantly greater serum estradiol concentrations compared to transdermal ET at these dosage levels. GH AUC and mean concentration were significantly increased to a similar extent with both routes of estrogen administration. Further, serum IGF-1 concentration was significantly reduced as a result oral and transdermal ET (33% and 26% decrease, respectively). GH dynamics were altered similarly with both routes of estrogen administration resulting in increased basal GH concentration, GH pulse height, and mean GH pulse area [51]. Again, these findings conflict with the reported decrease/no change in IGF-1 concentrations with transdermal ET described above, which begs further mechanistic exploration.

However, the decrease in IGF-1 concentration as a result of transdermal 17β-estradiol was not observed by investigators in a study utilizing a smaller dose. Postmenopausal women (n = 14, median age 49.5 years) were assigned to receive 6-month regimens of oral (2 mg/day days 1–22, 1 mg/day days 23–28) and transdermal (50 μg/day) 17β-estradiol in a random order. Note that this transdermal ET dose is only half of the dose in the previously discussed investigation. With oral ET, the women took 1 mg/day of oral norethisterone on days 13–22 of each 28-day therapy cycle and with transdermal ET, 250 μg/day of norethisterone were administered on days 15–28 of each 28-day therapy cycle. While oral ET resulted in a 16% decrease in IGF-1 concentrations, there was no change in IGF-1 concentrations as a result of 6 months of transdermal ET at this dose [56]. Because estrogen and the progestin in each cycle were both administered orally or both administered transdermally, the results support the hypothesis that oral hormone therapy results in decreased hepatic protein synthesis due to the first-pass effect and this is diminished with the transdermal route of hormone therapy administration, especially if a lower dose is used.

To evaluate the role of progestins in modulating the effect of oral and transdermal ET, four different progestins were investigated in postmenopausal women randomized to oral CEE (1.25 mg/day) (n = 10) or transdermal 17β-estradiol (100 μg/day) (n = 10). These two estrogen doses are proposed to be bioequivalent by measure of serum estradiol suppression [53], so the impact of different progestin administration could be isolated. Women received the 4 different progestin treatments in a random order during the first 12 days of each monthly ET cycle for 4 months. The progestins used were, in order of increasing androgenicity, 1) 5 mg cyproterone acetate (CA) (anti-androgenic activity), 2) 20 mg/day dydrogesterone (no androgenic activity), 3) 10 mg/day MPA (slight androgenic activity), and 4) 2.5 mg/day norethisterone (androgenic action). Overall, progestin administration resulted in significant increases in IGF-1 concentrations compared to unopposed estrogen administration in the women in the transdermal ET group ($p < 0.03$) but no change in IGF-1 concentration in the women in the oral ET group. When examining the different progestins individually, reductions in IGF-1 during

oral ET were still significant (−20 to −36%) for CA, dydrogesterone, and MPA use. However, norethisterone administration abolished the suppression in IGF-1 with oral CEE therapy. During transdermal ET, MPA and norethisterone resulted in 31 and 54% increases in IGF-1 concentration, respectively, while no significant changes in IGF-1 occurred with CA and dydrogesterone treatment [57]. Supportive data are reported in another longitudinal study of different progestins in postmenopausal women (n = 12, mean age 49.7 years) randomized to receive oral CEE (0.625 mg/day) or transdermal 17β-estradiol (50 μg/day). For the first 6 months of the intervention, dydrogesterone (10 mg/day) was administered on the last 12 days of each 28-day treatment cycle and for the second 6 months of the intervention, norethisterone acetate (5 mg/day) was administered during the last 12 days of each 28-day treatment cycle. The women taking oral CEE experienced a significant decrease in IGF-1 concentration when dydrogesterone was administered which was reversed with norethisterone administration. Further, in the women receiving transdermal ET, no significant change in IGF-1 concentrations were observed during dydrogesterone administration, but a slight increase in IGF-1 concentration was observed with norethisterone treatment [58]. These findings suggest that androgenic progestins (namely, norethisterone) may attenuate the negative effects of ET on IGF-1 concentrations. Future studies to investigate if this preservation of IGF-1 with ET plus androgenic progestin translates to maintenance of bone health are warranted.

