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Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials

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Running Title

Inositol for ovulation Induction in PCOS

Abstract

Background: Polycystic ovary syndrome is a common cause of anovulation and infertility, and a risk factor for development of metabolic syndrome and endometrial cancer.

Objectives: Systematic review and meta-analysis of randomised controlled trials that evaluated the effects of inositol as an ovulation-induction agent.

Search Strategy: We searched MEDLINE, EMBASE, Cochrane and ISI conference proceedings, Register and Meta-register for RCTs and WHO trials' search portal.

Selection Criteria: We included studies that compared inositol with placebo or other ovulation induction agents.

Data Collection and Analysis: Quality of studies was assessed for risk of bias. Results were pooled using random effects meta-analysis and findings were reported as relative risk or standardized mean differences.

Main Results: We included 10 randomised trials. Total women on inositol were 362 (myo-inositol = 257; di-chiro-inositol = 105), placebo were 179 and metformin were 60. Inositol was associated with significantly improved ovulation rate (RR 2.3; 95% CI 1.1, 4.7; I2 = 75%) and increased frequency of menstrual cycles (RR 6.8; 95% CI 2.8, 16.6; I2 = 0%)

compared with placebo. One study reported on clinical pregnancy rate with inositol compared with placebo (RR 3.3; 95% CI 0.4, 27.1), and one study compared with metformin (RR 1.5; 95% CI 0.7, 3.1). No studies evaluated live birth and miscarriage rates.

Conclusions: Inositol appears to regulate menstrual cycles, improve ovulation and induce metabolic changes in PCOS, however evidence is lacking for pregnancy, miscarriage or live birth. Further well-designed multicenter trial to address this issue to provide robust evidence of benefit is warranted.

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Key words: PCOS, inositol, ovulation induction, meta analysis.

A tweetable abstract: Inositols improve menstrual cycles, ovulation and metabolic changes in PCOS

Introduction

Absence of ovulation is a key problem in women who are diagnosed with polycystic ovary syndrome (PCOS), a condition also characterised by hyperandrogenaemia, hyperinsulinaemia and a typical sonographic ovarian morphology.[1] It affects up to 1 in 6 women, and is a major contributor to infertility.[2,3] In the long term, it increases the risk of endometrial hyperplasia and endometrial cancer.[4] Furthermore, it predisposes women to type 2 diabetes mellitus, and associated cardiovascular complications.[5,6] The obesity epidemic may be a contributory factor to the increasing numbers diagnosed with PCOS.[7]

Regarding fertility, lifestyle interventions targeting weight loss remain the primary therapy in PCOS, since reduction in weight of as little as 5% can restore regular menstruation and improve response to ovulation inducing agents, but it is known to be associated with low adherence and sustainability.[8,9] Pharmacological ovulation induction options include clomiphene citrate or metformin or a combination of both.[10,11] If clomiphene citrate is given, ultrasound monitoring is necessary in secondary care, to guide dose adjustment and monitor complications including multiple pregnancy, which is reported to be about 10%.[12] Moreover, it is not advisable to continue the treatment longer than 6 months (may be considered up to 12 months) for women who are taking clomiphene citrate.[13,14]

Inositols (myo-inositol and di-chiro inositol) are nutritional supplements, available over-the-counter. In PCOS women, a defect in tissue availability or altered metabolism of inositol and/or inositolphosphoglycans mediators (second messenger pathway in insulin signaling) has been suggested to contribute to insulin resistance.[15] Studies have also demonstrated a physiological role of inositol and its metabolites in human reproduction and supplementation has proposed to improve endocrine and reproductive outcome in these women, including ovulation, in women with PCOS, at low cost and potentially with fewer side effects.[16]

The primary studies on inositol are too small,[17] and existing reviews are narrative, without quantifiable estimates of effect on ovulation and live births.[18–20] We aimed to undertake a systematic review to assess the effects of inositol on ovulation induction and reproductive outcomes, as well as on hormonal and glycaemic profile, when compared to placebo and/or metformin or clomiphene, in women with PCOS.

Methods

Literature search

We searched MEDLINE (1950 to Aug 2016), EMBASE (1980 to Aug 2016), the Cochrane Library, ISI conference proceedings for randomised controlled trials on the effects of myo-inositol on ovulation, clinical pregnancy rate, miscarriage rate, live birth rate and hormonal and glycaemic profile in women with PCOS. We also searched for ongoing and archived RCTs using the International Standard Randomised Controlled Trial Number (ISRCTN) Register and Meta-register for RCTs (http://www.controlled-trials.com), and WHO trials' search portal (ICTRP, apps.who.int/trialsearch/Trial). We combined the Medical Subject Headings (MeSH) and text words for PCOS (PCOS; Polycystic ovary syndrome; polycystic ovar*; PCO)'and 'Inositol' (Inositol; myo-inositol; DCI; di-chiro-inositol). The reference lists of all known primary and review articles were examined for relevant citations not captured by the electronic searches. There were no language restrictions.

