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# Insulin Resistance Influences Central Opioid Activity in Polycystic Ovary Syndrome

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## Abstract

This pilot study describes a relationship between insulin resistance and μ-opioid neurotransmission in limbic appetite and mood-regulating regions in women with polycystic ovary syndrome, suggesting that insulin-opioid interactions may contribute to behavioral and reproductive pathologies of PCOS. We found that 1) insulin resistant PCOS patients (n=7) had greater limbic μ-opioid receptor availability (non-displaceable binding potential) than controls (n=5), 2) receptor availability was correlated with severity of insulin resistance, and 3) receptor availability normalized after insulin-regulating treatment.

### Keywords

Positron emission tomography; glucose regulation; mu-opioid receptors; neuroimaging; beta-endorphins; PCOS; insulin resistance



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Polycystic ovary syndrome (PCOS), the most common endocrine disorder in young women(1), affects up to 18% of reproductive-aged women(2). Reproductive and metabolic disruptions include hyperandrogenism and infertility, insulin resistance (IR), and a higher prevalence of impaired glucose tolerance and type 2 diabetes(3–9).The endogenous opioid system may contribute to PCOS pathogenesis, through central effects on gonadotropin secretion and peripheral effects on glucose metabolism(10–16). Endogenous opioidsmediate insulin's neuromodulatory effects, and almost 90% of cells containing insulin receptors are immunoreactive for the μ-opioid receptor (μOR) agonistβ-endorphin, suggesting interactions between insulin and opioid neurotransmission(17, 18).

Opioid abnormalities in PCOS and the impact of potential modulation by insulin are presently unknown, but evidence suggests decreased central opioid sensitivity in PCOS patients.Luteal phase luteinizing hormone (LH) pulsatility slowing by progesterone, which is largely absent in PCOS, is mediated by GnRH release inhibition by β-endorphin(19, 20). CNS opioid system changesin PCOS patientshave not been reported.

We evaluated IR effects on μORs in women with insulin resistant PCOS (IR-PCOS), and used positron emission tomography (PET) with the μ-receptor selective radiotracer [$^{11}$C] carfentanil(21, 22) to compare receptor availabilityto non-IR controls, and to assess the ability of the hypoglycemic agent metformin to restore μOR concentrations. We hypothesized decreased opioid activity in IR-PCOS, reflected by unbound μORs, and increased opioid activity after metformin treatment, in the β-endorphin projection regions nucleus accumbens/ventral pallidum and amygdala(23).

Women aged 21 – 40 years were recruited intoIR-PCOS patients (n=7) andnon-IRcontrols (n=5) groups. PCOS criteriawere irregular menstrual cycles/amenorrhea and hyperandrogenism(24). IR was estimated using the homeostatic model (HOMA2-IR; glycemia(mmol/1) × insulinemia(μU/ml)/22.5)(25), using HOMA2-IR sensitivity ≤60%(26). Controls had regular menstrual cycles, normal androgens and glucose tolerance,no hirsutism or acne, and HOMA2-IR sensitivity ≥80%.

Participants were healthy, right-handed, nonsmokers, excluded forsignificant illness including diabetes, hormones within 2 months, pregnancy within 6 months, centrally-acting medications, substance abuse,MRI contraindications, corticosteroid, cimetidine use, and opioid allergy.

Women underwent medical histories, physical exam, and fasting glucose, insulin, 2 hour 75g dextrose oral glucose tolerance test (OGTT), free and total testosterone, dehydroepiandrosterone sulfate (DHEAS), lipids, blood count, TSH, electrolytes, and liver function screening.

Procedures were approved by the University of Michigan Institutional Review Board and Radiation Safety Review Committee. Written informed consent was obtained.

MRI and [$^{11}$C]carfentanil PET scansoccurredduring the follicular phase, if cycling.. IR-PCOS patients subsequently began metformin (500mg titrated to1500mg daily). PET scan and OGTT were repeated after four months of treatment.

