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# SERUM INSULIN, GLUCOSE AND NON ESTERIFIED FATTY ACIDS AFTER ADMINISTRATION OF FOLLICLE-STIMULATING AND LUTEINIZING HORMONES IN BITCHES

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**Abstract** This paper reports the effect of the simultaneous administration of follicle-stimulating (FSH) and luteinizing hormones (LH) on serum glucose, insulin and nonesterified fatty acid responses after glucose or insulin challenge. The animals were originally at anestrous. FSH (dose 2.5 U/kg body wt.) and LH (0.27 U/kg body wt.) were sc injected on days 1, 4, 8 and 11. Vaginal smears were obtained daily. Six untreated controls at anestrous and six treated bitches reaching proestrous were used. Glucose tolerance tests were done with a dose of 1 g of glucose per kg of body weight. Bovine insulin was administered at the dose of 0.25 U/kg body wt. During these tests, neither serum glucose and nonesterified fatty acids nor glucose distribution space and glucose clearance were affected by the treatment. The serum insulin response to hyperglycemia was greatly increased. The distribution space and clearance rate of this hormone were not affected by FSH + LH treatment. We conclude that, in the bitch, FSH + LH treatment, at doses that trigger "sex seasons", increases the serum insulin response to glucose load and produces a moderate resistance to the hypoglycemic, lipogenic and antilipolytic insulin actions. These phenomena are evident during hyperglycemia.

**Key words:** FSH, LH, glucose, nonesterified fatty acids, insulin

**Resumen** ***Modificaciones de la glucemia, insulina y ácidos grasos no esterificados durante la sobrecarga*** **de glucosa o insulina en perras tratadas con hormona folículo-estimulante y luteinizante.** Este trabajo describe el efecto de la administración simultánea de FSH y LH sobre los niveles de glucemia e insulina y ácidos grasos no esterificados séricos luego de una sobrecarga de glucosa o insulina. Los animales se encontraban originalmente en anestro, controlado por extendidos vaginales diarios. FSH (2.5 U/kg peso corp./día y LH (0.27 U/kg peso corp./día) se inyectaron por vía subcutánea en los días 1, 4, 8 y 11 del tratamiento. Cada grupo experimental estaba formado por seis perros en anestro y seis en proestro. Las sobrecargas de glucosa (1g/kg peso corp.) fueron administradas por vía endovenosa rápida. Las concentraciones de glucosa en sangre o ácidos grasos no esterificados séricos durante los tests de sobrecarga, los espacios de distribución de glucosa en sangre e insulina sérica o sus *clearances* plasmáticos no se vieron afectados por el tratamiento. Concluimos que la secreción de insulina como respuesta a una sobrecarga de glucosa aumenta significativamente en perras en anestro tratadas con FSH + LH. Al mismo tiempo se observa una moderada resistencia a la insulina, en los efectos hipoglucemiantes, lipogénicos y antilipolíticos de esta hormona durante esta prueba.

**Palabras claves:** FSH, LH, insulina, glucosa, ácidos grasos no esterificados

Houssay and Biasotti[1] were the first to show the important role played by the hypophysis in the development of diabetes mellitus. Revision of the scientific literature reveals that the influence of FSH and LH on glucose homeostasis is still an undefined issue. Some studies in the bitch have demonstrated that there is a close relationship of the estrous cycle with the onset of diabetes mellitus and the insulin requirements during overt diabetes[2,3]. Ovariectomy greatly stimulates the insulin response to hyperglycemia in normal dogs[4]. This phenomenon does not appear to be a primary action of estrogen deficiency, though it is reverted by 17-beta-estradiol therapy probably by feed-back inhibition of gonadotrophin secretion[4].

On the other hand, the spontaneous activation of the hypothalamus-pituitary-ovarian axis in normal bitches at anestrous produces complete estrous cycles. In this state, the tolerance to glucose of these animals is not affected but hyperglycemia promotes an intense secretion of insulin[5], entirely different from those when the entire axis is in operation[6]. This phenomenon is particularly evident during the estrogenic phase.