2.5. Connections between estrogen therapy, IGF-1, and bone health

The menopausal transition is characterized by an acute, inevitable and unpreventable decrease in BMD, due largely to the loss of endogenous estrogen production, since, as is well described, estrogen has potent, anti-resorptive properties [59,60]. Changes in the GH/IGF-1 axis have during aging have also been characterized and it is suspected that the gonadotrophic and somatotropic dynamics are interrelated. Based on the observation of a low GH/low IGF-1 environment during the follicular (i.e. low estrogen) phase of the menstrual cycle, one might expect the menopausal loss of estrogen to result in a similar state [43,61–67]. Thus, it is conceptually plausible that exogenous estrogen administration, which increases circulating serum estradiol, should restore GH and IGF-1 concentrations and promote bone formation. Indeed, ET is typically effective in maintaining bone mass and preventing fractures in postmenopausal women [1,30–35], although the extent to which this is attributable to the positive effect of estrogen administration alone on bone versus the indirect impact that estrogen has on IGF-1 or other biochemical mediators of bone turnover are not clear. Further, the route of exogenous estrogen administration may dictate whether IGF-1 is playing a helping or hindering role in preventing bone loss. As previously discussed, oral ET is subject to first-pass effects of the liver, leading to suppressed hepatic IGF-1 production despite increased GH production, in which case the potential for IGF-1 to promote bone formation is not realized [6,25,26]. Conversely, transdermal ET avoids the first-pass effect by delivering estradiol directly to the circulation, and many studies have shown increases in IGF-1 as a result of transdermal ET [38,39,49,50,57,58]. Consequently, it would be expected that transdermal ET could promote bone formation and attenuate bone loss and this has, in fact, been reported [30,35,68,69]. The question that remains, however, is whether transdermal ET has the capability to preserve bone mass *beyond* that of oral ET due to the differences in IGF-1 production resulting from the two therapies.

Very few studies have compared bone health in women using oral vs. transdermal ET. In one retrospective analysis of postmenopausal women who had been using oral CEE (0.625 mg/day) (n = 46) or transdermal 17β-estradiol (100 μg/day) (n = 54) for two years. BMD increased to a similar extent regardless of the route of administration or progestin use, exhibiting 4.8% and 3.5% increases in lumbar spine and total hip BMD, respectively, in the oral ET group, and 4.9% and 4.2% increases in lumbar spine and total hip BMD, respectively, in the transdermal ET group. These changes were significantly different than the control group, in which women not receiving ET experienced decreases in BMD [30]. In a similar study, postmenopausal women (n = 50), receiving oral and transdermal ET resulted in non-significant increases in BMD after 1 and 2 years, but these changes did not differ between treatment groups [69]. In one of the only RCTs designed to examine oral vs. transdermal ET on direct bone outcomes, postmenopausal women (n = 66) were randomized to receive oral CEE (0.625 mg/day + 0.15 mg/day norgestrel on the last 12 days of each 28-day cycle) or transdermal 17β-estradiol (50 μg/day + 0.25 mg/day norethisterone acetate for the days 14–28 of each 28-day cycle). An additional, no-treatment control group was also recruited (n = 30). After 3 years, both ET groups experienced significant increases in lumbar spine, femoral neck, Ward's triangle, and trochanteric region BMD ($p < 0.02$) compared to the women not receiving ET [70]. From the data available, it appears that the route of administration, though it may dictate GH and IGF-1 response to exogenous estrogen therapy in postmenopausal women, may not impact bone outcomes. Clearly more research is warranted to clarify these outcomes.

3. Premenopausal women

3.1. GH/IGF-1 axis across the menstrual cycle

In order to better interpret the effects of exogenous estrogen on the GH/IGF-1 axis in premenopausal women, we must first establish a baseline understanding of the effects of endogenous estrogen. Here, we briefly summarize what is known regarding the regulation of the GH/IGF-1 axis across a normal menstrual cycle.

