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Acylation Stimulating Protein Increases During the Luteal Phase of the Menstrual Cycle and Correlates with Increased Progesterone levels

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Abstract:

Objective: The menstrual cycle represents a continuous state of change in terms of female sex steroid environment which are believed to affect fat storage and distribution in women. Progesterone is linked to increased fat storage while estrogen exerts anti-lipogenic effects. In this study, we investigate variations in the potent lipogenic factor, the acylation stimulating protein (ASP), and examine its association with hormonal alterations across the menstrual cycle.

Methods and design: Nineteen non-obese women with regular menstrual cycles were investigated longitudinally during the follicular, ovulatory and mid-luteal phases of the cycle. ASP, LH, FSH, progesterone, estradiol, insulin, lipid profile and apoproteins were measured and compared between different phases of the cycle.

Results: Repeated measures analysis-of-varience showed that ASP levels changed significantly throughout the menstrual cycle. Interestingly, these changes followed a similar pattern as variations in progesterone levels across the cycle as no significant change in ASP levels was seen across the follicular phases of the cycle, followed by a marked increase in the mid-luteal phase. Importantly, this increase in ASP levels correlated positively with progesterone levels normally elevated in the mid-luteal phase. No significant association of ASP levels was seen with estrogen or any other measured female hormone. Multiple regression analysis including BMI, age and lipid profile measures showed that progesterone was the only significant predictor of ASP levels in this study.

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Conclusion: Our findings suggest that ASP production is increased in the luteal phase associated with high progesterone levels, which may contribute to enhanced fat storage in women.

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Introduction:

Acylation stimulating protein (ASP) is an adipokine that was shown to affect lipid metabolism in humans and mice (1). It is a 8.9 kDa autocrine hormone produced through the alternate complement pathway by the interaction of complement factor C3 with factor B and factor D (also called adipsin), which results in the formation of C3a-des-Arg, also called ASP. *In vitro,* ASP increases triglyceride synthesis and storage in adipocytes through activation of diacylglycerol acyltransferase the rate limiting enzyme in triglyceride synthesis and by stimulating glucose uptake (2, 3). The effect of ASP on fat storage is supported by *in vivo* data showing that ASP administration increases triglyceride clearance in mice and that ASP-deficient (C3-/-) mice exhibit delayed postprandial lipid clearance and reduced adipose tissue depots (4-7). In humans, ASP released from adipose tissue increased postprandially in coordination with triglyceride clearance (8, 9). ASP levels decreased during fasting and after weight loss (10, 11), and increased in obesity (12) and dyslipidemic disorders (13, 14).

It is well established that fat storage and lipid metabolism are affected by hormonal changes in humans (15). Substantial amount of evidence indicates that sex steroids play a role in fat tissue regulation and distribution (15 -17). Differences in fat distribution and lipid profile exist between males and females of reproductive age. In females, these changes coincide with onset of ovarian production of estrogen and progesterone during puberty (18- 20) and with cessation of hormone production during menopause (21-23).

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Premenopausal women typically demonstrate subcutaneous lower body fat (gynoid) distribution. Marked changes in their lipid metabolism occur during pregnancy and in reproductive disorders in response to hormonal changes (16, 17, 25).

The menstrual cycle represents a continuous state of change in terms of female sex steroid environment. In an average "28 day" female cycle, day one indicates the start of menstruation where estrogen and progesterone released by the female follicles remain low (the follicular phase) until day 12 when an estrogen surge (pre-ovulatory phase) is followed by the release of lutinizing hormone (LH) from the pituitary gland. LH surge, along with release of follicular stimulating hormone (FSH) around day 14, mark the onset of ovulation. Post ovulation (luteal phase), progesterone is secreted from the corpus luteum and starts to rise substantially in blood after day 14 and remains elevated along the luteal phase with moderate elevations in estrogen levels spanning the period of day 14 to day 28. The decline in progesterone levels causes the onset of menstruation marked as day 1 of menses (26-27). Abundant *in vitro* and *in vivo* evidence show that progesterone is mainly linked to increased fat storage and adipocyte differentiation (28-30). Estrogen on the other hand, exhibits antilipolytic effects such as inhibition of lipoprotein lipase activity and reducing fat storage in adipocytes (31, 33).

The effects of menstrual cycle hormonal changes on factors linked to lipid metabolism are continuously investigated. These include studies on insulin and adipokines such as leptin and adiponectin. Leptin levels were shown to increase significantly during the luteal phase of the menstrual cycle (34-36). Adiponectin levels showed no significant

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change during the menstrual cycle (37). Several studies showed no significant differences in insulin levels between the different phases of the menstrual cycle and no correlations were found between insulin and estradiol or progesterone levels in serum (38, 39).

Limited evidence is available considering sex hormone effects on ASP levels or function. Some studies showed that ASP levels were higher in obese females compared to obese males (12). It was found to bind with higher affinity to subcutaneous fat cells freshly prepared from females than male subcutaneous and omental fat cells (40). Recently, ASP was found to markedly increase in pregnancy in association with high lipid levels (41), and was found to increase in females with polycystic ovary syndrome associated with dyslipidemia, independent of obesity (42).

In this study we aim to further investigate if female hormone changes *in vivo* are associated with changes in plasma ASP levels in women. For this purpose, we study the variation in ASP levels and their correlation with hormonal changes during different phases of the menstrual cycle including follicular, ovulatory and luteal phases. This is the first study to investigate physiological variations in ASP levels with regards to major hormonal alterations in healthy women.

