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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

The role of Kisspeptin levels in polycystic ovary syndrome: a systematic review and meta-analysis

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Abstract

Purpose Polycystic ovarian syndrome (PCOS) is a complex and not fully elucidated pathology. This prevalent endocrinopathy affects patients in reproductive age, impacts on estrogen-dependent diseases, as well as in infertility. In this context, Kisspeptin (KP) may be considered a potential biomarker for PCOS diagnosis and follow-up. Here, we aimed to verify the levels of KP in obese and non-obese patients with PCOS, their relationship with other hormones, in comparison to healthy controls.

Methods A systematic review and meta-analysis were performed according to the PRISMA guidelines. We searched MEDLINE, EMBASE, PsycINFO, Global Health, The Cochrane Library, Health Technology Assessment Database, and Web of Science for eligible studies. A random effects model meta-analysis of standardized mean difference (SMD) was conducted and the I^2 was used to assess heterogeneity. Meta-regression was conducted through mixed-effects model.

Results A total of 12 studies were included, comprising 660 PCOS patients and 600 controls. The KP levels were lower in the control group (0.76: 0.17–1.35; 95% CI). In the subgroup analyses, patients were divided in non-overweight/obese (BMI < 25) and overweight/obese (BMI ≥ 25) groups. The meta-regression revealed a difference between the obese and non-obese groups ($z = 2.81$; $p = 0.0050$).

Conclusions PCOS patients showed higher KP levels than control, and obese non-PCOS patients also showed altered KP levels. All studies had poor descriptions of sample collection, pre-analytical and analytical procedures, which is critical considering structural characteristics of the KP molecule.

Keywords Polycystic ovary syndrome · Kisspeptins · Human fertility · Meta-analysis · Obesity

Introduction

Polycystic ovarian syndrome (PCOS) is an heterogenous endocrinopathy associated with the presence of clinical and/or biochemical hyperandrogenism (a), oligo-anovulation (b) and the polycystic ovary morphology (c), characterized by multiple follicles observable by ultrasonography, being ≥ 12 follicles with 2–9 mm diameter, or ovarian volume > 10 ml in at least one ovary [1]. The Rotterdam ESHRE/ASRM criteria [2] recognizes four PCOS phenotypes, defined by the combination of these symptoms. Phenotype 1 (a + b + c), phenotype 2 (a + b), phenotype 3 (a + c) and phenotype 4 (b + c). In addition, there is a frequent association with obesity and insulin resistance [3] and, consequently, with an increased risk of type 2 diabetes and cardiovascular events [4]. Its approximate prevalence is 10%, which makes it the main endocrinopathy of women in reproductive age (6–10%) [3].

Increased levels of luteinizing hormone (LH) may occur in some patients with PCOS, although the criterion of their reversal with follicle-stimulating hormone (FSH) is no longer a diagnostic parameter [5]. In addition, there is currently a tendency to consider the four phenotypes of PCOS

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(full-blown, nonpolycystic ovaries, nonhyperandrogenic, and ovulatory phenotypes) by the likely distinct pathophysiology in each group [6].

However, the role of LH in the folliculogenesis is of paramount importance in all cases and highlighted since the theory of "two cells, two gonadotrophins" [7]. Its adequate action depends on a pulsatile release, whose pathophysiology is closely associated with the peptide hormone kisspeptin (KP). This was also associated with higher LH levels in PCOS patients, suggesting their contribution to LH hypersecretion [8, 9].

The KP is composed of 54 amino acids also presenting biological activity in the fractions with 14, 13 and 10 amino acids [4]. Its receptor, GPR54, plays a pivotal role in the regulation of the hypothalamic-pituitary–gonadal axis (HHG), controlling events associated with pubertal triggering in girls, and in the reproductive system [10]. In humans, point mutations or deletions in the receptor sequence lead to the reduction of its function, which was identified in patients with idiopathic hypogonadotrophic hypogonadism, characterized by the absence of, or incomplete, pubertal development [6]. Therefore, KP is essential for the function of the reproductive system [11] and its abnormal secretion or the abnormal release of GnRH induced by KP may result in different problems in pubertal development and in the HHG axis [11].

However, as no reference serum values have been established for KP, including in patients with PCOS, there are no data on the dimensions of increased concentration of this hormone.

Considering its relationship with LH, its potential as a biomarker in the diagnosis of PCOS, besides improving the understanding of its role in pathophysiology of this disease, the objective of the present study is to verify the levels of KP in patients with PCOS, its relationship with LH levels and the differences between non-obese patients, in comparison to healthy controls.

Methods

Search strategy

A systematic review and meta-analysis were performed according to the PRISMA statement [12]. We searched the following electronic bibliographic databases: MEDLINE, EMBASE, PsycINFO, Global Health, The Cochrane Library (Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, Cochrane Methodology Register), Health Technology Assessment Database, and Web of Science (science and social science citation index). The search strategy included only terms relating to or describing the intervention, adapted for use with other bibliographic databases in combination with database-specific filters for controlled trials (where these are available): ("kisspeptin*" OR "metastin*") AND ("dosage" OR "level*" OR "concentration*" OR "values*") and correlate terms to obtain the largest volume of articles involving KP dosage. No term was used for "PCOS" and the studies were obtained in the selection phase. The search included article in any language from the inception of the above-mentioned databases to April 30, 2019.

Selection of studies

Titles and/or abstracts of studies retrieved using the search strategy, and those from additional sources were screened independently by three authors (NPA, EM, RO) to identify studies that potentially met the inclusion criteria outlined below. The full text of these studies was retrieved and independently assessed for eligibility by two review team members (CMT, VZ). Any disagreement between them over the eligibility of particular studies was resolved through discussion with a third (external) collaborator.

