

Research: Complications

C-Reactive protein and insulin growth factor 1 serum levels during the menstrual cycle in adolescents with Type 1 diabetes

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Accepted 1 June 2015

Abstract

Aims To evaluate C-reactive protein, insulin growth factor 1 and lipid levels during the follicular and luteal phases in adolescents with Type 1 diabetes.

Methods Adolescents with Type 1 diabetes ($N = 40$) and healthy controls (C; $N = 43$) were studied during the follicular and luteal phases of their menstrual cycles. C-Reactive protein, insulin growth factor 1 and lipid levels were measured.

Results Adolescents with Type 1 diabetes exhibited higher C-reactive protein levels than the C group during the follicular ($P < 0.0001$) and luteal phases ($P < 0.01$). The elevation of C-reactive protein levels was more pronounced in overweight adolescents with Type 1 diabetes than in adolescents in the C group. More adolescents with Type 1 diabetes were classified as having an elevated risk of cardiovascular disease (C-reactive protein > 3 mg/l) in the luteal phase than in the follicular phase (37.5% and 17.5%, respectively); half of the overweight adolescents with Type 1 diabetes in the luteal phase reached this level. BMI was the only significant factor affecting follicular and luteal phase C-reactive protein levels in adolescents with Type 1 diabetes. Lower insulin growth factor 1 levels were observed during both phases of the menstrual cycle in adolescents with Type 1 diabetes compared with controls. An elevation in insulin growth factor 1 levels in the luteal phase relative to the follicular phase was observed in controls, but not in adolescents with Type 1 diabetes. Luteal insulin growth factor 1 and C-reactive protein exhibited an inverse correlation ($r = -0.4$, $P = 0.01$).

Conclusions Adolescents with Type 1 diabetes have higher C-reactive protein levels and lower insulin growth factor 1 levels relative to controls, especially during the luteal phase. Type 1 diabetes diminishes the natural elevation in insulin growth factor 1 levels observed during the luteal phase in controls. Excess weight exacerbates the subclinical inflammatory state observed during both phases of the menstrual cycle in adolescents with Type 1 diabetes.

Diabet. Med. 33, 70–76 (2016)

Introduction

Women with Type 1 diabetes exhibit an adverse profile for the risk of chronic microvascular and macrovascular complications and mortality relative to men with Type 1 diabetes [1–5]. The increased risk of complications observed in women with Type 1 diabetes is underscored by the presence of early cardiovascular disease in premenopausal women with Type 1 diabetes. These women appear to have lost the physiological protection against cardiovascular disease that is usually observed in young women [1]. The reasons for this

unfavourable risk profile in female patients with this condition are unclear, but may be associated with abnormalities in ovarian function, steroid hormone action or a higher prevalence of excess weight [4,6–9]. Variations in inflammatory markers during the menstrual cycle have not been investigated.

Patients with Type 1 diabetes frequently exhibit hyperglycaemia and decreased insulin sensitivity during the luteal phase [7]. In this context, we postulate that during this period of the menstrual cycle, C-reactive protein (CRP) and lipid levels may be elevated.

CRP is an acute-phase protein that is a marker of inflammation and a predictor of cardiovascular disease

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What's new?

- The pathophysiology underlying why women with Type 1 diabetes have a greater risk of chronic complications relative to men is unknown.
- This research evaluated the effect of the menstrual cycle on the metabolic milieu in adolescents with Type 1 diabetes and demonstrated that these patients exhibit higher levels of C-reactive protein relative to controls, especially during the luteal phase.
- Excess weight exacerbates the subclinical inflammatory state observed in Type 1 diabetes.
- Lower insulin growth factor 1 levels were observed in both phases of the menstrual cycle in adolescents with Type 1 diabetes compared with controls. Adolescents with Type 1 diabetes lack the physiological elevation of insulin growth factor 1 during the luteal phase observed in controls.

[10,11]. Mild elevations of serum CRP levels, as measured with high-sensitivity assays (hs-CRP), are characteristic of a subclinical inflammatory process and have been associated with endothelial dysfunction in Type 1 diabetes [12]. Clinically, elevated hs-CRP levels have been shown to be linked to cardiovascular events in healthy adult women and in patients with Type 2 diabetes [13,14]. In adolescents, elevated hs-CRP levels have been associated with high blood pressure [15] and insulin resistance [16].

