Published in final edited form as:

Am J Hum Biol. 2014 ; 26(1): 95–98. doi:10.1002/ajhb.22465.

Ovarian Function in Samoan Women Shows Stronger Association with Signals of Energy Metabolism than Fat Reserves

Diana S. Sherry,

Department of Human Evolutionary Biology, Harvard University, Cambridge, MA 02138

Stephen T. McGarvey,

Department of Epidemiology and International Health Institute, Brown University, Providence, RI 02912

Margaret L. Sesepasara, and

Department of Public Health, American Samoa, Pago, Pago American Samoa

Peter T. Ellison

Department of Human Evolutionary Biology, Harvard University, Cambridge, MA 02138

Diana S. Sherry: dsherry@fas.harvard.edu; Stephen T. McGarvey: Stephen_McGarvey@brown.edu; Margaret L. Sesepasara: misepasara@yahoo.com; Peter T. Ellison: pellison@fas.harvard.edu

Abstract

Objectives—The relative influence of prominent energetic hormones such as insulin and leptin on ovarian steroid production has yet to be determined and demonstrated consistently *in vivo*. This study reports preliminary findings on the relationship between insulin, leptin, and estradiol, a major ovarian steroid, in a sample of Samoan women.

Methods—Participants were 34 regularly cycling, premenopausal women in the follicular phase of their menstrual cycle with indicators of normal glucose tolerance. Fasting serum samples provided one-time, cross-sectional measures of glucose, insulin, leptin, and estradiol. Main statistical analyses consisted of Student's t-tests, used to determine significant differences in mean estradiol level between contrasting groups of insulin and leptin.

Results—Relatively high insulin levels within the normal range of variation showed a positive association with estradiol levels whereas relatively high leptin levels did not. The relationship between insulin and estradiol appeared to conform to a step-like categorical function -- with the highest insulin levels exerting the greatest positive effect -- rather than a dose-response linear function.

Conclusions—The current study adds to the growing evidence that peripheral regulation of ovarian function likely involves permissive signals that emphasize a state of energy surplus,

Correspondence to: Diana S. Sherry, dsherry@fas.harvard.edu.

Declaration of interests: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

related primarily to energy metabolism rather than energy reserves, and warrant more extensive study.

Keywords

ovarian function; estradiol; insulin; leptin; energetics; Samoa

In recent decades, reproductive ecologists have established that ovarian function operates along a graded response continuum in relation to female energetic condition (Ellison 2001). Ovarian sensitivity likely includes a peripheral mechanism regulating ovarian steroid production directly at the level of the ovary independent of gonadotropin release, yet the precise physiological pathways involved remain poorly understood.

Two main models have been advanced to explain the general relationship between ovarian function and female energetic condition. The “lipostatic” model emphasizes the role of body fat and the absolute level of energy reserves available for reproduction (Caro and Sellen 1990; Frisch 2002). Leptin, a hormone secreted exclusively by adipose tissue, provides a likely signal of energy reserves. Leptin shows a strong correlation with fat mass in well-nourished populations generally and has been associated with several aspects of female reproduction (Moschos et al. 2002). In contrast, the “metabolic” model emphasizes the dynamics of energy availability as the body shifts between states of energy storage and utilization (Wade and Schneider 1992; Lipson 2001). Insulin, in particular, emerges as a prominent signal of shifts in energy balance. Insulin facilitates glucose uptake, promotes energy storage and has been shown to augment ovarian steroid production *in vitro* (Griesen et al. 2001). In addition, clinical research has shown that insulin may augment leptin production (Cammisotto et al. 2005).

Although fat reserves accumulate as a consequence of positive energy balance, the relative influence of high levels of energetic hormones such as insulin and leptin to stimulate ovarian steroid production has yet to be demonstrated consistently *in vivo*. As Lipson (2001) points out, the link between ovarian function and female energetic condition has been shown most often in terms of energy deficits. Data on ovarian function at the upper end of the energetic condition spectrum in the absence of pathologies remain sparse, yet have global relevance for women's health (Pollard 2008).