Materials and Methods:

Subjects

Nineteen women aged 20- 24 years old were enrolled in this longitudinal study. All of them were single students and had regular menstrual cycles of an average of 28 days. They were non-obese, never pregnant or have taken oral contraceptives. All participants

were non smokers and non alcohol consumers. They were not receiving any medications and had no disorders that may affect hormonal function such as polycystic ovary syndrome. Women with menstrual abnormalities and abnormally low hormone levels that suggest anovulatory cycles were removed from the study. The subjects for this study come from a considerably homogeneous Omani population, which is characterized by large tribes of extended families with similar lifestyles.

The study was approved by the Sultan Qaboos University ethical committee. Informed consent forms were filled out by all the women participating in the study.

Blood collection:

Fasting blood samples were collected by venipuncture at days 1, 7, 12, 14 and 21days. These times were chosen to represent different phases of an average "28 day" menstrual cycle. Days 1 and 7 marked the early follicular (EF) and mid follicular (MF) phases respectively. Day 12 marked the late follicular (LF) phase (i.e. pre-ovulatory phase). Day 14 marked the ovulatory (OV) phase. The mid-luteal phase (ML) was represented by Day 21. Female hormone levels of all samples were measured to verify the phase at which samples were taken.

Analysis:

Venous blood samples were collected in plain tubes with no anti-coagulant for serum lipid and hormone measurements and EDTA tubes for ASP measurements. The samples were put on ice and immediately centrifuged. The serum and plasma were stored at –80^{o}C until analysis.

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Lipid profile measurements

Samples were analyzed for the fasting lipid profile at different phases of the menstrual cycle including: triglycerides (TG), total cholesterol (T-CHOL), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). Analysis was performed using automated Roche Integra 800's analyzer, Switzerland-Germany. ApoA1 and apoB were measured on the Beckman Coulter Immage 800 Immunochemistry System, U.S.A in the central routine laboratory of the University hospital

Hormone measurements:

Hormone levels including estrogen, progesterone, lutinizing hormone (LH), follicular stimulating hormone (FSH) and insulin were measured at different phases of the menstrual cycle using an automated clinical chemistry analyzer Beckman Coulter ACCESS 2 Immunoassay System, analyzer , Chaska, Minnesota (U.S.A) in the central routine laboratory of the University hospital

Sandwich ELISA for ASP determination:

ASP levels at the different phases of the menstrual cycle were measured using a sensitive in-house ELISA assay (8). The human plasma EDTA samples, controls, and standard ASP were pretreated with polyethylene glycol PEG 8000 to precipitate C3 thus preventing any artefactual generation of ASP as described in detail previously (8).

Statistical analysis:

Results were expressed as mean ± SEM (standard error of the mean) of variables at each phase of the menstrual cycle. Within-group differences at different phases were assessed

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by one way repeated-measures analysis of variance (RM ANOVA). Once it was determined that differences exist among the means, pair-wise comparisons were performed by paired-T test to compare the means of variables at the different phases. The Wilcoxon signed-rank test was performed for pair-wise comparisons of variables with skewed distributions. Correlations between plasma ASP levels and the all measured variables in the different phases of the cycle were examined by bivariate analysis using *Pearson coefficients. Spearman correlation* coefficients were used for parameters with skewed distributions. Stepwise multiple linear regression analysis was performed to determine the factors that significantly associated with variations in ASP levels. Logarithmic transformations were applied for parameters that did not follow a normal distribution. Significance was set at $p< 0.05$. Analysis was computer assisted using SPSS software.

Results:

The anthropometric measures for the women are shown in **Table 1**. Fasting ASP, progesterone, estradiol, progesterone/estrogen ratio and insulin levels are shown in **Figures 1 and 2**. LH, FSH, insulin, TG, T-CHOL, LDL-C, HDL-C, apoA1 and apoB levels are shown in **Table 2**. All parameters were measured at the early follicular (EF), mid follicular (MF) late follicular (LF), ovulatory (OV) and mid-luteal (ML) phases.

Hormone level changes during the menstrual cycle

Figure 1 and Table 2 showed fluctuations in estradiol, progesterone, LH and FSH levels as expected in a typical regular 28 day menstrual cycle of healthy women. Repeated measures ANOVA showed a significant overall change during the menstrual cycle for

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LH, FSH, progesterone and estradiol (P< 0.0001). Pairwise comparisons showed a significant surge in LH and FSH in the OV phase (day 14) **(Table 2)**. Progesterone remained low in the EF, MF, LF (days 1, 7 and 12) and OV (day 14) phases then markedly increased (P< 0.0001) in the ML phase **(Figure 1**). Estradiol showed a pre-ovulatory surge in the LF phase (pre-ovulatory) (day 12) (P< 0.0001) which slightly decreased during the OV phase (P=NS) and continued to increase in the ML phase along with the increase in progesterone levels.

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Plasma ASP and insulin level changes during the menstrual cycle

Figure 2 shows that fasting ASP levels change significantly during the different phases of the menstrual cycle (RM ANOVA, P< 0.05). Pairwise comparisons showed that at the onset of menses at day 1 (EF phase), ASP levels were (18.7 ± 1.9 nM). No significant change was seen in the MF (17.3 ± 1.8 nM) and LF (14.5 ± 1.5 nM) phases. A significant increase from the LF phase occurred in the OV phase (18.6 ± 2.3 nM) (P< 0.05) followed by a 2-fold increase during the ML phase (29.6 ± 6.6 nM) compared to the LF phase (P< 0.02). The luteal increase in plasma ASP levels coincided with the increase in progesterone levels and progesterone/estrogen ratio (P: E) in the luteal (ML) phase **Figure 2B.**

In contrast, fasting insulin concentrations did not show any significant change during the different phases (RM ANOVA, P= NS) and pairwise comparisons of insulin levels at different phases of the cycle were all non significant (Figure 3).