Patients with Type 1 diabetes exhibit diminished insulin growth factor 1 (IGF-1) levels, which are associated with microvascular complications and a detrimental metabolic profile in adults without diabetes [17,18]. However, healthy women exhibit elevated IGF-1 during the luteal phase of the menstrual period [19]. It is not known, however, whether the different phases of the menstrual cycle affect the serum levels of this growth factor in adolescents with Type 1 diabetes. We postulated that adolescents with Type 1 diabetes exhibit higher and lower levels of hs-CRP and IGF-1, respectively, and elevated lipid levels during the menstrual cycle.

Methods**Patients**

Non-obese post-menarcheal adolescents with Type 1 diabetes who were younger than 20 years of age were recruited from two public hospitals in Santiago, Chile ($N = 40$). Type 1 diabetes was diagnosed by the presence of severe insulinopenic diabetes treated with insulin from the time of diagnosis. All patients were diagnosed at least one year prior to this study. The exclusion criteria were as follows: having Type 2 or another type of diabetes or obesity; being in the honeymoon period, defined as a daily insulin requirement

below 0.5 IU/kg/day and $HbA_{1c} < 53$ mmol/mol ($< 7\%$) or > 108 mmol/mol ($> 12\%$); having abnormal thyroid function; having elevated creatinine levels; using oral contraceptives, steroids or any other type of medication; and having other chronic conditions (celiac disease, renal, liver or cardiac disease, or undernourishment) or genetic syndromes.

We included 43 healthy and non-obese adolescents who had regular menstrual cycles, defined as a cycle length between 21 and 45 days [20]. All had a normal birthweight and normal pubertal development. The patients were recruited from schools in downtown Santiago, which is a middle-class area. The exclusion criteria were as follows: moderate-to-severe acne, hirsutism [Ferriman-Gallwey (FG) score ≥ 7], chronic diseases, chronic use of medications (oral contraceptives, steroids or any other type of medication that might affect ovarian function) and obesity.

The protocol was performed according to the Helsinki declaration and approved by the Institutional Review Board of the Servicio de Salud Metropolitano Central and San Borja Arriaran Hospital. Parents of adolescents younger than 18 years provided signed consent, and participants gave their written assent before entering the study. Adolescents older than 18 years signed the consent form themselves.

Study protocol

A complete clinical and physical examination was performed in patients and controls. Gynaecological age was defined as the number of years elapsed from menarche until the time of the study. Standard deviations (SD) were calculated for BMI (BMI-SD), weight, and height using WHO standard curves [21]. Overweight and obesity were defined as a BMI > 1.036 and 1.97 SD above the mean BMI using the World Health Organization Standard [21]. Estimated glucose disposal rate and daily insulin dose (reported as the daily insulin dose/body surface), which are appropriate measures of insulin sensitivity in adolescents with Type 1 diabetes, were calculated as described previously [22].

An early-morning blood sample was obtained during the follicular and luteal phases (days 1–7 and 21–23 of the menstrual cycle, respectively) after an overnight fast and not later than 9 a.m. Ovulation was determined by a serum progesterone level > 4 ng/ml on the days 21 to 23 of the menstrual cycle. HbA_{1c} levels were measured using a commercially available automatic system (DCA 2000, Bayer Diagnostics, Tarrytown, NY, USA).

Blood samples were centrifuged, and the serum was stored at -20 °C until use. Samples were collected into a 1.5-ml serum-separating tube and allowed to clot for 1 h at room temperature. The samples were subsequently centrifuged for 10 min at 4 °C, and the serum was aliquoted and stored at -80 °C until further use.

hs-CRP was measured by an ultrasensitive assay using a sandwich ELISA produced by Biovendor (Brno, Czech Republic) that has a detection limit of 0.02 μ g/ml and intra- and

interassay coefficient of variation (CV) of 4.4 and 5.7%, respectively. Samples from adolescents with Type 1 diabetes and group C participants were analysed in duplicate and run together in the same assay. As described previously, an hs-CRP level > 3 mg/l was considered a marker of high cardiovascular risk [23]. Serum IGF-I levels were determined using a locally developed radioimmunoassay requiring sample extraction as a first step. The sensitivity of this assay is 5 ng/ml. The intra- and interassay CVs are 8.6% and 10.2%, respectively. Serum IGF binding protein 3 concentrations were determined using a commercial IRMA (Diasource, Nivelles, Belgium). The sensitivity of the assay is 0.1 mg/l, the intra-assay CV is 1.1%, and the interassay CV is 1.8%, as described previously [24].