Study selection

Studies were selected in a two-step process by two independent researchers (JP, DP). In the first step, we reviewed the abstracts of identified studies for potential eligibility. The full texts of studies that were considered to be relevant were retrieved in the second stage for detailed evaluation. Any disagreements about inclusion were resolved by consensus or arbitration by a third reviewer (ST). We included studies if the target population was women with polycystic ovary syndrome undergoing treatment for ovulation induction. The intervention was inositol (myo- or di chiro isomers) compared with placebo, clomiphene and/or metformin. We also included studies that compared the effects of the two isomeric forms of inositol. The primary outcomes were rates of ovulation induction, and clinical pregnancy and live births. We considered menstrual regularisation as a surrogate marker of ovulation. Secondary outcomes were changes in hormonal (total androgens, total testosterone, free testosterone, Dehydroepiandrosterone, and Sex hormone-binding globulin levels) and glycaemic (fasting insulin, fasting glucose, glucose/insulin ratio, homeostatic model assessment (HOMA - a method used to quantify insulin resistance) profiles. In cases of duplicate publication, the most recent or complete versions were selected. We excluded observational studies.

Assessment of study quality and data extraction

Two independent reviewers completed data extraction (DP and MA) and quality assessment (JP and DP). The qualities of included studies were assessed using the Cochrane risk of bias tool. We obtained information on adequacy of randomisation, allocation concealment, blinding, intention-to-treat analysis, incomplete outcome data, selective outcome reporting, follow-up rates and other potential sources of bias. Data were extracted in 2x2 tables for dichotomous outcomes, and as 1 x 2 tables for continuous outcomes.

Analysis

We estimated the relative risk (RR) for dichotomous outcomes, and standardized mean difference (SMD) with 95% CI for each study. The estimates were pooled using random effects meta-analysis. We considered $P < 0.05$ to be statistically significant. The results from individual studies were pooled using either a fixed effect [21] or random effects model as appropriate [22]. We evaluated the statistical Heterogeneity of the exposure effects graphically using forest plots [23] and statistically using the I^2 statistic [24]. All statistical analyses were performed using RevMan 5.2.7 software (Cochrane Collaboration, Oxford, UK). A funnel plot was produced to assess publication bias for the primary outcome measure.

Results

From 107 potential citations, we included 10 studies (601 women) in the review. Fig 1 provides the details of study identification and selection. The list of included and excluded studies is provided in Table S3 and search strategy is provided as Appendix S1. Inositol (myo-inositol or di-chiro-inositol) was compared with placebo in seven trials,[25, 26, 27, 28, 29, 30, 31] myo-inositiol was compared with di-chiro-inositol and with placebo in a three arm trial,[32] myo-inositiol was compared with di-chiro-inositol in one trial,[33] and one trial compared myo-inositol with metformin.[34] We found no randomised controlled trials which compared myo-inositol with clomiphene. Total women included on inositol were 362 (myo-inositol = 257; di-chiro-inositol = 105), on placebo were 179 and on metformin were 60.

Characteristics and quality of the included studies

One study involved obese women with PCOS [31], and others did not pre-specify or included women of any BMI. All the studies used pre-defined criteria for PCOS, and the population was relatively homogeneous by meeting the Rotterdam diagnostic criteria for PCOS.[35] Eight trials studied myo-inositol in doses ranging from 1.2 to 4 g; two evaluated di-chiro inositol with doses from 600 mg to 1.2 g. Seven trials evaluated the effects of inositol on reproductive, hormonal, and glycaemic outcomes. Pregnancy rates with myo-inositol was reported in two trials,[29,34] five studies reported ovulation induction.[26,29–31,34] Three trial reported on the improved frequency of menstrual cycles,[28,32,33] and six trials reported the effects of on hormonal profile such as serum total androgens, total testosterone, free testosterone, dehydroepiandrosterone, and sex hormone-binding globulin levels, and on glycaemic outcomes such as serum fasting insulin, fasting glucose, glucose/insulin ratio, homeostatic model assessment to quantify insulin resistance, glucose area under the curve and insulin area under the curve.[28,25–27,30,31] The details of the study characteristics are provided in Table S1.

The risk of bias in selection for randomisation was low in half the trials (5/10), and 20% (2/10) had low risk of allocation concealment. The risk of bias in performance was low in 70% studies (7/10), which blinded participants and/or health care providers and the outcome assessors. There was no attrition bias in any of the studies. The quality of the included studies is provided in Fig S1 and Table S2.

Effects of inositol on reproductive outcomes

Ovulation induction

In anovulatory women with PCOS, treatment with inositol significantly increased the ovulation rate (RR 2.3; 95% CI 1.1, 4.7; I^2 = 75%) compared with placebo (Fig 2a). One small study (120 women) that compared the effects of myo-inositol and metformin found no differences between the groups (RR 1.5; 95% CI 0.7, 3.1).[34]

In women diagnosed with PCOS and known to oligo or amenorrhoea, inositol increases frequency of menstrual cycles 6 fold (RR 6.8; 95% CI 2.8, 16.6; I^2 = 0%) compared with placebo. There was no difference in cycle regularisation between myo-inositol and di-chiro- inositol (RR 1.0; 95% CI 0.8, 1.3) (Fig 2b). Sensitivity analysis by including studies on menstrual regularisation as a surrogate for ovulation induction showed a three-fold increase in the effect with inositol than placebo (RR 3.2; 95% CI 1.4, 7.1) (Figure S2).

