DOI: 10.1111/dme.13078

Research: Complications

Oestrogen activity of the serum in adolescents with Type 1 diabetes

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Accepted 19 January 2016

Abstract

Aims To assess serum oestrogen levels and oestrogenic activity in adolescents with Type 1 diabetes compared with a healthy control group.

Methods We conducted a cross-sectional study that evaluated adolescents with Type 1 diabetes (n = 38) and healthy adolescents (control group; n = 32). Serum oestrogens, urinary oestrogen metabolites and serum oestrogenic activity were assessed. Oestrogenic activity was evaluated in an *in vitro* cell proliferation assay using a modified E-screen assay with MCF-7/BUS cells.

Results Adolescents with Type 1 diabetes had lower oestrogenic activity levels in both phases of the menstrual cycle compared with the control group (follicular phase: 76 vs 94%; luteal phase: 97 vs 131%; $P < 0.01$), even after adjusting for BMI, oestradiol and oestrone levels. Postmenarcheal adolescents with Type 1 diabetes had lower oestradiol levels compared with control subjects in the follicular phase (63.3 pmol/l vs 89.4 pmol/l; $P < 0.01$) and higher oestrone levels compared with controls in the luteal phase (196 vs 151.9 pmol/l; $P < 0.05$).

Conclusions Adolescents with Type 1 diabetes had lower levels of serum oestrogenic activity, and these were lower than expected based on their serum oestradiol levels. We postulate that changes in the serum milieu of oestrogens in patients with Type 1 diabetes may explain their decreased oestrogenic activity and may play a role in their adverse metabolic profile.

Diabet. Med. 33, 1366–1373 (2016)

Introduction

During recent decades, significant changes in the reproductive abnormalities associated with Type 1 diabetes have been observed in association with improvements in the metabolic control of this condition. Currently, hypogonadism is rarely observed in women with Type 1 diabetes, but to date a thorough investigation of oestrogen action and metabolism has not been performed in patients with Type 1 diabetes [1]. Clinical conditions related to oestrogen deficiency, such as osteoporosis [2] and cardiovascular disease, may represent a serious burden in women with Type 1 diabetes [3,4]. The first manifestations of these abnormalities occur during adolescence, and puberty has been regarded as a critical period in the pathophysiology of complications [5,6]. We postulate that variations in serum oestrogen levels or in serum oestrogenic activity, which have not been previously evaluated, might be present in these patients after the onset of puberty.

Women with Type 1 diabetes have an adverse profile of chronic complications during early adulthood compared with men. Even during adolescence, female patients with Type 1 diabetes have a higher incidence of cardiovascular disease and higher mortality rates [3,4]. By contrast, cardiovascular disease is rarely present in women without diabetes during their reproductive years [7], which has been explained by the possible protective role of serum oestrogens [8–10].

Serum oestrogenic activity depends mainly on oestradiol (E2) levels but is modulated by a mixture of oestrogen metabolites derived from the enzymatic conversion of E2 and oestrone (E1) [11]. The main E2 metabolic pathways are based on the action of CYP1A2, a hepatic enzyme that converts oestrogens to 2-hydroxyoestrogens, which are less potent than E2 [12].

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What's new?

- Clinical evidence of hypoestrogenism has been found in women with Type 1 diabetes, but there have been no systematic studies evaluating oestrogen profile in such patients.
- We studied the overall serum oestrogenic activity and oestrogenic profile in adolescents with Type 1 diabetes.
- This study showed that adolescents with Type 1 diabetes have lower levels of serum oestrogen activity, and these levels were lower than expected based on measurement of their serum oestradiol levels.
- We postulate that low serum oestrogenic activity levels may play a role in the pathophysiology of chronic complications in women with Type 1 diabetes.

Serum oestrogen profiles have not been thoroughly studied in patients with Type 1 diabetes. Whether adolescents with Type 1 diabetes have lower levels of serum E2 and E1 and urinary oestrogen metabolites as well as lower serum oestrogenic activity levels is unknown. We postulated that adolescents with Type 1 diabetes have lower serum oestrogen levels and oestrogenic activity. The aim of the present study was to evaluate the oestrogen profile of adolescents with Type 1 diabetes, including serum steroid and urinary metabolite levels, and to assess overall serum oestrogenic activity during the follicular and luteal phases of the menstrual cycle.