Subjects underwent one (controls) or two (PCOS patients) 70 minute PET scans (HR$^{+}$ scanner;Siemens, Knoxville) in 3-dimensional mode (reconstructed full-width/half-maximum resolution, ≈5.5mm in plane, 5.0mm axially), with septa retracted and scatter correction, collecting 28 increasing duration frames. Tracer quantity [$^{11}$C]carfentanil was administered (10–15mCi, ≤0.03μg/kg) via intravenous line (50% in initial bolus and remainder continuously infused for constant concentrations). [$^{11}$C]carfentanil was

synthesized athigh specific activity by the reactionof [$^{11}$C]methyl iodide and a normethyl precursor(27).

Images were decay-corrected and reconstructed, and the dynamic frames coregistered to each other and transformed into tracer transport ($K_1$ ratio) and receptor-related($BP_{ND}$, binding potential) measures, using the occipital cortex as reference region, which lacks μORs,calculated using a modified Logan graphical analysis(28). After 5–7 minutes of radiotracer administration, the Logan plot becomes linear with slope=$BP_{ND}$+1, which is proportional to μORconcentration ($B_{max}$)/receptor radiotracer affinity ($K_d$) (Bmax/Kd ≈ $BP_{ND}$).

Anatomical MR images were acquired axially with a 3T scanner (GE, Milwaukee) with a spoiled gradient recalled 3D volumetric acquisition (repetition time= 9.6, echo time=3.3, inversion recovery preparation=200ms, flip angle=17°, bandwidth=15.63, 24-cm field-of-view, 1.5mm slice thickness, 106–110 slices, 256×256 matrix, 2 excitations). T1-weighted MR and PET images were coregistered to each other and the International Consortium for Brain Mapping/Montreal Neurological Institute (ICBM/MNI) template(29).

PET images were analyzed usingSPM2(Wellcome Cognitive Neurology, London) and SPSS (SPSS Inc., Chicago). Groupcomparisons were performed using unpaired (control versus PCOS) or paired (pre- versus post-metformin) T tests on μOR $BP_{ND}$ data extracted from the nucleus accumbens/ventral pallidum and amygdala. Significance was set at $p<0.001$ uncorrected with *a priori* hypotheses. Relationships between μOR$BP_{ND}$ and IRwere determined using Pearson correlations at $p<0.05$.

Demographic and clinical information is provided in Table 1A. Mean (SD)age was 26.1 (3.5). PCOS women had oligomenorrhea (cycle length>35 days), and had higher BMI, weight, waist circumference, total and free testosterone, insulin, and HOMA-IR compared to controls. Improvements following metformin treatment did not reach statistical significance.

Baseline μ OR $BP_{ND}$ was greater in IR-PCOS women than controls in the nucleus accumbens/ventral pallidum (coordinates, *x,y,z* (mm), left −6,−4,−12, cluster size 3088mm$^3$, p=0.009; right 10,0,−10, cluster size 2912mm$^3$, p=0.012) and amygdala, bilaterally (left −26,−8,−24, cluster size 896mm$^3$, p=0.045; right 18,−4,−22, cluster size 416mm$^3$, p=0.161).

After 4 months of metformin treatment, $BP_{ND}$ in IR-PCOS women was reduced in the nucleus accumbens/ventral pallidum by15.2(10.5)% (left, t=3.95, p=0.008) and 12.2(7.7)% (right, t=4.26, p=0.005) and in the amygdala by 8.7(7.2)% (left, t=3.07, p=0.022) and 7.1(7.7)% (right, t=2.66, p=0.038). $BP_{ND}$ values were not statistically different from controls.

Baseline regional μOR$BP_{ND}$ was correlated with IR (fasting insulin, HOMA% sensitivity and HOMA-IR) for the nucleus accumbens/ventral pallidum bilaterally and the left amygdala (table 1b). The change in μOR$BP_{ND}$ after treatment was not significantly correlated withIR improvements.

