

# Beta Endorphin Levels in PCOS Women: Relationship With Insulin Secretion

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## Abstract

**Background:** An understanding of the relationship between beta endorphin ( $\beta$ E) with insulin, glucose and gonadotropins may explain how non-pharmacologic treatment options such as exercise and acupuncture may be beneficial to women with Polycystic Ovary Syndrome (PCOS). The objective was to examine the correlation of  $\beta$ E with insulin, glucose and gonadotropins, controlling for exercise and body mass index (BMI).

**Methods:** 40 untreated women with a confirmed diagnosis of PCOS (NIH criteria) were tested. Height, exercise frequency and exercise intensity were self-reported; weight was measured by nurses. Blood samples were collected for the biological measures of interest. The setting was an academic US medical center. Statistical analyses consisted of Spearman correlations and partial correlations with an alpha = 0.05. The main outcome measures included plasma  $\beta$ E, insulin and glucose (fasting and AUC) from a 2-hour oral glucose tolerance test, serum gonadotropins.

**Results:** The mean  $\beta$ E level was 7.92 pmol/mL (sd = 4.0, range 1.92 - 18.7, 25%/75% interquartile range 4.56 - 11.29).  $\beta$ E was associated with log<sub>e</sub> AUC-insulin (P = 0.04) after adjustment for

exercise as measured via energy equivalents; this correlation was unaffected by additional control of BMI. No relationship between  $\beta$ E and luteinizing hormone, the luteinizing hormone/follicle stimulating hormone ratio, or glucose was seen after accounting for BMI and exercise.

**Conclusions:**  $\beta$ E was positively correlated with AUC-insulin after accounting for exercise. Future studies could investigate the affects of  $\beta$ E-potentiating therapies, such as exercise and acupuncture, in women with PCOS to determine how these changes in  $\beta$ E are related to insulin/glucagon balance.

**Keywords:** Beta endorphin; Polycystic ovary syndrome; Insulin; Exercise

## Introduction

Polycystic ovary syndrome (PCOS), the most common endocrinopathy in women, is a heterogeneous condition which can present with varied clinical and biochemical characteristics [1]. Common features of PCOS, though not a requirement for diagnosis, include hyperandrogenism, oligomenorrhea, anovulation, an increased ratio of luteinizing hormone (LH) to follicle stimulating hormone (FSH) [2], insulin resistance, glucose intolerance, hirsutism, and a cystic appearance of the ovaries as seen with ultrasound.

There is increasing support for the concept that dysregulation of the endogenous opioid system contributes to the metabolic as well as the reproductive hormone abnormalities seen in PCOS. Normal  $\beta$ E values in females with regular menses has been reported to be 4.4 - 4.73 by one of the authors [3] and 2.9 - 4.3 by others [4, 5] depending on whether the women were exercisers or sedentary. Several studies report higher levels of peripheral  $\beta$ E in women with PCOS, especially in those who are obese [6], compared with women who do not have PCOS [7-9].  $\beta$ E has been implicated in blood glucose control during both rest [10, 11] and exercise [12-14] as  $\beta$ E and opiate receptors have been isolated from sites that are involved in glucoregulation [15, 16].  $\beta$ E, an important part of the opioid system, was shown

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to increase in the circulation with exercise of sufficient intensity and duration [17, 18]. Additionally,  $\beta$ E appears to play a role in metabolic regulation during exercise [13, 19, 20].

An intravenous bolus of beta endorphin ( $\beta$ E) has been reported to result in an acute and simultaneous increase in both insulin and glucagon in normal and diabetic men and women [21, 22], although this only held true in obese subjects in articles that stratified by BMI [23, 24]. Other studies have shown that administering an opioid antagonist results in a decrease in the insulin response to an oral glucose tolerance test in hyperinsulinemic, but not normoinsulinemic, women with PCOS [25-27]. Studies suggest that  $\beta$ E is involved in the regulation of insulin secretion and carbohydrate metabolism in hyperandrogenic, hyperinsulinemic women [25, 28, 29].

In terms of the relationship between  $\beta$ E and gonadotropin secretion, in healthy individuals  $\beta$ E is considered a hypothalamic neurotransmitter which suppresses GnRH release and thus inhibits the release of LH [26]. In contrast, women with PCOS are observed to have increased peripheral  $\beta$ E levels [9] in concert with elevated LH levels [30]. Administration of an opioid antagonist leads to a normalization of the LH response to a GnRH challenge, although this effect seems to be limited to hyperinsulinemic women [29, 31], a category into which many PCOS patients fall.