We have included studies comparing women with PCOS versus a control group which had evaluated blood KP concentration. Thus, the inclusion criteria were women with PCOS diagnosis, a healthy control-group and KP dosage. The exclusion criteria were comorbidities and incomplete data report. In addition, we excluded reviews, case-reports, and animal studies, in silico or molecular prediction models.

Data extraction

A standardized prepiloted form was used to extract data from the included studies for assessment and evidence synthesis. Three review authors extracted data independently (NPA, CMT, VZ); discrepancies were identified and resolved through discussion (with an external collaborator where necessary). Missing data were requested from study authors when required.

The primary pieces of information retrieved were: first author, publication year, sample size for case and control groups as well as KP concentration measured. Secondary information included age, phase of the menstrual cycle, daytime for sample collection, KP fraction measured, analytical procedure, kit manufacturer, BMI, and finally LH and FSH for each case and control group. KP concentrations were converted to pmol/mL to estimate the number of active molecules per volumetric unity. We considered the molar mass according to the KP fraction mentioned in the study obtained in the PubChem Compound Database [13]. When this information was not available, we considered the authors used the KP-54 fraction, formerly called metastin, which is commonly used among manufacturers. Measures reported

were transformed to mean and standard deviation if reported otherwise according to previous studies [14].

Risk of bias assessment

We used a modified version of the Newcastle–Ottawa Scale (NOS) for observational studies [15] to include patient selection, pre-analytical and analytical parameters which could interfere on KP dosage. Patient selection comprises description of the experimental setting, declaration of the hour of sample collection, and day of the menstrual cycle of the population studied. Pre-analytical parameters consider the description of the sample treatment before analysis, comprising description of tube for sample collection (e.g. EDTA, protease inhibitor), time lapse between sample collection and processing, sample temperature maintenance until processing, processing protocol (e.g. centrifugation, separation, fraction obtained) and protein purification techniques for KP fraction isolation. Analytical data included technique (analytical tool, e.g. ELISA), dosage (protocol adopted), parameters (calibration curve, sensitivity, e.g. ROC curve) and interferents (potential limiters of the process). Three review authors assessed the studies (NPA, CMT, VZ) considering low, medium or high risk. Discrepancies were identified and resolved through discussion (with an external collaborator where necessary).

Statistical analysis

The meta-analysis was performed using a random-effect model. Due to very large variations in the kisspeptin concentrations, we used the standardized mean difference (SMD) for pooling estimates. For the same reason, the Hedges' g (bias corrected standardized mean difference) was adopted [16]. Heterogeneity was tested with Q test and I^2 statistic [17], considering estimates between 50 and 75% as moderate, and greater than 75% was considered high and not explained by chance. The association of KP levels with LH within the case and control groups was analyzed and calculated separately [18]. The Egger test [19] and the Baujat plot [20] were used to detect sources of heterogeneity. An analysis of influence was performed by single study removal [21]. Studies that changed the SMD or I^2 would be considered for further analysis. Results are displayed as forest plots showing SMD and 95% CIs.

Meta-regression analysis was conducted with mixed-effects model. Relationships between groups were tested when possible. The results of the meta-regression analysis are given as regression coefficients with 95% CIs.

Statistical analyses were performed with RStudio (version 1.1.383), using the meta package for pooled estimates, forest