Research design and methods

Subjects

Postmenarcheal adolescents with Type 1 diabetes and healthy postmenarcheal adolescents (control group) were recruited (Type 1 diabetes group, $n = 38$ and control group, $n = 32$). Because maturation of the gonadal axis continues after menarche, adolescents were further divided into two groups according to gynaecological age, < 2 years of menarche or > 2 years of menarche.

Adolescents with Type 1 diabetes were recruited from two hospitals in Santiago, Chile ($n = 38$). Type 1 diabetes was diagnosed by the presence of severe insulinopenia treated with insulin from the time of diagnosis. All the patients were diagnosed at least 2 years before the study commenced. Exclusion criteria were: Type 2 or another type of diabetes; honeymoon period, defined as a daily insulin requirement below 0.5 IU/kg/day and an HbA_{1c} < 7% or > 12%; abnormal thyroid function; elevated creatinine levels; moderate to severe acne, hirsutism (Ferriman–Gallwey score ≥7), use of oral contraceptives, steroids or any other type of regular medication; and other chronic conditions (coeliac, renal, liver or cardiac disease or undernourishment) and genetic syndromes.

We also included 32 healthy adolescents who had regular menstrual cycles, defined in adolescents by the American Academy of Pediatrics as a cycle length of 21–45 days [13]. All of the adolescents in the control group had normal birth weights and normal pubertal development. The adolescents were recruited from schools in Santiago. Exclusion criteria were the same as those for the group with Type 1 diabetes.

The study was performed according to the Declaration of Helsinki and approved by the Institutional Review Board of *Servicio de Salud Metropolitano Central* and *Hospital San Borja Arriarán* in Santiago, Chile. The parents of girls aged < 18 years and adolescents aged > 18 years provided written consent before the study. Girls younger than 18 years signed written assent.

Study protocol

A complete physical examination was performed by one of the investigators (P.M. or D.M.). Standard deviation scores were calculated for height, weight and BMI using current National Center for Health Statistics curves.

Blood samples were obtained during the follicular (days 3–5) and luteal (days 21–23) phases of the same menstrual cycle. Samples were obtained between 08:00 and 10:00 h after at least 8 h of fasting. Serum was separated by centrifugation, and aliquots were subsequently stored at −80 °C.

An overnight urine sample was collected over a 12-h period to measure urinary oestrogen metabolites and creatinine. The sample was obtained the night preceding the collection of the blood sample.

Hormone assay

Serum E2 and E1 levels were determined by radioimmunoassay (see Appendix S1 for coefficients of variation). Sex hormone-binding globulin (SHBG) levels were measured using an immunoradiometric assay.

Free E2 was calculated according to the method described by Södergård *et al.* [14], which considers the measured E2 and SHBG levels, assuming a fixed albumin concentration (4.0 mg/dl), as described by Frank Stanczyk and Catherine Kim (personal communication, University of Southern California, Los Angeles, CA, and University of Michigan, Ann Arbor, MI, respectively) [15].

Urinary 2-hydroxyestrone (2-OHE1) (sensitivity =0.5 nmol/l) and 16-hydroxyestrone (16-OHE1) (sensitivity = 0.3 nmol/l) levels were measured using an Estramet 2/16 ELISA kit (Immuna Care Corp., Bethlehem, PA, USA) and were reported after normalization to the urine creatinine concentration.

HbA_{1c} levels were measured using a commercially available automatic system (DCA 2000; Bayer Diagnostics, Tarrytown, NY, USA).

E-screen bioassay

A modified E-screen technique was developed to assess total serum oestrogenic activity. Soto *et al.* [16] reported the original technique, which was designed to evaluate the oestrogenic activity of environmental compounds by determining proliferative effects in the MCF-7 cell line. Subsequently, the assay showed better sensitivity and reproducibility using the MCF-7/BUS subline [17,18].

Experiments were performed using the modified E-screen technique on the MCF-7/BUS cells at cell passages 75-85. Mycoplasma contamination was excluded by PCR.

Before performing the E-screen assay, MCF-7/BUS cells were seeded in 25 cm^2 bottles (Corning-Costar, Sigma-Aldrich, St Louis, MO, USA) at a density of 1200 cells/mm^2 with 4 ml of pre-culture medium (Appendix S1). The pre-culture phase was maintained until the cells reached 70% confluence.