Prolactin has been reported to have no effect on metabolic variables during glucose or insulin tolerance tests[7].

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The simultaneous administration of FSH and LH triggers estrous cycles in canines[8, 9]. This paper reports the effects of these hormones on serum insulin, non esterified fatty acids and blood sugar during glucose and insulin tolerance tests.

## Materials and Methods

*Animals.* Twenty mongrel, disinfested, female dogs, of unknown ages, weighing 7.3-16.0 kg, were used in these experiments. They were housed in metabolic cages, fed on dog chow pellets and "ad libitum" water, for 3 months. During this period they were at anestrous. It was expected that the high progesterone levels of the early stages of the preceding metadiestrous would prevent the occurrence of new estrous cycles[10].

Six out of the fourteen treated bitches (43%) responded to FSH + LH treatment producing proestrous. Later, for a few days, these bitches showed atypical vaginal smears, and then returned to anestrous. This reaction was taken as evidence for the adequacy of the dose of gonadotrophins.

Two groups were then formed, receiving (on days 1, 4, 8 and 11 of the experiments) either 1 ml saline (six control bitches) or a mixture of FSH and LH, in 1 ml saline (14 treated bitches). The dose of FSH was 2.5 U/kg body wt. and that of LH 0.27 U/kg body wt. FSH was from Serono Laboratory, Argentina, highly purified of human origin. LH was from UIAMDD (Batch 13, biopotency: 15 N.I.H. U/mg protein; negligible contamination with pituitary GH, TSH and PRL). Only the six bitches reaching proestrous just after treatment completion and the six saline treated controls were submitted to the tests described below. Anestrous and occurrence of estrous cycles were checked by vagina smears according to Schutte[9].

The bitches were to be tested on the following day fasted overnight for 18-22 h. They had free access to tap water.

*Tests.* Every dog was submitted to glucose tolerance tests and insulin tolerance tests on consecutive days, in that order.

*Glucose tolerance tests.* Glucose solution (200 g/L) was administered i.v. at the dose of 1 g/kg body weight. Glucose was administered as a bolus injection through a peripheral vein (external saphena, median or radial). Blood samples were drawn from a peripheral vein not used for injection, before and at 5, 15, 25, 45, 60 and 90 minutes after glucose load.

*Insulin tolerance Tests.* Insulin, (from ox origin, crystalline, potency: 27.5 IU/mg protein, glucagon-free) was dissolved in 0.005 N HCl, pH 2.4, at a concentration of 1 mg/mL, and further diluted with saline so that the total dose per animal (0.25 IU/kg body wt.) was contained in a final volume of 5.0 ml. Blood samples was drawn from a peripheral vein (not used for injection) before and at 15, 20, 25, 30 and 35 minutes after insulin challenge.

Blood was allowed to clot for two hours at room tempe-rature and centrifuged for 5 minutes at 3000 g. Serum was stored at -25 °C. Glucose was measured in a Technicon Autoanalyzer[11]. Serum insulin was measured with a commer-cial kit (C.N.E.A.) and according of the cross-reaction between dog, ox and pork insulins[12, 13]. Nonesterified fatty acids were measured according to Itaya and Ui method[12].

Equations for the regressions between the natural logarithms of glucose[14] and insulin[15] concentrations as a function of time were calculated. The general form of these equations was $Y = a \times e^{-kt}$, where Y = concentration of the variable at time t, a = concentration at time zero, k = time coeficient. Glucose and insulin half-life times in blood stream were calculated according to $t_{1/2} = \ln 2 / k$. Their distribution spaces were calculated as: total amount of glucose or insulin divided by a.

*Statistical analysis.* An analysis of variance (ANOVA) of two factors per variable -dog, group (G), time (T) -with repeated measures on one factor (T) was performed[16].

As a significant G-effect on a variable was found, the mean data per group at every time were compared with the respective basal value, and between groups, following one-tailed, Dunnett test[16]. The same test was used to assess the significance of G X T. The significance of deviations from the mean regression line per group was tested[16]. The G effects on the respective $t_{1/2}$ or distribution spaces were assessed by ANOVA of one factor (G). A difference was considered not significant (NS) as $P > 0.05$.