This is the first study to evaluate the link betweenopioid neurotransmission and IR in PCOS.We focused our analysis on the amygdala and nucleus accumbens, limbicβ-endorphin neurotransmission projection areaswith reproductive, metabolic, appetite, and mood function, all of which can be disordered in PCOS(8, 30, 31). We found that IR-PCOS patientsshowed greater μORavailability in these regions thannon-IRcontrols. Four months of metformin treatment decreased receptor availability to levels similar to controls. These resultssupport insulin modulation of central opioid activity in PCOS.

Insulin receptorsare expressed in proopiomelanocortin (POMC) containing neurons, the precursor of β-endorphin. Arcuate nucleus POMC neurons project to the amygdala, nucleus accumbens and hypothalamus, where they regulate mood, reward processing, including food reward, and the hypothalamic-adrenal and hypothalamic-gonadal axes(32–35). Detection of altered opioid receptor measures in IR-PCOS patients in these regions provides information about PCOS pathophysiology, and suggests a mechanism to explain neuroendocrine dysregulation, increased incidence of depression, and appetite dysregulation and obesity associated with PCOS(8, 30, 36).

A classic neuroendocrine feature of PCOS is rapid and high amplitude LH pulses(37, 38). Opioids are believed to mediate sex steroids negative feedback on gonadotropin release via the hypothalamus and amygdala(21, 39).Although we did not test for presynaptic opioid release, altered μORavailability in the IR-PCOS subjects may indicate reduced central opioid activity and compensatory receptor upregulation. Consistent with the hypothesized relationships between μORavailability and insulin, we observed positive correlations between baseline μORBP$_{ND}$ andIR.Metformin treatment normalized μORBP$_{ND}$ in IR-PCOS patients, suggesting that improved insulin sensitivity restores dysregulated opioid neurotransmission, consistent with research showing that chronic opioid receptor antagonism has metabolic and reproductive effects only in PCOS patients with IR(10–12, 40–43).

Altered opioid function in IR-PCOS may contribute to appetite and mood difficulties observed in PCOS(8, 30, 36, 44). Opioid transmission in the ventral pallidum/nucleus accumbens regulates reward processing and hedonic function/motivation, and μORagonistsstimulate food-intake and appetite for high-fat/high-glucose foods through amygdala and nucleus accumbens reward circuitry(34, 35, 45–47). The ventral basal ganglia and amygdala's rich population of μORsregulate affective states(32). In women, major depression is associated with opioid system dysregulation in the amygdala(33). Abnormal glucose metabolism is also linked to depression, and diabetesprevalence is 2–3 times higher in depressed individuals (48–50).

Study strengths include intrasubject design and control comparison.Limitationsare sample size, lack of placebo, and no comparison with non-IR PCOS women.Hypothalamic nucleiare not easily assessed with PET because of size, limitedresolution,and averaging of activity with surrounding areas. In future studies, a challenge intervention to induce endogenous opioid release would allow presynaptic function evaluation, and distinguish isolated changes in receptor availability from alterations in presynaptic endogenous opioid tone(22, 29, 51).

The endogenous opioid systemmay represent an essential link in the interface between the reproductive and metabolic systems. While further study is required to understand the complex relationship between insulin function and opioid transmission in PCOS, this pilot study provides strong initial evidence of altered metabolic-opioid system interactions in IR women with PCOS. We found altered μORavailability prior to metformin treatment in regions that regulate appetite and mood, and modulation of receptor sites after metformin treatment. Our results suggest that insulin-opioid interactions contribute to behavioral and reproductive pathologies of PCOS, which should be explored in comprehensive larger-scale studies.