After the pre-culture phase, MCF-7/BUS cells were seeded in 96-well plates (Nunc®, cat no. 165305; Sigma-Aldrich) as described in Appendix S1. The MCF-7/BUS cells were plated at 2500 cells per well (counted in a Neubauer chamber). The cells were cultured with 200 µl of pre-culture medium for 48 h to allow cell adhesion. After this, the medium was replaced with 199 µl of stimulation medium (Appendix S1).

The patient and control serum samples were added to the plates in groups of six, and the cells were cultured for 6 days without changing the medium. Serum aliquots from each subject were thawed and used immediately in a single assay. All the cell cultures were maintained in a 5% CO_2/95% air atmosphere under saturating humidity at 37 °C.

Immediately after culturing, the 96-well E-screen assay plates containing adherent cells were frozen at −80 °C for a minimum of 24 h and a maximum of 7 days. Subsequently, cell growth was determined by measuring the cellular DNA content with a CyQUANT® GR Cell Proliferation Assay Kit (cat no. C7026; Invitrogen, Carslbad, CA, USA) according to the manufacturer's protocol with a multimode microplate reader (Synergy 2; Bio-Tek, Winooski, VT, USA).

Data are presented as the mean fluorescence compared with a standard serum pool. The intra- and interassay coefficients of variation were 10.9 and 18%, respectively. Assays were acceptable if four out of six standard serum pool samples had a standard deviation of < 20%.

Based on data of standard curves of serum pool (Appendix S1), we defined the optimum sensitivity of our assay using serum samples and serum pool similarly diluted to 0.5%; therefore, we simultaneously tested each serum sample with 0.5% serum pool, which was used as a reference standard to normalize the results. Accordingly, using these conditions, the E-screen results correlated with the serum E2 concentrations of healthy adolescents in the follicular (r^2 = 0.5, P = 0.02) and luteal phases of the menstrual cycle (r^2 = 0.79, P = 0.001).

Statistical analysis

Clinical and laboratory data are presented as mean ± sd values. The normality of the distribution was evaluated using the Kolmogorov–Smirnov test. The oestrogenic activity bioassay passed the normality test, and parametric statistics were used to analyse these data statistically; however, most of the serum hormone and urinary metabolite levels did not exhibit normal distributions, therefore, non-parametric statistical tests were used for these variables.

Serum oestrogenic activity and clinical characteristics were evaluated using unpaired and paired Student's *t*-tests. Mann–Whitney's *U*-test and the Wilcoxon test for related samples were used to analyse serum hormone and urinary oestrogen metabolite levels. Correlations between serum oestrogenic activity, serum levels of E2 and E1 and urinary levels of 16-OHE1 and 2-OHE1 were evaluated with Spearman's correlation coefficient. Regression analysis was used to evaluate the effect of diabetes on overall serum oestrogenic activity after adjusting for BMI, E2, E1, SHBG, HbA_{1c} and glucose levels. The slopes of the two regression lines were compared using ANCOVA with GraphPad Prism version 6 (San Diego, CA, USA). The remaining statistical analyses were performed using SPSS version 19.

Results

The clinical characteristics of the study cohort are shown in Table 1. Adolescents with Type 1 diabetes and those in the control group had similar chronological and gynaecological ages, but adolescents with Type 1 diabetes had higher BMI-Z scores (P < 0.01) compared with adolescents in the control group. The proportions of adolescents with > 2 years of menstruation were 58 and 56% in the Type 1 diabetes and control groups, respectively (P = 0.89).

Table 1 Clinical characteristics of adolescents with Type 1 diabetes and healthy control subjects

	Type 1 diabetes group	Control group
No. of subjects	38	32
Age, years	14.9 ± 1.8	14.9 ± 2.3
Height, m	1.57 ± 0.1	1.59 ± 0.1
BMI, Z score	0.9 ± 0.7*	0.3 ± 0.7
Time since menarche, years	2.5 ± 1.7	2.6 ± 1.9
Glucose, mg/dl	188.7 ± 95**	76.5 ± 5.5
Glucose in luteal phase, mg/dl	173.7 ± 94**	83.0 ± 7.2
HbA1c, mmol/mol	70 ± 11	
HbA_{1c}, %	8.6 ± 1.3	
Duration of Type 1 diabetes, years	6.6 ± 4.3	

Values are mean ± sd.
*P < 0.05 Type 1 diabetes group vs control group.
**P < 0.01 Type 1 diabetes group vs control group.