## Results

### *Glucose Load*

As shown in Fig.1, the levels of blood glucose in control and LH + FSH treated bitches did not differ significantly before or after glucose load ($G \times T$, $P > 0.05$ ). The results of both bitches groups were pooled. Hyperglycemia was then observed at 5, 15 and 25 minutes; the basal values were again reached at 45 minutes.

The rate of glucose disappearance from blood in both groups, estimated between 5 and 45 minutes, decayed following the function stated in *Material and Methods*[14]. The $t_{1/2}$ of glucose did not significantly differ between groups (Controls: 22.03 ± 1.73 minutes, Treated bitches: 30.91 ± 4.85; $F_{1;10} = 2.97$, mean ± S.E.M.).

Glucose distribution space (mean ± S.E.M.)was not significantly different between groups (Controls dogs: 36.92 ± 4.52 % body wt., Treated bitches: 70.70 ± 22.82; $F_{1,10} = 2.11$, $P > 0.05$).

As expected, glucose administration increased insulin serum levels ($T$, $P < 0.01$). Gonadotrophins treatment did not affect the basal serum insulin ($P > 0.05$) but it did increase the mean serum insulin ($G$, $P < 0.01$) and the shape of serum insulin profile ($G \times T$, $P < 0.05$) during the test.

Since G x T interaction is significant, the effect of time alone was assessed. Gonadotrophins treatment further increased insulin levels at 5 ($P < 0.01$), 15 ($P < 0.01$), 25 ($P < 0.01$) and 45 ($P < 0.05$) minutes.

As expected, glucose administration reduced the serum concentration of nonesterified fatty acids. ($T$, $P < 0.01$). Gonadotrophins treatment affected neither the basal concentration of this variable ($P > 0.05$), their mean concentrations during the test ($G$, $P > 0.05$) nor the shape of the curve.

### *Insulin Load*

Insulin administration produced the expected hypoglycemic response ($T$, $P < 0.01$) in both dogs groups, without significant differences between them ($G$, $P > 0.05$). Gonadotrophins treatment did not affect the basal

