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Original article

Female sex hormones in relation to insulin resistance after hysterectomy: A pilot study

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SUMMARY

Background & aim: Surgery causes development of insulin resistance. Women undergoing hysterectomy have different female sex hormonal status, ranging from premenopausal to postmenopausal. The aim of the study was to explore the relation between the female sex hormones and insulin resistance (IR%) after hysterectomy.

Methods: A secondary analysis from a randomised controlled single-centre study at the Department of Obstetrics and Gynaecology, Örebro University Hospital, Sweden. Twenty women were randomised to robot-assisted laparoscopic or abdominal hysterectomy. Blood were drawn before and after surgery for measurement of oestrogens, progesterone, and gonadotropins alongside determination of insulin sensitivity using the hyperinsulinemic normolycaemic clamp.

Results: Female sex hormonal status was not correlated to insulin sensitivity before operation. Premenopausal women developed more IR% than postmenopausal women ($p = 0.012$). Premenopausal women also showed a significant decrease in absolute levels of oestradiol (E2) ($p = 0.016$), and the relative decrease in E2 from preoperative to postoperative values (E2%) was significantly higher ($p = 0.001$). There was a significant positive correlation in the entire study population between E2% and IR% ($r = 0.72$, $p = 0.001$, r^2 0.51) that remained when adjusted for age ($p = 0.028$), BMI ($p = 0.001$), and preoperative insulin sensitivity ($p = 0.011$) separately.

Conclusions: Premenopausal women developed a higher degree of postoperative insulin resistance that was associated with a parallel relative change in oestradiol levels compared with the postmenopausal women. It remains unclear whether these are independent phenomena in the overall stress response or whether a causal relationship exists.

1. Introduction

When the body is subjected to trauma, either acute or planned (as in elective surgery), a number of reactions will follow, including a complex system of inflammatory and neuroendocrine response. Different types of surgery thus place the body into a metabolic stress situation where the normal insulin actions are blocked and insulin resistance develops [1]. This operative stress is believed to prolong recovery and to be a risk factor for complications, especially infectious complications [2,3].

We have previously conducted an open randomised single-centre trial comparing hysterectomies with two different surgical techniques, robot-assisted laparoscopic hysterectomy (RTLH) and open abdominal hysterectomy (AH) [4]. Laparoscopic techniques are generally considered less invasive, and the hypothesis was that less operative stress, and as a consequence less development of insulin resistance, would therefore be present after minimally-invasive surgery compared with the traditional abdominal approach [5]. As expected, that study revealed a favourable clinical outcome and a less pronounced inflammatory response in the RTLH group compared with the AH group. However, we could not demonstrate a difference in development of insulin resistance after

Abbreviations: IR%, Insulin resistance; E2, Oestradiol; BMI, Body Mass Index; M Pre, Preoperative insulin sensitivity; M Post, Postoperative Insulin sensitivity; RTLH, Robot-assisted total laparoscopic hysterectomy; AH, Open abdominal hysterectomy; E1, Oestrone; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

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Table 1
Baseline data.

	All patients	RTLH	AH
Age, years	50 (41–67)	52 (41–66)	50 (41–67)
BMI, kg/m^2	26 (18–38)	26 (21–38)	26 (18–38)
M Pre,mg/kg × min	6.1 (2.2–11.5)	6.3 (2.2–11.5)	6.1 (2.3–9.1)
M Post,mg/kg × min	3.5 (1.9–5.4)	3.6 (1.9–5.3)	3.2 (1.9–5.4)
IR%	44 (10–69)	44 (11–69)	40 (10–66)

BMI = body mass index, M Pre = measure of preoperative insulin sensitivity, M Post = measure of postoperative insulin sensitivity, IR% = insulin resistance, % remaining insulin sensitivity postoperatively. No statistically significant difference was observed between groups in any of the variables. Data are given as median and range.

surgery between groups. A significant insulin resistance developed in both groups, but with considerable variation within each group. This result is in contrast to other studies of the development of insulin resistance after surgery [5,6].