Pregnancy outcomes

When compared to placebo, there were no differences in the rates of clinical pregnancy with myo - inositol (RR 3.30; 95% CI 0.40, 27.13) in one study involving 92 women, but the study was underpowered for this outcome.[29] There was no difference in clinical pregnancy rate between myo-inositol and metformin in another small study of 120 women (RR 1.64; 95% CI 0.85, 3.16).[34] No studies evaluated live birth and miscarriage rates as an outcome. (Figure 2c)

Effects of inositol on hormonal profile

Treatment with inositol in anovulatory women with PCOS showed a significant decrease in levels of total androgen (Standardised Mean Difference (SMD)-1.6; 95% CI -2.5, -0.6; p =0.001), total testosterone (SMD -3.3; 95% CI -5.1, -1.5; p =0.0004), free testosterone (SMD -4.4; 95% CI -9.0, 0.2; p = 0.06) and serum DHEA (SMD -3.2; 95% CI -5.7, -0.6; p =0.02) compared to placebo. The levels of SHBG were significantly increased (SMD 1.3; 95% CI 0.9, 1.7; p <0.00001) (Figure 3).

Effects of inositol on glycaemic parameters

Treatment with inositol in anovulatory women with PCOS significantly decreased levels of serum fasting insulin **(**SMD -2.1, 95% CI -3.2, -0.9; p =0.0003), fasting glucose (SMD -1.0 95% CI -1.7, -0.2; p =0.01), HOMA (SMD -1.8; 95% CI -2.6, -1.0; p<0.00001) and insulin area under the curve (AUC) (SMD -1.6; 95% CI -2.8, -0.4; p=0.01). The decrease in glucose area under the curve was not significant (SMD -2.7; 95% CI -5.5, 0.1; p=0.06). Glucose/Insulin ratio was significantly higher with inositol compared with the placebo group (SMD 2.9; 95% CI 2.2, 3.6; p<0.00001) (Figure 4).

The shape of the funnel plot for each indicator of the ovulation and metabolic factors did not reveal any asymmetry (Figure S3).

Discussion

Main Findings

In women with PCOS, inositol supplementation appears to increase the rates of ovulation and frequency of menstrual cycles. Two trials evaluated CPR with inositol, and both showed no differences to placebo or metformin respectively, although studies were underpowered and no studies reported live birth or miscarriage rates. There was a consistent improvement in glycaemic parameters such as fasting glucose, insulin levels and insulin resistance with inositol compared with placebo. The levels of total androgens, serum testosterone and DHEA were lowered significantly, and levels of SHBG were improved with inositol. There were no differences in the performance of di-chiro or myo-inositol for any of the above outcomes.

Strengths and Limitations

To our knowledge, this is the first systematic review to provide quantitative estimates on the effects of inositol polymers on ovulation and explore them in pregnancy rates in women with PCOS. We adopted stringent inclusion criteria and included only RCTs to remove potential bias. We did not have any language restrictions. All studies used pre-defined Rotterdam diagnostic criteria for PCOS,[35] however they were heterogeneous by BMI status and ethnicity. We studied the effects of myo-inositol on both clinical and laboratory parameters. In addition to inositol vs. placebo, we compared the performances of the inositol polymers against each other, and against metformin.

The meta-analysis included small numbers of studies with relatively small sample sizes. This contributed to the imprecision in estimates. Studies varied in the type of outcomes reported, and used inconsistent and proxy measures for insulin resistance to assess them. There was variation in the dose and type of inositol and the duration of follow-up in these studies, leading to heterogeneity in the findings. Very few studies reported on clinical pregnancy rates, none were powdered for this outcome and none reported on the clinically relevant outcome of live birth. We analysed outcomes of ovulation rate and menstrual regularisation separately, and also performed sensitivity analysis by combining these together considering menstrual regularisation as a proxy for ovulation. Both these outcomes individually and in combination showed significant improvement with inositol.

Interpretation

The Cochrane review[36] included only two studies [31, 37] on the effects of insulin sensitising agents on ovulation induction suggested a potential benefit with di-chiro inositol which was not significant. Other systematic reviews on inositol in PCOS did not provide summary estimates of benefit.[17,18] In comparison, we have included additional studies, [25, 26, 27, 28, 29, 30, 32, 33, 34] with improved precision in estimates for ovulation induction. Only two studies reported on clinical ~~pregancny~~ pregnancy rate comparing with placebo and metformin respectively.[29,34] None of the studies compared inositol with clomifene and none reported on miscarriage or live birth rates. Randomised trials on myo-inositol in pregnancy have also shown preliminary beneficial effects in reducing the risk of gestational diabetes[38] which is likely to be mediated through an improvement in insulin sensitisation.