Lipid profile and apoprotein levels during the menstrual cycle

Table 2 shows the fluctuations in TG, T-CHOL, LDL-C, HDL-C, apoA and apoB during the different phases of the menstrual cycle. Minor but significant changes were shown during the different phases of the cycle. Significant overall changes during the cycle were shown for LDL-C (RM-ANOVA, P< 0.001) and apoB levels (RM-ANOVA, P< 0.005). Pairwise comparisons showed decreases in LDL-C and apoB levels at the LF and ML phases compared to EF phase. TG and VLDL levels were shown to decrease during the OV phase compared to the EF phase **(Table 2)**. No significant change was seen for HDL-C or apoA1 levels throughout the different phases of the cycle.

Correlation of ASP with hormones and lipids during the mid luteal phase (ML)

Increased ASP levels in the ML phase showed a significant positive correlation with progesterone levels (r = 0.562, P =0.012) and with the progesterone/estrogen ratio (r = 0.600, P = 0.007). ASP also positively correlated with BMI (r = 0.471, P = 0.042). A multiple regression model was set in order to determine factors that predicted ASP levels in the ML phase. Plasma ASP (log transformed) was set as the dependent variable. Hormone, lipid, and subject characteristics were entered into the model as predictors including progesterone, estradiol, LH, FSH, insulin, TG, LDL-C, HDL-C and VLDL-C and apoproteins, in addition to BMI and age. The results showed that progesterone significantly associated with ASP levels (ß= 0.516, P = 0.028) and entered this model as the only significant predictor. BMI was excluded from the model as non-significant in the presence of progesterone. Progesterone levels significantly accounted for 22 % variation in fasting (as determined by adjusted R^2). All other measured hormones, lipid parameters and age were excluded from the model as non significant. This correlation was found in

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the ML phase and no correlation was found between ASP levels and corresponding hormone and lipid parameters in other phases of the menstrual cycle.

In contrast, although insulin levels in ML phase correlated positively with BMI ($r = 0.44$, $P < 0.05$), it showed no significant correlation with progesterone, estrogen levels or progesterone/estrogen ratio throughout the menstrual cycle phases.

Discussion

In this study we showed expected reproductive hormonal alterations during different phases of an average 28 day menstrual cycle.

The key finding in this study was that ASP levels changed significantly across the phases of the menstrual cycle showing a similar pattern of change as progesterone levels and (progesterone/estrogen ratio). ASP levels were low across the follicular phases and increased significantly in the OV phase followed by a marked (2-fold) increase in the ML phase. Importantly, this increase in ASP levels correlated positively with the normally elevated progesterone levels in the mid-luteal phase, whereas no significant association of ASP was seen with estrogen levels or any other reproductive hormone. The correlation of ASP levels with the progesterone/estrogen ratio was similar to the correlation of ASP with progesterone levels alone further indicating that estrogen levels had no association with plasma ASP levels. ASP levels correlated with BMI of the females in this study which is consistent with previous findings in Omani women (41), however a stronger correlation was found for ASP with progesterone levels. Multiple regression analysis

including all measured variables in the study showed that progesterone was the only significant predictor of ASP levels and excluded BMI as non significant from the regression model. This may be due to the fact that all females included in this study were non-obese, highlighting the association between ASP and progesterone levels independent of weight gain. The rest of the female hormones, insulin and lipid parameters measured in the study were also excluded as non-significant. Although insulin, which is a major fat storage factor, correlated positively with BMI ($P< 0.05$), it did not show any significant changes during the cycle and did not correlate with progesterone or any of the reproductive hormones in this study. This agrees with several findings in the literature where no significant changes in insulin levels were seen across the cycle in healthy females (38, 39).

Recent studies support our findings of increased ASP levels in the luteal phase of the menstrual cycle. These studies, although not in plasma, showed that C3 and factor B (precursors of ASP) are produced in the human endometrium in a cyclic specific manner. It was found that luteal phase endometrium synthesize complement C3 de novo, whereas proliferative endometrium produces little or no C3. Likewise factor B, which is critical to the activation of the complement altenative pathway “which leads to ASP production”, has been shown to be present only in the luteal phase endometrium and not in the follicular phase. Also, factor B was found to be synthesized in the endometrial cells of patients treated with exogenous progesterone therapy. Therefore, these precursors are found in the presence of high progesterone/estrogen ratio characterizing the luteal phase and not in the follicular phase which is marked with low progesterone and estrogen levels (43, 44).

Although progesterone is suggested exert lipogenic effects in females, controversy still exists as to whether it mediates these effects directly or indirectly. Some studies suggested direct anabolic effects or by effecting on insulin action, others suggest an action through specific transcription factors (28-31, 45). This study is of particular interest as it is the first study to show a link between levels of ASP, which is a potent fat storage factor, and progesterone which is known for its lipogenic effects in females. The significant positive association between luteal phase ASP and progesterone, as the main predictor of ASP levels in this study, may suggest a role for progesterone on ASP production and its lipogenic effects in women.

No correlation was seen between ASP levels and the lipid profile of these healthy women, however, small but highly significant decreases were seen in the second half of the cycle which is consistent with previous studies that showed that lipid levels decrease during the luteal phase of the cycle suggesting to explain anti-atherogenic effects seen in females of reproductive age (46-48). This may be associated with hormonal effects that enhance fat clearance from the circulation during that phase.

This is the first study showing variations in ASP levels during the menstrual cycle and its correlation with progesterone levels, and may therefore contribute to further understanding of the mechanism of ASP regulation regarding fat storage and distribution in women. These findings also lend to the importance that menstrual cycle phases should be taken into consideration when performing studies on ASP and lipid levels in women of reproductive age.