However, our population was a mixture of women in the pre-, peri-, and postmenopausal states, and the indication for hysterectomy varied from benign conditions related to the pre- or perimenopausal period, such as bleeding disorders and fibroids, to dysplasias and endometrial cancer. Hence, the women's hormonal status also varied. Physiological levels of oestradiol in premenopausal women have been linked to favourable insulin sensitivity status, while oestrogen deficiency has been associated with the development of insulin resistance [7,8]. However, the underlying mechanisms are not fully understood. Previous literature has tried to explore the relation between menstrual cycle and insulin sensitivity, but the data are contradictory and the issue is still not clarified [9–12].

In this study, we wanted to explore whether there was any relationship between hormonal status and the development of insulin resistance after surgery that could help explain the variation in insulin resistance. This has to our knowledge not been explored previously.

2. Materials and methods

The study population comprised 20 patients in a randomised trial scheduled for and operated with hysterectomy using either robot-assisted laparoscopic or abdominal technique. Details of the RCT, including study design, CONSORT flow chart, and method have been published previously [4]. The study protocol was approved by the Regional Ethics Board (ref: Uppsala 2014/235), and informed consent was obtained from each participant. The study was registered in the ClinicalTrials.gov Protocol Registration System (NCT02291406).

2.1. Analysis of insulin resistance

Insulin resistance was measured by the hyperinsulinemic normoglycaemic clamp method, as described in detail elsewhere [4,13]. In short, insulin was infused intravenously at a steady pace to achieve physiological elevations of insulin, and at the same time glucose was infused intravenously to maintain a normal blood glucose level. The amount of glucose needed reflects the insulin sensitivity, and is presented as the M-value, defined as mg glucose/(kg body weight × minutes). The procedure was performed preoperatively 1–13 days before planned surgery, to measure the basal level of insulin sensitivity, and a second time on the morning after the operation. The relative change in insulin sensitivity (100-(M postoperative/M preoperative × 100)) represents the development of insulin resistance (IR%).

Before onset of each clamp, blood was drawn for the analyses of HbA1c and hormones. Plasma was isolated and stored at −80 °C for later batch analysis. One batch of samples was sent to the Mayo Clinic Immunochemical Laboratory, Rochester, MN, USA, for analyses of oestrogens, and another batch was sent to the laboratory at the Uppsala Department of Chemistry, BMC, Sweden, for analyses of gonadotropins.

2.2. Analysis of oestrogens

17 β-oestradiol (E2) and oestrone (E1) were extracted with methylene chloride. After derivatization with dansyl chloride, high-pressure liquid chromatography (HPLC) was used prior to introduction of the derivatized sample extract into the tandem mass spectrometry (LC-MS/MS; Agilent Technologies, Inc., Santa Clara, CA 95051) [14]. Intra-assay coefficient of variations (CV) for E1 ranged from 17.8% at a concentration of 0.30 pg/ml (1.11 pmol/L) to 1.2% at 389 pg/ml (1440 pmol/L). For E2, intra-assay CV ranged from 11.8% at a concentration of 0.23 pg/mL (0.84 pmol/L) to 1.4% at 405 pg/ml (1489 pmol/L). Inter-assay CVs ranged from 12.0% to 6.6% at 0.25–355 pg/ml (0.93–1315 pmol/L) for E1 and 10.8%–4.8% at 0.29–382 pg/ml (1.07–1404 pmol/L) for E2. Minimal detectable

Table 2
Hormone levels (oestrogens and gonadotropins), and pre- and postoperative insulin sensitivity for premenopausal and postmenopausal patients, respectively.