Insulin resistance and hyperinsulinemia is an intrinsic feature of both obese and non-obese PCOS women. It is observed in up to 95% of obese women and 75% of lean PCOS women,[39] and is considered to play a key role in the pathogenesis of anovulation, increased ovarian testosterone production and development of various features of metabolic syndrome.[40] Moreover, women with PCOS have an increased risk and prevalence of obesity, which further exacerbates the intrinsic PCOS related insulin resistance and worsens clinical features.[39,41,42] Insulin ~~sentisers~~ sensitisers and lifestyle have been used to treat PCOS, however they fail to normalise insulin resistance and further interventions are needed.[36,39,43]

In anovulatory women diagnosed with PCOS, a defect in tissue availability, or altered metabolism of inositol and/or inositolphosphoglycans mediators (involved in the second messenger pathway of insulin signaling) have been suggested to contribute to insulin resistance.[15] Inositol, a vitamin B complex nutritional supplement is available as an over-the-counter product. Epimerization of the six hydroxyl groups of inositol leads to the formation of up to nine stereoisomers. Of these myo-inositol and di-chiro-inositol, may have a potential role in improving endocrine and reproductive outcome in women with PCOS, because of their involvement, as second messengers of insulin resulting in insulin sensitisation.[16,31]

PCOS has clinical implications throughout a woman's lifespan and it is also relevant to family members with an increased risk for metabolic conditions reported in first-degree relatives. From a public health point of view, it has a huge economic burden on the health care system due to both reproductive issues and long term chronic morbidity effecting later part of the life.[4,44] The costs of evaluating for PCOS and its associated morbidities, and treating the long-term morbidities, exceed $4 billion annually in 2004 dollars in the United States alone.[4] Furthermore, these women also have obstetrical complications including high risk of gestational diabetes.[45,46]

Until now, insulin-sensitizing compounds, such as metformin, pioglitazone and troglitazone, have been considered to induce ovulation and improve features of metabolic syndrome in women with PCOS.[36] Of these, thiazolidinedione, category C drug, is associated with significant adverse effects such as myocardial infarction[47] weight gain, and adverse effects in animal studies in pregnancy. Therefore they are unlikely to have a major clinical role in treating women with PCOS. While metformin has a role in reducing insulin resistance, it does not normalise insulin resistance in PCOS and has limited efficacy in infertility and its use is limited by mild gastrointestinal side effects possibly reducing compliance.

Our systematic review has shown a clear benefit with inositol in improving ovulation rate and on the hormonal and glycaemic profile in women with PCOS. Whether this translates into clinical benefit with improved pregnancy and increased live birth rate and into reduced development of metabolic complications including gestational diabetes, type II diabetes or metabolic disease is yet to be shown. If found to be effective at improving primary clinical outcomes, inositol supplementation, alongside life style advice could become a first line treatment to improve fertility in women with PCOS. By regularising menstrual cycles, it also has the potential to also reduce the burden of endometrial hyperplasia and malignancy in these women. With no significant side effects, and easy accessibility, it is likely to result in high compliance. Unlike clomiphene citrate, the supplement could be provided in primary care settings and does not require expensive specialist review and monitoring.

There is a clear need for a large randomised trial to compare the effects of inositol alongside lifestyle advice compared with placebo and life style advice as a first line of treatment for reproductive outcomes across ovulation induction, pregnancy rates and and live birth rates in women with PCOS. The possibility of addition of clomifene and/or metformin in both arms after a set period of trial with the above needs be further evaluated. Likewise, longer term studies on the effect of inositol on metabolic outcomes and pregnancy outcomes is also warranted.

Conclusion

Inositol appears to significantly improve the ovulation rate, metabolic and hormonal profile in women with PCOS compared to placebo. There is a need to assess its effect on pregnancy and live birth rates and on longer term metabolic health outcomes. This review shows promising but preliminary favorable results with myo-inositol in women with polycystic ovary syndrome and further well-designed and well-conducted multicenter trial to address this issue to provide robust evidence of benefit is warranted before its widespread use can be recommended.

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Contribution to authorship

JP - Conception, planning, carrying out, analysing and writing up

DP - Data extraction, Literature search

PS – Data extraction

LS –Revising the article critically for important intellectual content

PB - Revising the article critically for important intellectual content

HT - Revising the article critically for important intellectual content

AC - Revising the article critically for important intellectual content

ST – Conception, Planning, Revised the article critically for important intellectual content.

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Table/figure Caption List

Online supporting information

Figure S1: Risk of bias for studies on Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials

Figure S2: Forest plot of comparison; Inositol vs Placebo, outcome: Ovulation Induction and menstrual regularisation used as a surrogate.

Figure S3: Funnel plot of comparison: Inositol vs Placebo, outcome: Ovulation.

Table S1: Characteristics of the studies included in the review of Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials

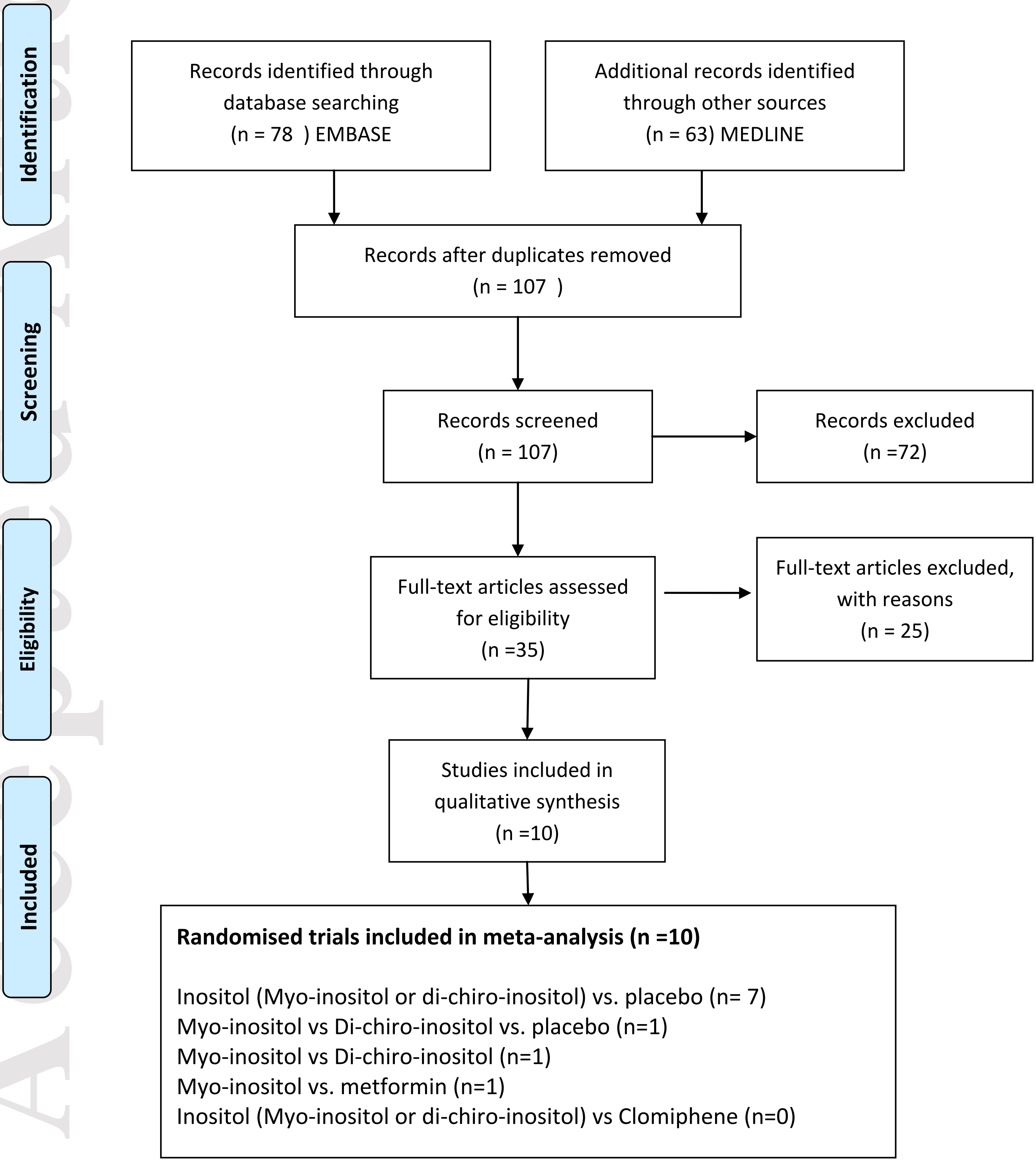
Table S2: Quality of studies included in the review of Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials

Table S3: Excluded and included studies

Appendix S1: Search Strategy

PRISMA 2009 Flow Diagram

Inositol treatment of anovulation in women with polycystic ovary syndrome: a meta-analysis of randomised trials



Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Random, 95% CI
1.1.1 Di-Chorio-inositol						
Iuorno 2002	6	10	2	10	15.9%	3.00 [0.79, 11.44]
Nestler 1999	19	22	6	22	26.8%	3.17 [1.57, 6.39]
Subtotal (95% CI)		**32**		**32**	**42.6%**	**3.13 [1.68, 5.83]**
Total events	25		8			
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.94); I² = 0%						
Test for overall effect: Z = 3.60 (P = 0.0003)						
1.1.2 Myo-inositol						
Costantino 2009	16	23	4	19	22.7%	3.30 [1.33, 8.22]
Gerli 2007	37	45	30	47	34.6%	1.29 [1.00, 1.66]
Subtotal (95% CI)		**68**		**66**	**57.4%**	**1.89 [0.69, 5.18]**
Total events	53		34			
Heterogeneity: Tau² = 0.43; Chi² = 4.68, df = 1 (P = 0.03); I² = 79%						
Test for overall effect: Z = 1.24 (P = 0.21)						
Total (95% CI)		**100**		**98**	**100.0%**	**2.32 [1.14, 4.73]**
Total events	78		42			
Heterogeneity: Tau² = 0.36; Chi² = 12.11, df = 3 (P = 0.007); I² = 75%						
Test for overall effect: Z = 2.32 (P = 0.02)						
Test for subgroup differences: Chi² = 0.69, df = 1 (P = 0.40), I² = 0%						

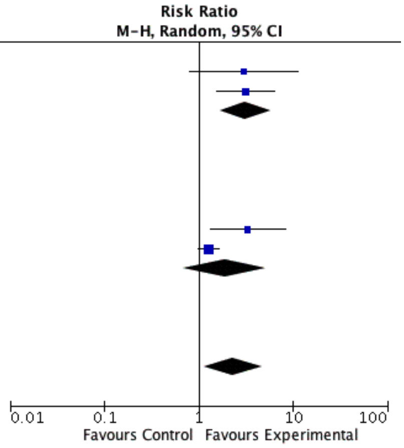


Fig 2a. Forest plot of comparison; Inositol vs Placebo, outcome: Ovulation.