It is apparent that growth hormone dynamics and IGF-1 production are modulated by circulating estrogen and, therefore, demonstrate changes over the course of the menstrual cycle. Several investigators seeking to characterize GH and IGF-1 at multiple time points throughout the menstrual cycle in premenopausal women have produced consistent findings [43,61–67]. For the purposes of summarizing that reported in the literature, this discussion will focus on 3 phases of the menstrual cycle, defined as 1) early follicular (EF), comprising the onset of menses until day 5 of the cycle, 2) midcycle (MC), comprising day 6 of the follicular phase to 3 days post-ovulation, and 3) luteal (LU), comprising the day after ovulation until the day before the onset of the subsequent menses. Estrogen levels are typically lowest during the EF phase and rise to a peak during MC. Increases in GH and IGF-1 parallel the increase in estrogen from the EF phase to MC. Specifically, mean GH concentration and 24 h GH AUC have been reported to increase 55–67% from the EF to MC [61,63,64,67,71], while IGF-1 concentration exhibits increases of 3–21% from the EF phase to MC [65–67]. GH dynamics have also been characterized as a function of menstrual cycle phase. Increases in the number of GH secretory bursts, shorter interburst intervals, increased maximum GH peak, and increased total GH pulse area have been observed during MC compared to the EF phase [64,67]. However, while increases in GH pulse height are observed with increased MC estrogen, basal GH is similar across all phases of the menstrual cycle [67], indicating that estrogen likely provides a stimulus for GH secretory burst activity. Additionally, arginine administration to stimulate GH production results in significantly greater increases in GH concentrations during MC than it does during the EF phase, further suggesting an augmenting role of estrogen in GH production [62]. Consistently, estrogen exhibits significant positive correlations with GH concentration, GH peak height, and IGF-1 concentration [64,66,67,72]. Note that the positive correlations between endogenous estrogen concentration and GH are in contrast with findings in postmenopausal women in which a decrease GH responsivity to bolus GHRH injection is observed as transdermal estradiol dose increases [47].

The magnitude of change in GH throughout the menstrual cycle exhibits changes with increasing reproductive age. In a prospective study, the GH/IGF-1 response to estrogen fluctuations across the menstrual

cycle in older (n = 8, age 42–46 years) and younger (n = 8, age 19–34 years) premenopausal women were characterized. Beginning in the EF phase of the menstrual cycle, daily blood samples were collected for the analysis of ovarian steroid and gonadotropin production. While the older women had significantly higher estradiol concentrations than the younger women, average 12 h GH AUC was significantly lower in the older women. There was a trend toward lower IGF-1 concentrations in the older than younger women as well [27]. This evolution of the relationship between endogenous estrogen and GH and IGF-1 production should be kept in mind when considering the impact of hormonal contraceptive use, as age of use will likely modify the effects on the GH/IGF-1 axis and subsequent bone outcomes.

To better identify the mechanism underlying endogenous estrogen's modulation of the GH/IGF-1 axis, an IGF-1 Generation Test was performed. In a group of premenopausal women (n = 9, mean age 38 years), significantly lower IGF-1 concentrations were measured during the EF phase compared to the MC and LU phases, consistent with the findings above. However, an injection of GH resulted in greater IGF-1 generation during the EF phase than during the MC and LU phases. Indeed, serum estradiol was a significant negative predictor of percentage increase in IGF-1 in response to exogenous GH stimulus, indicating decreased peripheral responsiveness to GH with increasing endogenous estrogen [43]. This finding warrants closer scrutiny of the previously conducted investigations that reported increases in GH and IGF-1 with increases in estrogen. The relationship between GH and IGF-1 is not linearly dependent on estrogen levels and reported increases in IGF-1 are dissimilar in magnitude compared to increases in GH that occur with MC increases in estrogen. In the two investigations that have reported changes in both GH and IGF-1 at different phases of the menstrual cycle, increases in GH were more pronounced and disproportionate to the increases in IGF-1 [65,67], highlighting the likelihood that increases in estrogen result in decreased peripheral sensitivity to GH stimulus. This understanding informs the interpretation of the following discussion of the effects of exogenous estrogen on the GH/IGF-1 axis.