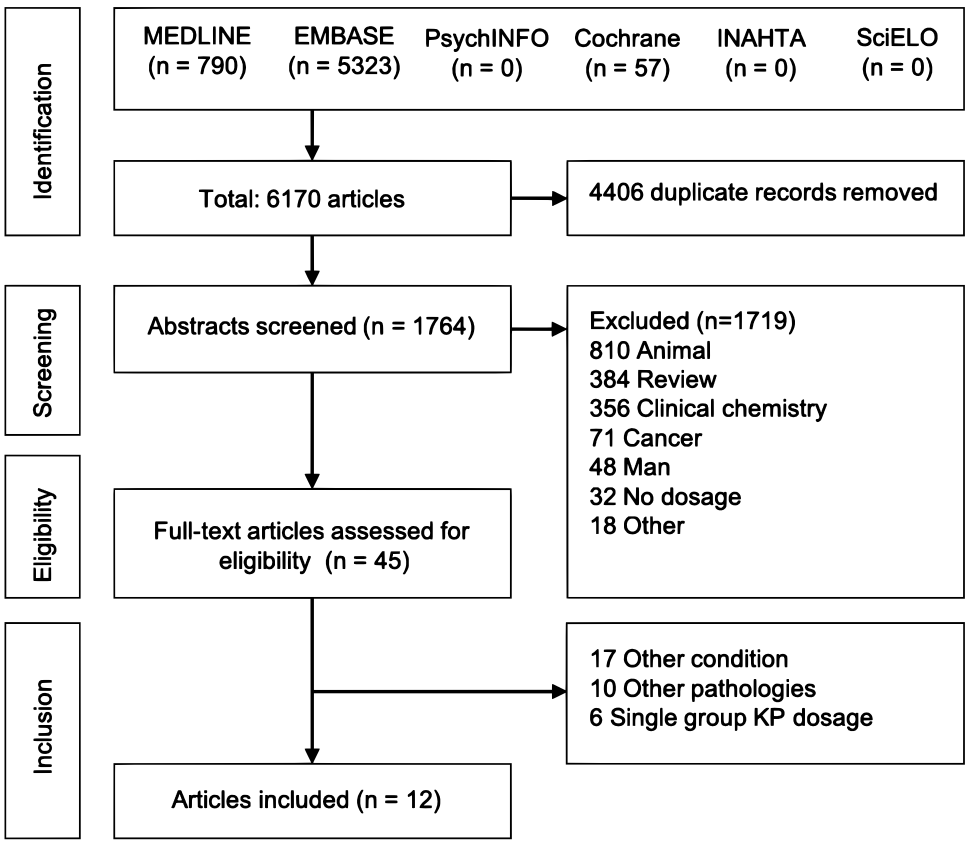

Fig. 1 The PRISMA flowchart of study eligibility

Table 1 The summary of evidence of the studies included in the meta-analysis

Author, year	Country	Study type	Groups	KP serum level[a]		Unity	Age		Main findings
				Case	Control		Case	Control	
Panidis et al. 2006 [26]	Greece	Clinical study	Obese PCOS and obese controls	0.24 ± 0.02	0.36 ± 0.05	fmol/mL	23.64 ± 0.58	26.85 ± 1.06	KP level is not directly involved in the hypersecretion of LH in PCOS
Chen et al. 2010 [27]	China	Clinical study	PCOS and controls	0.23 ± 0.18	0.19 ± 0.21	fmol/mL	17.89 ± 1.24	18.6 ± 0.68	KP level increased in patients with PCOS, and relationship with LH and Testosterone levels
Jeon et al. 2013 [28]	South Korea	Clinical study	Obese PCOS and obese controls	10.12 ± 5.81	6.51 ± 3.13	pmol/L	23.88 ± 4.86	24.92 ± 2.94	Increased KP levels in obese patients with PCOS, and positive relationship with free androgen levels
Yilmaz et al. 2014 [29]	Turkey	Clinical study	Obese PCOS and obese controls	2.06 [0.67–4.43][b]	1.08 [0.45–2.00][b]	ng/mL	22.9 ± 4.61	23.49 ± 4.84	Increased KP levels in patients with PCOS, regardless of BMI
Emekci Ozay et al. 2016 [30]	Turkey	Case–control	PCOS and controls	1.92 ± 1.29	1.49 ± 1.46	ng/mL	23.99 ± 4.63	24.43 ± 4.39	Positive correlation among KP, LH and leptin levels in PCOS
Nyagolova et al. 2016 [31]	Bulgaria	Observational cohort	Obese PCOS and obese controls	0.28 ± 0.04	0.32 ± 0.14	ng/mL	24.13 ± 0.6	27.5 ± 1.21	GALP and KP levels are increased in PCOS and positively associated with hyperandrogenism
Gorkem et al. 2017 [32]	Turkey	Observational cohort	Infertile PCOS and infertile with adequate ovarian reserve	5.76 ± 2.11	4.65 ± 2.16	ng/mL	27.7 ± 4.65	27.92 ± 4.67	Increased KP levels in PCOS, negative relation with FSH, and positive with total Testosterone and DHEAS levels.
Albalawi et al. 2018 [9]	Saudi Arabia	Case–control	PCOS and controls	0.43 ± 0.15	0.39 ± 0.07	pg/mL	29.4 ± 3.93	26.7 ± 3.6	KISS1 variant contribute with the development of PCOS; increased KP levels in PCOS
Daghestani et al. 2018 [33]	Saudi Arabia	Observational cohort	PCOS and controls	0.39 ± 0.08	0.39 ± 0.07	fmol/mL	24.05 ± 4.41	25.43 ± 4.8	No difference between groups
Kaya et al. 2019 [34]	Turkey	Observational cohort	PCOS and controls	525.9 ± 164.17	354.31 ± 111.38	pg/mL	28.75 ± 3.49	30.55 ± 4.66	Increased KP levels in patients with PCOS

Table 1 (continued)

Author, year	Country	Study type	Groups	KP serum level[a]		Unity	Age		Main findings
				Case	Control		Case	Control	
Wang et al. 2019 [35]	China	Case–control	Obese PCOS and obese controls	265.85 ± 214.67	219.11 ± 165.69	pg/mL	–	–	KP levels positively correlated with LH levels, negatively correlated with glucose, insulin, and triglyceride levels
Branavan et al. 2019 [24]	Sri Lanka	Case–control	PCOS and controls	4.873 ± 0.238	4.127 ± 0.132	nmol/L	24.67 ± 0.88	33.8 ± 0.53	GPR54 and KISS1 variations unlikely to be associated with PCOS

PCOS polycistic ovarian syndrome, *KP* kisspeptin, *LH* luteinizing hormone, *BMI* body mass index, *GALP* galanin-like peptide, *DHEAS* dehydroepiandrosterone, *GPR54* KISS1 receptor gene or KISS1R gene, *KISS1* KISS-1 metastasis suppressor gene

[a]All studies measured the kisspeptin-54 fraction by ELISA and the KP concentrations were expressed as mean and standard deviation

[b]Kisspeptin concentration was expressed as median and 95% confidence interval

plots and publication bias assessment [22], and the metafor package for the meta-regression [23].

Results

A total of 12 studies were included in the meta-analysis, comprising 660 PCOS patients and 600 controls. Figure 1 outlines our study selection process in a PRISMA flowchart. The summary of evidence is presented in Table 1. When data from the same authors were published twice with slight modifications [24, 25], only the first study was considered.

Kisspeptin levels in PCOS

As shown in Fig. 2, the KP levels were lower in the control group (0.76: 0.17–1.35; 95% CI), except in only one study [26], which presented the opposite from the expected according to the literature and included obese controls. The heterogeneity was $I^2 = 93\%$ (considered high).