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Myo-Inositol versus Placebo						
Formuso 2015	34	51	4	38	6.6%	6.33 [2.46, 16.33]
Genazzani 2008	5	10	0	10	0.7%	11.00 [0.69, 175.86]
Subtotal (95% CI)		**61**		**48**	**7.4%**	**6.79 [2.78, 16.62]**
Total events	39		4			
Heterogeneity: Chi² = 0.14, df = 1 (P = 0.71); I² = 0%						
Test for overall effect: Z = 4.20 (P < 0.0001)						
Myo-inositol versus D-chiro-inositol						
Formuso 2015	34	51	31	48	46.3%	1.03 [0.78, 1.37]
Pizzo 2014	34	51	31	48	46.3%	1.03 [0.78, 1.37]
Subtotal (95% CI)		**102**		**96**	**92.6%**	**1.03 [0.84, 1.26]**
Total events	68		62			
Heterogeneity: Chi² = 0.00, df = 1 (P = 1.00); I² = 0%						
Test for overall effect: Z = 0.31 (P = 0.76)						

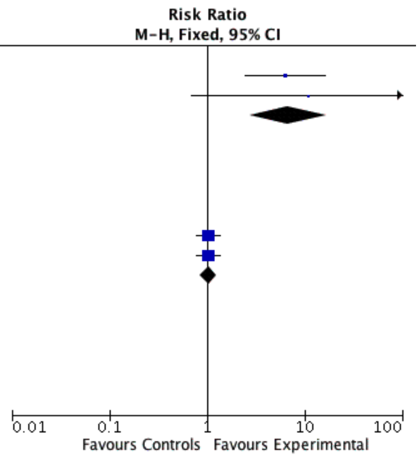


Fig 2b. Forest plot of comparison; Myo-Inositol vs Placebo; Myo-Inositol vs Di-Chorio-Inositol, outcome: Menstrual cycle regularisation

Study or Subgroup	Myo-Inositol Events	Myo-Inositol Total	Control Events	Control Total	Weight	Risk Ratio M-H, Fixed, 95% CI
Myo-Inositol versus Placebo						
Gerli 2007	4	23	1	19	9.1%	3.30 [0.40, 27.13]
Subtotal (95% CI)		**23**		**19**	**9.1%**	**3.30 [0.40, 27.13]**
Total events	4		1			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.11 (P = 0.27)						
Myo-Inositol versus Metformin						
Raffone 2010	18	60	11	60	90.9%	1.64 [0.85, 3.16]
Subtotal (95% CI)		**60**		**60**	**90.9%**	**1.64 [0.85, 3.16]**
Total events	18		11			
Heterogeneity: Not applicable						
Test for overall effect: Z = 1.46 (P = 0.14)						

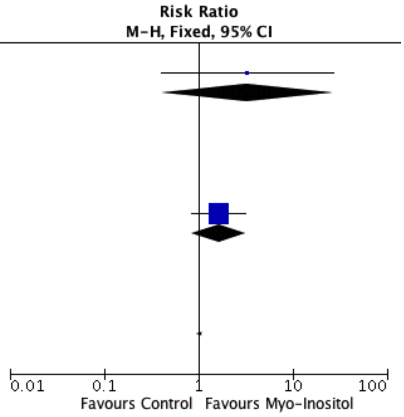


Fig 2c. Forest plot of comparison; Myo-Inositol vs Placebo; Myo-Inositol vs Metformin, outcome: Clinical Pregnancy Rate.