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## REFERENCES

1. Franks S. Polycystic ovary syndrome: a changing perspective. Clin Endocrinol (Oxf). 1989; 31(1): 87–120. [PubMed: 2513151]
2. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010; 25(2):544–551. [PubMed: 19910321]
3. Ehrmann DA, Liljenquist DR, Kasza K, Azziz R, Legro RS, Ghazzi MN. Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. The Journal of clinical endocrinology and metabolism. 2006; 91(1):48–53. [PubMed: 16249284]
4. Ehrmann DA, Barnes RB, Rosenfield RL, Cavaghan MK, Imperial J. Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. Diabetes Care. 1999; 22(1):141–146. [PubMed: 10333916]
5. Legro RS, Gnatuk CL, Kunselman AR, Dunaif A. Changes in glucose tolerance over time in women with polycystic ovary syndrome: a controlled study. The Journal of clinical endocrinology and metabolism. 2005; 90(6):3236–3242. [PubMed: 15797965]
6. Legro RS, Kunselman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. The Journal of clinical endocrinology and metabolism. 1999; 84(1):165–169. [PubMed: 9920077]
7. Norman RJ, Masters L, Milner CR, Wang JX, Davies MJ. Relative risk of conversion from normoglycaemia to impaired glucose tolerance or non-insulin dependent diabetes mellitus in polycystic ovarian syndrome. Hum Reprod. 2001; 16(9):1995–1998. [PubMed: 11527911]
8. Teede H, Deeks A, Moran L. Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med. 8:41. [PubMed: 20591140]
9. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. Hum Reprod Update. 2008; 14(4):367–378. [PubMed: 18499708]
10. Fulghesu AM, Lanzone A, Cucinelli F, Caruso A, Mancuso S. Long-term naltrexone treatment reduces the exaggerated insulin secretion in patients with polycystic ovary disease. Obstet Gynecol. 1993; 82(2):191–197. [PubMed: 8336863]
11. Guido M, Romualdi D, Lanzone A. Role of opioid antagonists in the treatment of women with glucoregulation abnormalities. Curr Pharm Des. 2006; 12(8):1001–1012. [PubMed: 16533167]
12. Lanzone A, Apa R, Fulghesu AM, Cutillo G, Caruso A, Mancuso S. Long-term naltrexone treatment normalizes the pituitary response to gonadotropin-releasing hormone in polycystic ovarian syndrome. Fertility and sterility. 1993; 59(4):734–737. [PubMed: 8458488]
13. Lanzone A, Fulghesu AM, Fortini A, Cutillo G, Cucinelli F, Di Simone N, et al. Effect of opiate receptor blockade on the insulin response to oral glucose load in polycystic ovarian disease. Hum Reprod. 1991; 6(8):1043–1049. [PubMed: 1666896]
14. Cumming DC, Reid RL, Quigley ME, Rebar RW, Yen SS. Evidence for decreased endogenous dopamine and opioid inhibitory influences on LH secretion in polycystic ovary syndrome. Clin Endocrinol (Oxf). 1984; 20(6):643–648. [PubMed: 6088126]
15. Barnes RB, Lobo RA. Central opioid activity in polycystic ovary syndrome with and without dopaminergic modulation. The Journal of clinical endocrinology and metabolism. 1985; 61(4): 779–782. [PubMed: 3928677]
16. Eyvazzadeh AD, Pennington KP, Pop-Busui R, Sowers M, Zubieta JK, Smith YR. The role of the endogenous opioid system in polycystic ovary syndrome. Fertility and sterility. 2009; 92(1):1–12. [PubMed: 19560572]

17. Kleinrok Z, Sieklucka-Dziuba M, Sek A, Rodziewicz E, Latus U. The effect of repeated administration of insulin on pain threshold and gamma-aminobutyric acid level in mouse brain. Acta Physiol Pol. 1987; 38(6):477–482. [PubMed: 3331234]

18. Unger JW, Lange W. Insulin receptors in the pituitary gland: morphological evidence for influence on opioid peptide-synthesizing cells. Cell Tissue Res. 1997; 288(3):471–483. [PubMed: 9134860]

19. Berga SL, Yen SS. Opioidergic regulation of LH pulsatility in women with polycystic ovary syndrome. Clin Endocrinol (Oxf). 1989; 30(2):177–184. [PubMed: 2532984]