This report presents preliminary findings on the relationship between circulating levels of insulin, leptin and estradiol, a major ovarian steroid, in a small study group of Samoan women. The populations of American Samoa and (formerly Western) Samoa have both undergone rapid modernization in recent decades, leading to a high prevalence of obesity, diabetes and metabolic syndrome (Keighley et al. 2007) as well as a wide range of naturally occurring variation in the absence of pathologies. Characterizing the relationship between prominent energetic hormones and estradiol in a population like Samoa thus adds breadth to our understanding of ovarian function at the upper end of the energetic condition spectrum.

Methods

Materials and methods are described at length elsewhere (Sherry 2002; Chin-Hong and McGarvey 1996) and derive originally from dissertation research conducted in collaboration with a large, prospective study on cardiovascular disease (CVD). Research protocols, including procedures for informed consent, received initial approval by the Institutional Review Board (IRB) of The Miriam Hospital, Providence, Rhode Island and additional authorization by The Human Subjects Committee at Harvard University.

Data presented here were collected in 1995 and included measures of height, weight, age, reproductive status, and hormonal measures based on a fasting serum sample. Height and weight were used to calculate Body Mass Index (BMI) according to the standard formula: weight (kg) divided by height (m)². Measurements of glucose and insulin, and leptin were performed, in 1995 and 1997, respectively, at the Lipid Research Laboratory at The Miriam Hospital in Providence, RI. Glucose level was determined with an automatic analyzer, Beckman CX4. Insulin was measured with a direct radioimmunoassay (RIA) kit from Diagnostic Products Corporation. Leptin was measured with an RIA kit from ALPCO. Estradiol (E2) was measured at the Reproductive Ecology Laboratory at Harvard University using a time-resolved fluoroimmunoassay (FIA) kit from Wallac.

At the time the blood sample was drawn, each woman identified on a calendar the first day (day 1) of her last menses. Day 1 was then used to count forward and identify women in the follicular phase of their menstrual cycle. The “forward count” method, although less precise than hormonal profiles, offers a fairly accurate means of identifying the follicular phase for one-time, cross-sectional data such as these. To control for variation in E2 level attributed to cycle day, we identified women whose E2 values fell within the normal reference range for follicular levels prior to the midcycle peak established by the assay manufacturer. The typical E2 trajectory is marked by relatively low levels at the beginning of the cycle followed by a gradual increase and a rapid rise at midcycle. Linear correlation analysis showed no significant relationship between cycle day and E2 level in our study sample ($r = .188$, $p = .252$, $n = 34$).

Participants were 34 regularly cycling, premenopausal women aged between 32 and 49, of whom 26 were overweight (BMI $> 26 \text{ kg/m}^2$, adjusted for Pacific Islanders based on Swinburn et al. 1999). The sample tended to consist of relatively older overweight women, yet all participants showed indicators of normal glucose tolerance, with physiological values in the normal range of variation for a well-nourished population: fasting glucose levels $< 105 \text{ mg/dl}$; insulin $< 20 \text{ uU/ml}$; leptin $< 30 \text{ ng/ml}$.

Data Analysis

Data analyses involved standard parametric techniques. We used the Pearson product moment coefficient to determine the degree of correlation between continuous variables, followed by regression analysis to identify significant linear relationships. Multiple regression was used *ad hoc* to evaluate the regulatory effect of insulin on leptin independent of adiposity.

Our initial analyses revealed no significant correlations between estradiol and any other variable -- insulin, leptin, glucose, BMI, or age -- indicating that 1) adipose tissue and age did not represent potentially confounding variables in our study sample and 2) energetic signals potentially affecting estradiol production did not conform to a linear type of relationship. Therefore, we conducted two main categorical analyses to examine the relationship further between the energetic hormones and estradiol.

First, we set up a step-like comparison by separating each energetic hormone into an “elevated” group based on the upper tertile of the data set and an “average” group based on the lower two tertiles. Student's t-test (two-tailed, unpaired) was used to compare mean estradiol level between the two groups. In the second analysis, following a method utilized by Doucet et al. (2000) given a small sample size, we generated ten pairs of study subjects matched for similar BMI (within a margin of 1.0 kg/m²) and leptin level, with a maximal difference in insulin level. These “matched pairs” thus represented contrasting metabolic profiles independent of adiposity and leptin level. Student's t-test (two-tailed, paired) was used to compare the mean difference in estradiol level within pairs. (It was not possible to repeat the analysis for leptin because the data set contained too few matched pairs with similar insulin levels and contrasting leptin levels.)