PCOS is a complex disorder, and the pathogenesis is not fully understood. To date, there have been no other reports that considered the potential effects of exercise on  $\beta$ E/metabolic/gonadotropin relationships in women with PCOS. There is only one prior study reporting  $\beta$ E's relationship with the LH/FSH ratio [32]. This study aimed to expand upon the limited reports on the possible relationships of  $\beta$ E and insulin, glucose, LH, LH/FSH ratio in a cohort of PCOS women with an assessment of potential confounding from exercise and BMI.

## Methods

The population studied was comprised of  $n = 40$  untreated women diagnosed with PCOS who had participated in a randomized clinical trial [33]. This publication is a cross-sectional analysis of baseline data collected prior to any intervention. The University of Virginia (UVA) Institutional Review Board approved this protocol and all study participants provided an informed consent.

### Enrollment criteria

PCOS was diagnosed using NICHD criteria [34]; namely, women had oligomenorrhea and hyperandrogenism (e.g., elevated free testosterone, hirsutism, or acne). Subjects had at least one menses in the previous six months, but no more than eight periods in the previous 12 months. Additional in-

clusion criteria were an age between 18 and 43 years, and a weight of 250 pounds (113 kg) or less. All women had normal serum concentrations of prolactin, thyroid stimulating hormone, and 17-hydroxyprogesterone. No woman had used any hormonally-active intervention (e.g., metformin, hormonal contraceptives) in the prior 60 days. Free testosterone was calculated from testosterone and sex hormone binding globulin levels (SHBG) [35] using " $> 6.8$  pg/mL" as the definition of an elevated level among females [36]. Exclusion criteria were: a) fasting blood glucose level  $> 125$  mg/dL, and b) hemoglobin A1C (HgbA1C) level  $> 6.0\%$ .

### Data sources

Demographic, lifestyle and gravidity data were self-reported via questionnaire. A standard 2-hour oral glucose tolerance test was administered using a 75-gram glucose load (either glucola drink or jelly beans [37]) in a fasting state. The pre-intervention serum samples for LH and FSH were collected within 7 days of the self-reported last menstrual period (LMP) for 3 subjects; two women had mid-cycle samples drawn and were removed from the LH and LH/FSH data analysis. For the remaining 35 women, the median menstrual cycle day (CD) was 31 with an interquartile range of 18 - 38 days. Blood draws by trained nursing staff were taken at times 0, 30 (allowing 10 minutes for glucose consumption), 60, 90 and 120. Androgens were assayed on fasting blood samples collected for diagnostic purposes. All assays (except HgbA1C and  $\beta$ E) were run by the UVA General Clinical Research Center Core Lab using standard chemiluminescent enzyme-linked immunoassays. The analytical sensitivity of insulin was 2.0 mIU/mL with an intra-assay assay coefficient of variation (CV) of 2.3% and an inter-assay CV of 8.0%. The analytical sensitivity of FSH was 0.1 mIU/mL with a coefficient of variation (cv) of 5.6% among normally ovulating females (mean 4.85 mIU/mL) and 6.3% cv for perimenopausal females (mean 15.4 mIU/mL). The analytical sensitivity of LH was 0.05 mIU/mL with a 7.4% cv at normal follicular phase levels (mean 11.3 IU/L). HgbA1c was run by the UVA Clinical Lab. Serum insulin samples were stored at  $-80$  °C and subsequently batch assayed with a RIA kit on the Immulite 2000 instrument. The minimum detection for insulin was 2.0 mIU/mL. Height and weight were measured by a research nurse for all study participants.

