J. Physiol. Biochem., 53 (2), 211-216, 1997
Revista española de Fisiología

# Influence of gonadectomy in eSS diabetic rats

M. C. Tarrés[1,2], S. M. Martínez[1,2], S. M. Montenegro[1,2], N. S. Figueroa[1], A. E. D'Ottavio[1,2] and J. C. Picena[1]

[1]Cátedra de Biología, Facultad de Ciencias Médicas, and [2]Consejo de Investigaciones, Universidad Nacional de Rosario, Santa Fe, 3100-2000 Rosario (Argentina)

(Received on July 15, 1996)

M. C. TARRÉS, S. M. MARTÍNEZ, S. M. MONTENEGRO, N. S. FIGUEROA, A. E. D'OTTAVIO and J. C. PICENA. *Influence of gonadectomy in eSS diabetic rats.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (2), 211-216, 1997.

The influence of gonadectomy on some variables related to the diabetic syndrome was studied in the eSS line of rats, a nonobese model of spontaneous non-insulin-dependent diabetes, whose biochemical and histopathological manifestations are more severe in males than in females. Rats were gonadectomized at 90 days of age. Spayed animals showed higher body weight, impaired intolerance to glucose at 9 and 12 months of age, lower insulinemia and a decreased number of large pancreatic islets. Castrated rats revealed lower body weight when compared with controls. However, those males did not evidence impairment in the intolerance to glucose, changes in insulinemia or remarkable modifications in endocrine pancreas histology. In kidneys, a lower cellular area in superficial proximal convoluted tubules was noticed. Despite the lower biomass registered in orchidectomized animals, their diabetic evolution was not modified. Conversely, ovariectomy appeared to be a worsening factor.

Key words: Diabetic rats, Gonadectomy, Glucose tolerance.

The IIM/Fm eSS rat is a nonobese model of human spontaneous non-insulin-dependent diabetes developed and bred in the School of Medicine of Rosario, Argentina (12, 13, 19, 22). Male rats show early onset slowly progressive glucose intolerance with age as well as glycosuria. During the second year of life a significant decrease in islets of Langerhans, glomerulosclerotic lesions and presence of cataracts are prominent in these animals (11, 13, 21, 22). Conversely, female rats reveal lower biomass and fewer conspicuous biochemical and histopathological manifestations (19, 20).

Lower diabetic syndrome severity demonstrated in eSS females has also been described in other murine models (8, 10,

Correspondence to M. C. Tarrés (TEL/FAX: 54 41 25 37 53).

16). Sexual hormones seem to play an important role, though the problem is not yet fully clarified (5, 8).

The present study was undertaken to investigate the influence of gonadectomy on some variables related to the diabetic syndrome in male and female eSS rats.

## Materials and Methods

Thirty five female and sixteen male eSS rats were fed ad libitum since weaning with a commercial balanced diet containing per 100 g food the following: protein 25.1 g, fat 3.5 g, carbohydrate as cornstarch 43 g, fiber 6 g, minerals 8 g, vitamins 1.9 g and moisture 12.5 g. The caloric density was 304 Kcal/100 g.

Gonadectomy was performed at the age of 3 months under ether anesthesia through scrotal incision in seven males and laparatomy in nine females. Thirteen intact females were mated with males of the same line, while the other thirteen remained virgins.

Body weight was recorded prior to castration or mating and at 6, 9 and 12 months of age.

Basal glycemia (G0) and glycemia at 30 (G30), 60 (G60) and 120 min (G120) after a load of 10% glucose (200 mg/100 g body weight) via stomach tube were determined at 6, 9 and 12 months of age.

At 13 months of age basal glycemia and insulinemia (G0, I0) and 120 min after the oral glucose load (G120, I120) were evaluated.

Blood samples were obtained by tail puncture. Plasma glucose was analyzed by enzymatic method using a commercial kit (Wiener Laboratories, Argentina) while insulin levels were determined by double-antibody radioimmunoassay (15).

Animals were sacrificed by ether overdose at 13 months of age. Kidneys and pancreas were excised, immediately fixed in 10 % neutral formalin and embedded in paraffin.

The pancreas were longitudinally cut to include pieces of head, body and tail in histological sections. Kidneys were horizontally cut at the renal pelvis level. Specimens were cut 6 µm thick and stained with hematoxylin-eosin (HE). Kidneys were also stained with Periodic Acid-Schiff (PAS).

Large and small islets of Langerhans per microscopic field were counted at 100 x (11).