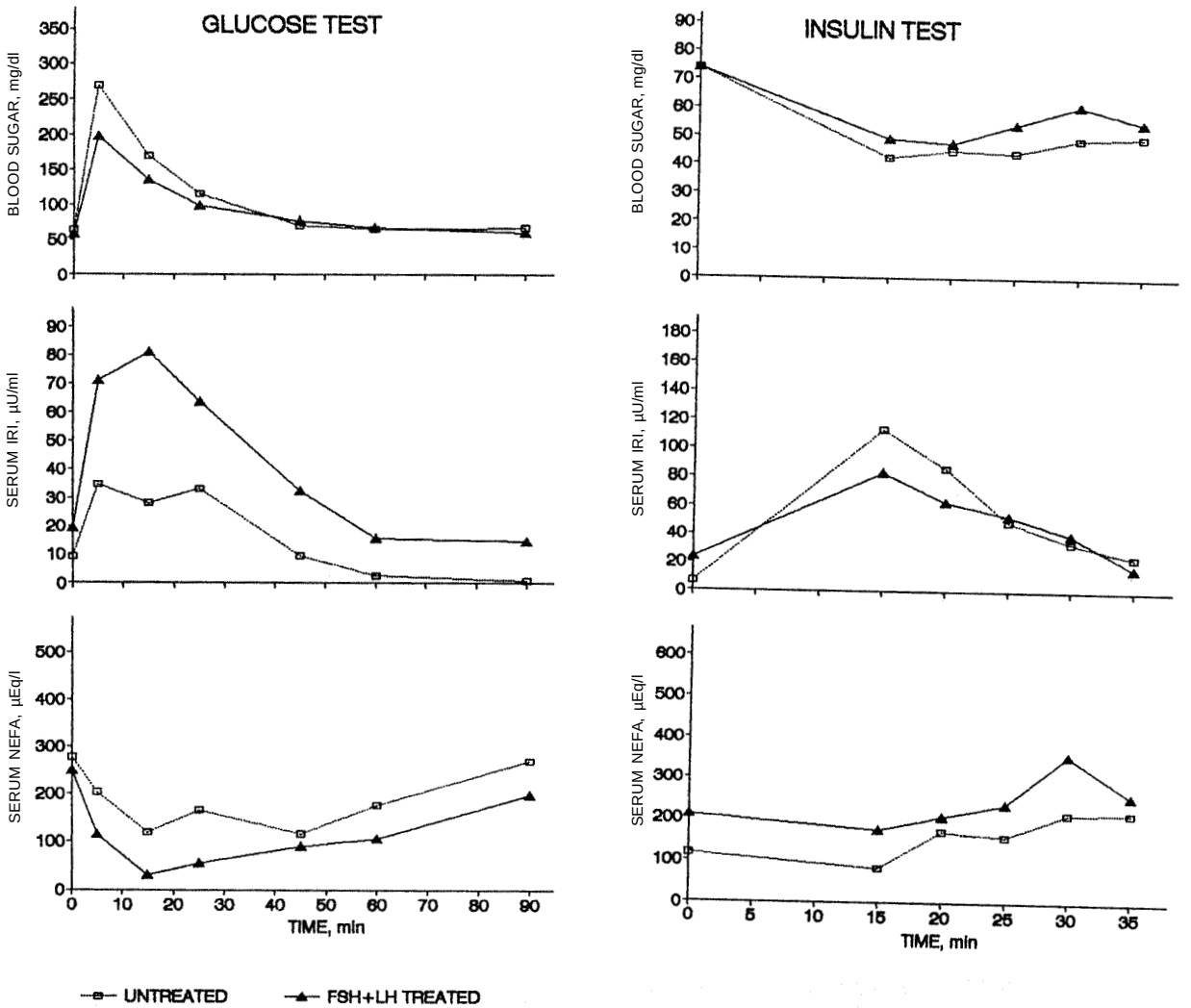


Fig.1.– Influence of previous treatment with FSH + LH on serum glucose, insulin and free fatty acids after a glucose load (left panels) and insulin load (right panels). Six animals per group.

insulin levels nor the concentration of this hormone during the tests ($P > 0.05$).

During this test, the rate of disappearance of serum insulin followed the function stated in *Material and Methods*, with good fitting between 15 and 30 minutes after insulin injection. The F value for respective deviations from the regression line was very low ($F_{2,20} = 0.29$, $P > 0.05$). Half life time of insulin in blood stream did not differ between groups (Controls = 8.94 ± 1.19 minutes, Treated = 30.67 ± 12.76 minutes; $F_{1,9} = 3.51$, $P > 0.05$).

The distribution space of insulin was not affected by treatment (Controls = 31.04 ± 7.85% body wt. (mean + S.E.M.); Treated = 111.26 ± 64.75 ($F_{1,9} = 2.08$, $P > 0.05$).

The insulin load increased significantly serum nonesterified fatty acids levels (T, $P < 0.05$), without difference between groups.

## Discussion

The bitches treated with FSH + LH share some metabolic features characteristic of obese women with polycystic ovaries. These patients show increased serum levels of LH together with normal or low serum FSH concentration[17] and exhibit variable degrees of peripheral resistance to insulin.

The serum concentrations of FSH and LH were not measured in this study. Literature reports indicate for the bitch at anestrous, low or negligible serum FSH, LH, progesterone and estrogens concentrations[10, 18-20]. This hormonal state provokes ovary quiescence and sex rest.

FSH[20] and LH[19] administration are known to promote the secretion of estrogens and progesterone in the bitch. These hormones characterize early spontaneous proes-

trous in these animals, which could be reasonably extended to our bitches at proestrous triggered by FSH + LH treatment. These two groups of bitches exhibit at proestrous similar pictures of the vaginal epithelium, but these pituitary hormones fail to evoke further estrous and metadiestrous, then leading to atypical, highly misleading vagina smear pictures.