3.2. Changes in the GH/IGF-1 axis with hormonal contraceptive use

3.2.1. Section 1. Combined oral contraception (COC)

The effect of exogenous estrogen administration in the form of hormonal contraception on the GH/IGF-1 axis in premenopausal women has been investigated in a limited number of studies. Results from these investigations are consistent in suggesting alterations in GH pulse dynamics and an overall decrease in circulating IGF-1 with COC use [73–78]. In the first study designed to examine 24 h GH pulse dynamics longitudinally as a result of monophasic COC use, premenopausal women (n = 9, age 22–31 years) underwent serial blood sampling every 30 min for a 24 h period both before and after 2–3 months of COC treatment with either 30 μg ethinyl estradiol (EE) + 150 μg desogestrel or 30 μg EE + 150 μg levonorgestrel. While there were no differences observed in total mean GH concentration after therapy, COC use resulted in decreases in mean GH pulse amplitude (11.2 to 7.3 mU/L, $p < 0.05$), mean GH peak area (24.1 to 14.1 mU/L, $p < 0.05$), and interpeak interval (4.3 to 3.0 h, $p < 0.05$) indicating increased GH pulse frequency [73]. In another RCT, two different formulations of COC on GH dynamics and IGF-1 concentration in premenopausal women were studied [74]. Women were randomly assigned to take one 21-day cycle of either 30 μg EE + 2 mg dienogest (n = 9) or 30 μg EE + 0.125 mg levonorgestrel (n = 9) and blood sampling was timed to day 21 of a control menstrual cycle and day 21 of COC treatment. Similar to the findings of Karlsson et al. [73], neither group experienced changes in mean GH concentration after COC use. However, GH AUC was significantly greater after COC therapy compared to baseline in the women taking the levonorgestrel formulation (a second generation progestin). Within the levonorgestrel group, *lower* baseline GH concentration was associated with *higher* post-treatment mean GH concentration ($p = 0.025$) [74]. Most importantly, both COC formulations resulted in decreases in mean IGF-1 concentrations ($p < 0.007$) as well as IGF-1 AUC ($p < 0.028$) [74]. However, the reduction in IGF-1 AUC was significantly more pronounced in the women taking the dienogest (a third generation progestin) formulation compared to the women taking the levonorgestrel (a second generation progestin) formulation (32% vs. 14%, $p = 0.003$). No differences in IGFBP-3 were reported after COC therapy [74]. These findings indicate that the type of progestin used in the COC may modulate the response of the GH/IGF-1 axis to exogenous oral EE.

Broader cross-sectional studies also provide an informative picture of the impact of COC use on the GH/IGF-1 axis. In a small cross-sectional analysis of premenopausal women, no significant differences in GH or IGF-1 concentrations were observed in regularly menstruating women (n = 14) versus women taking COC (n = 10) (20–35 μg EE + varying concentrations of second generation progestins). However, GHBP was significantly higher in the women taking COC [75]. It must be noted that the time during the menstrual cycle/contraceptive pill cycle that the blood samples were obtained was not controlled in this study, which may have contributed to variability in the measurements, especially in the women not taking contraception. In a much larger cross section study in 311 white women, of which 50% were current COC users (EE + various progestins), lower IGF-1 concentrations and higher IGFBP-3 concentrations were observed in the users compared to the nonusers ($p < 0.006$) [76]. This cross-sectional analysis also revealed a dose-response relationship between daily average EE dose and IGF-1 concentrations, with increasing dosage from 20 to 35 μg EE resulting in progressively lower IGF-l levels. Like the previously mentioned analysis by Massa et al. [75], the investigators did not control the day of the contraception cycle on which the blood samples were collected. However, the larger sample size allowed for a unique analysis in which the women were secondarily grouped according to the day during the contraceptive cycle that the blood sample was collected. Markedly higher IGF-1 concentrations were observed in the women who had the blood drawn during the 'placebo' week of the contraceptive cycle, i.e. when no active hormone was being administered, and lower for all other phases during the 'active' contraceptive use, demonstrating a clear, acute interaction between exogenous estrogen and IGF-1 production [76].

Many women vary their use of hormonal contraception throughout the reproductive years for various reasons, including family planning. Thus, it is necessary to consider how different patterns of COC use over time impact IGF-1 concentrations in order to better evaluate the impact of these usage patterns on long term bone health. Current COC users (n = 17) were compared with former users (n = 16) and never users (n = 10) and blood samples were taken between days 5 and 10 of the menstrual cycle/contraceptive cycle to reflect the follicular phase and between days 18 and 23 of the menstrual cycle/contraceptive cycle to reflect the luteal phase. While there were no differences in IGF-1 concentration among the groups in the follicular phase, present COC users had lower IGF-1 concentrations during the "luteal phase" than the former users and never users ($p = 0.0013$). Further, while there were no significant differences in the change in IGF-1 concentration from the follicular phase to the luteal phase between former users and never users, current COC users had smaller changes in IGF-1 (i.e. more constant IGF-1 concentration across the month) compared to the other groups ($p = 0.0002$) [77].