Study or Subgroup	Inositol Mean	Inositol SD	Inositol Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Random, 95% CI
Total Androgens ng/dl								
Artini 2013	167.5	29	25	189	24	25	5.3%	-0.80 [-1.37, -0.22]
Costantino 2009	196	26	23	306	41	19	5.0%	-3.21 [-4.16, -2.27]
Dona 2012	316.05	61.89	18	418.91	11.17	8	5.0%	-1.90 [-2.90, -0.90]
Genazzani* 2008	170.5	29	10	191	24	10	5.1%	-0.74 [-1.65, 0.18]
Iuorno 2002	193	26	10	303	41	10	4.7%	-3.07 [-4.45, -1.69]
Nestler 1999	173	50	22	186	53	22	5.3%	-0.25 [-0.84, 0.35]
Subtotal (95% CI)			**108**			**94**	**30.3%**	**-1.58 [-2.53, -0.63]**
Heterogeneity: Tau² = 1.18; Chi² = 39.09, df = 5 (P < 0.00001); I² = 87%								
Test for overall effect: Z = 3.27 (P = 0.001)								
Total Testosterone ng/dl								
Artini 2013	53.8	6.2	25	54.2	9.1	25	5.3%	-0.05 [-0.61, 0.50]
Costantino 2009	34.8	4.3	23	109	7.5	19	3.3%	-12.22 [-15.03, -9.41]
Dona 2012	41	7	18	56	8	8	5.0%	-1.99 [-3.01, -0.97]
Genazzani* 2008	54.8	6.2	10	55.2	9.1	10	5.1%	-0.05 [-0.93, 0.83]
Iuorno 2002	34	4.3	10	108	7.5	10	2.3%	-11.59 [-15.70, -7.49]
Nestler 1999	61	33	22	79	39	22	5.3%	-0.49 [-1.09, 0.11]
Subtotal (95% CI)			**108**			**94**	**26.2%**	**-3.28 [-5.10, -1.46]**
Heterogeneity: Tau² = 4.36; Chi² = 105.54, df = 5 (P < 0.00001); I² = 95%								
Test for overall effect: Z = 3.53 (P = 0.0004)								
Free Testosterone ng/dl								
Costantino 2009	0.24	0.03	23	0.85	0.13	19	4.5%	-6.65 [-8.26, -5.04]
Iuorno 2002	0.22	0.03	10	0.83	0.13	10	3.8%	-6.19 [-8.51, -3.88]
Nestler 1999	0.5	0.5	22	0.8	0.4	22	5.3%	-0.65 [-1.26, -0.04]
Subtotal (95% CI)			**55**			**51**	**13.5%**	**-4.43 [-9.03, 0.18]**
Heterogeneity: Tau² = 15.85; Chi² = 62.10, df = 2 (P < 0.00001); I² = 97%								
Test for overall effect: Z = 1.88 (P = 0.06)								
Dehydroepiandrosterone (DHEA) mcg/dl								
Costantino 2009	188	24	23	320	35	19	4.9%	-4.40 [-5.56, -3.24]
Iuorno 2002	187	24	10	319	35	10	4.4%	-4.21 [-5.91, -2.51]
Nestler 1999	274	91	22	421	179	22	5.2%	-1.02 [-1.65, -0.39]
Subtotal (95% CI)			**55**			**51**	**14.5%**	**-3.15 [-5.71, -0.59]**
Heterogeneity: Tau² = 4.73; Chi² = 32.21, df = 2 (P < 0.00001); I² = 94%								
Test for overall effect: Z = 2.41 (P = 0.02)								
Sex Hormone-Binding Globulin (SHBG) nmol/l								
Costantino 2009	198	24	23	163	26	19	5.2%	1.38 [0.70, 2.06]
Iuorno 2002	196	24	10	161	26	10	5.0%	1.34 [0.35, 2.33]
Nestler 1999	132.4	60.7	22	77.3	24.8	22	5.2%	1.17 [0.52, 1.81]
Subtotal (95% CI)			**55**			**51**	**15.5%**	**1.28 [0.86, 1.70]**
Heterogeneity: Tau² = 0.00; Chi² = 0.21, df = 2 (P = 0.90); I² = 0%								
Test for overall effect: Z = 5.93 (P < 0.00001)								

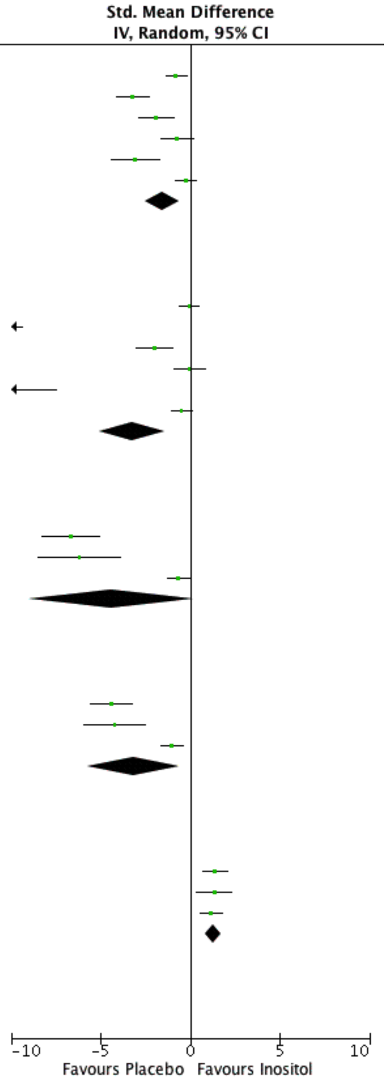


Figure 3: Forest plot of comparison; Inositol vs Placebo, outcome: Hormonal factors.