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Figure Legends:

Figure 1: **(A)** Progesterone and **(B)** Estradiol levels at the EF: early follicular, MF: mid follicular, LF: late follicular, OV: ovulatory and ML: mid luteal phases. Data at each phase is shown as the mean ± SEM (n=19). Pairwise comparisons of the means were made by paired sample T-Test. The Wilcoxon signed-rank test was used when values of progesterone were skewed in the EF and OV phases and estrogen in the EF phase. Progesterone: *** P< 0.001: ML phase compared to all phases. ** P< 0.05: OV phase compared to the EF phase. Estradiol: *** P< 0.001 in ML, OV and LF compared to EF and MF phases. *P< 0.02 MF compared to the EF phase.

Figure 2: **(A)** ASP levels at the EF: early follicular, MF: mid follicular, LF: late follicular, OV: ovulatory and ML: mid luteal phases. Data at each point (phase) is shown as mean ± SEM (n=19). Pairwise comparisons of the means were made by paired sample T-Test. The Wilcoxon signed-rank test was used when ASP levels were skewed in the ML phase.** P< 0.02: ML higher than LF and MF phases. * P< 0.05: LF lower than OV phase **(B)** Corresponding levels of progesterone to estrogen ratio in the different phases are shown. *** P< 0.001 ML phase compared to all phases. ****** P < 0.05: LF compared to EF and P< 0.001 compared to the MF phase, *P< 0.001: MF compared to EF phase.

Figure 3: Insulin levels at different phases of the menstrual cycle: EF: early follicular, MF: mid follicular, LF: late follicular, OV: ovulatory and ML: mid luteal. Data at each point (phase) is shown as mean ± SEM (n=19). Pairwise comparisons of the means were made by paired sample T-Test. No significant differences were found between any of the means in the different phases of the cycle.

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List of Abbreviations

ASP: Acylation Stimulating Protein, EF: early follicular, MF: mid follicular, LF: late follicular, OV: ovulatory, ML: mid luteal, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, T-CHOL: Total cholesterol, TG: Triglyceride, BMI: body mass index. RM-ANOVA: repeated measures analysis of varience, P/E ratio: progesterone/estradiol ratio

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References

1. Sniderman AD, Maslowska M, Cianflone K. Of mice and men (and women) and the acylation-stimulating protein pathway. Currunt Opinion on Lipidology 2000 **11** 291–296.
2. Cianflone K, Roncari DAK, Maslowska M, Baldo A, Forden J, Sniderman AD The adipsin/acylation stimulating protein system in human adipocytes: regulation of triacylglycerol synthesis. Biochemistry 1994 **33** 9489–9495.
3. Cianflone K, Maslowska M. Differentiation induced production of ASP in human adipocytes. European Journal of Clinical Investigation 1995 **25** 817–825.
4. Murray I, Havel PJ, Sniderman AD, Cianflone K. Reduced body weight, adipose tissue, and leptin levels despite increased energy intake in female mice lacking acylation-stimulating protein. Endocrinology 2000 **141** 1041–1049.
5. Murray, I., Sniderman AD, Havel PJ, Cianflone K. Acylation stimulating protein (ASP) deficiency alters postprandial and adipose tissue metabolism in male mice. Journal of Biological Chemistry 1999 **274** 36219–36225.
6. Murray IA, Sniderman AD, Cianflone K. Enhanced triglyceride clearance with intraperitoneal human acylation stimulating protein (ASP) in C57Bl/6 mice. American Journal of Physiology Endocrinology and Metabolism 1999 **277** E474–E480.
7. Saleh J, Blevins JE, Havel PJ, Barrett JA, Gietzen DW, Cianflone K. Acylation stimulating protein (ASP) acute effects on postprandial lipemia and food intake in rodents. International Journal of Obesity and Related Metabolic Disorders 2001 **25** 705–713.

For Review Only

8. Saleh J, Summers LKM, Cianflone K, Fielding BA, Sniderman AD, and Frayn KN. Coordinated release of acylation stimulating protein (ASP) and triacylglycerol clearance by human adipose tissue in vivo in the postprandial period. Journal of Lipid Research 1998 **39** 884–891.
9. Cianflone K, Zakarian R, Couillard C, Delplanque B, Despres JP, Sniderman A Fasting acylation- stimulating protein is predictive of postprandial triglyceride clearance. Journal of Lipid Research 2004 **45** 124-131.
10. Cianflone K, Sniderman AD, Kalant D, Marliss EB, Gougeon R. Response of plasma ASP to a prolonged fast. International Journal of Obesity 1995 **19** 604–609.
11. Sniderman AD, Cianflone K, Eckel RH. Levels of acylation stimulating protein in obese women before and after moderate weight loss. International Journal of Obesity 1991 **15** 333–336.
12. Maslowska M, Vu H, Phelis S, Sniderman AD, Rhode BM, Blank D, Cianflone K Plasma acylation stimulating protein, adipsin and lipids in non-obese and obese populations. European Journal of Clinical Investigation 1999 **29** 679–686.
13. Cianflone K, Zhang XJ, Genest Jr J and Sniderman AD. Plasma acylation stimulating protein in coronary artery disease. Arteriosclerosis and Thrombosis Vascular Biology 1997 **17** 1239–1244.
14. Cianflone KM, Maslowska MH, Sniderman AD. Impaired response of fibroblasts from patients with hyperapobetalipoproteinemia to acylation-stimulating protein. Journal of Clinical Investigation 1990 **85** 722–730.