20. Khoury SA, Reame NE, Kelch RP, Marshall JC. Diurnal patterns of pulsatile luteinizing hormone secretion in hypothalamic amenorrhea: reproducibility and responses to opiate blockade and an alpha 2-adrenergic agonist. The Journal of clinical endocrinology and metabolism. 1987; 64(4): 755–762. [PubMed: 2880864]

21. Smith YR, Zubieta JK, del Carmen MG, Dannals RF, Ravert HT, Zacur HA, et al. Brain opioid receptor measurements by positron emission tomography in normal cycling women: relationship to luteinizing hormone pulsatility and gonadal steroid hormones. The Journal of clinical endocrinology and metabolism. 1998; 83(12):4498–4505. [PubMed: 9851799]

22. Smith YR, Stohler CS, Nichols TE, Bueller JA, Koeppe RA, Zubieta JK. Pronociceptive and antinociceptive effects of estradiol through endogenous opioid neurotransmission in women. J Neurosci. 2006; 26(21):5777–5785. [PubMed: 16723535]

23. Mansour A, Fox CA, Thompson RC, Akil H, Watson SJ. mu-Opioid receptor mRNA expression in the rat CNS: comparison to mu-receptor binding. Brain research. 1994; 643(1–2):245–265. [PubMed: 8032920]

24. Zawadski, JK.; Dunaif, A. Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In: Dunaif, A., editor. Polycystic Ovary Syndrome. Boston: Blackwell Scientific; 1992. p. 377-384.

25. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412–419. [PubMed: 3899825]

26. Mohlig M, Spranger J, Ristow M, Pfeiffer AF, Schill T, Schlosser HW, et al. Predictors of abnormal glucose metabolism in women with polycystic ovary syndrome. Eur J Endocrinol. 2006; 154(2):295–301. [PubMed: 16452544]

27. Jewett DM. A simple synthesis of [11C]carfentanil using an extraction disk instead of HPLC. Nucl Med Biol. 2001; 28(6):733–734. [PubMed: 11518656]

28. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab. 1996; 16(5):834–840. [PubMed: 8784228]

29. Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, et al. Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science. 2001; 293(5528):311–315. [PubMed: 11452128]

30. Farrell K, Antoni MH. Insulin resistance, obesity, inflammation, and depression in polycystic ovary syndrome: biobehavioral mechanisms and interventions. Fertility and sterility. 2010; 94(5): 1565–1574. [PubMed: 20471009]

31. Esch T, Stefano GB. The neurobiology of pleasure, reward processes, addiction and their health implications. Neuro Endocrinol Lett. 2004; 25(4):235–251. [PubMed: 15361811]

32. Zubieta JK, Ketter TA, Bueller JA, Xu Y, Kilbourn MR, Young EA, et al. Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. Archives of general psychiatry. 2003; 60(11):1145–1153. [PubMed: 14609890]

33. Kennedy SE, Koeppe RA, Young EA, Zubieta JK. Dysregulation of endogenous opioid emotion regulation circuitry in major depression in women. Archives of general psychiatry. 2006; 63(11): 1199–1208. [PubMed: 17088500]