The regulatory role played by the estrogens on the variables studied here has been analyzed elsewhere[21]. The data indicate that estrogenic mediation in the effect of FSH + LH on serum insulin during the glucose tolerance tests is deemed unlikely.

That FSH + LH are directly responsible for the strong stimulation of insulin secretion is only a hypothesis. However, Bailey and Matty[22] have observed raised serum insulin responses to glucose in ovariectomized rats treated with *Pergonal*.

Althouth the pituitary gonadotrophins actions on the metabolism of ovarian and testicular cells have been extensively studied during the last past years, the role played by FSH and LH in the regulation of lipid and carbohydrate metabolism *in vivo* is largely unknown. Our experiments show clearly the lack of action of FSH + LH on glucose homeostasis of intact bitches during glucose and insulin load. These findings agree with observations made in ketoacidotic human patients[23] and also in ovariectomized rats[22]. Gonadotrophins did not exert a direct lipolitic action on isolated fat cells of rats and rabbits as incubated *in vitro*[24-26].

It can be concluded that the results reported here show that FSH + LH treatment increases the serum insulin response to hyperglycemia in the bitch at anestrous. However, the treatment fails to affect two major extrapancreatic factors regulating serum IRI: the distribution space and the insulin rate of clearance from the blood stream. These observations suggest that the treatment with FSH + LH would stimulate the *in vivo* secretion of insulin without affecting the blood sugar and serum nonesterified fatty acid levels. These findings are interpreted as evidence for the development of a moderate resistance to the hypoglycemic, lipogenic and antilipogenic actions of insulin in these animals.

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## References

1. Houssay BA, Biassotti A. Acción diabetógena de diversas hormonas hipofisarias. *Revta Soc Argent Biol* 1938; 297A-307A.
2. Tishler SA. The effect of the estrous cycle on diabetes mellitus in the dog. *J Am An Hosp Assn* 1974; 10: 122-5.
3. Eigenman JE, Peterson ME. Diabetes mellitus associated with other endocrine disorders. *Vet Clin North Am Small An Pract* 1981; 2: 837-51.
4. Renauld A, Pérez RL, Rodríguez RR. Pancreas cytology and metabolic adjustments in short and long-term ovariectomized dogs. 17 Beta-estradiol replacement therapy. *Com Biol* (Buenos Aires) 1991; 9: 275-91.
5. Renauld A, Sverdlik RC, Agüero A, Rodríguez RR, Foglia VG. Serum insulin, free fatty acids and blood sugar during the estrous cycles in dogs. *Horm Metab Res* 1982; 14: 4-7.
6. Renauld A, Sverdlik RC.Agüero A, Pérez RL. Influence of estrogen-progesterone sequential administration on pancreas cytology, serum insulin and metabolic adjustments in female dog. *Acta diabetol Lat* 1990; 27: 315-27.
7. Renauld, Sverdlik RC, Andrade LL, Rodríguez RR. The effect of chronic prolactin administration upon the blood sugar, insulin and free fatty acid response to a glucose load in the dog. *Acta diabetol Lat* 1973; 10: 1286-99.
8. Evans LE. Induction of estrus in the bitch. In: Morrow DA, editor. Current therapy in theriogenology: Diagnosis, treatmet and prevention of reproductive diseases in animals. Philadelphia: WB Saunders Co Press. 1980.
9. Shutte AP. Canine vaginal cytology. I) Technique and cytological morphology, II) Cycle changes, III) Compilation and evaluation of cellular indices. *J Small An Pract* 1967; 8: 301-17.
10. Christie DW, Bell ET.Endocrinology of the oestrous cycle in the bitch. *J Small An Pract* 1971; 12: 383-9.
11. Renauld A, Andrade LL, Lindental DM, García Ben MS. Micrométodo automatizado para la medición de la glucosa. Estudio comparativo de conservadores. *Rev Asoc Bioquím Argent* 1975; 40: 171-5.
12. Itaya K, Ui M. Colorimetric determination of free fatty acids in biological fluids. *J Lipid Res* 1965; 6: 16-20.
13. Goetz FG, Greenberg BZ, Ells J, Meinert C. A simple immunoassay for insulin: Aplication to human and dog plasma. *J Clin Endocr Metab* 1963; 23: 1237-46.
14. Conard V. Mesure de l´assimilation du glucose. Bases théoriques et applications cliniques. *Acta gastro-enter belg* 1975; 18: 803-45.
15. Campell J, Rastogi KS. Effect of growth hormone on the rate of disappearance of insulin from blood in depancreatized and Houssay dogs. *Metabolism* 1967; 16: 562-71.
16. Winer BJ. Statistical principles in experimental design. New York: McGraw-Hill Book Co Press. 1990.
17. Jialal I, Naiker P, Reddi K, Moodley J, Joubert SM. Evidence for insulin resistance in nonobese patients with polycystic ovarian disease. *J Clin Endocr Metab* 1987; 64:1066-9.
18. Mellin TN, Orczyk GP, Hichens M, Behrman HR. Serum profiles of luteinizing hormone, progesterone and total estrogens during canine estrous cycle. *Theriogenology* 1976; 5: 175-87.
19. Concannon PW, Hansel W, Mcentee K. Changes in LH, progesterone and sexual behavior associated with preovulatory luteinization in the bitch. *Biol Reprod* 1977; 17: 604-13.
20. Concannon PW, Hansel W, Visek WJ. The ovarian cycle of the bitch. Plasma estrogen, LH and progesterone. *Biol*