For Review Only

15. Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC, Sheffield-Moore M, Mauras N and Bowers CY. Endocrine Control of Body Composition in Infancy, Childhood, and Puberty. Endocrine Reviews. 2004 **26** (1) 114-146

16. Norton M, Buchanan TA, Kitzmiller J. Endocrine Pancreas and Maternal Meatbolism. In Maternal-Fetal Endocrinology, edn 2, pp 48-77. Eds. D Tulchinsky & A Brian Little, Philadelphia, Pennsylvania: W.B Saunders company; 1994.

17. Blackburn ST, Loper DL. Carbohydrate, Fat, and Protein Metabolism. In: Maternal, Fetal and Neonatal Physiology, A clinical Perspective. pp 583-613. Ed. T Eoyang, Philadelphia, Pennsylvania: W.B Saunders company. 1992.

18. de Ridder CM, Bruning PF, Zonderland ML, Thijssen JH, Bonfrer JM, Blankenstein MA, Huisveld IA and Erich WB. Body fat mass, body fat distribution, and plasma hormones in early puberty in females. Journal of Clinical Endocrinology and Metabolism 1990 **70** 888-893.

19. Chumlea WC, Roche AF, Siervogel RM, Knittle JL and Webb P. Adipocytes and adiposity in adults. American Journal of Clinical Nutrition 1981 **34** 1798-1803.

20. Chumlea WC, Knittle JL, Roche AF, Siervogel RM and Webb P. Size and number of adipocytes and measures of body fat in boys and girls 10 to 18 years of age. American Journal of Clinical Nutrition 1981 **34** 1791-1797.

21. Ley CJ, Lees B and Stevenson JC. Sex- and menopause-associated changes in body-fat distribution. American Journal of Clinical Nutrition 1992 **55** 950-954.

22. Tchernof A, Poehlman ET, Després JP. Body fat distribution, the menopause transition, and hormone replacement therapy. Diabetes Metabolism 2000 **26** (1) 12-20.

For Review Only

23. Changes in energy balance and body composition at menopause: a controlled longitudinal study. Annals of Internal Medicine 1995 **123** 673-675.

24. Berneis K, Rizzo M, Lazzarini V, Fruzzetti F, Carmina E. Atherogenic lipoprotein phenotype and low-density lipoproteins size and subclasses in women with polycystic ovary syndrome. Journal of Clinical Endocrinology and Metabolism. 2007 **92**(1) 186- 189.

25. Michel Ferin, Raphael Jewelewicz, and Michelle Warren. The Menstrual Cycle: Physiology, Reproductive Disorders, and Infertility. New England Journal of Medicine 1994 **330** (15) 1097.

26. Landgren BM, Unden, AL and Diczfalusy E. Hormonal Profile of the Cycle in 68 Normally Menstruating Women. Acta Endocrinology Copenhagen 1980 **94** 89–98.

27. Horton TJ, Miller EK, Glueck D, and Tench K. No effect of menstrual cycle phase on glucose kinetics and fuel oxidation during moderate-intensity exercise. American Journal of Physiology Endocrinoogy and Metabolism 2002 **282**: E752–E762

28. Lobo MJ, Remesar X, Alemany M. Effect of chronic intravenous injection of steroid hormones on body weight and composition of female rats. Biochemistry & Molecular Biology International 1993 **29** (2) 349-358.

29. Mendes AM, Madon RJ and Flint DJ. Effects of cortisol and progesterone on insulin binding and lipogenesis in adipocytes from normal and diabetic rats. Journal of Endocrinology 1985 **106** (2) 225-231.

30. Kalkhoff RK. Metabolic effects of progesterone. American Journal of Obstetreics and Gynecology 1982 **142** (6 Pt 2):735-738.

For Review Only

31. Homma H, Kurachi H, Nishio Y, Takeda T, Yamamoto T, Adachi K, Morishige K-i, Ohmichi M, Matsuzawa Y and Murata Y. Estrogen Suppresses Transcription of Lipoprotein Lipase Gene. *Existence of a unique estrogen* response *element on the lipoprotein lipase promoter*. Journal of Biological Chemistry 2000 **275** (15) 11404-11411.

32. Cooke PS, and Naaz A. Role of Estrogens in Adipocyte Development and Function. Experimental Biology and Medicine 2004 **229** 1127-1135

33. Cucinelli F, Paparella P, Soranna L, Barini A, Cinque B, Mancuso S, Lanzone A. Differential effect of transdermal estrogen plus progestagen replacement therapy on insulin metabolism in postmenopausal women: relation to their insulinemic secretion. European Journal of Endocrinology 1999 **140** (3) 215-223.

34. Hardie L, Trayhurn P, Abramovich D, Fowler P. Circulating leptin in women: a longitudinal study in the menstrual cycle and during pregnancy. Clinical Endocrinology 1997 **47** (1) 101-106.

35. Cella F, Giordano G, Cordera R. Serum leptin concentrations during the menstrual cycle in normal-weight women: effects of an oral triphasic estrogen-progestin medication European Journal of Endocrinology 2000 **142** (2) 174-178.

36. Ludwig M, Klein HH, Diedrich K and Ortmann O. Serum leptin concentrations throughout the menstrual cycle Archives of Gynecology and Obstetrics 2000 **263** (3) 99-101.

37. Kleiblová P, Springer D and Haluzík M The influence of hormonal changes during menstrual cycle on serum adiponectin concentrations in healthy women. Physiological Research 2006 **55** (6) 661-666.

For Review Only

38. Juul A, Scheike T, Pedersen AT, Main KM, Andersson AM, Pedersen LM and Skakkebaek NE. Changes in serum concentrations of growth hormone, insulin, insulin-like growth factor and insulin-like growth factor-binding proteins 1 and 3 and urinary growth hormone excretion during the menstrual cycle. Human Reproduction 1997 **12** (10) 2123-2128.