		Premenopausal n = 11[a]	Postmenopausal n = 9[b]	*p*-value
E1	Preop	222 (118–589)	115 (48–204)	0.010
	Postop	218 (63–682)	81 (52–270)	0.131
E2	Preop	279 (48–1456)	18 (7–118)	<0.001
	Postop	40 (19–577)	22 (6–51)	0.031
FSH	Preop	6 (3–12)	81 (17–125)	<0.001
	Postop	6 (2–13)	70 (26–122)	<0.001
LH	Preop	10 (5–24)	65 (30–102)	<0.001
	Postop	13 (5–29)	60 (33–101)	<0.001
Progesterone	Preop	0.8 (<0.6–35.0)	<0.6 (<0.6–0.6)	0.038
	Postop	<0.6 (<0.6–8.9)	<0.6 (<0.6–1.5)	0.503
E2%		74 (44–92)	4 (-43-69)	0.001
M Pre		7 (2–12)	5 (2–10)	0.230
M Post		3 (2–5)	4 (2–5)	0.456
IR%		51 (17–69)	21 (10–56)	0.012

E1 = oestrone (pmol/L), E2 = oestradiol (pmol/L), FSH = follicle stimulating hormone (IU/L), LH = luteinizing hormone (IU/L), progesterone (nmol/L), M Pre = measure of preoperative insulin sensitivity, M Post = measure of postoperative insulin sensitivity (mg/kg × min), IR = insulin resistance, E2% = relative decrease in E2 from preoperative to postoperative value.

[a] One patient operated with bilateral salpingo-oophorectomy (BSO), and one patient with hormonal intrauterine device and oestradiol patch.

[b] Four patients operated with BSO.

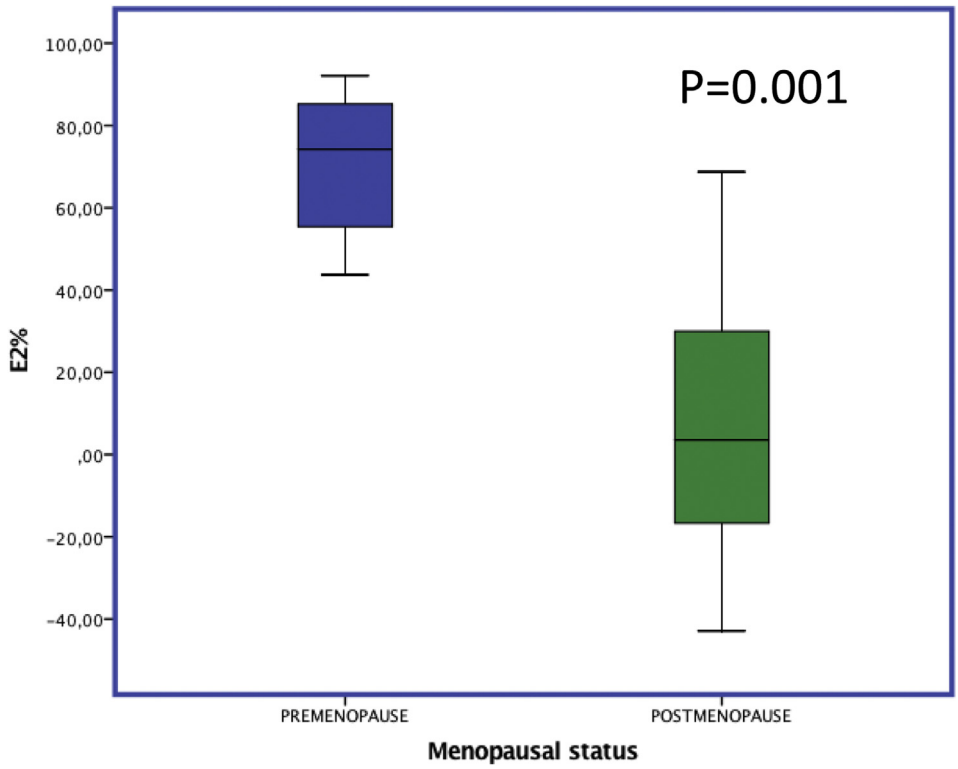

Fig. 1. Boxplot with correlation between pre- and postmenopausal status (blue and green, respectively) and E2%. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

level was 1.0 pg/mL (3.7 pmol/L) for E1 and 0.3 pg/mL (1.1 pmol/L) for E2.