Table 2 shows the serum oestrogen and urinary oestrogen metabolite levels. Adolescents with Type 1 diabetes had lower levels of E2 ($P < 0.01$) and free E2 ($P < 0.01$) than those in the control group during the follicular phase. In addition, adolescents with Type 1 diabetes had higher E1 levels during the luteal phase compared with the control group ($P < 0.01$). Luteal sex steroid levels were higher than follicular sex steroid levels in both groups of adolescents.

Urinary oestrogen metabolite levels were similar in adolescents with Type 1 diabetes and those in the control group in both phases of the menstrual cycle. Higher urinary 16-OHE1 levels in the luteal phase compared with the follicular phase were observed in the Type 1 diabetes group only. Urinary 2-OHE1 levels were higher in the luteal phase than in the follicular phase in adolescents with Type 1 diabetes and in the control group.

Serum oestrogenic activity is shown in Fig. 1. Adolescents with Type 1 diabetes had lower serum oestrogenic activity levels compared with adolescents in the control group in the luteal phase [95.9 vs 125.3; $P < 0.001$ (Fig. 1b)]. Moreover, adolescents with Type 1 diabetes with > 2 years of menstruation exhibited lower oestrogenic activity levels in the follicular and luteal phases compared with the control group [76.2 ± 12.5 vs 93.7 ± 20.6; $P < 0.001$, and 97.4 ± 25.1 vs 130.8 ± 25.9; $P < 0.001$, respectively (Figs 1c and d)].

Regression analysis showed that Type 1 diabetes was a significant factor that affected serum oestrogenic activity levels during the luteal phase, even after adjusting for E2, E1, SHBG and BMI Z-score (Table 3). Model 1 showed that Type 1 diabetes had a significant effect on luteal serum oestrogenic activity levels, even after adjusting for BMI Z-score and E2. Model 2 showed that Type 1 diabetes was significantly associated with luteal serum oestrogenic activity levels after adjusting for BMI Z-score and E1. Similar results were observed when the covariate obesity replaced BMI Z-score.

Linear regression analysis did not detect any association of serum oestrogenic activity or oestrogen levels (17β-oestradiol, free E2 or E1) in adolescents with Type 1 diabetes in both menstrual phases with any clinical variable (daily insulin dose or diabetes duration) and any characteristics of metabolic control (glucose or HbA_{1c} levels).

Serum oestrogenic activity in adolescents in the control group correlated with serum E2 levels in the follicular phase [r = 0.55, $P = 0.02$ (Fig. 2a)] and luteal phase [r = 0.79 $P < 0.001$ (Fig. 2b)]; however, in adolescents with Type 1 diabetes, serum oestrogenic activity did not correlate with E2 in the follicular phase (r = -0.1, $P = 0.5$, Fig. 2a) but did exhibit a positive correlation with E2 in the luteal phase (r = 0.51, $P < 0.001$, Fig. 2b). No relationship was observed between E1 and oestrogenic activity in the follicular phase (Fig. 2c), but a positive correlation was observed between oestrogenic activity and E1 in the luteal phase in the control group (Fig. 2d). The slopes of the regression curves for serum follicular E2 and luteal E1 were significantly different between the adolescents with Type 1 diabetes and the adolescents in the control group (Figs 2a and 2d).

Discussion

The present study evaluated oestrogen levels and *in vitro* oestrogen bioactivity in adolescents with Type 1 diabetes compared with healthy control subjects. The results showed that adolescents with Type 1 diabetes have lower serum oestrogenic activity levels that were not explained by E2 levels. This finding was especially evident in adolescents with > 2 years of menstruation; these adolescents exhibited lower serum oestrogenic activity levels in both phases of the menstrual cycle and lower E2 levels in the follicular phase.