39. Riad-Gabriel M, Jinagouda SD, Sharma A, Boyadjian R and Saad M. Changes in plasma leptin during the menstrual cycle. European Journal of Endocrinology 1998 **139** 528–531.

40. Saleh J, Christou N, Cianflone K. Regional Specificity of ASP binding in human adipose tissue. American Journal of Physiology 1999 **276 (5):** E815- E821

41. Saleh J, Cianflone K, Chaudhary T, Al-Riyami H, Al-Abri Aand Bayoumi R. Increased Plasma Acylation-Stimulating Protein Correlates with Hyperlipidemia at Late Gestation. Obesity 2007 **15** 646–652.

42. Wu Y, Zhang J, Wen Y, Wang H, Zhang M, Cianflone K. Increased acylation-stimulating protein, C-reactive protein, and lipid levels in young women with polycystic ovary syndrome. Fertility and Sterility 2008 Epub ahead of print.

43. Hasty LA, Lambris JD, Lessey BA, Pruksananonda K, Lyttle CR. Hormonal regulation of complement components and receptors throughout the menstrual cycle. American Journal of Obstetric and Gynecology 1994 **170** (1 Pt 1) 168-175.

44. Hasty LA, Brockman WW, Lambris JD, Lyttle CR. Hormonal regulation of complement factor B in human endometrium. American Journal of Reproductive Immunology 1993 **30** (2) 363-367.

For Review Only

45. Lacasa D, Le Liepvre X, Ferre P, Dugail I. Progesterone stimulates adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression. potential mechanism for the lipogenic effect of progesterone in adipose tissue. Journal of Biological Chemistry 2001 **276** (15) 11512-11516.

46. Barnett JB, Woods MN, Lamon-Fava S, Schaefer EJ, McNamara JR, Spiegelman D, Hertzmark E, Goldin B, Longcope C and Gorbach SL. Plasma Lipid and Lipoprotein Levels during the Follicular and Luteal Phases of the Menstrual Cycle. Journal of Clinical Endocrinology and Metabolism 2004 **89** (2) 776-782.

47. Woods M, Schaefer EJ, Morrill A, Goldin BR, Longcope C, Dwyer JD, Gorbach SL. Effect of menstrual cycle phase on plasma lipids. Journal of Clinical Endocrinology and Metabolism 1987 **65** 321-323.

48. Muesing RA, Forman MR, Graubard BI, Beecher GR, Lanza E, McAdam PA, Campbell WS and Olson BR. Cyclic changes in lipoprotein and apolipoprotein levels during the menstrual cycle in healthy premenopausal women on a controlled diet. Journal of Clinical Endocrinology and Metabolism 1996 **81** 3599-3603.

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Table 1: Anthropometric measures

	Age (years)	BMI Kg/m^2	Waist/Hip ratio	Menstrual cycle length days
Mean ± SEM	**22.5 ± 0.56**	**21.5 ± 0.74**	**0.74 ± 0.01**	**28.7 ± 1.4**

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Table2: Hormone and lipid profile levels shown at different phases of the menstrual cycle of healthy women.

Variable (n= 19)	EF	MF	LF	OV	ML
LH (IU/L)	4.0 ± 0.4	6.0 ± 0.5	20.2 ± 4.6 [1]	23.9 ± 5.3 [1]	4.5 ± 0.69
FSH (IU/L)	7.0 ± 0.67	7.1 ± 0.4	7.3 ± 0.79	9.2 ± 0.73 [2]	3.5 ± 0.35
TG (mM)	0.85 ± 0.05	0.85 ± 0.07	0.76 ± 0.06 [3]	0.68 ± 0.04[4]	0.75 ± 0.07
T-CHOL (mM)	5.2 ± 0.16	5.2 ± 0.15	4.9 ± 0.18	4.97 ±0.18	5.1 ± 0.13
LDL-C (mM)	3.3 ± 0.18	3.3 ± 0.18	3.0 ± 0.18[5]	3.15± 0.17	2.98 ± 0.17[5]
VLDL-C (mM)	0.38 ± 0.02	0.38 ± 0.03	0.34 ± 0.03	0.31± 0.02[6]	0.33 ± 0.03
HDL-C (mM)	1.7 ± 0.08	1.8 ± 0.09	1.8 ± 0.09	1.8 ± 0.11	1.87 ± 0.1
apoAI (g/L)	1.56 ± 0.06	1.6 ± 0.07	1.57 ± 0.07	1.59 ± 0.07	1.58 ± 0.05
apoB (g/L)	0.94 ± 0.04	0.9 ± 0.04	0.80 ± 0.03[7]	0.88 ± 0.03	0.81 ± 0.03[8]

Results are shown as mean ± standard error of the mean. Abbreviations: LH: leutinizing hormone, FSH: follicular stimulating hormone, TG: triglycerides, LDL: low density lipoprotein, VLDL: very low density lipoprotein, HDL; high density lipoprotein, T-CHOL: total cholesterol, apoA: apoprotein A, apoB: apoprotein B, GLU: glucose, EF: Early follicular, MF: Mid follicular, LF: Late follicular, OV: Ovualtory, ML: mid luteal. Significance is indicated by superscript numbers as shown below:

LH: [1] OV and [1] LF higher than EF, MF and ML phases (P< 0.005).
FSH: [2] OV higher than EF, MF (P< 0.02) and ML phases (P< 0.001).
TG: [3] LF lower than MF (P< 0.05). [4] OV lower than EF (P< 0.01) and MF phases (P< 0.05).
LDL-C: [5] LF and [5]ML lower than EF (P< 0.02) and MF phases (P≤ 0.01).
VLDL-C: [6] OV lower than EF phase (P< 0.05).
apoB: [7] LF lower than EF and MF phases (P< 0.05). [8] ML lower than EF and MF phases (P< 0.01).