### Lab assay technology – Beta endorphin

The  $\beta$ E samples were assayed in the Exercise Physiology Research Laboratory at the University of North Carolina Greensboro under the direction of Dr. Goldfarb. For these assays, exactly 5 ml blood was collected in a pre-chilled 6 ml lavender tube containing 0.5 ml Trasylol (aprotinin), the sample was cold centrifuged at 1600 rpm for 15 min, and then the plasma samples were frozen at  $-80$  °C. The plasma

**Table 1.** Demographics and Behavioral Characteristics of the Study Population (n = 40)

Factor	N (%), or Mean (sd) and range
Age	27.5 (sd = 6.61), 18 - 43 years
Race	
White	32 (80%)
Black or African-American	4 (10%)
Asian or American Indian	2 (5%)
Mixed	2 (5%)
Hispanic	2 (5%)
Prior pregnancy history	
Nulligravida	30 (75%)
1 prior pregnancy	3 (7.5%)
2 prior pregnancies	7 (17.5%)
Smoke	5 (13%)
BMI	29.4 (sd = 6.40), range 18.6 - 43.1
Number of periods in the most recent 12 months without medication	5.1 (sd = 2.23)
Self-reported acne	19 (48%)
Self-reported hirsutism	30 (75%)
Exercise (38)	
None	6 (15%)
Some but less than national guidelines	19 (48%)
Meets national guidelines	15 (37%)

samples (2 ml aliquot) were loaded onto pre-treated C-18 columns (acetone 2 ml and 10 ml DD H<sub>2</sub>O). The column was washed 2 times with 2 ml of 4% acetic acid in distilled water. The endorphin was eluted off the column with 5 ml of 0.2 M hydrochloric acid/acetone (25/75) in polypropylene tubes and taken to dryness in a speed vacuum centrifuge. The pellet was then resuspended in 0.300 µl βE assay buffer and the Elisa for this assay from ALPCO was followed. All procedures were according to ALPCO and compared to known standards ([www.alpco.com/single.asp?CatNumber=13-RB%20301](http://www.alpco.com/single.asp?CatNumber=13-RB%20301)).

### Exercise classification

Through a self-administered questionnaire, participants were asked six questions about their physical activity other than employment-related activities during the past month. The questions were based on the Behavioral Risk Factor Surveillance System survey [38]. The exercise intensity and average minutes of that physical activity were used to categorize

each participant into one of three groups: those that had no physical activity outside of work, those who met the Department of Health and Human Services guidelines of “physically active” [39], and those that had some exercise but that did not meet the national guidelines. These three levels of daily exercise were coded as: 1 = none, 2 = some exercise but at a level below the recommendations, and 3 = at or above recommended level.

These data were also transformed into metabolic equivalents, or METs, using a published compendium of physical activities [40]. For activities where the compendium provided a range of MET values, the midpoint was used. That classification was conducted independently by two authors, and reviewed by a third author in cases of disagreement. Similar to the method described by Mudd [41], the total exercise energy expenditure per week was calculated as the MET for that activity multiplied by the frequency per week and the duration of the activity (relative to 1 hour); the two most common physical activities per week were included in this calculation.