34. Pecina S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci. 2005; 25(50):11777–11786. [PubMed: 16354936]

35. Smith KS, Berridge KC. Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. J Neurosci. 2007; 27(7):1594–1605. [PubMed: 17301168]
36. Rassi A, Veras AB, Dos Reis M, Pastore DL, Bruno LM, Bruno RV, et al. Prevalence of psychiatric disorders in patients with polycystic ovary syndrome. Compr Psychiatry. 2010; 51(6): 599–602. [PubMed: 20965306]
37. Balen AH. Hypersecretion of luteinizing hormone and the polycystic ovary syndrome. Hum Reprod. 1993; 8 Suppl 2:123–128. [PubMed: 8276945]
38. Blank SK, McCartney CR, Marshall JC. The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Hum Reprod Update. 2006; 12(4):351–361. [PubMed: 16670102]
39. Parvizi N, Ellendorff F. Gonadal steroids in the amygdala--differential effects on LH. Brain research. 1980; 195(2):363–372. [PubMed: 7397506]
40. Hadziomerovic D, Rabenbauer B, Wildt L. Normalization of hyperinsulinemia by chronic opioid receptor blockade in hyperandrogenemic women. Fertility and sterility. 2006; 86(3):651–657. [PubMed: 16901484]
41. Lanzone A, Fulghesu AM, Cucinelli F, Ciampelli M, Caruso A, Mancuso S. Evidence of a distinct derangement of opioid tone in hyperinsulinemic patients with polycystic ovarian syndrome: relationship with insulin and luteinizing hormone secretion. The Journal of clinical endocrinology and metabolism. 1995; 80(12):3501–3506. [PubMed: 8530590]
42. Ahmed MI, Duleba AJ, El Shahat O, Ibrahim ME, Salem A. Naltrexone treatment in clomiphene resistant women with polycystic ovary syndrome. Hum Reprod. 2008; 23(11):2564–2569. [PubMed: 18641399]
43. Fruzzetti F, Bersi C, Parrini D, Ricci C, Genazzani AR. Effect of long-term naltrexone treatment on endocrine profile, clinical features, and insulin sensitivity in obese women with polycystic ovary syndrome. Fertility and sterility. 2002; 77(5):936–944. [PubMed: 12009347]
44. Douglas CC, Norris LE, Oster RA, Darnell BE, Azziz R, Gower BA. Difference in dietary intake between women with polycystic ovary syndrome and healthy controls. Fertility and sterility. 2006; 86(2):411–417. [PubMed: 16762348]
45. Adam TC, Epel ES. Stress, eating and the reward system. Physiol Behav. 2007; 91(4):449–458. [PubMed: 17543357]
46. Denbleyker M, Nicklous DM, Wagner PJ, Ward HG, Simansky KJ. Activating mu-opioid receptors in the lateral parabrachial nucleus increases c-Fos expression in forebrain areas associated with caloric regulation, reward and cognition. Neuroscience. 2009; 162(2):224–233. [PubMed: 19422884]
47. Napier TC, Mitrovic I. Opioid modulation of ventral pallidal inputs. Annals of the New York Academy of Sciences. 1999; 877:176–201. [PubMed: 10415650]
48. Adriaanse MC, Bosmans JE. Diabetes prevalence, diabetes regimen and co-morbidity in depressed patients compared with non-depressed patients in primary care in the Netherlands. Diabet Med. 2010; 27(6):718–722. [PubMed: 20546295]
49. Egede LE, Ellis C. Diabetes and depression: global perspectives. Diabetes Res Clin Pract. 2010; 87(3):302–312. [PubMed: 20181405]
50. Golden SH. A review of the evidence for a neuroendocrine link between stress, depression and diabetes mellitus. Curr Diabetes Rev. 2007; 3(4):252–259. [PubMed: 18220683]
51. Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, et al. mu-opioid receptor-mediated antinociceptive responses differ in men and women. J Neurosci. 2002; 22(12):5100–5107. [PubMed: 12077205]

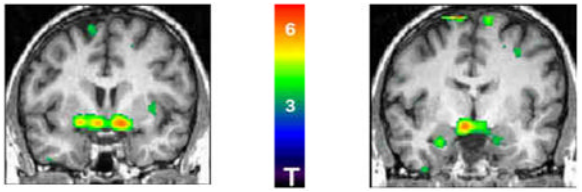
**A. Demographic and clinical measures**