2.3. Analysis of progesterone

Progesterone was measured by a two-site immunoenzymatic sandwich assay on the Roche Cobas e411 (Roche Diagnostics, Indianapolis, IN 46250). Inter-assay CVs were <5%, and the limit of sensitivity was 0.15 ng/ml (0.48 nmol/L).

2.4. Analysis of gonadotropins

Serum hormone levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined by enzyme-linked immunosorbent assay (ELISA) through the FSH (Human) ELISA Kit (Abnova, CA, USA) and the LH (Human) ELISA Kit (Abnova), respectively, according to the manufacturer's instructions. FSH and LH concentrations were determined by measuring the optical density at 450 nm in a Model 680 microplate reader (Bio-Rad, Marnes-la-Coquette, France). Minimal detectable level was 2.5 IU/L for FSH and 1 IU/L for LH.

2.5. Statistics

The power calculation for sample size was based on the primary outcome of the original trial, insulin resistance. Data are presented as median and ranges. Variables were analysed using the Mann–Whitney *U* test for two independent groups. The characteristics of distribution were tested using the Shapiro–Wilk test, and when the data were not normally distributed they were log-transformed (ln) before the analyses. Correlation was tested by calculating the Pearson's correlation coefficient and coefficient of determination (r^2). For non-normally-distributed variables, Spearman's rank correlation test was also used to confirm the results. A multivariate linear regression was used to test the independent association, adjusted for potential covariates. A *p*-value of 0.05 was considered statistically significant. Statistical data were analysed using the version 24 of IBM SPSS for Macintosh (IBM Corp., Armonk, NY, USA).

3. Results

Baseline data are presented in Table 1. More details on clamp results and characteristics of the study population have been reported previously [4].

3.1. Female sex hormones and type of surgery

Levels of pre- and postoperative oestrone (E1) and oestradiol (E2) showed a large variation but did not differ between the surgery groups; progesterone also did not differ. Statistically significant differences were seen in FSH pre- and postoperatively and LH preoperatively, indicating the uneven distribution in menopausal status between the groups (data not shown). In terms of hormonal

Table 3
Preoperative variables with significant correlation to IR%. Unadjusted linear regression. Dependent variable IR%.

	B-coefficient	R^2	*p*-value
E2	0.027	0.3	*
M Pre	5.416	0.5	***
BMI	−1.819	0.3	*
Age	−1.554	0.4	**

*p < 0.05 **p < 0.01 ***p < 0.001. IR%, Insulin resistance; E2 Oestradiol; BMI, Body Mass Index; M Pre, Preoperative insulin sensitivity.

levels, a total of nine patients were in the postmenopausal hormonal state, defined as FSH >25 IU/L (Six operated with RTLH and three with AH).

3.2. Female sex hormone levels in the pre- and postmenopausal groups

Hormone levels (oestrogens and gonadotropins), and pre- and postoperative insulin sensitivity are presented in Table 2 for premenopausal and postmenopausal patients, respectively. In the premenopausal group, there was a significant decrease in absolute levels of E2 after operation ($p = 0.016$); this was not seen in the postmenopausal group ($p = 0.730$). In addition, the relative decrease in E2 from preoperative to postoperative values (E2%) was significantly different between the pre- and postmenopausal groups, as seen in Table 2 and Fig. 1. While there was a clear reduction in the premenopausal group, there was no change in the postmenopausal women. None of the other hormones analysed (E1, progesterone, FSH, and LH) differed between pre- and postoperative values.

3.3. Correlation between female sex hormones and IR%

There was no correlation between any of the oestrogens, progesterone, or gonadotropins, and preoperative insulin sensitivity (M Pre). In the entire population, age ($p = 0.006$), BMI ($p = 0.020$), and preoperative M-values ($p < 0.001$) were all correlated with IR% (Table 3). There was a significant positive correlation in the entire study population between preoperative E2 and IR% ($r = 0.55$, $p = 0.013$, $r^2 = 0.3$), which remained significant when adjusted for operation type ($p = 0.010$) or BMI ($p = 0.016$) respectively, but was not significant when adjusted for age or M Pre. None of the other preoperative hormone levels correlated with IR%.