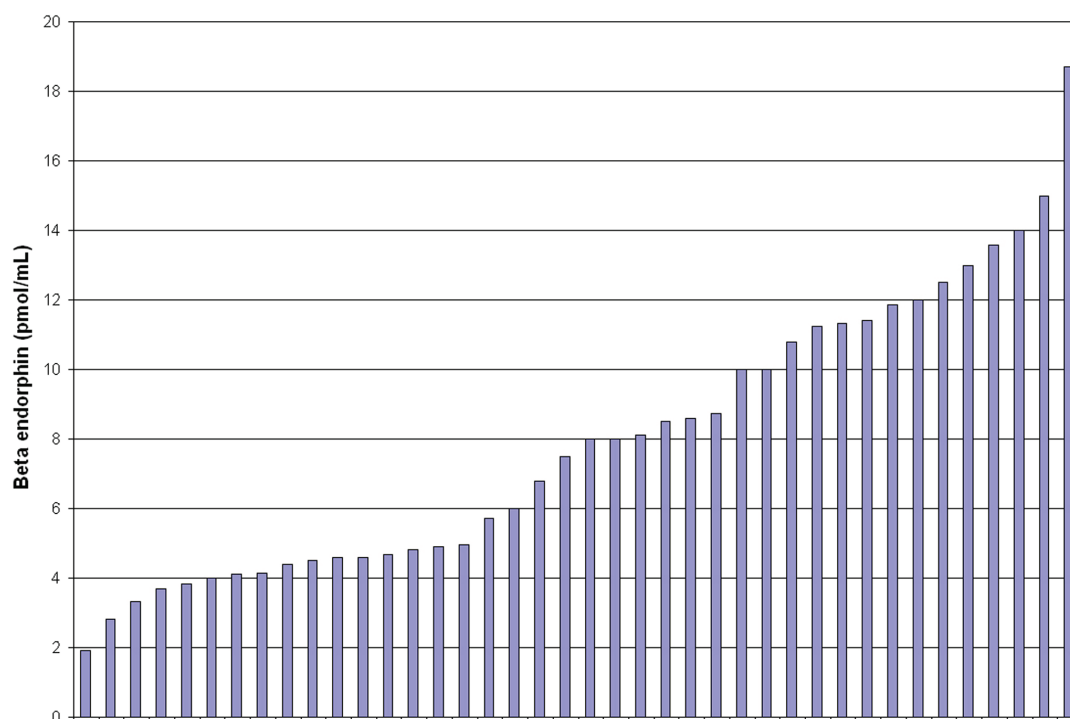


Figure 1. Beta Endorphin levels in 40 women with PCOS.

## Statistical analyses

Spearman rank correlations were calculated to assess the relationship between  $\beta E$  with the six biological parameters of interest (LH, the LH/FSH ratio, fasting insulin, area under the curve (AUC) insulin, fasting glucose, and AUC glucose). Graphs with lines-of-best-fit were developed. Based on limited prior research that indicated  $\beta E$  appeared to be greater in obese women with PCOS than non-obese women with PCOS [6, 32], the partial correlations were run controlling for BMI. As previously described, research has also demonstrated an influence on  $\beta E$  levels from exercise, hence partial correlations were also run controlling for exercise. Finally, partial correlations were calculated controlling for both BMI and exercise.

All analyses were performed with SAS (version 9.2, Cary, NC). An alpha of 0.05 was considered statistically significant, and P-values between 0.05 and 0.10 were considered marginally significant. Given the relatively modest size of this cohort, marginally significant associations were noted in the text as areas for future investigation.

## Results

This cohort contained a wide age range (18 to 43 years old) of women with PCOS, most of whom were Caucasian (Table 1). A broad BMI range was represented, with 37% ( $n = 15$ )

in the lean BMI category ( $BMI < 25$ ), 12% ( $n = 5$ ) considered overweight ( $25 < BMI < 30$ ), and the remaining 51% obese ( $BMI > 30$ ) [42]. The frequency of exercise in this cohort ranged from 15% without any regular exercise to 38% who met the US fitness guidelines.

As evidence of hyperandrogenemia, the androgen profile of the study participants was as follows. The mean testosterone was 58.2 ng/dL ( $sd = 28.52$ ), and the mean DHEAS was 176.4  $\mu g/dL$  ( $sd = 96.69$ ). The mean free testosterone 13.28 pg/mL ( $sd = 7.83$ ), and the mean SHBG was 30.09 nmol/liter ( $sd = 15.07$ ).

The mean  $\beta E$  level was 7.92 pmol/mL ( $sd = 4.0$ ) for the entire cohort. The range was 1.92 - 18.7 pmol/mL with a 25%/75% interquartile range of 4.56 - 11.29. Figure 1 displays the distribution of all the individual  $\beta E$  levels. Among the non-obese women ( $n = 20$ ), the mean  $\beta E$  level was 7.69 pmol/mL ( $sd = 4.0$ ) with the same interquartile range as for the full cohort. Among the obese sub-cohort ( $n = 20$ ), the mean  $\beta E$  level was 8.14 pmol/mL ( $sd = 4.1$ ) with a 25%/75% interquartile range of 4.61 - 10.71.  $\beta E$  was not correlated with BMI (as a continuous measure) in this cohort ( $P = 0.66$ ).