The lower serum oestrogenic activity levels observed in adolescents with Type 1 diabetes was evident even after adjusting for BMI, E2 and E1. The total oestrogenic effect includes the action of all oestrogens and their metabolites, as well as other factors that regulate the final response to oestrogens in target tissues. In this regard, the E-screen

Table 2 Hormonal profile of adolescents with Type 1 diabetes and control adolescents in the luteal and follicular phases

	Follicular phase		Luteal phase	
	Type 1 diabetes group	Control group	Type 1 diabetes group	Control group
17β-oestradiol, pmol/l	63.3 ± 30.9*,§	89.4 ± 46§	219.6 ± 172.1	265.6 ± 294.2
Oestrone, pmol/l	124.4 ± 60.2§	119.3 ± 135.1§	196 ± 95.4*	151.9 ± 103.1
SHBG, nmol/l	50.3 ± 21.8	50.3 ± 18.3	52.4 ± 21.6	51.9 ± 19.8
Free oestradiol, pmol/l	0.4 ± 0.2*§	0.6 ± 0.3§	1.4 ± 1.2	1.7 ± 1.7
Testosterone, nmol/l	2.08 ± 0.69§	1.73 ± 0.69§	2.43 ± 0.69	2.08 ± 1.04
Free androgen index	4.5 ± 2.3§	4.1 ± 2.6§	5.4 ± 2.6	4.8 ± 3
16-OHE1, nmol/l/mg creatininuria	26.5 ± 22.3§	26.1 ± 17.8	39 ± 33.8	32.4 ± 21.9
2-OHE1, nmol/l/mg creatininuria	29.3 ± 23.7§	35.2 ± 36.2§	61.3 ± 61.3	69 ± 99.6
2-OHE1/16-OHE1	1.6 ± 1.4	1.7 ± 1.4	1.9 ± 2.2	4.3 ± 8.5

Values are mean ± sd.
*$P < 0.05$ Type 1 diabetes group vs control group.
§$P < 0.05$ follicular phase vs luteal phase.
OHE1, hydroxyestrone; SHBG, sex hormone-binding globulin.