However, development of insulin resistance differed when analysed according to menopausal status; the premenopausal group developed significantly more insulin resistance than the postmenopausal group (Fig. 2).

There was also a significant positive correlation between E2% and IR% in the whole study population ($r = 0.72$, $p = 0.001$, $r^2 = 0.51$) (Fig. 3). This association remained significant when adjusted for age ($p = 0.028$), BMI ($p = 0.001$), and M Pre ($p = 0.011$) separately, but not quite significant when adjusted for all three possible covariates together ($p = 0.07$) (Table 4). None of the inflammatory markers in the original article [4] (C-reactive protein, white blood cell count, interleukin 6, and cortisol) had any correlation with the development of IR% and they did not impact the association between IR% and E2%, when adjusted for all inflammatory markers in a multiple regression analyses ($p = 0.033$).

In the original study, the development of IR% did not differ significantly between the different types of surgery. When adjusted for menopausal status, there was still no statistically significant difference in the development of insulin resistance between the RTLH and the AH group ($p = 0.404$; CI: -23-10).

4. Discussion

In this explorative study, we examined hormonal status in relation to insulin sensitivity and development of insulin resistance after hysterectomy. We could not find any relation between any of the hormonal levels and preoperative insulin sensitivity. However, in the stressed state after surgery, the premenopausal women developed a greater degree of insulin resistance than the postmenopausal women, and in addition the reduction in E2 levels after

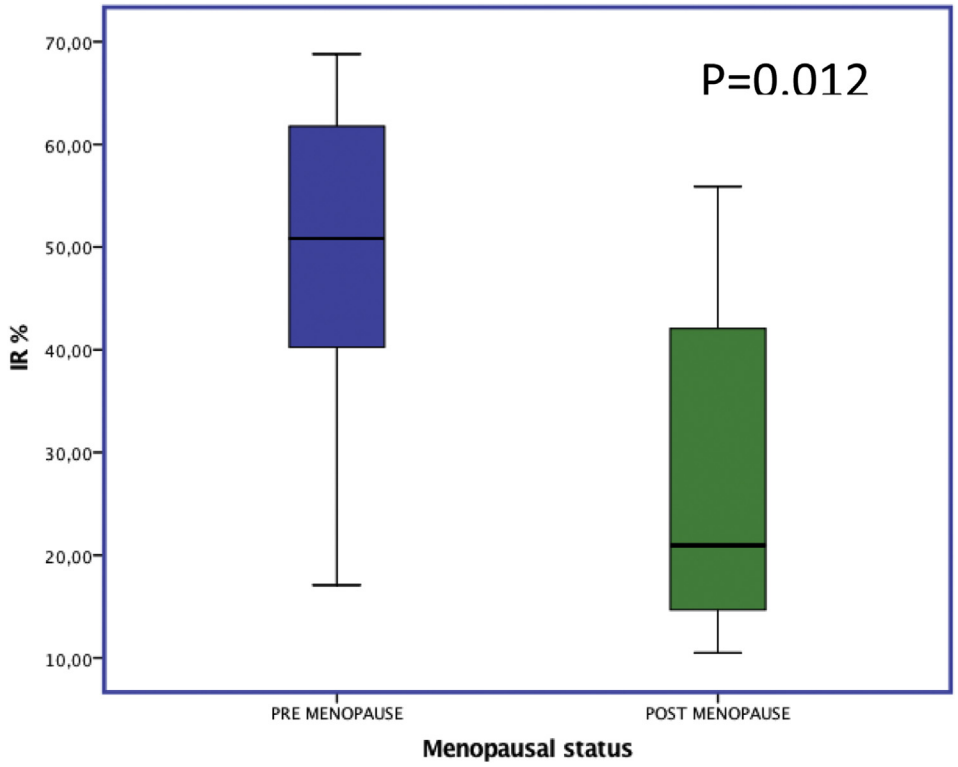

Fig. 2. Boxplot with correlation between pre- and postmenopausal status (blue and green, respectively) and IR%. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