*Reprod* 1975; 13: 111-21.
21. Renauld A, Von Lawzewitsch I, Pérez RL, Agüero A, Foglia VG, Rodríguez RR. Effect of estrogens on blood sugar, serum insulin and serum free fatty acids and pancreas cytology in female dogs. *Acta Diabet Lat* 1983; 20: 47-56.
22. Bailey CJ, Matty AJ. The effect of sex hormones on plasma insulin and glucose tolerance in the female rat. *J Endocr (London)* 1971; 51: xxi-xxii.
23. Barnes AJ, Kohner EM, Bloom SR. Importance of pituitary hormones in the aethiology of diabetic ketoacidosis. *Lancet* 1978; 1: 1171-4.
24. Heald PJ, Rookledge KA.Effect of gonadal hormones, gonadotrophins and thyroxine on plasma free fatty acids in the domestic fowl. *J Endocr (London)* 1964; 30: 115-30.
25. Gospodarowicz D. Lipolytic activity of the luteinizing hormone (LH) and of its two subunits. *Endocrinology* 1971; 89: 571-5.
26. Farmer SW, Ramachandran J, Papkoff H. Lipolytic activity of gonadotrophins and their subunits. *Endo-crinology* 1972; 91: 543-8.

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*I believe that everyone participating in a discovery, regardless of the stage at which he or she contributed, should be pleased by its outcome. Unfortunately, as scientific knowledge increases, the temporal impact of important early discoveries is frequently forgotten because they become incorporated into a foundation of facts that serves as the basis for ongoing research. Although it is difficult to accept, each of us should realize that science constantly moves on. ...I became a scientist because I found science to be exceptionally exciting. I loved having the opportunity to make original contributions. Aiding young scientists to find their way proved to be an extraordinary bonus I did not anticipate.*

Creo que cada uno de los involucrados en un descubrimiento, independientemente de la etapa en que haya contribuido, debería alegrarse del resultado. Desgraciadamente, a medida que el conocimiento científico aumenta, el impacto temporal de los muy importantes descubrimientos iniciales es frecuentemente olvidado porque se ven rápidamente incorporados en una colección de datos que sirven de base para la etapa siguiente de la investigación. Aunque es difícil de aceptar, cada uno debe reconocer que la ciencia sigue progresando... Yo me dediqué a la investigación porque encontré la ciencia especialmente atrayente. Me encantó tener la oportunidad de hacer trabajos originales. Ayudar a los jóvenes investigadores a encontrar su camino me dio una satisfacción muy superior a la jamás anticipada.

Charles Yanofsky

Advancing our knowledge in biochemical genetics, and microbiology through studies on tryptophan metabolism. *Ann Rev Biochem* 2001; 70: 37