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Table 3 Bivariate correlation between ASP levels in the ML: mid-luteal phase with anthropometric, hormone and lipid profile levels. *(* P* = Spearman correlation significant, two tailed).

n=19	ASP	Sig (2-tailed)
BMI	r = 0.52 *	*P = 0.019*
Age	r = -0.005	*P= 0.98*
Progesterone	r = 0.48 *	*P= 0.029*
Estradiol	r = 0.261	*P= 0.25*
P/E ratio	r = 0.49 *	*P= 0.023*
LH	r = -0.023	*P= 0.92*
FSH	r = -0.358	*P= 0.11*
Insulin	r = 0.038	*P= 0.87*
TG	r = -0.88	*P= 0.71*
T-CHOL	r = 0.27	*P= 0.26*
VLDL-C	r = -0.067	*P= 0.77*
LDL-C	r = 0.32	*P= 0.16*
HDL-C	r = 0.10	*P= 0.66*
apoB	r = 0.421	*P= 0.58*
apoA1	r = -0.03	*P= 0.88*

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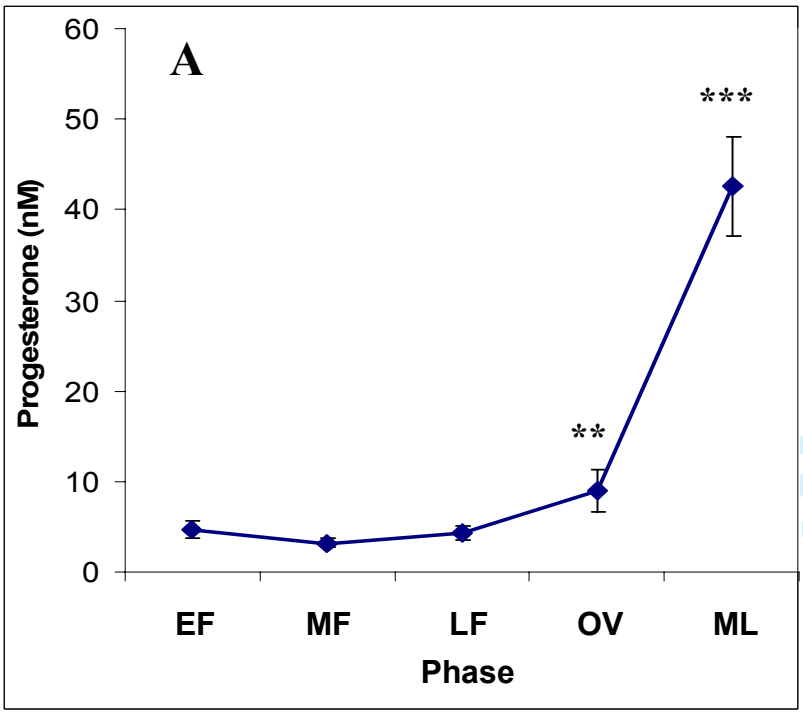
Figure 1