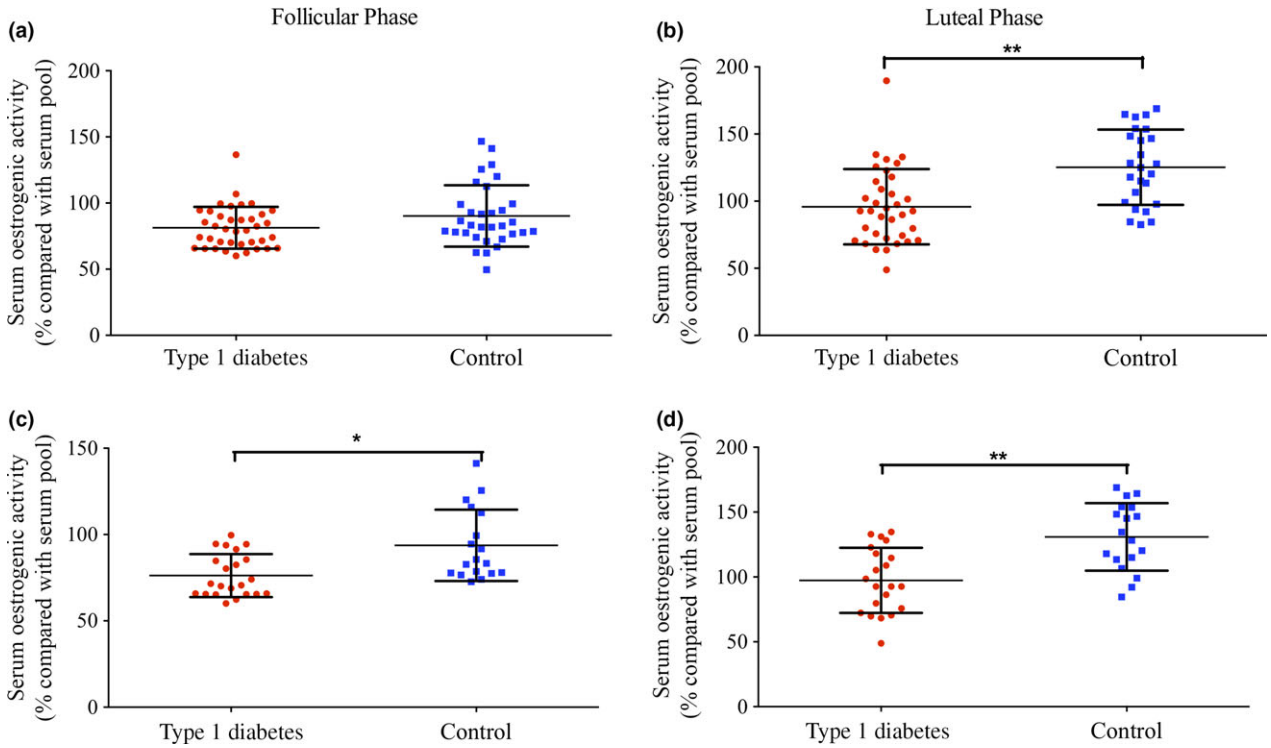

FIGURE 1 Serum oestrogenic activity in postmenarcheal adolescents with Type 1 diabetes and a control group of healthy adolescents. Serum oestrogenic activity during the follicular (a) and luteal (b) phases in all postmenarcheal adolescents and in adolescents who were > 2 years postmenarche (c and d). *P = 0.002, Type 1 diabetes vs control group. **P = 0.0002, Type 1 diabetes vs control group.

Table 3 Factors associated with serum oestrogenic activity during luteal phase

	Serum oestrogenic activity		
	β	SE of β	P
Model 1			
Type 1 diabetes	−24.8	7.5	0.002
BMI Z-score	5.6	5.2	0.28
17β-oestradiol, pmol/l	0.18	0.06	0.003
SHBG, nmol/l	0.19	0.17	0.26
Model 2			
Type 1 diabetes	−30.5	7.4	0.0001
BMI Z-score	3.6	5.3	0.5
E1, pmol/l	0.41	0.15	0.007
SHBG, nmol/l	0.2	0.17	0.25

Regression analysis of factors that are associated with serum oestrogenic activity during the luteal phase. The non-standardized β is presented. Model 1 shows the effects of Type 1 diabetes, BMI Z-score, oestradiol and SHBG in postmenarcheal adolescents with Type 1 diabetes and the healthy control group. Model 2 shows the effects of Type 1 diabetes, BMI Z-score, E1 and SHBG in postmenarcheal adolescents with Type 1 diabetes and control group adolescents.
E1, oestrone; SHBG, sex hormone-binding globulin.

method enables the evaluation of the interactions between all circulating oestrogenic compounds on the oestrogen receptor [19]. The low serum oestrogenic activity in adolescents with Type 1 diabetes documented in the present study suggests that oestrogen metabolism is affected by diabetes. Healthy adolescents, but not those with Type 1 diabetes, exhibited a positive correlation between serum E2 levels and *in vitro* oestrogen activity, as was expected because previous studies had shown that E2 was the most potent serum oestrogen [11]. It is plausible, therefore, that oestrogen activity in adolescents with Type 1 diabetes is affected by diabetes beyond the effects shown by serum E2 and E1 levels.

Adolescents with Type 1 diabetes had lower levels of E2 and free E2 during the follicular phase of the menstrual cycle, and in the case of adolescents with Type 1 diabetes who were 2 years postmenarche, serum oestrogen (E2 and E1) levels were diminished in both phases of the menstrual cycle compared with those in adolescents in the control group. Previously, Salonia *et al.* [15] showed that adult women with Type 1 diabetes had lower E2 levels in both phases of the menstrual cycle; therefore, a plausible hypothesis is that, with advancing postmenarcheal age and established ovulatory function, decreased E2 levels become apparent during the luteal phase. The mechanisms underlying the decreased E2 levels in women with Type 1 diabetes could include diminished GnRH secretion by hypothalamic neurons as a result of hyperglycaemia, insulin deficiency and metabolic stress [1,20,21]. Recently, decreased kisspeptin signalling was shown to be an important pathophysiological mechanism for hypogonadism in animals with diabetes [1]. Ovarian steroidogenesis may also be affected by Type 1 diabetes, as