Since increasing age independently contributes to alterations in the GH/IGF-1 axis, the age of women during COC use throughout the reproductive years appears to further modulates the IGF-1 response to exogenous estrogen. A large cross-sectional study investigated the effects of different patterns of COC use on IGF-1 and BMD in premenopausal women as a function of age. Among the 18 to 21-year-old women studied (n = 180), women who had ever used COC had significantly lower IGF-1 concentrations than never-users. While there was no relationship between age at first COC use, time since last COC use, or total duration of COC use and IGF-1, having ever used COC explained 9% of the variance in

IGF-1 concentration. Similarly, in 31 to 40-year-old women (n = 148), there was no correlation between time since last COC use or duration of COC use and IGF-1 concentrations, but current COC users had similar IGF-1 levels to those who had never used COC. In addition, the women in the older group who reported using COC starting in 1995 or later had the highest IGF-1 levels compared to never-users, indicating that using a 3rd generation progestin may result in higher IGF-1 levels than the 2nd generation progestin [78]. This is in contrast to the findings observed by Karlsson et al., in which there was a more pronounced *reduction* in IGF-1 in the women using the third generation progestin [73]. Further investigation to this effect is necessary.

The prevailing theme throughout these premenopausal investigations is the dichotomous relationship between endogenous versus oral exogenous estrogen on GH dynamics and IGF-1 production. While increasing endogenous estrogen (for example, during the MC phase of the menstrual cycle) results in increases in GH and IGF-1, the increase in IGF-1 is less marked than the increase in GH. However, while exogenous oral estrogen administration similarly results in increases in GH, the impact of exogenous oral estrogen is a decrease in IGF-1 production. This finding is likely attributable to the first-pass effect by which the metabolism of exogenous estrogen suppresses hepatic IGF-1 production. If the first-pass effect is responsible for these changes, then an alternative route of estrogen administration should theoretically prevent the decrease in IGF-1.

3.2.2. Section 2. Transdermal Hormonal Contraception

Transdermal hormonal contraceptives provide EE directly to the systemic circulation, thereby avoiding the first-pass EE exposure to the liver that oral contraceptives provide. Thus, hepatic protein synthesis, including that of IGF-1, may not be compromised to the same extent with transdermal contraceptive use, in which case the implications for bone health may be more favorable. The limited research published to date supports this hypothesis. In a small study of adolescent (age 12–21) girls, changes in BMD and markers of bone metabolism were examined in girls randomized to transdermal Ortho Evra patch (20 μg EE + 150 μg norelgestromin) (n = 5) or a control group using no hormonal contraception (n = 5) for one year [79]. Measurements at baseline, 6 months, and 12 months revealed that IGF-1 concentrations were not different at any time point between the two groups or as a function of time, nor were any differences observed for markers of bone turnover (serum BAP and osteocalcin, urinary NTX). However, while increases in whole body BMC, lumbar spine and total hip BMD were observed in girls in the control group ($p < 0.044$), there were no significant changes at any site in the girls using Ortho Evra [79]. Indeed, although limited by a small sample size, based on these findings, it appears that transdermally-delivered exogenous EE may not impact liver protein synthesis to the degree that orally-administered exogenous EE seems to. Differences in BMC and BMD between the groups despite similar levels of IGF-1 and bone turnover markers may reflect increased bone resorption in the face of lower and more constant serum estradiol levels in the girl administered transdermal EE, though serum estradiol was not reported in the current study. Future studies to confirm these findings and investigate additional mechanisms by which bone health is altered in premenopausal girls and women using transdermal contraception are warranted.