No bivariate correlation was observed between  $\beta E$  and any of the six biological parameters of interest (Table 2). The scatterplots and lines-of-best-fit (Fig. 2), however, indicated a linear association between  $\beta E$  and the natural log-transformed AUC-insulin results. Partial correlations controlling for BMI did not meaningfully improve the correlations. Partial correlations controlling for exercise (coded as a tri-level

**Table 2.** Spearman Rank Correlations and Partial Correlations ( $r_s$ , P-value) for the Association Between Beta Endorphin With LH, LH/FSH Ratio, Fasting Insulin, AUC Insulin, Fasting Glucose, and AUC Glucose

Biological Parameter	Bivariate Correlation	Partial correlation controlling for BMI	Partial correlation controlling for exercise (3-level variable)	Partial correlation controlling for exercise(energy expenditure continuous variable)
LH	0.18 (0.28)	0.20 (0.24)	0.18 (0.28)	0.20 (0.23)
LH/FSH Ratio	0.15 (0.38)	0.14 (0.39)	0.14 (0.40)	0.16 (0.37)
$I_0$	0.003 (0.98)	-0.08 (0.63)	-0.004 (0.98)	-0.01 (0.94)
AUC <sub>I</sub>	0.24 (0.14)	0.25 (0.13)	0.28 (0.09)*	0.34 (0.04)**
$G_0$	0.09 (0.59)	-0.08 (0.65)	-0.04 (0.79)	-0.06 (0.74)
AUC <sub>G</sub>	-0.01 (0.94)	0.07 (0.69)	0.09 (0.58)	0.11 (0.53)

\*0.05 < P < 0.10; \*\*P < 0.05; n = 40 for all measurements except LH and LH/FSH ratio where the sample size was n = 38.

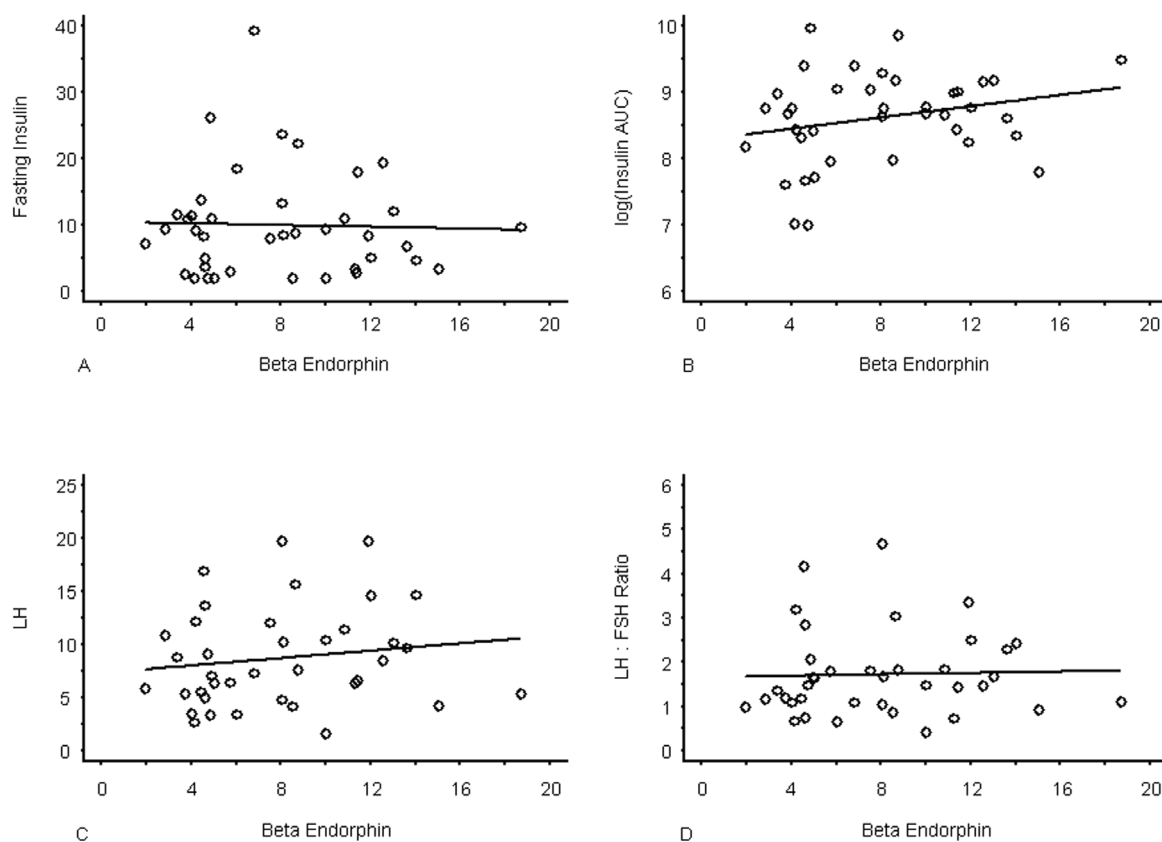
variable) increased the relationship between  $\beta E$  and AUC insulin into the marginal significance range ( $P = 0.09$ ), and this association was not strengthened when BMI was additionally controlled. When exercise was measured as the continuous energy expenditure variable, the partial correlation was strengthened between  $\beta E$  and AUC insulin ( $P = 0.04$ ), and this association was maintained when BMI was additionally controlled.