| *n: 5 controls & 7 PCOS patients* | Control | | PCOS *pre-metformin* | | p[a] *control vs. pre-metformin* | PCOS *post-metformin* | | p[b] *pre- vs. post-metformin* |
|---|---|---|---|---|---|---|---|---|
| | Median (IQR) | | Median (IQR) | | | Median (IQR) | | |
| **Age (yr)** | 26 | (8) | 25 | (6) | 0.742 | | | |
| **Education (yr)** | 16 | (4) | 17 | (4) | 0.743 | | | |
| **Weight (lb)** | 123.7 | (35.3) | 203.9 | (85.8) | 0.007 | 187.1 | (98.6) | 0.499 |
| **BMI** | 23.0 | (3.1) | 35.3 | (16.2) | 0.019 | 31.7 | (15.4) | 0.108 |
| **Waist circumference (cm)** | 72.0 | (13.5) | 102.0 | (27.6) | 0.004 | 94.5 | (27.6) | 0.176 |
| **Free testosterone (pg/ml)** | 0.5 | (0.2) | 1.5 | (0.9) | 0.006 | | | |
| **Total testosterone (ng/ml)** | 0.36 | (0.32) | 0.70 | (0.51) | 0.073 | | | |
| **DHEAS (ug/dL)** | 189 | (88) | 200 | (88) | 0.927 | | | |
| **Total cholesterol (mg/dl)** | 179 | (60) | 157 | (46) | 0.073 | | | |
| **HDL (mg/dl)** | 76 | (24) | 47 | (17) | 0.073 | | | |
| **Triglycerides (mg/dl)** | 72 | (37) | 74 | (42) | 0.416 | | | |
| **Fasting insulin (uU/ml)** | 8.1 | (0.9) | 21.4 | (5.1) | 0.004 | 18.7 | (11.5) | 0.398 |
| **Fasting glucose (mg/dl)** | 89 | (7) | 87 | (15) | 0.683 | 94 | (14) | 0.400 |
| **HOMA % sensitivity** | 95.4 | (12.8) | 36.6 | (9.3) | 0.004 | 43.6 | (42.2) | 0.398 |
| **HOMA insulin resistance** | 1.0 | (0.2) | 2.7 | (0.6) | 0.004 | 2.4 | (1.5) | 0.225 |

**B. *In vivo* μ-opioid receptor availability before and after metformin treatment**

**PCOS pre-metformin >**

**PCOS post-metformin**[c]




| μ-**Opioid binding potential** (mean ± SD)[d] | **Left Nucleus Accumbens** *Mean* ± SD | **Right Nucleus Accumbens** *Mean* ± SD | **Left Amygdala** *Mean* ± SD | **Right Amygdala** *Mean* ± SD |
|---|---|---|---|---|
| **PCOS** (*pre-metformin*) | 1.95 ± 0.17 | 2.40 ± 0.11 | 2.28 ± 0.17 | 2.28 ± 0.23 |

**B. *In vivo* μ-opioid receptor availability before and after metformin treatment**

| | | | | |
|---|---|---|---|---|
| **PCOS** (*post-metformin*) | 1.65 ± 0.27 | 2.11 ± 0.21 | 2.08 ± 0.19 | 2.11 ± 0.22 |
| **Control** | 1.53 ± 0.28 | 2.05 ± 0.28 | 2.03 ± 0.07 | 2.06 ± 0.26 |
| **Binding potential correlation** (R(p))[e] | *R (p)* | *R (p)* | *R (p)* | *R (p)* |
| **Fasting insulin (mU/mL)** | .633 (.027) | .706 (.010) | .618 (.032) | .303 (.339) |
| **HOMA % sensitivity** | −.689 (.013) | −.737 (.006) | −.610 (.035) | −.315 (.319) |
| **HOMA insulin resistance** | .643 (.024) | .720 (.008) | .634 (.027) | .306 (.333) |

[a] Mann-Whitney test between controls and PCOS patients pre- -metformin treatment

[b] Wilcoxon signed ranks test between PCOS patients before and after metformin treatment

[c] Regions with greater μ-opioid receptor binding potential in pre-treated patients (T test, compared to post-treatment)

[d] μ-Opioid binding potential expressed as $B_{max}/K_d$; Bmax = receptor concentration and Kd = receptor affinity for radiotracer

[e] Pearson correlation coefficient and 2-tailed significance level across controls and PCOS patients pre-insulin regulation

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