The lipid profile [total cholesterol (TC), HDL-C and triglycerides] was determined by Reflotron® Plus (Roche Diagnostics SL., Barcelona, Spain). LDL-C was determined as described previously [Friedewald formula: LDL-C = TC - HDL-C - (TRIGLYCERIDES/5)] [25].

Data analysis

The normality of the distribution was evaluated using the Shapiro-Wilk test. Data that did not exhibit a normal distribution, which included hs-CRP, were logarithmically transformed to the natural logarithm. A Student's *t*-test was performed to evaluate differences between adolescents with Type 1 diabetes and group C participants. Differences between the luteal and follicular phases were assessed with the Wilcoxon paired test. The association of clinical characteristics and the hormonal profile with hs-CRP levels was evaluated with regression analysis. The interaction of overweight and Type 1 diabetes on fluctuation of hs-CRP levels was evaluated with ANCOVA models for repeated measures estimated through mixed models. Correlation analysis was performed with Spearman's rank correlation coefficient. Differences in the proportion of overweight adolescents were evaluated with a chi-squared test.

The sample size was calculated based on the hs-CRP levels observed in patients with Type 1 diabetes reported by Hayashi *et al.* [26], which demonstrated that to reach a significance level of 5% and a power of 80% with a one-tailed test, the smallest appropriate sample size was 41 subjects in each group.

All statistical calculations were performed using SPSS for Windows, v. 19. A significance level of 5% was employed. The data are presented as the mean ± SD.

Results

The clinical and anthropometric characteristics of the adolescents with Type 1 diabetes and the healthy adolescents are presented in Table 1. Adolescents with Type 1 diabetes and those in the C group exhibited similar chronological ages, gynaecological ages, anthropometric characteristics, proportions of overweight and ovulatory cycles.

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

	Type 1 diabetes (N = 40)	Control (N = 43)
Age; years	15.1 ± 1.8	15.6 ± 2.2
Gynaecological age; years	2.7 ± 2.0	3.5 ± 2.0
Cycle length; days	31.4 ± 7.6	30.5 ± 4.6
Ovulation during the studied cycle; %	30.0	35.7
Weight; kg	56.9 ± 5.6	55.4 ± 7.4
Height; m	1.58 ± 5.9	1.59 ± 6.0
BMI; kg/m ²	22.8 ± 2.5	21.9 ± 2.5
Overweight; N (%)	16 (40)	9 (21)
Duration of Ttype 1 diabetes; years	6.3 ± 4.3	
HbA _{1c} (mmol/mol)	70 ± 10.6	
HbA _{1c} (%)	8.6 ± 1.3	
Daily insulin injections; N	3.5 ± 0.8	
Daily insulin dose; U/kg/day	1.1 ± 0.4	

Serum levels of hs-CRP, growth factors and lipids in both phases of the menstrual cycle are presented in Table 2. Adolescents with Type 1 diabetes had higher hs-CRP levels compared with the C group during both phases of the menstrual cycle. An assessment of hs-CRP levels in the luteal phase relative to the follicular phase revealed that both the participants with Type 1 diabetes and those in the C group exhibited an elevation of this inflammatory marker in the sample obtained on days 21–23 of the menstrual cycle compared with the baseline sample obtained on days 1–7 (Table 2).

Overweight adolescents with Type 1 diabetes had higher hs-CRP than overweight adolescents in the C group and normal weight adolescents with Type 1 diabetes and normal weight adolescents in the C group. Fifty per cent of overweight adolescents with Type 1 diabetes exhibited an hs-CRP level > 3 mg/l during the luteal phase (Fig. 1a). Overweight adolescents with Type 1 diabetes had higher hs-CRP than normal weight adolescents with Type 1 diabetes ($P < 0.05$ for the follicular phase), overweight adolescents in the C group ($P = 0.002$ for the follicular phase and $P = 0.049$ for the luteal phase, Fig. 1a) and normal weight adolescents in the C group ($P < 0.001$ for the follicular phase and $P < 0.01$ for the luteal phase). The elevation of hs-CRP levels during the luteal phase relative to the follicular phase was observed in all groups, except for overweight adolescents in the C group (Fig. 1a and b). The magnitude of the elevation of hs-CRP levels was more pronounced in overweight adolescents with Type 1 diabetes than those in the C group. However, no significant interaction among overweight and variations in Type 1 diabetes and hs-CRP during the menstrual cycle were shown.