## Discussion

This study found that  $\beta E$  was significantly associated with AUC-insulin (after log transformation to ensure normality), after controlling for exercise expenditure levels in this PCOS cohort. This association was unaffected by control of BMI levels, and was weakened when exercise was measured as a categorical variable rather than as a continuous energy expenditure. There was no association between  $\beta E$  and LH, the LH/FSH ratio, fasting insulin, fasting glucose, or AUC glucose. This is the first known report to include exercise as a potential confounder of these relationships. As reported previously by others [6-9], in our sample of women with PCOS the  $\beta E$  levels were higher than in women without PCOS (published norms [4, 5] were used in this analysis for comparison).

The primary limitation of this study is the lack of a non-PCOS, BMI-matched control population. If a comparison population had been available, the analysis would have been able to comment on potential disease-mediated relationships separate from the effect of weight. A second limitation is that peripheral concentrations of BE were measured, rather than central levels, thus this study is not measuring central opioid tone. The latter is likely to have a stronger biological connection with the endocrine markers of interest than peripheral BE. Our sample size of 40 women is a strength relative to the literature, as is the wide range of BMI. There have been twelve prior publications on  $\beta E$  levels in women with PCOS characteristics [6-9, 32, 43-49]. With two exceptions [32, 49], these reports had fewer than 20 PCOS study participants. An additional strength of our study is that endogenous  $\beta E$  was measured instead of an exogenous infusion of  $\beta E$ , thus examining a normal physiologic state in women with PCOS versus an exogenous infusion. Furthermore, we also studied the relationship of  $\beta E$  with insulin, LH, and the LH/FSH ratio along a continuum of BMI's rather than stratifying groups as "obese" versus "non-obese" thereby observing the effects of differing levels of obesity on  $\beta E$ , insulin, LH, LH/FSH ratio in women with PCOS.

The previous reports on  $\beta E$  in women with PCOS overall found that  $\beta E$  levels were higher in women of greater body mass, both within PCOS and non-PCOS populations [6, 7, 32], and our findings concur (significant positive correlation between  $\beta E$  and BMI,  $P = 0.0009$ ). In addition, we found a



**Figure 2.** Graphs of Individual Observations of Beta endorphin by LH, LH/FSH Ratio, Insulin and log AUC-Insulin with Line-of-Best-Fit. N = 40 for all measurements except LH and LH/FSH ratio where the sample size was n = 38.

positive correlation between  $\beta E$  and AUC-insulin even after controlling for BMI. This suggests that  $\beta E$  itself could be potentiating the release of insulin after a glucose load. Our data is consistent with Laatkainen et al who observed this relationship in a sample size of eight women with PCOS [45].

To explore this further, we must first expound on recent understandings of insulin secretion. Rorsman et al discovered that  $\beta$ -islet cells studied in vitro showed insulin secretion as biphasic, the first phase being an acute degranulation occurring  $\sim 1$  minute after extracellular addition of glucose, 5 - 10% of the total insulin was released in this fashion. The second phase starts at  $\sim 5$  minutes with a steady stream of insulin secretion over a longer time course [50]. Rat studies have shown that  $\beta E$  causes transient increases in insulin after glucose load  $>150$  mg/dl with effects lasting for 3 - 4 minutes. This suggests that  $\beta E$  may target first phase insulin release at sufficient glucose loads [51]. A mild correlation between  $\beta E$  and AUC-insulin, such as our data shows, would be expected given that only 5 - 10% of circulating insulin in humans is released during the first phase. The relationship of  $\beta E$  and opioid receptors on insulin release, specifically the

K-ATPase/voltage gated Ca-channel pathway, could be explored via future research. Should an underlying pathophysiology be found explaining the relationship between  $\beta E$  and AUC-Insulin secretion, alternative treatments that modulate  $\beta E$  such as exercise or acupuncture [52, 53] may delay the need for or potentiate the effect of medications such as metformin.