Figure 3a, b, c present the funnel plot, Egger plot and Baujat plot, respectively. In details, the funnel plot reveals asymmetry; the Baujat plot shows the contribution of each study to the overall Q test statistic for heterogeneity and confirms that one study [26] is a strong source of heterogeneity. The values for the linear regression test of funnel plot asymmetry are $t = 0.65601$, df = 8, p value = 0.5302, sample estimates for bias = 1.4373, SE = 2.1911 and slope = 0.2154.

Risk of bias

The NOS scale [15] presented low variations among the studies (Table 2). All studies showed poor descriptions of the sample collection, pre-analytical and analytical procedures, which is critical considering structural characteristics of the KP molecule.

Subgroup analysis

Subgroup analyses were performed through the transformation of BMI as a dichotomic variable. Thus, the groups were divided as non-overweight (BMI < 25) and overweight/obese (BMI ≥ 25). This first subgroup analysis consisted of obese-PCOS versus obese-Control and non-obese-PCOS versus non-obese Control group. The forest plot with these subgroups is presented in Fig. 4.

The subgroup analyses showed in the test of residual heterogeneity a QE (df = 14) = 223.2605 and p value < 0.0001, and in the test of moderators (coefficients: 2), F (df1 = 1,

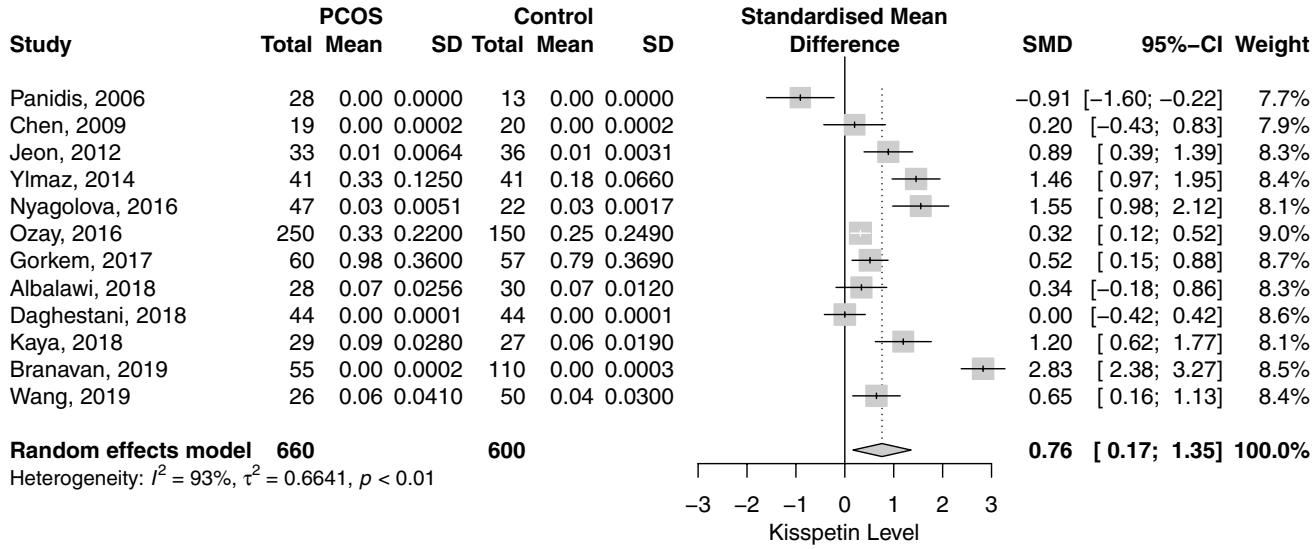

Fig. 2 Forest plot of all included studies

df2 = 14) = 0.2028 and p value = 0.6593. The metaregression showed a mean difference (inverse variance method) of 0.83 (0.25–1.42; 95% CI), $z = 2.81$ and $p = 0.0050$, revealing that there is difference among the obese and non-obese group.

A second subgroup analysis (Fig. 5) consisted of a within-group comparison between LH and KP levels. The meta-analysis was performed comparing LH levels versus KP levels in PCOS group and LH levels versus KP levels in Control group. The purpose of such analysis was to set a baseline through LH values since KP has no reference value defined. The result of LH versus KP in the case group (PCOS patients) was 3.26 (2.06–4.46; 95% CI) comprising 51.4% of the weighted sample, and the result for the control group was 4.33 (2.17–6.48; 95% CI) comprising 48.6% of the weighted sample. The LH/KP ratio for Case/Control was 0.75 indicating a higher LH/KP ratio on PCOS patients.

The third subgroup analysis (Fig. 6) consisted of the same within-group comparison between LH and KP levels, following the same principles above mentioned. However, as obesity was found to influence the KP levels, we formed four groups of patients: obese PCOS, non-obese PCOS, obese controls and non-obese controls. The ratio of LH versus KP in non-obese PCOS group (4.83: − 0.03–6.40; 95% CI) and obese PCOS group (5.68: 2.26–9.11; 95% CI) was 0.85, indicating a higher LH/KP ratio in obese patients. Worth note that this difference is even higher in the obese control group (7.78:4.16–11.40; 95% CI).

One study [35] did not supply data for age, BMI, LH or FSH separately for each group, and one study [24] did not supply LH data for obese control group.