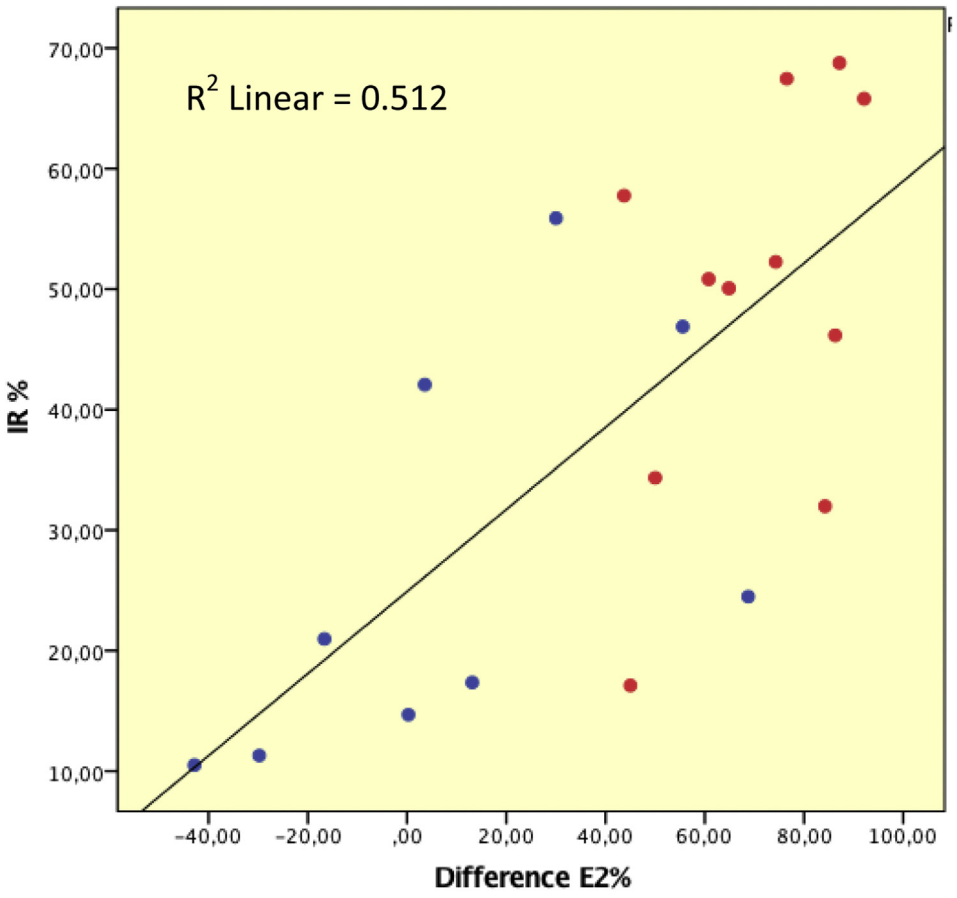

Fig. 3. Scatter plot showing relative reduction of E2% and IR%. Red dots: premenopausal, blue dots: postmenopausal. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

surgery was prominent in premenopausal women but absent in the group of postmenopausal women.

From studies both in animal models and in humans, there is evidence linking sex steroids, mainly oestrogens, to the regulation of glucose homeostasis. Physiological levels of oestradiol in premenopausal women are linked to favourable insulin sensitivity status, while oestrogen deficiency is associated with the development of insulin resistance. However, the underlying mechanisms of this relation are complex and not fully understood [7,8]. Clinical studies have explored insulin sensitivity in the non-stressed state in normal healthy premenopausal women in relation to potential changes during the menstrual cycle, but the results have been inconclusive, either finding no difference between phases [9,11] or showing decreased sensitivity in the luteal phase [10,12].

Previous studies on humans and rodents have linked oestrogen to the regulation of glucose homeostasis, showing premenopausal women to be more insulin sensitive and more resistant to develop insulin resistance compared with men and postmenopausal women, and display increased expression of GLUT4 [7,8]. In contrast, we could not find any relation between levels of sex hormones and insulin sensitivity in the non-stressed preoperative situation. This could be due to our study design, with a small population.