Superficial and juxtamedullary renal corpuscles (i.e.: capsule plus glomerular tufts) as well as proximal convoluted tubules (CPT) were measured (18). Corpuscular diameters and CPT cellular areas were determined.

Comparison among groups were performed through analysis of variance and multiple comparisons (6). The number of large and small pancreatic islets per microscopic field were analyzed by using the Kruskal-Wallis analysis of variance and the Mann-Whitney U Test (17).

## Results

There was a significant decrease of body weight in orchidectomized animals and a significant increase of it in spayed rats when compared with controls (fig 1).

Since virgins and mothers did not differ in any of the studied variables, they were considered as one group.

Tables I and II show that G120 was higher in gonadectomized females than in controls at 9, 12 and 13 months of age whilst no differences were detected between castrated and uncastrated animals. Female controls resulted significantly less intolerant to glucose than intact males.

Table II puts into evidence that ovariectomized animals registered lower

Table I. *Glycemia 120 min after glucose load (mg/dl) in gonadectomized and control eSS rats at different ages.*
Values are means ± SD. **: p<0.01; ***: p<0.001; ns: not significant.

| Groups | 6 months | 9 months | 12 months |
|---|---|---|---|
| Gonadectomized females | 164 ± 17 | 316 ± 169 | 286 ± 38 |
| Control females | 168 ± 8 | 177 ± 10 | 199 ± 20 |
| Gonadectomized males | 189 ± 28 | 257 ± 63 | 280 ± 89 |
| Control males | 213 ± 44 | 218 ± 47 | 304 ± 88 |
| Comparisons among: | | | |
| • all groups | ** | ** | *** |
| • females | ns | *** | *** |
| • males | ns | ns | ns |
| • controls | *** | ns | *** |
| • gonadectomized animals | ** | ns | ns |

Table II. *Plasma glucose (mg/dl) and plasma insulin ( U/ml) in fasting state and 120 min after glucose load in gonadectomized and control eSS rats at 13 months of age.*
All values are means ± SD. *: p<0.05; ***: p<0.001; ns: not significant.

| Groups | G0 | I0 | G120 | I120 |
|---|---|---|---|---|
| Gonadectomized females | 159 ± 8 | 18.6 ± 6.06 | 360 ± 56 | 22.88 ± 11.02 |
| Control females | 150 ± 10 | 31.04 ± 7.28 | 237 ± 81 | 24.14 ± 6.31 |
| Gonadectomized males | 157 ± 11 | 13.42 ± 8.43 | 288 ± 90 | 13.26 ± 4.74 |
| Control males | 154 ± 18 | 9.25 ± 3.46 | 301 ± 65 | 18.25 ± 7.25 |
| Comparisons among: | | | | |
| • all groups | ns | *** | * | ns |
| • females | -- | *** | * | -- |
| • males | -- | ns | ns | -- |
| • controls | -- | *** | * | -- |
| • gonadectomized animals | -- | ns | ns | -- |

I0 than female controls and do not differ from castrated rats; female controls revealed higher values than male ones. G0 and I120 were similar among groups.

Islets of Langerhans were round-shaped and evidenced regular borders, especially in females. The number of large islets per microscopic field in female controls was higher than in uncastrated males (0.87 ± 0.29 vs 0.46 ± 0.14; $p < 0.001$). In contrast, ovariectomized animals had a smaller number of large islets than controls (0.59 ± 0.19; $p < 0.05$) while no differences were observed between castrated and uncastrated animals (0.41 ± 0.28 vs 0.46 ± 0.14; $p > 0.05$). On the other hand, no differences were seen in the number of large pancreatic islets in any of the studied groups ($p > 0.05$). Neither lymphocytic infiltrates nor necrosis were visualized.

No corpuscular lesions were described in renal parenchyma. Notwithstanding, orchidectomized animals showed foci of interstitial chronic nephritis and tubular dilatation in medullary zone as well as focal lymphocytic infiltrates. In some cases, protein cylinders were observed.

Corpuscular diameters were not significantly different between males and females (gonadectomized and controls).