Although hormonal variables typically require log transformation prior to data analyses to improve linearity, our analyses gave the same results for both the log-transformed and untransformed data set. For easier interpretation, we present all results based on the untransformed data. In analyses involving leptin, four values were missing due to insufficient serum volume following a series of lipid measurements for the CVD project. For all other variables, sample size consisted of the entire data set (n = 34).

All means are reported with standard errors unless otherwise indicated. Probability values < .05 were considered statistically significant.

Results

Linear Analyses

The strong correlation between BMI and leptin ($r = .740$, $p = .002$, $n = 30$) and the weak correlation between BMI and insulin ($r = .257$, $p = .143$, $n = 34$) confirmed our designation of leptin primarily as a signal of energy reserves and insulin as a signal of energy metabolism and underscored the physiological distinction between the two hormones. The moderate correlation between insulin and leptin ($r = .454$, $p = .012$, $n = 30$) reinforced that the two hormones did not function as entirely independent variables. In multiple regression analysis, BMI was the major predictor of leptin level ($\beta = .674$, $p < .0001$), yet insulin emerged as a significant independent factor as well ($\beta = .310$, $p = .014$) -- consistent with the proposition that insulin exerts a regulatory effect on leptin production. Combined, BMI and insulin explained 64% of the variance in leptin.

Elevated versus Average Comparison

Mean insulin level for the Elevated Insulin (EI) group came to 11.7 ± 0.9 uU/ml ($n = 12$) and 5.7 ± 0.3 uU/ml ($n = 22$) for the Average Insulin (AI) group, according well with similar

designations within the normal range of variation used in clinical research (Carmina et al. 1999). As shown in Figure 1, mean estradiol for the EI group came to $.356 \pm .031$ nmol/L versus $.272 \pm .024$ nmol/L for the AI group ($p = .046$), representing a 30% increase in estradiol for the EI group.

Mean leptin level for the Elevated Leptin (EL) group came to 20.5 ± 1.1 ng/ml ($n = 10$) and 10.2 ± 0.9 ng/ml ($n = 20$) for the Average Leptin (AL) group with no significant difference in mean estradiol: $.307 \pm .031$ nmol/L versus $.291 \pm .029$ nmol/L respectively ($p = .732$).

Matched Pairs Comparison

The contrasting insulin pairs matched for similar BMI and leptin level showed a significant mean difference in estradiol level within pairs ($p = .040$, $n = 10$). As expected, the matched pairs also showed a highly significant difference in insulin level within pairs ($p = .0002$) and no significant difference in leptin level ($p = .139$). Mean estradiol level for the High and Low insulin pairs as a group came to $.359 \pm .033$ nmol/L versus $.244 \pm .034$ nmol/L respectively (Fig. 2), representing a 47% increase in estradiol for the High insulin group.

Discussion

The findings in this preliminary report showed that ovarian steroid production was tied more closely to circulating levels of insulin than leptin in a study sample of Samoan women. Relatively high insulin levels within the normal range of variation showed a positive association with estradiol levels whereas relatively high leptin levels did not. The relationship between insulin and estradiol appeared to conform to a step-like categorical function -- with the highest insulin levels exerting the greatest positive effect -- rather than a dose-response linear function, indicating a possible guidepost for future research protocols. Our results also supported the proposed regulatory effect of insulin on leptin production. Together, these findings suggest that peripheral regulation of ovarian function likely involves pathways related primarily to energy metabolism rather than energy reserves.

Relatively high insulin levels within the range of normal variation clearly signal a metabolic state of energy surplus (above maintenance requirements) and the potential for energy storage. This type of signal aligns well with studies in human reproductive ecology that point to an adaptive physiology designed to maximize female fecundity when prevailing ecological conditions generate surplus energy available for reproduction (Ellison 2001). Jasienska and Ellison (2004), for example, found that heavy workloads during the harvest season in a population of rural, well-nourished Polish women lowered progesterone levels, in the absence of significant weight loss and negative energy balance, by prohibiting energy surplus. Energy surplus in association with rising insulin levels was also implicated in the resumption of ovarian function following lactational amenorrhea in a population of well-nourished Toba women in Argentina (Valeggia and Ellison 2004). The current study of Samoan women thus adds to the growing evidence that peripheral regulation of ovarian function likely involves signals that emphasize a metabolic state of energy surplus. The precise physiological pathways involved warrant more extensive study utilizing hormonal profile measures with larger sample sizes.