3.3. Connections between COC, IGF-1, and bone health

While decreases in IGF-1 concentrations after oral COC use have been consistently reported, it is unclear whether this actually contributes to BMD impairments reported in adolescent girls and young women as a result of COC. Building upon the findings of Jernstrom and Olsson described above [77], another investigator reported relationships between patterns of COC use, IGF-1 concentrations, and BMD in women age 20–40 years. Current COC users (n = 43) had lower IGF-1 concentrations than past users (n = 41) ($p = 0.033$), which held true after adjusting for age and BMI, but there was no difference between past users and never users. Further, while total body T-score was not different among the groups, current users had a lower average femur T-score than the past users and lower forearm and spine T-scores than both past users and never-users ($p < 0.03$). These findings remained significant after adjusting for age and BMI. Individual subgroup analyses revealed a significant correlation between IGF-1 concentration and bone density in past users and never users, but not in current users [80]. This raises the speculation that during COC use, the GH/IGF-1 axis may be suppressed, thereby diminishing the potential for IGF-1 to augment bone formation in this age group. There was also a wide range of COC use duration represented by the participants in this study. Among current users, the mean duration of use was 64.3 ± 56.85 months, but the range of use was 5–204 months. Similarly, the mean duration of COC use in past users was 56.4 ± 47.74 months with a range of 6–198 months. Regardless, there was not a significant relationship between duration of COC use (past or current) and IGF-1 concentration or BMD [80]. Thus, while it is apparent that IGF-1 concentration is associated with BMD, it is still unclear as to whether the transient impact that COC use has on the GH/IGF-1 axis contributes to long-term effects of COC use on bone health. This gap in understanding underscores the necessity of identifying the mechanisms bridging COC use and changes in bone turnover in order to inform future contraceptive formulations.

In an effort to elucidate the individual and interactive mechanisms by which exogenous estrogen and IGF-1 impact bone health, an RCT was conducted in osteopenic women with anorexia nervosa (n = 60, age 18–38 years). Women were randomized to receive recombinant human IGF-1 (rhIGF-1, 30 μg/kg 2×/day), COC (35 μg/d EE + 0.4 mg/day norethindrone), both therapies, or neither (control), for a total of 4 separate groups. Factorial analysis revealed that after 9 months, the women receiving rhIGF-1 experienced significant increases in spine BMD, whereas COC use had no significant effect on BMD. However, the effect of rhIGF-1 therapy *plus* COC was significant, resulting in increased lumbar spine BMD compared to the control group. Further, while those not receiving rhIGF-1 experienced a significant decrease in PICP, a marker of bone formation, those receiving rhIGF-1 had no change in PICP. NTX, a marker of bone resorption, decreased in response to COC use compared to non-use. [81]. Thus, in the face of undernutrition, where bone resorption is likely upregulated and formation is hindered, COC therapy and IGF-1 administration, respectively, have the potential to counter these physiologic pathways and preserve bone mass.

4. Interactions between estrogen, GH, and bone health

In both pre- and postmenopausal women, the relationships between estrogen, the GH/IGF-1 axis, and bone health are present, but not well understood to date. Indeed, the ways in which these three factors interact to impact one another vary as a function of age, estrogen dose, route of administration, and the presence and type of progestin in the formulation. Thus, these variables must be considered when interpreting research examining the impact of exogenous estrogen on the GH/IGF-1 axis and bone health. Despite the complexities associated with such interpretations, the literature published to date is largely consistent within pre- and postmenopausal women.

Premenopausal women using COC therapy experience no change or increases in GH concentrations and no change or decreases in IGF-1 concentrations. To our knowledge, only two studies have examined BMD changes in association with changes in the GH/IGF-1 axis after COC use. Elkazaz and Salama [80] reported significantly lower IGF-1 concentration and lower lumbar spine, forearm, and femur T-scores in current COC users compared to past users. The conclusion (though further studies are needed to replicate this result) is that COC therapy may be detrimental to bone, perhaps due to a reduction in IGF-1 production in response to exogenous estrogen metabolism. Conversely, in the sample

of young women with anorexia nervosa studied by Grinspoon and colleagues [81], those who received rhIGF-1 and COC therapy in combination experienced *increases* in IGF-1 concomitant with *increases* in lumbar spine BMD, but no BMD changes were observed as a result of rhIGF-1 therapy or COC therapy individually. Markers of bone turnover were reported as well, and rhIGF-1 treatment successfully maintained PICP (bone formation marker); whereas, COC treatment resulted in decreased NTX (bone resorption marker) [81]. Thus, it appears that in the absence of COC therapy, IGF-1 promotes bone formation and, in those with presumably low endogenous estrogen, COC therapy may can reduce bone resorption. This combination may lead to improved bone mass. Additional studies examining this interaction are warranted.