The lack of difference between the development of insulin resistance in the minimally-invasive group and the group operated with open surgery, which is in contrast to prior studies in other types of surgery [5,6], could not be explained by the uneven distribution of menopausal status between surgery groups. However, our results suggest that premenopausal women develop significantly more insulin resistance after hysterectomy, in comparison to postmenopausal women.

A prominent finding in the premenopausal group was that oestradiol decreased significantly after surgery. Since there was a median of five days between first and second clamp session, one explanation could have been that women in this group had changed menstrual cycle phase. When examining the individual

Table 4
Correlation between E2% and IR%. Linear regression. Dependent variable IR%. Rows with b-coefficients.

	Unadjusted	Adjusted for Mpre	Adjusted for Mpre, BMI	Adjusted for Mpre, BMI, age
b-coefficients				
E2%	0.341***	0.221*	0.241**	0.213
M Pre		3.588**	2.384	2.189
BMI			−.829	−0.910
Age				−0.213
R^2	0.512	0.678	0.712	0.714

*p < 0.05 **p < 0.01 ***p < 0.001. IR%, Insulin resistance; E2 Oestradiol; BMI, Body Mass Index; M Pre, Preoperative insulin sensitivity.

data for the combined hormonal profile, we could not confirm that this was the case, and so the postoperative drop in oestradiol levels was likely caused by the surgical procedure. There is some evidence in the literature that women tend to enter menopause two to four years earlier after hysterectomy, compared to a non-operated population with intact uterus [15,16]. It is not fully clear whether the cause of this early menopause is the surgery per se, with disruption of ovarian blood flow from the uterine side or the loss of paracrine or endocrine signals from the uterus, or if it is the diseases leading to the surgery [15,16]. Due to findings of similar levels of anti-Müllerian hormone prior to surgery as control, the former explanation is suggested to be the most likely [17]. The effects of hysterectomy on hormone levels have often been measured months after surgery, and data from the immediate postoperative period are sparse. However, Wang et al. [18] showed a reduction of anti-Müllerian hormone as early as two days after surgery, and Xiangying et al. [19] reported a decrease in oestradiol levels five days after hysterectomy. One suggested mechanism for a fall in oestradiol levels is the effect on the ovaries of the impaired blood flow caused by the surgery limiting the blood flow from the uterine arteries [16,19]. This suggestion is supported by studies showing a decreased blood flow after hysterectomy [19], and this mechanism is congruent with the drastic fall found in our data from only one day after the operation.

The association between the acute drop in oestradiol levels and the parallel development of insulin reduction in our study has to our knowledge not previously been reported in humans. Given the known association between menopausal status and the development of metabolic diseases such as type 2 diabetes mellitus and the metabolic syndrome (i.e. long-term consequences), it is an intriguing question whether acute changes in oestradiol levels have a corresponding effect on insulin resistance in a short-term perspective. However, the present study cannot reveal whether this is a parallel phenomenon, where both variables are influenced by other stress mechanisms, or whether they have a causal relationship.

This was a small exploratory study, and the limited sample size may have influenced our results. Since this is a secondary analysis from a prior RCT, our findings should be interpreted with caution and need further confirmation.

5. Conclusion

In this exploratory study, female sex hormonal status was not correlated to insulin sensitivity in the non-stressed preoperative situation. Postoperatively, the premenopausal group developed a higher degree of insulin resistance, which was associated with a parallel relative change in oestradiol levels compared with the postmenopausal women. It remains unclear whether these are independent phenomena in the overall stress response, or whether a causal relationship exists.

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Conflict of interest

OL has an advisory appointment with Danone research, a commercial company that produces the carbohydrate drink used in this study. The other authors report no conflict of interest.

Statement of authorship

All authors (LW, KN, OL) participated in the planning of this study. LW did all the data analyses and was the primary author of the manuscript. All authors (LW, OL, KN) contributed substantially to the manuscript, revised and approved the final version.

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