Acknowledgments

We thank Susan Lipson for laboratory assistance and Meredith Reiches for comments and suggestions on an earlier version of the manuscript.

This research was supported in part by a grant from NIH (AG09375) to STM, and by the Mellon Foundation and Department of Human Evolutionary Biology, Harvard University.

Literature Cited

- Cammisotto PG, Gelinas Y, Deshaies Y, Bukowiecki LJ. Regulation of leptin secretion from white adipocytes by insulin, glycolytic substrates, and amino acids. *Am J Physiol Endocrinol Metab*. 2005; 289:E166–71. [PubMed: 15741239]
- Carmina E, Ferin M, Gonzalez F, Lobo RA. Evidence that insulin and androgens may participate in the regulation of serum leptin levels in women. *Fertil Steril*. 1999; 72:926–31. [PubMed: 10561001]
- Caro TM, Sellen DW. The reproductive advantages of fat in women. *Ethol Sociobiol*. 1990; 11:51–66.
- Chin-Hong PV, McGarvey ST. Lifestyle incongruity and adult blood pressure in Western Samoa. *Psychosom Med*. 1996; 58:130–7.
- Doucet E, St-Pierre S, Almeras N, Mauriege P, Depres JP, Richard D, Bouchard C, Tremblay A. Fasting insulin levels influence plasma leptin levels independently from the contribution of adiposity: evidence from both a crosssectional and an intervention study. *J Clin Endocrinol Metab*. 2000; 85:4231–7. [PubMed: 11095459]
- Ellison, PT. *On fertile ground: a natural history of human reproduction*. Cambridge: Harvard University Press; 2001. p. 358
- Frisch, RE. *Female fertility and the body fat connection*. Chicago: University of Chicago Press; 2002. p. 194
- Griesen S, Ledet T, Ovesen P. Effects of androstenedione, insulin and luteinizing hormone on steroidogenesis in human granulosa and luteal cells. *Hum Reprod*. 2001; 16:2061–5. [PubMed: 11574492]
- Jasienska G, Ellison PT. Energetic factors and seasonal changes in ovarian function in women from rural Poland. *Am J Hum Biol*. 2004; 16:563–80. [PubMed: 15368604]
- Keighley, ED.; McGarvey, ST.; Quested, C.; McCuddin, C.; Viali, S.; Maga, UA. Nutrition and health in modernizing Samoans: temporal trends and adaptive perspectives. In: Ohtsuka, R.; Ulijaszek, SJ., editors. *Health change in the Asia-Pacific region: biocultural and epidemiological approaches*. Cambridge: Cambridge University Press; 2007. p. 147–191.
- Lipson, SF. Metabolism, maturation and ovarian function. In: Ellison, PT., editor. *Reproductive ecology and human evolution*. New York: Aldine de Gruyter; 2001. p. 235–244.
- Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: a review. *Fertil Steril*. 2002; 77:433–44. [PubMed: 11872190]
- Pollard, TM.; Unwin, N. Impaired reproductive function in women in Western and Westernizing populations. In: Trevathan, WR.; Smith, EO.; McKenna, JJ., editors. *Evolutionary medicine and health: new perspectives*. 2008. p. 169–180.
- Sherry, DS. Ph D Thesis. Cambridge, MA: Harvard University; 2002. *Reproductive seasonality in chimpanzees and humans: ultimate and proximate factors*.
- Swinburn BA, Ley SJ, Carmichael HE, Plank LD. Body size and composition in Polynesians. *Int J Obes*. 1999; 23:1178–1183.
- Valeggia C, Ellison PT. Lactational amenorrhoea in well-nourished Toba women of Formosa, Argentina. *J Biosoc Sci*. 2004; 36:573–95. [PubMed: 15446353]
- Wade GN, Schneider JE. Metabolic fuels and reproduction in female mammals. *Neurosci Biobehav Rev*. 1992; 16:235–72. [PubMed: 1630733]

Fig. 1.
Mean estradiol level for Samoan women with Elevated versus Average insulin levels.

Fig. 2. Mean estradiol level for matched pairs of Samoan women with High versus Low insulin levels. Pairs were matched for similar BMI and leptin level to isolate the effect of insulin.