Differences emerge when exogenous estrogen is delivered through the transdermal route in premenopausal women. Though the data are limited, transdermal EE seems to produce no change in IGF-1 and no change in BMD in adolescents and young women. However, in the sole sample of women studied, whole body BMC and lumbar spine/total hip BMD increased in the women not using transdermal contraceptive therapy but did not change in the women using transdermal contraception [79], indicating that despite having a minimal impact on the GH/IGF-1 axis, exogenous estrogen may still impact bone turnover and bone mass.

In postmenopausal women, the impact of exogenous estrogen on the GH/IGF-1 axis and how that affects bone mass appears to be less dependent on the route of administration, but research is similarly limited. Three studies have compared BMD after oral versus transdermal estrogen therapy. Two of these studies have reported increases in lumbar spine and total hip BMD after 2–3 years of oral or transdermal estrogen therapy [30,70]. The remaining study reported a small, non-significant decrease in lumbar spine BMD after 2 years of oral or transdermal estrogen therapy [69]. None of these investigations reported GH/IGF-1 concentrations, so conclusions about the role of those hormones in these BMD outcomes cannot be made.

In contrast to BMD, markers of bone metabolism may respond differently to exogenous estrogen depending on the route of administration, though data are limited and reflect one short-term (2 month) study. Specifically, postmenopausal women using oral estrogen therapy experienced increases in GH and decreases in IGF-1 concomitant with decreases in osteocalcin, a marker of bone formation. Conversely, women using transdermal estrogen therapy experienced increases in IGF-1 and parallel increases in osteocalcin and procollagen I and III, all indicators of bone formation [38,55]. Due to the short duration of the study, changes in BMD were not investigated.

We consider estrogen to work on bone resorption while IGF-1 contributes to bone formation; however, exogenous estrogen crosses over to impact bone formation due to the impact it has on IGF-1 production, presumably in the liver. In healthy, premenopausal women, COC therapy and transdermal contraceptive therapy may pose a detriment to bone due to a relative decrease in circulating estrogen leading to an upregulation of bone resorption and, in the case of COC therapy, a decrease in IGF-1 concentration leading to a downregulation of bone formation. In postmenopausal women and premenopausal women with anorexia nervosa, estrogen therapy results in a relative increase in systemic estrogen which may decrease bone resorption and lead to increased bone mass, despite a decrease in IGF-1 that may occur due to the metabolism of oral estrogen administration.

5. Summary

The interdependent relationship between the HPO and the GH/IGF-1 axes has been characterized in women of all ages. In postmenopausal women, oral ET results in increases in GH concentrations and altered GH dynamics, but decreases in circulating IGF-1 [31,36–39,42,44,82]. While GH concentration may be similarly increased with transdermal ET, IGF-1 is typically unchanged or increased as a result of transdermal ET [47–50]. Similar to oral ET in postmenopausal women, exogenous estrogen administration in the form of COC in premenopausal women produces increases in in GH concentrations, altered GH dynamics, and decreases in circulating IGF-1 concentrations [73,74,76,77]. While the data are limited, transdermal hormonal contraception appears to preserve IGF-1 concentrations in premenopausal girls and women [79].

Because bone turnover depends on both estrogen and IGF-1, the way in which these hormones impact one another is of interest, as changes in either of these hormone concentrations can have downstream consequences on bone that influence BMD, the development of osteoporosis, and fracture risk. In postmenopausal women, while circulating IGF-1 is decreased with oral ET and unchanged or increased with transdermal ET, this doesn't seem to translate to changes in BMD. Indeed, BMD at the lumbar spine and hip are similarly maintained or increased in women using either oral or transdermal ET [1,30–35,68,69]. However, in premenopausal women, both routes of hormonal contraception have negative effects on bone density, despite disparate changes in IGF-1 concentrations depending on route of administration [80].

In summary, exogenous estrogen administration impacts the GH/IGF-1 axis differently across the lifespan. These differences are modified by age and reproductive status of the individual, the type of estrogen administered, the presence and type of progestin administered with estrogen, the duration of use, and the route of administration. The implications for bone health as a result of these changes are further modified by age and reproductive status and future research to explore the relationship between estrogen, GH/IGF-1, and resultant bone health are warranted.

Conflict of interest statement

There are no conflicts of interest to report.

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