Adolescents with Type 1 diabetes exhibited lower IGF-1 levels and higher IGF binding protein 3, total cholesterol and HDL-C levels relative to those in the C group in the follicular phase and the luteal phase (Table 2). IGF-1 serum

Table 2 Hormonal profile and hs-CRP levels during the menstrual cycle in both groups

	Type 1 diabetes (N = 40)		Control (N = 43)	
	Follicular Phase	Luteal Phase	Follicular Phase	Luteal Phase
hs-CRP (mg/l)	2.4 ± 1.4***	3.0 ± 2.0* ^{§§}	1.5 ± 1.0	2.3 ± 1.7 ^{§§}
hs-CRP > 3 mg/l (%)	17.5	37.5 [†]	7.0	18.6
IGF-1 (ng/ml)	281.1 ± 62.5***	286.8 ± 61.8***	329.6 ± 46.5	340.9 ± 54.7 [§]
IGFBP-3 (mg/l)	2.7 ± 0.5***	2.7 ± 0.5*	2.4 ± 0.4	2.4 ± 0.4
Progesterone (ng/ml)		3.0 ± 3.5		3.5 ± 3.8
Total cholesterol (mg/dL)	164.4 ± 34.7**	164.4 ± 37.1**	145.6 ± 24	142.4 ± 26.1
HDL-C (mg/dl)	52.7 ± 12.3**	54.1 ± 15.6*	44.9 ± 8.9	45.5 ± 9.9
LDL-C (mg/dl)	93.5 ± 29	93.6 ± 28.7 [†]	83.2 ± 21.1	81.6 ± 21.2
TC/HDL-C ratio	3.2 ± 0.7	3.3 ± 1.5	3.2 ± 0.7	3.2 ± 0.8
Triglycerides (mg/dl)	90.2 ± 42	85 ± 31.2	88 ± 41.6	76.8 ± 29.7 ^{§§}

Differences of Type 1 diabetes compared with C group in the same phase of the menstrual cycle are described by *. **P* < 0.05 Type 1 diabetes vs. C. ***P* < 0.01 Type 1 diabetes vs. C. ****P* < 0.0001.

Differences in follicular (FP) and luteal phase (LP) within the same group of adolescents are described by [§]. [§]*P* < 0.05 FP vs. LP. ^{§§}*P* < 0.01 FP vs. LP. ^{§§§}*P* < 0.0001 FP vs. LP.

[†]*P* = 0.06 luteal phase in Type 1 diabetes vs. C.