Study or Subgroup	Inositol Mean	Inositol SD	Inositol Total	Placebo Mean	Placebo SD	Placebo Total	Weight	Std. Mean Difference IV, Random, 95% CI
Insulin(fasting) µU/ml								
Artini 2013	5.5	1.1	25	10.1	1.1	25	4.7%	−4.12 [−5.12, −3.11]
Costantino 2009	26	8	23	38	7	19	5.0%	−1.56 [−2.26, −0.86]
Dona 2012	5.16	2.61	18	7.75	0.76	8	4.8%	−1.12 [−2.02, −0.23]
Genazzani* 2008	6.5	1.1	10	11.3	1.1	10	4.0%	−4.18 [−5.87, −2.49]
Iuorno 2002	24	8	10	36	7	10	4.7%	−1.53 [−2.55, −0.51]
Nestler 1999	22	21	22	42	52	22	5.1%	−0.50 [−1.10, 0.11]
Subtotal (95% CI)			**108**			**94**	**28.3%**	**−2.06 [−3.18, −0.94]**
Heterogeneity: $Tau^2 = 1.68$; $Chi^2 = 46.96$, df = 5 ($P < 0.00001$); $I^2 = 89\%$								
Test for overall effect: Z = 3.62 (P = 0.0003)								
Glucose(fasting) mg/dl								
Costantino 2009	81.6	4	23	88	4	19	5.0%	−1.57 [−2.27, −0.87]
Dona 2012	83.7	5.6	18	86.2	2.3	8	4.9%	−0.50 [−1.34, 0.35]
Iuorno 2002	80	4	10	87	4	10	4.7%	−1.68 [−2.73, −0.63]
Nestler 1999	90	19	22	95	24	22	5.1%	−0.23 [−0.82, 0.37]
Subtotal (95% CI)			**73**			**59**	**19.6%**	**−0.95 [−1.70, −0.20]**
Heterogeneity: $Tau^2 = 0.43$; $Chi^2 = 11.32$, df = 3 ($P = 0.01$); $I^2 = 73\%$								
Test for overall effect: Z = 2.47 (P = 0.01)								
Glucose/Insulin ratio								
Artini 2013	16.5	2.9	25	8.4	2.6	25	4.9%	2.89 [2.08, 3.71]
Genazzani* 2008	17.4	2.9	10	8.6	2.6	10	4.3%	3.06 [1.69, 4.43]
Subtotal (95% CI)			**35**			**35**	**9.2%**	**2.94 [2.24, 3.64]**
Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 0.04$, df = 1 ($P = 0.84$); $I^2 = 0\%$								
Test for overall effect: Z = 8.25 (P < 0.00001)								
HOMA-IR Index (Insulin Resistance))								
Artini 2013	1.1	0.3	25	2.4	0.7	25	5.0%	−2.38 [−3.11, −1.64]
Dona 2012	1.06	0.62	18	1.66	0.16	8	4.8%	−1.10 [−1.99, −0.20]
Genazzani* 2008	1.4	0.3	10	2.5	0.7	10	4.6%	−1.96 [−3.06, −0.85]
Subtotal (95% CI)			**53**			**43**	**14.4%**	**−1.83 [−2.63, −1.03]**
Heterogeneity: $Tau^2 = 0.28$; $Chi^2 = 4.70$, df = 2 ($P = 0.10$); $I^2 = 57\%$								
Test for overall effect: Z = 4.50 (P < 0.00001)								
Glucose Area Under Curve (AUC)								
Costantino 2009	10,452	414	23	12,992	793	19	4.6%	−4.06 [−5.15, −2.96]
Iuorno 2002	10,052	414	10	12,592	793	10	4.1%	−3.85 [−5.44, −2.25]
Nestler 1999	12,656	4,316	22	14,014	3,089	22	5.1%	−0.36 [−0.95, 0.24]
Subtotal (95% CI)			**55**			**51**	**13.8%**	**−2.70 [−5.50, 0.09]**
Heterogeneity: $Tau^2 = 5.76$; $Chi^2 = 43.41$, df = 2 ($P < 0.00001$); $I^2 = 95\%$								
Test for overall effect: Z = 1.89 (P = 0.06)								
Insulin Area Under Curve (AUC)								
Costantino 2009	5,535	1,792	23	9,100	1,162	19	4.9%	−2.27 [−3.06, −1.48]
Iuorno 2002	5,335	1,792	10	8,600	1,161	10	4.6%	−2.07 [−3.20, −0.94]
Nestler 1999	5,158	6,714	22	9,210	7,840	22	5.1%	−0.55 [−1.15, 0.06]
Subtotal (95% CI)			**55**			**51**	**14.6%**	**−1.59 [−2.82, −0.36]**
Heterogeneity: $Tau^2 = 0.99$; $Chi^2 = 13.55$, df = 2 ($P = 0.001$); $I^2 = 85\%$								
Test for overall effect: Z = 2.53 (P = 0.01)								

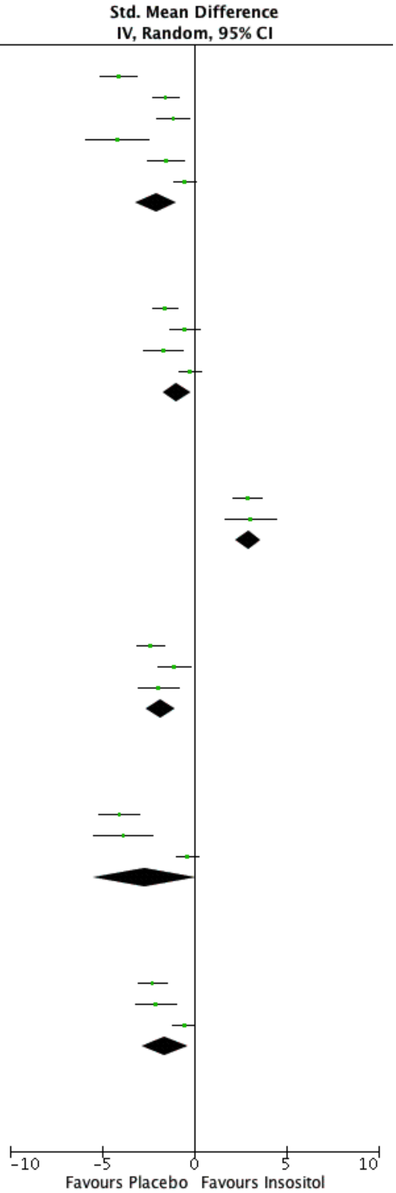


Figure 4: Forest plot of comparison; Inositol vs Placebo, outcome: Glycaemic factors.