Discussion

Our data analysis showed that the KP concentration is higher in PCOS patients, although the elevated heterogeneity observed. This could be explained by the significantly high differences on clinical, biochemical and ultrasound features among PCOS phenotypes, with reported higher levels of FSH levels in phenotype 3 and its partial role in ovulatory dysfunction [36]. Moreover, associations of higher serum Anti-Müllerian Hormone (AMH), which is related to ovulatory dysfunction, with the PCOS main symptoms (especially the polycystic ovary morphology) was reported [37]. Finally, even probable different etiologies for PCOS could be not discarded as potential causes of high heterogeneity for KP level [38]. Considering that the recommendation for PCOS phenotyping is recent, most of the available studies included in this systematic review and meta-analysis show the absence of such subgrouping.

On the other hand, the heterogeneity was found lower in the overweight/obese group. It is known that these patients show greater insulin resistance, and the existence of ovarian insulin receptors could justify the ovarian blockade. This could explain the greater expression of KP and the reduced heterogeneity of the analyzed group. Animal studies also showed that female mice knockout for KP receptor gene (*Kiss1r −/−*) shows elevated body weight, high adiposity and impaired glucose tolerance [39]. In the same study, the authors found that *Kiss1r −/−* female mice in comparison to controls presented lower locomotor activity, diminished respiratory rate and energy expenditure unrelated to impairment on thyroid hormone secretion. Remarkably, ovariectomized *Kiss1r −/−* female mice developed the same symptoms and

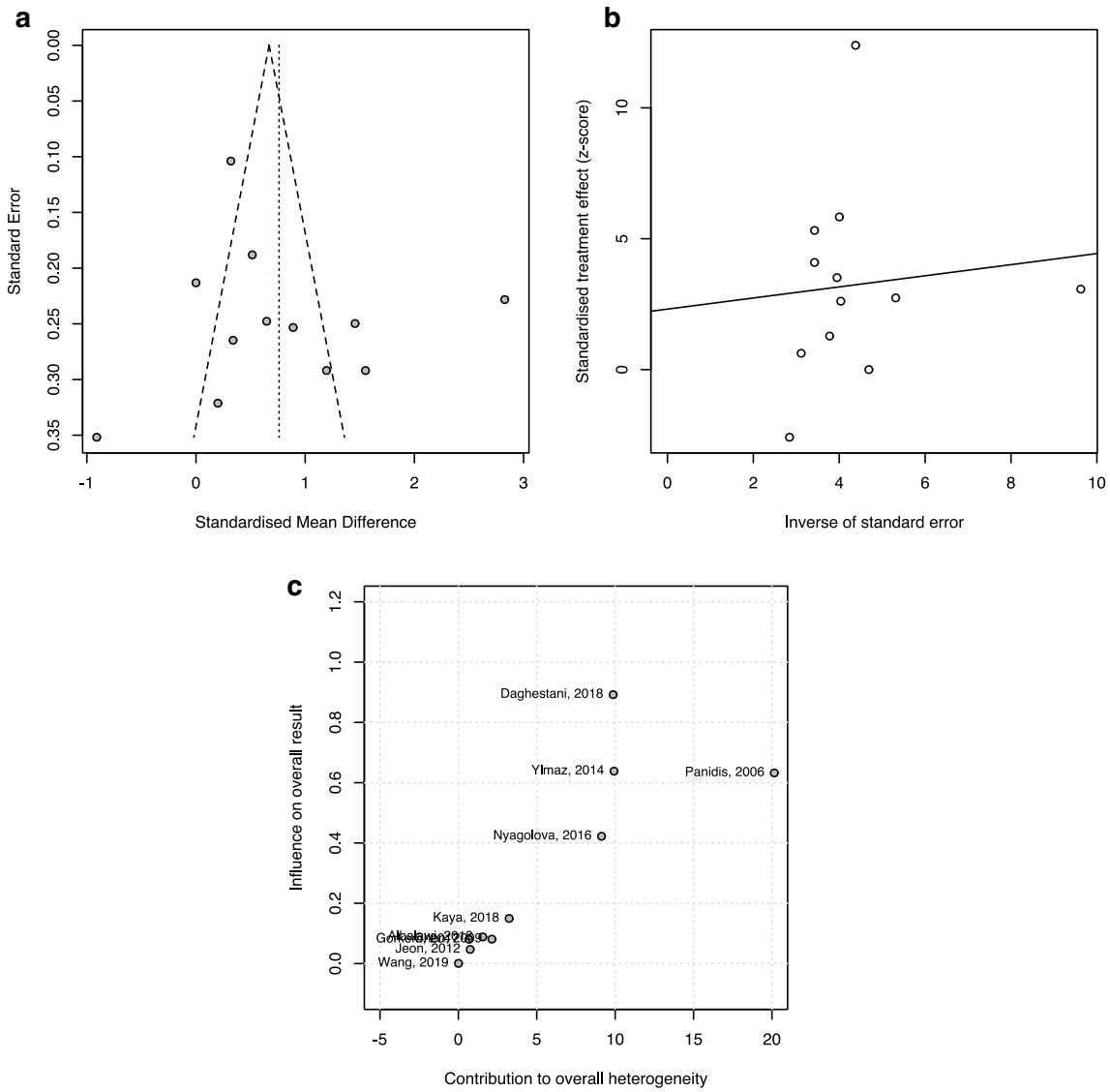

Fig. 3 **a** is the funnel plot for the included studies. **b** shows the heterogeneity of the studies. **c** is the Baujat plot, which shows the studies contribution to overall heterogeneity and the overall result

in higher proportion when compared to ovariectomized controls, suggesting clear roles for KP signaling on obesity, diabetes, and metabolic dysfunctions [40]. In addition, estrone that is produced by visceral fat could contribute to feedback to the HHG axis [11], consisting of one more factor to increase the homogenization of this group.