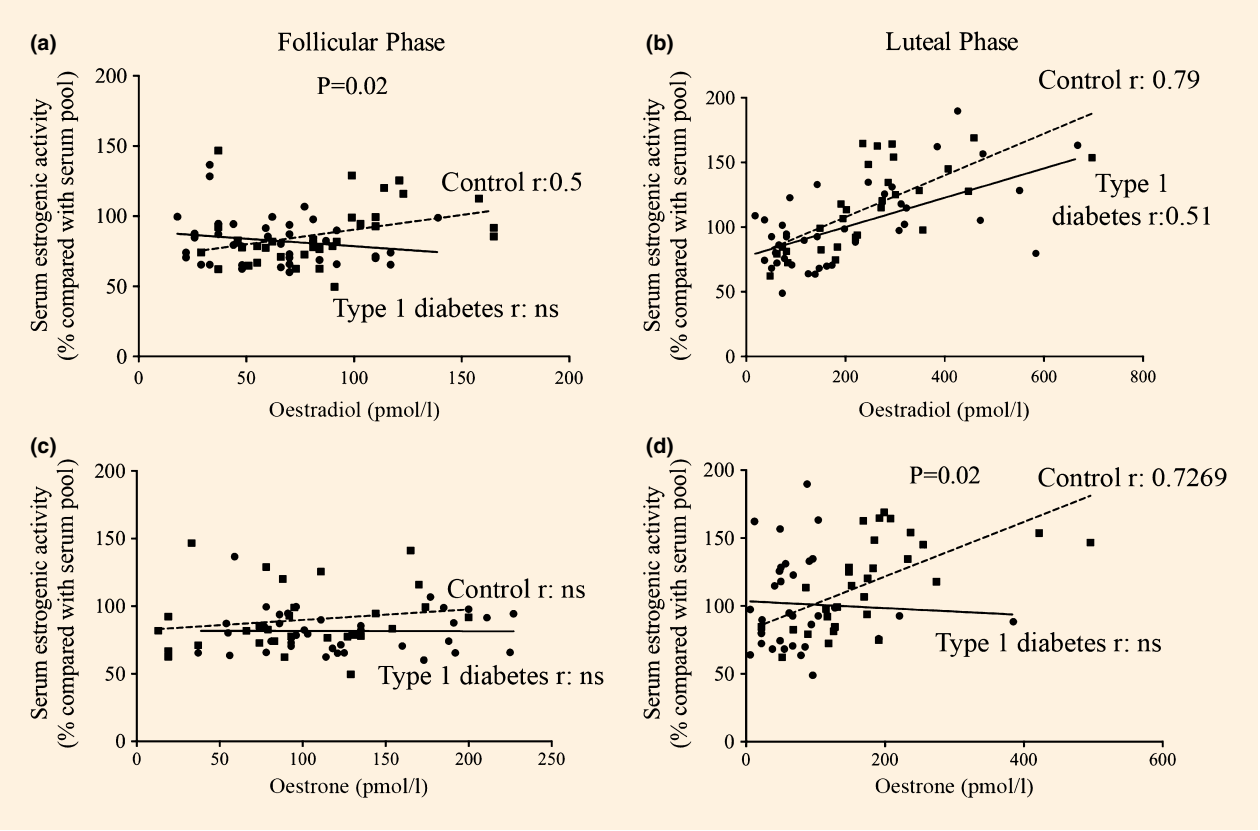

FIGURE 2 Correlations between serum oestrogenic activity, oestradiol and oestrone in the follicular (a and c) and luteal (b and d) phases in adolescents with Type 1 diabetes and a control group of healthy adolescents. The reported *P* values are for the differences in the slopes of the regression lines (a and d). Significant *r* values are reported. ns, not significant.

previously shown by a diminished response to luteinizing hormone-releasing hormone agonist stimulation in patients with Type 1 diabetes [22].

To the best of our knowledge, the present study is the first to evaluate E1 levels in patients with Type 1 diabetes. Higher serum E1 levels in the luteal phase were observed in adolescents with Type 1 diabetes compared with the control group. A possible explanation for this finding may be reduced conversion of E1 to E2. A recent study found that the expression of isoform 5 of 17β-hydroxysteroid dehydrogenase (AKR1C3) was decreased in the skin of women with Type 1 diabetes [23]. Because this enzyme participates in the conversion of its substrate E1 into E2 and is involved in the reductive detoxification of oxidative stress products, this observed decrease in AKR1C3 isoform 5 expression could partly explain the higher levels of E1.