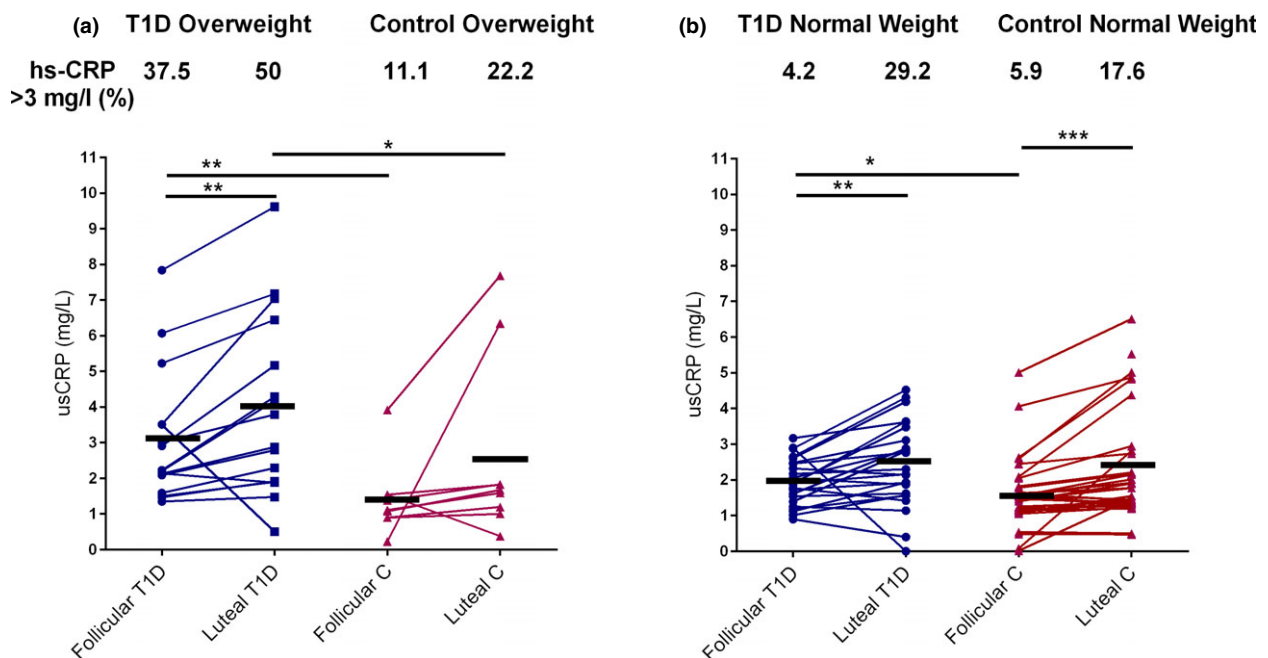


FIGURE 1 hs-CRP levels in overweight and normal-weight adolescents. (a) hs-CRP levels in overweight adolescents. (b) hs-CRP levels in normal-weight adolescents. **P* < 0.05, ***P* < 0.01. The proportion of adolescents who exhibited an hs-CRP level >3 mg/l in each phase of the menstrual cycle is presented in the upper portion of the figure.

levels, however, increased in the luteal phase compared with the follicular phase in the C group only. The serum levels of IGF binding protein 3, total cholesterol, LDL-C and HDL-C were similar in both groups during the two stages of the menstrual cycle. Triglyceride levels decreased in the luteal phase vs. the follicular phase only in the C group.

A correlation analysis revealed a positive relationship between BMI and hs-CRP levels in the follicular phase and the luteal phase in adolescents with Type 1 diabetes, but not in those in the C group (see Supporting Information). Luteal

hs-CRP levels were inversely correlated with IFG-1 levels ($r = -0.4$, $P = 0.01$). Estimated glucose disposal rate, insulin dose and HbA_{1c} did not exhibit a significant correlation with hs-CRP or with IGF-1 levels.

A regression analysis revealed that Type 1 diabetes was a significant factor affecting follicular hs-CRP levels, even after adjustment for BMI (Table 3, Model 1). In the case of luteal hs-CRP levels, BMI was a significant factor affecting the level of this inflammatory marker in adolescents with Type 1 diabetes (Model 2) but not adolescents in the

Table 3 Regression analysis evaluating factors associated with hs-CRP and IGF-1 levels

	Follicular Phase			Luteal Phase		
	β	<i>P</i>	ANOVA <i>P</i>	β	<i>P</i>	ANOVA <i>P</i>
Model 1: hs-CRP in all adolescents			0.002			0.002
Type 1 diabetes	0.87	0.001			ns	
BMI		ns		0.25	0.002	
Ovulation					ns	
Model 2: hs-CRP in adolescents with T1D			0.048			0.006
HbA _{1c}		ns			ns	
BMI	0.22	0.017		0.4	0.002	
Ovulation					ns	
Model 3: hs-CRP in Control adolescents			ns			ns
BMI						
Ovulation						
Model 4: IGF-1 in all adolescents			0.001			< 0.0001
Type 1 diabetes	-46.6	< 0.0001		-52.6	< 0.0001	
BMI		ns			ns	
Ovulation		ns			ns	

Model 1 demonstrates the effect of Type 1 diabetes, BMI and the presence of ovulation on follicular and luteal usCRP levels. Model 2 presents the effect of HbA_{1c}, BMI and ovulation on follicular and luteal usCRP levels in adolescents with Type 1 diabetes. Model 3 presents the effect of BMI and ovulation on follicular and luteal hs-CRP levels in control adolescents. Model 4 presents the effect of Type 1 diabetes, BMI and the presence of ovulation on follicular and luteal IGF-1 levels. ANOVA *P*: *P*-value of the *F*-test of the ANOVA of the regression analysis; β , Non-standardized β value of regression is shown.

C group (Model 3). In adolescents with Type 1 diabetes, the only significant factor affecting hs-CRP in the follicular phase and the luteal phase was BMI (Model 2); the remaining variables (insulin sensitivity, gynaecological age, the presence of ovulation, duration of diabetes, HbA_{1c} levels and daily insulin dose) were not associated with hs-CRP in either phase of the menstrual cycle. No clinical factors were associated with hs-CRP levels in the C group. The presence of Type 1 diabetes was associated with IGF-1 levels in the follicular phase and the luteal phase, even after adjustment for BMI (Model 4). Neither metabolic control nor BMI was associated with IGF-1 levels in adolescents with Type 1 diabetes (data not shown).