Accumulating evidence suggests that neuroendocrine impairments in PCOS affect the coupling of KP with LH pulses and potentially worsen as the disease progresses, becoming unequivocally evident in oligomenorrheic PCOS patients [41].

Nevertheless, we should consider that KP measurement does not respect robust standards nor have studies that allow precise estimation and provides a very broad spectrum of values, reaching differences from the order of 10^3 even when converted to the same unity. Probably this could be due, at least in part, to the short KP half-life of 50 s [42] and the time elapsed for sample processing, which plays a critical role in the sample processing. In the included studies, only sparse data about pre-analytical procedures are available, and analytical procedure information is confined to vague descriptions referring to the manufacturer instructions.

In addition, all studies in this systematic review and meta-analysis were conducted with convenience sample and are generally performed in small and numerically unbalanced groups, worsening the overall heterogeneity. In particular, Jeon et al. [28] report an increase in the concentrations of KP-54 fractions in adult patients with PCOS when compared

Table 2 Quality assessment of the studies included in the meta-analysis according to Modified Newcastle–Ottawa scoring guide [15]

Author	Case definition	Selection of control	Definition of control	Sample collection		Pre-analytic	Analytic				Score
				Hour	Day of cycle		Technique	Dosage	Parameters	Interferents	
Panidis et al. 2006 [26]	2	2	2	1	1	0	2	1	2	1	14
Chen et al. 2009 [27]	2	2	2	1	1	0	2	2	1	1	14
Jeon et al. 2012 [28]	2	2	2	1	1	0	2	1	1	1	13
Ylmaz et al. 2014 [29]	2	2	2	0	1	0	2	1	1	1	12
Emekci Ozay et al. 2016 [30]	2	2	2	1	1	1	0	0	2	1	12
Nyagolova et al. 2016 [31]	1	2	1	0	1	0	1	1	1	0	8
Gorkem et al. 2017 [32]	2	2	0	1	1	1	2	1	2	0	12
Albalawi et al. 2018 [9]	1	1	2	1	1	1	2	1	2	0	12
Daghestani et al. 2018 [33]	2	2	2	0	1	1	2	0	1	0	11
Kaya et al. 2018 [34]	2	2	0	1	1	1	2	1	1	0	11
Wang et al. 2019 [35]	2	2	1	0	1	0	2	0	0	1	9
Branavan et al. 2019 [24]	2	2	1	0	0	0	2	1	0	0	8

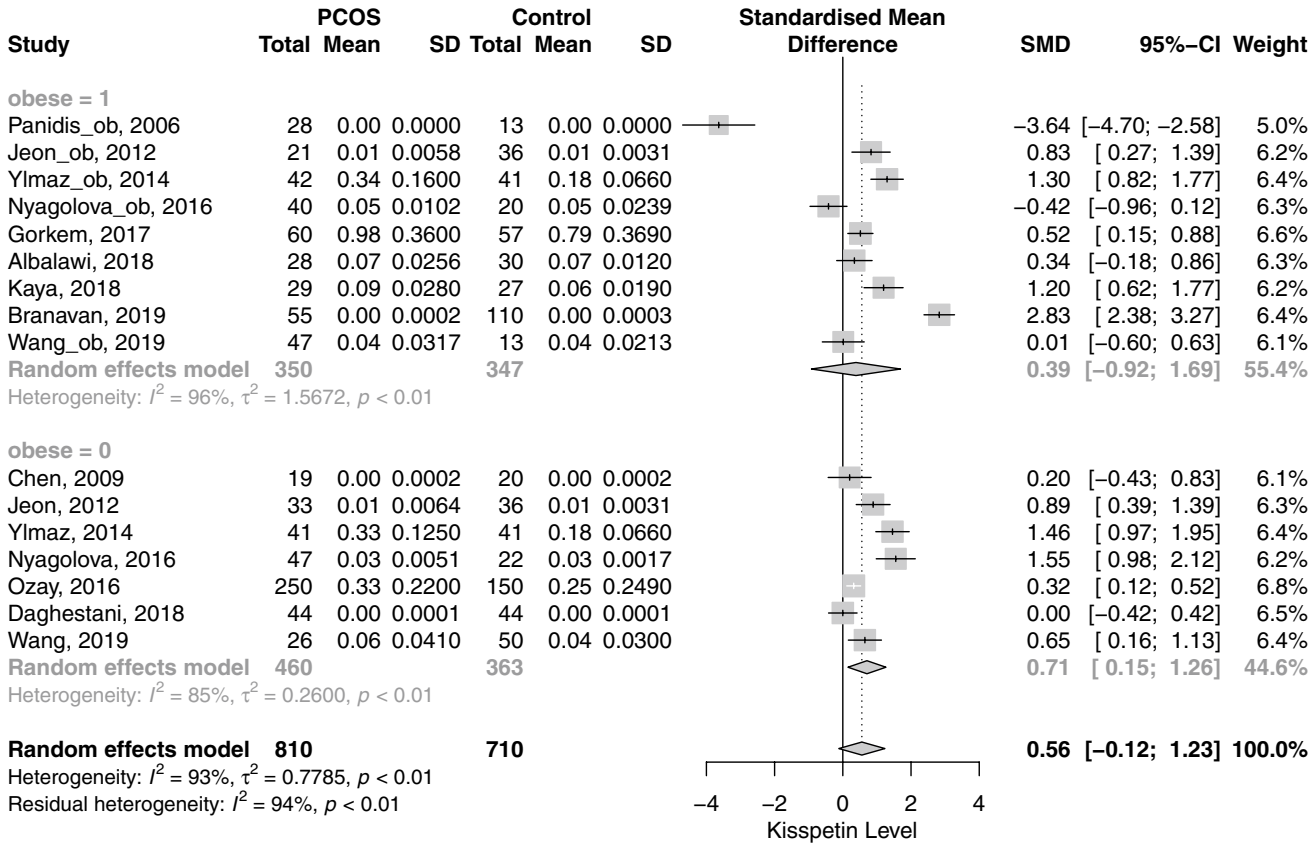

Fig. 4 Forest plot of the subgroups obese (obese = 1) and non-obese (obese = 0); data summary for each subgroup indicates a lower heterogeneity (I^2 = 85%) in the non-obese group