Our results may suggest that at rest  $\beta E$  exerts a potentiating effect on AUC-insulin. Several human and animal studies indicate that  $\beta E$  also exerts an effect on both insulin and glucagon at rest [11, 21, 54]. Previous rat studies [13] have shown that during exercise, a 6 - 7 fold increase in  $\beta E$  actually resulted in higher plasma glucose levels.  $\beta E$  maintenance infusion exacerbated the glucagon rise in exercised rats more so than in exercised rats that received a saline infusion. Clearly, the relationship between  $\beta E$  and glucose control is multi-factorial, and  $\beta E$  may be involved in both physiologic insulin and glucagon maintenance. A direct effect of  $\beta E$  on glucose homeostasis is implied by studies that found that an intravenous bolus of  $\beta E$  resulted in an acute and simultaneous increase in both insulin and glucagon in normal and dia-

betic [21, 22] men and women, although this only held true in obese subjects in studies that stratified by BMI [23, 24]. It may be worthwhile to study the effects of varying intensities of exercise on  $\beta$ E, and its subsequent effect on insulin and LH. This may lead to the development of therapeutic exercise regimens specifically designed for women with PCOS.

One prior study examined the relationship between  $\beta$ E with both insulin and LH in a single cohort of 137 women with PCOS (117 lean and 50 non-lean) [32]. They reported a significant inverse association between  $\beta$ E and LH ( $r = -0.173$ ,  $P = 0.025$ ) and a significant positive correlation between  $\beta$ E and fasting insulin ( $r=0.266$ ,  $P = 0.002$ ). They did not investigate AUC-insulin. Our cohort does not concur with their observations. Similarly, a different study with 64 subjects with PCOS [49] found an inverse association between  $\beta$ E and the LH to FSH ratio ( $r = -0.32$ ;  $P = 0.015$ ) whereas we found no correlation between the two. These differences could be due to the increased power of those studies, but, alternatively or additionally, our control of BMI and exercise level may underlie the variation in results across studies.

The association between  $\beta$ E and AUC-insulin only after controlling for exercise is worthy of additional discussion. Circulating  $\beta$ E increases have been reported in response to both aerobic and anaerobic exercise [19]. This increase in circulating  $\beta$ E depends on exercise intensity [17, 55] and duration [17, 56], which supports the fact that the statistical significance of our observation strengthened when exercise energy equivalents were measured. A critical minimum intensity of  $> 60\%$  of  $\text{VO}_2$  max appears to be needed to result in  $\beta$ E elevation with aerobic exercise [17, 57, 58]. Resistance exercise of sufficient intensity and volume (workload) can also result in a transient increase in  $\beta$ E levels within the circulation [55]. It should be noted that these elevations in  $\beta$ E levels are transitory and return to baseline levels in a relatively short time after the exercise is completed. Further studies could be conducted to determine how these changes in  $\beta$ E observed during exercise are related to the insulin/glucagon balance, particularly investigating what affect different exercise regimens have on the relationship between  $\beta$ E with insulin and glucagon in women with PCOS.

## Conclusion

As the most common endocrinopathy effecting women, very little is known about the underlying mechanisms of PCOS. Evidence shows that  $\beta$ E is involved in the regulation of insulin secretion and carbohydrate metabolism in hyperandrogenic, hyperinsulinemic women. Our study aimed to observe what, if any, relationship  $\beta$ E had on insulin, glucose, LH, and the LH/FSH ratio in women with PCOS. After accounting for exercise energy expenditures,  $\beta$ E was found to be significantly positively correlated with AUC-insulin ( $P = 0.04$ ). In light of the relatively new understanding of the delivery of

insulin, we hypothesize  $\beta$ E may be potentiating first phase, post-prandial insulin secretion. No relationship between  $\beta$ E and LH, the LH/FSH ratio, or glucose was seen after accounting for BMI and exercise frequency. As discussed, our findings provide several future avenues of research that may relate to therapeutic exercise regimens, or modalities such as acupuncture with a documented impact on  $\beta$ E, to improve the endocrine and metabolic profile of women with PCOS.

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## Disclosure Statement

The authors have nothing to disclose.

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