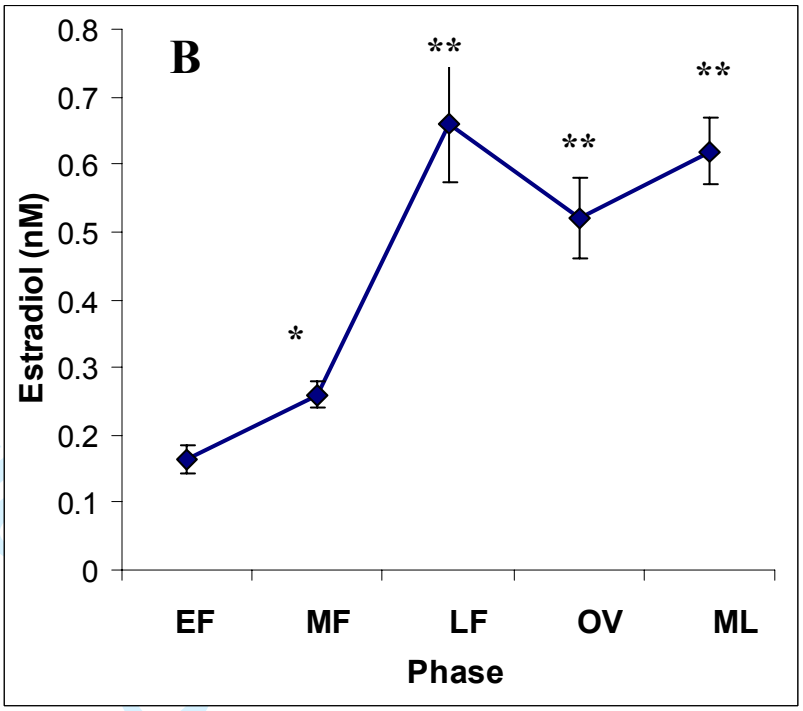


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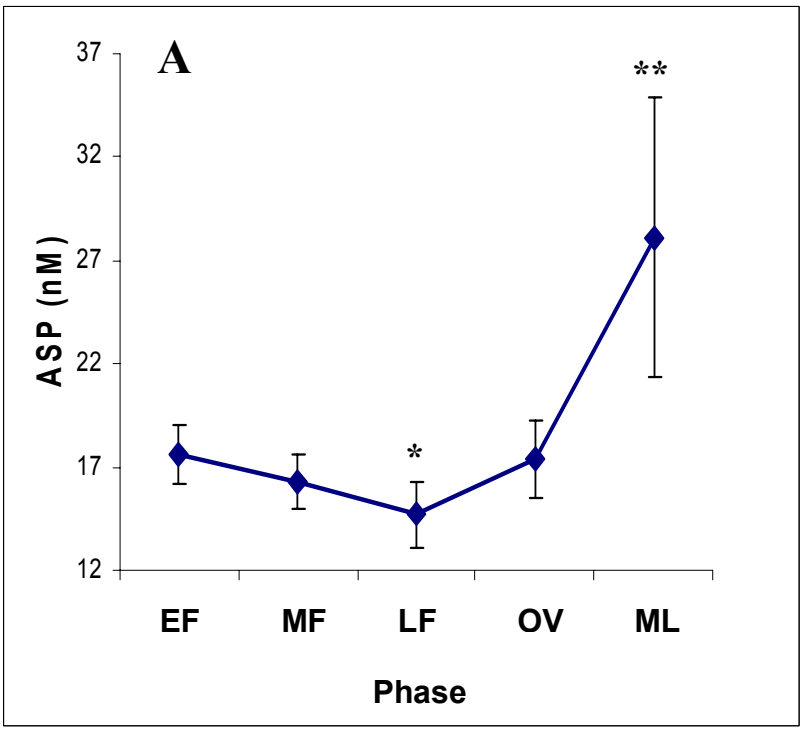
Figure 2


A
ASP (nM)
37
32
27
22
17
12
*
**
EF
MF
LF
OV
ML
Phase

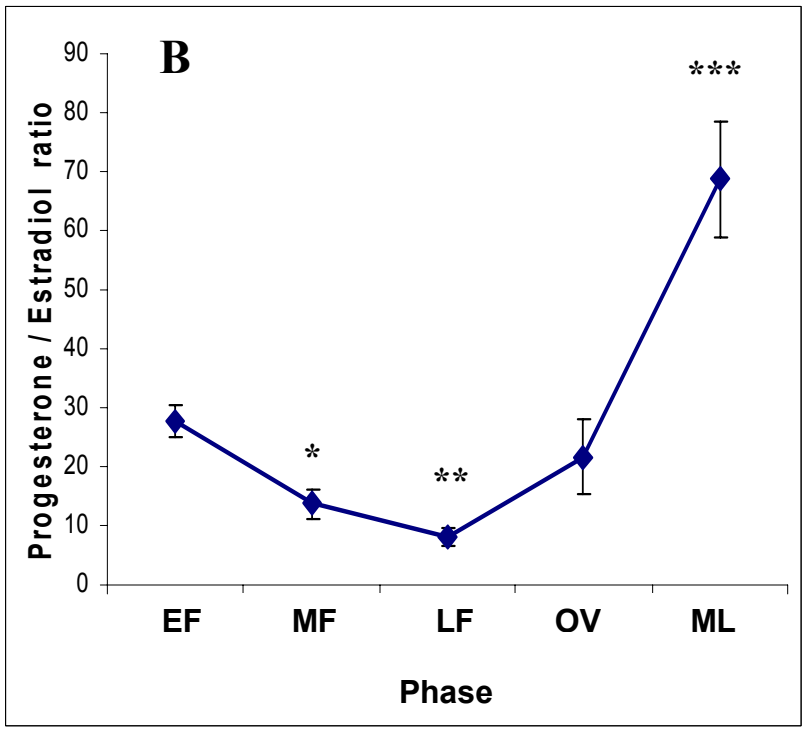
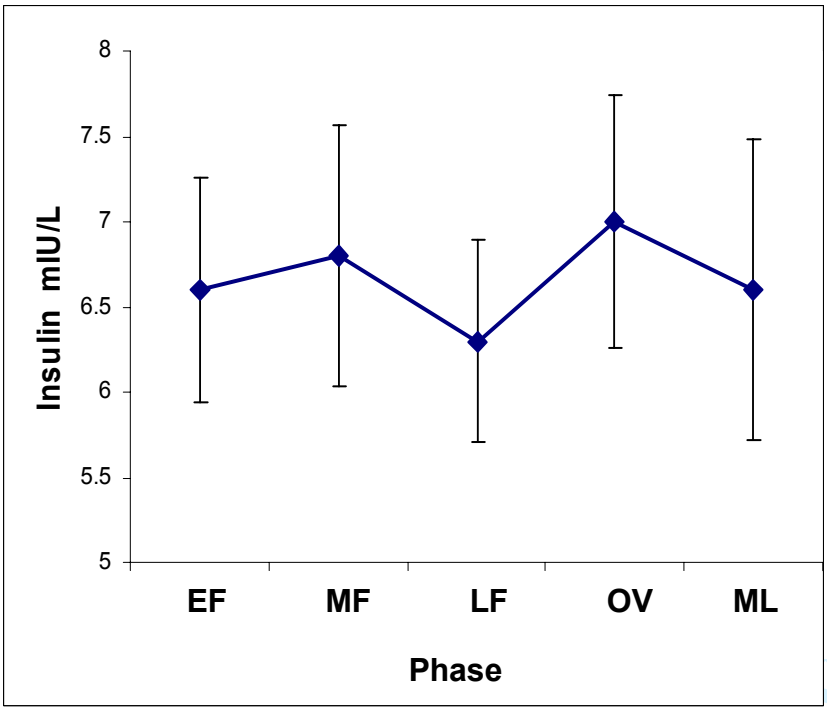

B
Progesterone / Estradiol ratio
90
80
70
60
50
40
30
20
10
0
*
**
***
EF
MF
LF
OV
ML
Phase

Figure 3



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