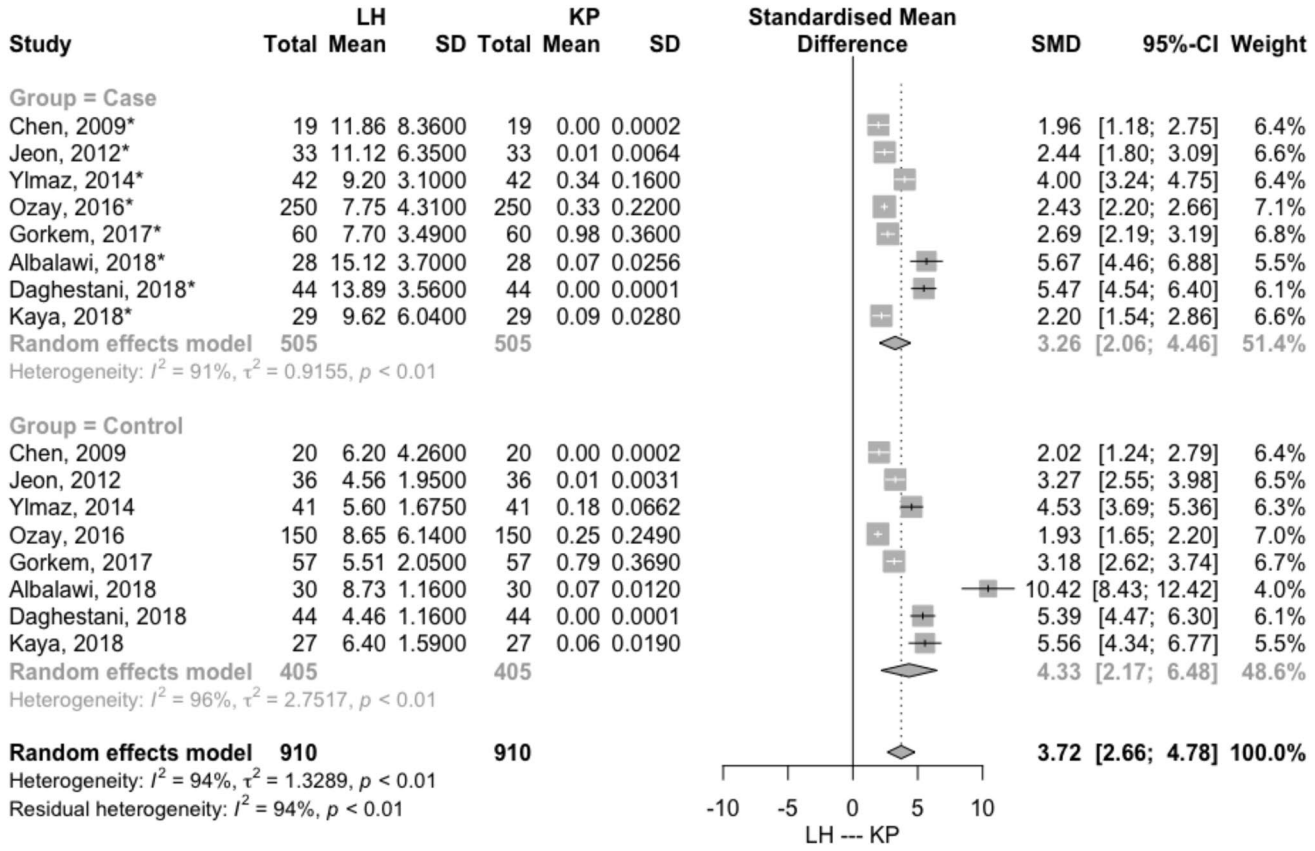

Fig. 5 Forest plot of the comparison between LH versus Kisspeptin for case (PCOS) and control groups

to the control group of the study. In addition, this study included non-obese and obese PCOS groups, but not non-obese control, whereas only non-obese groups were considered in the calculations. Moreover, other authors [29] presented the metabolic profile of the participants with PCOS, with positive correlation between BMI and LH levels, although no positive correlation was observed between KP-54 (metastin) and insulin resistance indices compared to the control group. Finally, Panidis et al. [26] included non-obese case group against obese control group, but only the obese group was considered for the calculations (all groups were paired considering BMI).

The not yet fully elucidated complex pathophysiology of PCOS urges for useful biomarkers for this prevalent endocrinopathy, especially for patients in reproductive age and in human infertility. Even though, clinical evaluation of KP levels must be cautiously considered in the next future as potential biomarker of PCOS diagnosis and follow-up. The observation of critical issues in the pre-analytical and analytical phases are demanding. Moreover, the KP dosage requires protocol validation following well-defined guidelines and reference values for being fully considered in daily clinical practice. Nevertheless, this dosage should be kept into account with a careful evaluation of PCOS phenotypes and patient's characteristics, since KP was shown to be influenced by BMI variations and insulin resistance [43].