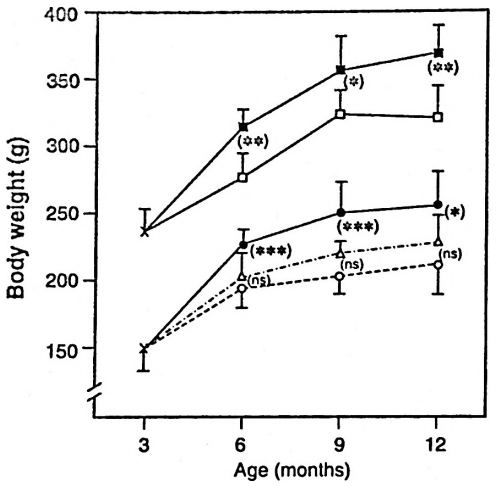


Fig. 1. *Body weights of gonadectomized and control male and female eSS rats from 3 to 12 months of age.* ●: gonadectomized females; △: mother controls; ○: virgin controls; (□ gonadectomized males; ■, control males. Mean ± SD; *p < 0.05; ** p < 0.01; ***p < 0.001; ns: not significant.

Superficial CPT cellular area was lower in castrated rats (1483 ± 174 $\mu m^2$ vs 1997 ± 543 $\mu m^2$, $p < 0.05$) but did not differ in female groups (1448 ± 418 $\mu m^2$ vs 1545 ± 351 $\mu m^2$ $p > 0.05$).

Juxtamedullary CPT shows no significant differences in male and female groups.

## Discussion

In spite of the verified adverse effects of diabetes on the female fertility of eSS line (13), pregnancy appears to have no effects on the time course of glucose homeostasis, as indicated by the fact that G120 and plasma insulin values as well as the number of large islets were the same as those of virgin controls.

Gonadectomy had a remarkable effect on the body weight of male and female eSS rats. Lower biomass in males may be associated to the lack of anabolic action of testosterone and it matches with findings in other murine diabetic model (7). Otherwise, the increase of body weight in ovariectomized eSS animals might be justified taking into account that estrogens inhibit body weight gain in rodents (7).

Considering that eSS females usually show lower intolerance to glucose and lower biomass than males (12, 13), it is interesting to outline that biomass in ovariectomized eSS rats was lower than in castrated animals although their tolerance to glucose appeared similar. Despite the demonstrated relationship between a higher body weight and the severity of hyperglycemia in male rats (21), neither lower biomass nor the lowering of sexual hormones could improve the diabetic syndrome. These data might point out that testosterone is not a main determinant factor in glucose intolerance of eSS male rats. However, the eventual influence of adrenal androgens cannot be excluded as seen in Cohen's rats after gonadectomy (1).

The impairment of glucose intolerance in spayed rats might be related with the loss of certain estrogenic effects on pancreas as revealed in pancreatectomized rats (4), in cyclophosphamide-induced type I diabetic rats after ovariectomy (14) and in human polycystic ovarian disease whose glucose intolerance appears reversible with estrogen administration (2). Treatment of ovariectomized eSS rats with estrogen could show whether this hormone acts to improve their diabetic status.

As it occurs in human type II diabetes when G0 increases between 80 and 140 mg/dl (3), the pancreas in eSS control females seemed to react to higher levels of G0 with higher values of I0. On the contrary, the pancreas loses such capacity in spayed animals as well as in males (castrated and controls) showing lower values of I0 with similar values of G0. Ovariec-

tomized rats also evidenced diminution in the number of large islets of Langerhans which, at least in humans, carry out the bulk of endocrine function (9). This fact is naturally characteristic in male rats of this line (11, 13).

IKEDA *et al.* reported that lack of hyperglycemia in female Wistar fatty rats would not obey to sexual hormones since ovariectomy performed during adulthood (8) and even during perinatal period (10) glycemia was not augmented. Although the reason of sexual dimorphism in Wistar fatty rats remains unclear, this feature could be influenced by higher food intake in males (8). Thus, it cannot be discarded that higher body weight as well as the impairment of glucose intolerance in spayed eSS rats might be due to a higher food intake induced by gonadectomy.

It has been repeatedly verified that renal lesions of older eSS males are similar to those found in long-term human diabetes (11, 12, 22). This fact is related to the intensity of hyperglycemia and the age of onset of the diabetic syndrome (21). The failure to induce a clear worsening of glucose intolerance should be considered a key factor in understanding why orchidectomy did not lead to an increase in glomerular lesions. On the other hand, tubular differences in superficial and juxtamedullary nephrons may be due to a distinct sensitivity to the deprivation of testosterone although the possible participation of adrenal androgens must also be kept in mind.

In sum, it might be said that only in females of the eSS diabetic line glucid homeostasis may be influenced, at least in part, by the presence of gonadal hormones.