Discussion

This study demonstrated that young women with Type 1 diabetes exhibit elevated hs-CRP levels during both phases of the menstrual cycle, which becomes more evident during the luteal phase with Type 1 diabetes. A significant proportion of overweight adolescents with Type 1 diabetes exhibited hs-CRP levels during the menstrual cycle that have been associated with high cardiovascular risk. In addition, we observed that compared with controls, adolescents with Type 1 diabetes exhibited lower IGF-1 levels that did not increase during the luteal phase. However, the lipid profile did not change significantly during the menstrual cycle in these patients.

Adolescents with Type 1 diabetes exhibited higher hs-CRP levels compared with controls throughout the menstrual cycle, with a significant elevation in the luteal phase in excess of the already increased basal levels that were present in the

follicular phase. Moreover, 37% of the adolescents with Type 1 diabetes exhibited hs-CRP levels during the luteal phase that have been associated with cardiovascular events. This proportion corresponds to twice the prevalence observed in the control group and four-fold the prevalence previously reported in healthy adults [27].

Higher hs-CRP levels were observed in overweight adolescents with Type 1 diabetes compared with lean adolescents with Type 1 diabetes and controls throughout the menstrual cycle. Half of the overweight patients with Type 1 diabetes exhibited hs-CRP levels in the luteal phase that have been associated with a high cardiovascular risk. Previously, studies that evaluated hs-CRP levels in adults with Type 1 diabetes reported that elevations of this inflammatory marker were observed in overweight individuals but not in lean individuals with Type 1 diabetes [28]. We hypothesize that a subclinical inflammatory condition may be observed in patients with Type 1 diabetes and is exacerbated during the menstrual cycle, especially in overweight women, which is in agreement with the lower life expectancy observed in overweight women with Type 1 diabetes [9].

We observed that young adolescents with Type 1 diabetes exhibit lower IGF-1 levels in both phases of the menstrual cycle, and these concentrations did not increase during the luteal phase, as was observed in control adolescents. Previous studies evaluating IGF-1 levels in the menstrual cycle of adolescents without diabetes have demonstrated that a physiological elevation of this growth factor occurs in the luteal phase relative to the follicular phase [19]. Decreased IGF-1 levels have been associated with kidney disease in women with Type 1 diabetes [17] and with insulin resistance, an adverse metabolic profile and a higher risk of

cardiovascular disease or death in the general adult population in some studies [18,29]. Therefore, the decreased IGF-1 levels that were present in our adolescents with Type 1 diabetes and that do not exhibit a physiological elevation during the luteal phase may be part of a constellation of abnormal metabolic elements that are present in women with Type 1 diabetes. In agreement with this concept, a negative correlation between luteal IGF-1 and hs-CRP levels was observed in our Type 1 diabetes patients.

Lipid levels were not affected by the menstrual cycle. We observed mild fluctuations in triglyceride levels during the luteal phase in the control group that were not observed in the adolescents with Type 1 diabetes. Consequently, our data suggest that the menstrual cycle does not significantly affect lipid levels in adolescents with Type 1 diabetes.

The main limitations of this study were that only one menstrual cycle was studied and that the adolescents who were studied had a maturing gonadal axis. Therefore, these data need to be replicated in adult women with Type 1 diabetes.

In summary, adolescent women with Type 1 diabetes exhibit elevated hs-CRP levels that become further increased during the luteal phase, particularly in overweight patients. Type 1 diabetes diminishes the natural elevation in IGF-1 levels observed in the luteal phase of controls. The association between elevated hs-CRP and decreased IGF-1 levels during the luteal phase may represent a mechanism that leads to metabolic complications in women with Type 1 diabetes. Excess weight may exacerbate these abnormalities and may represent an important modifiable element in the prevention of cardiovascular disease in women with Type 1 diabetes.

Funding sources

This work was funded by the Fondo Nacional de Ciencia y Tecnología (Fondecyt Grant 1100123) given by the Chilean Government.

Competing interests

None declared.

Acknowledgements

We are grateful to Mrs Alejandra Avila for excellent nursing care and to all the adolescents who participated in the study.

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Supporting Information

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

Table S1. Correlation analysis between clinical parameters and hs-CRP, growth factors and lipid levels.