Despite the reported success in the peripheral administration of KP-10 and KP-54 to trigger egg maturation in women undergoing fertility treatment [44], so far the findings are still much more promising than an actual option in clinical practice. Although promising data indicates potential suitability for use in assisted reproduction, the requirements for both patients and clinics are unwieldy [45]. Furthermore, outcomes related to indication, dose and routes of delivery requires further studies to verify efficacy and safety of KP versus the current triggers adopted in clinical practice [44].

In conclusion, PCOS patients showed lower KP levels than control, and obese non-PCOS patients also showed altered KP levels. All studies had poor descriptions of sample collection, pre-analytical and analytical procedures, which is critical considering structural characteristics of the KP molecule. In this scenario, further studies for standardization of serum KP measurement are needed to consider it as a reliable biomarker.

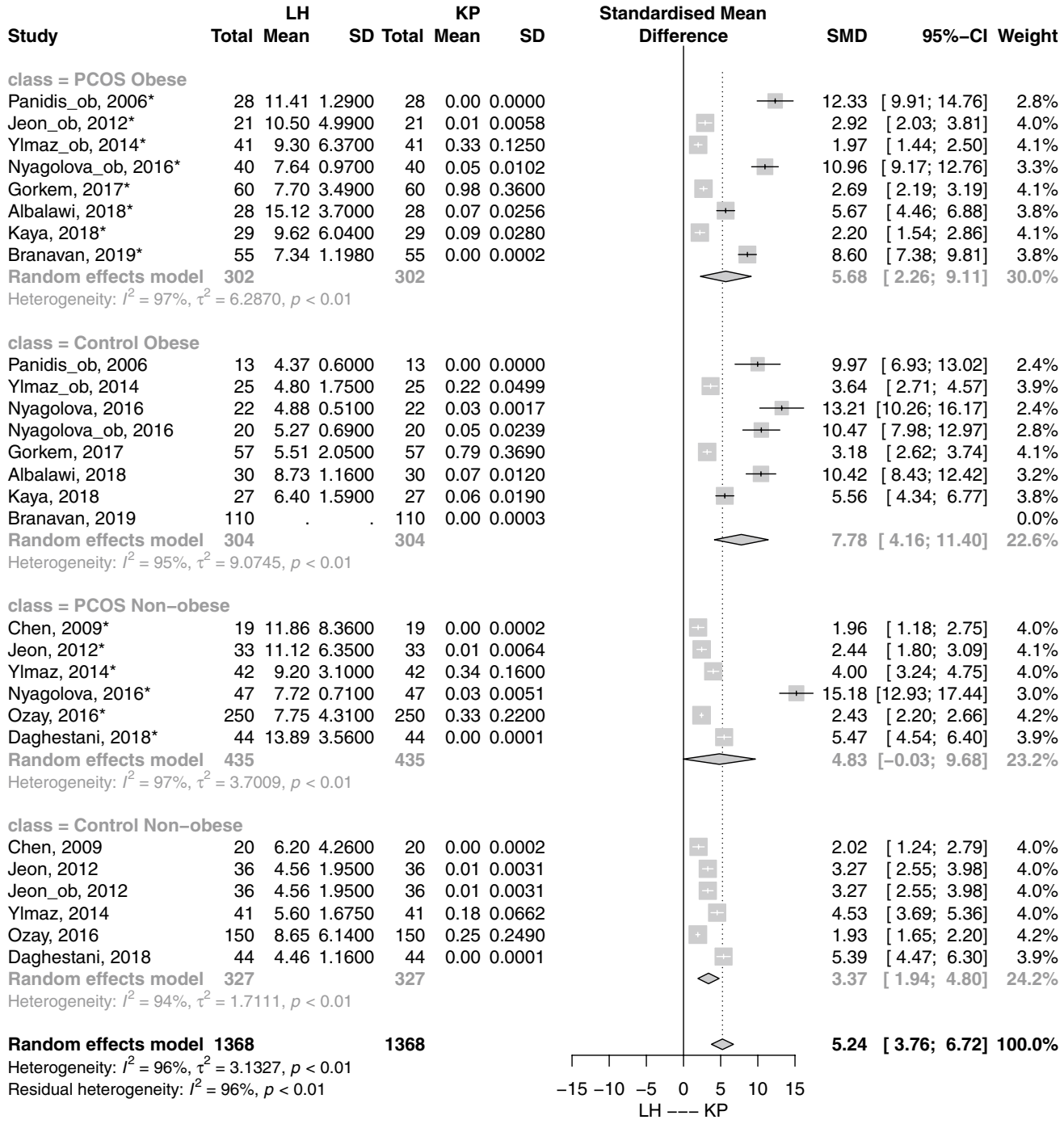

Fig. 6 Forest plot of the comparison between LH versus Kisspeptin for obese and non-obese PCOS patients, and obese and non-obese controls

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Authors' contribution NP Assis Rodrigues: design of the study, literature review, data collection, data analysis and manuscript writing; AS Laganà: data analysis, manuscript writing and editing; V Zaia: data collection, data analysis and review of the manuscript; A Vitagliano: data analysis and review of the manuscript; CP Barbosa: review of the manuscript; R Oliveira: data analysis, review and manuscript writing; CM Trevisan: data collection, data analysis and review of the manuscript; E Montagna: design of the study, literature review, data analysis and manuscript writing/review. All authors read and approved the final version of the manuscript.

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Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

Informed consent Not applicable.

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