An additional factor that may explain the lower levels of E2 and serum oestrogen activity in patients with Type 1 diabetes may be higher E2 metabolism. We observed that the levels of urinary 16-OHE1 were increased in the luteal phase of postmenarcheal adolescents with Type 1 diabetes, but not in adolescents in the control group. In addition, Mauras *et al.* [24] suggested that 16-OHE1 is related to the inflammatory profiles of obese prepubertal adolescents.

The mechanism by which oestrogen metabolism may be altered in Type 1 diabetes has not been elucidated. A study in female rats with streptozotocin-induced diabetes showed increased expression of CYP1A2, which is involved in the metabolism of oestrogen into 2-OHE1 and 16-OHE1 metabolites [25]. Matzke *et al.* [26] evaluated the metabolism of antipyrine by CYP1A2 in patients with diabetes and showed that these patients exhibit increased metabolism of antipyrine because of increased CYP1A2 activity, which is consistent with our findings of elevated 16-OHE1 in adolescents with Type 1 diabetes. Animal models of Type 1 diabetes have shown a 4.5-fold increase in levels of CYP1B1, which is involved in the conversion of E2 and E1 into 4-hydroxyoestrogens, suggesting that increased metabolism of the aforementioned active hormones may play a role in decreasing serum oestrogen levels [25].

The leading cause of mortality in women with Type 1 diabetes is cardiovascular disease, even among premenopausal women [3,4]. These data raise the question of why women with Type 1 diabetes lose physiological protection against cardiovascular disease during their premenopausal years. Chronic hyperglycaemia is the main mechanism involved in the pathophysiology of Type 1 diabetes complications, but hypoestrogenism may also play

a role [22,27]. Oestrogens play a protective role against cardiovascular disease through several mechanisms, including increased vasodilatory tone mediated by nitric oxide [9], decreased proliferation and contractility of vascular smooth muscle cells [10,28], decreased pro-inflammatory cytokines and reduced oxidative stress [29]. In an animal model of Type 1 diabetes, Han *et al.* [30] showed that E2 supplementation improves vascular reactivity, decreases vascular remodelling variables and decreases oxidative damage.

The strengths of the present paper include its detailed evaluation of oestrogen levels and oestrogenic activity, which were determined in a large group of adolescents with Type 1 diabetes and in a healthy control group. In addition, for the first time oestrogen bioactivity was determined by an *in vitro* assay that evaluates the proliferative effect of all circulating serum oestrogens. A limitation of this study, however, is that oestrogenic activity was evaluated based on the proliferation of a clonal breast cancer cell line (MCF-7/BUS), and this assay may not reflect the effects of oestrogen on other target tissues, such as the vascular endothelium and bone. Another limitation is that we cannot extrapolate enzymatic activity based on precursor and product concentrations. In addition, we did not measure a wide range of oestrogen metabolites that may potentially affect serum oestrogenic activity; however, oestrogenic activity was significantly associated with serum oestrogen levels, particularly with E2, which is consistent with this steroid being primarily responsible for the proliferation of MCF-7/BUS cells, as has been reported previously [19].

In conclusion, the present data suggest that postmenarcheal adolescents with Type 1 diabetes have decreased serum oestrogen activity and that this decrease becomes more pronounced as ovarian function matures. We postulate that the mild hypoestrogenism in adolescents with Type 1 diabetes may be secondary to an imbalance in various serum oestrogens, oestrogen metabolites and/or other oestrogen-modulating factors. Future studies should evaluate the mechanism by which decreased serum oestrogenic activity in adolescents with Type 1 diabetes may be involved in the pathophysiology of chronic complications in women with Type 1 diabetes.

Funding sources

This work was supported by a grant from FONDECYT 1100123.

Competing interests

None declared.

Acknowledgements

We are grateful to Alejandra Avila for excellent nursing care and to Dr Claudia Godoy for her help in recruiting the subjects who participated in this study. We thank all the adolescents who participated in this study. MCF-7/BUS cells for the modified E-screen technique were kindly provided by Drs Ana Soto and Carlos Sonnenschein (Tufts University School of Medicine, Boston, MA, USA).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Coefficient of variation of the hormonal assays and details of the E-screen technique, including media, detachment protocol and standardization.