*Acknowledgements*

We are indebted to Wiener Laboratories for providing part of the reagents used in this study. This research was partly supported by CIUNR (National University of Rosario Research Council) and Banco Bisel.

M. C. TARRÉS, S. M. MARTÍNEZ, S. M. MONTENEGRO, N. S. FIGUEROA, A. E. D'OTTAVIO y J. C. PICENA. *Influencia de la gonadectomía sobre las ratas diabéticas eSS.* J. Physiol. Biochem. (Rev. esp. Fisiol.), 53 (2), 211-216, 1997.

Se valora el efecto de la gonadectomía realizada a los 90 días de edad en machos y hembras de la línea de ratas eSS. Se trata de un modelo no obeso de diabetes insulino independiente cuyas manifestaciones bioquímicas e histopatológicas son más evidentes en los machos. Las hembras ovariectomizadas muestran, respecto de las testigos, incremento de peso, mayor intolerancia a la glucosa a los 9 y 12 meses de edad, menores niveles de insulina plasmática y reducción del número de islotes de Langerhans de mayor tamaño. Los machos castrados tienen menor peso que los testigos, sin mostrar empeoramiento de la intolerancia a la glucosa, cambios en la insulinemia, ni modificaciones pancreáticas. En el riñón se observa menor área celular en los túbulos contorneados proximales superficiales. Se concluye que la gonadectomía, a pesar de la menor biomasa de los machos castrados, no modifica la evolución de su diabetes. En las hembras eSS la ovariectomía parece constituir un factor agravante.

Palabras clave: Ratas diabéticas, Gonadectomía, Tolerancia glúcida.

## References

1. Cohen, A. M. and Rosenmann, E. (1984): *Diab. Metab.* (Paris), 10, 199-205.
2. Cole, C. and Kitabchi, A. E. (1978): *Clin. Res.*, 26, 412A.
3. De Fronzo, R. (1992): *Diabetes Care*, 15, 318-367.
4. Foglia V. G., Borghelli, D. D. S., Chieri, R. A., Fernández-Collazo, E. L., Spindler, I. and Wesely, O. (1963): *Diabetes*, 12, 231-237.
5. Foglia, V. G., Basave, J. C. and Chieri, R. A. (1969): *Diabetologia*, 5, 258-263.

6. Gabriel, K. R. (1964): *Biometrics*, 20, 459-462.
7. Hawkins, T., Gala, R. and Dunbar, J. (1993): P.S.E.B.M., 202, 201-205.
8. Ikeda, H., Shino, A., Matsuo, T., Iwatsuka, H. and Suzuoki, Z. (1981): *Diabetes*, 30, 1045-1050.
9. Kaihoh, T. T., Masuda, T., Sasano, N. and Takahashi, T. (1986): *Tokohu J. Exp. Med.*, 149, 1-10.
10. Kava, R. A., West, D. B., Lukasik, V. A., Wypijewski, C., Wojnar, Z. and Johnson, P. R. (1992): *Int. J. Obes.*, 16, 103-111.
11. Martínez, S. M., Tarrés, M. C., Liborio, M. M., Robledo, H. A., Picena, J. C., Rabasa, S. L. (1984): *Medicina*, 44, 145-152.
12. Martínez, S. M., Tarrés, M. C., Montenegro, S. M., Milo, R., Picena, J. C., Figueroa, N. and Rabasa, S. L. (1988): *Acta diabetol. lat.*, 25, 303-313.
13. Martínez, S. M., Tarrés, M. C., Picena, J. C., Montenegro, S. M., Gagliardino, J. J., Gómez-Dumm, C. L. A., D'Ottavio, A. E., Naves, A. and Rabasa, S. L. (1993): Lessons from animal diabetes. IV. (E. Shafrir, ed.). Smith-Gordon, London, pp. 75-90.
14. Mizuno, A., Iwami, T., Sano, T., Shi, K., Noma, Y. and Shima, K. (1993): *Metabolism*, 42, 865-869.
15. Morgan, C. R. and Lazarow, A. (1963): *Diabetes*, 12, 115-122.
16. Nakama, K., Shichinohe, K., Kobayashi, K., Naito, K., Uchida, O., Yasuhara, K. and Tobe, M. (1985): *Acta diabetol. lat.*, 22, 335-338.
17. Siegel, S. (1954): McGraw-Hill Book Company, Tokyo.
18. Tanner, G. A. and Evan, A.P. (1989): *Kidney Int.*, 36, 1050-1060
19. Tarrés, M. C., Martínez, S. M., Liborio, M. M. and Rabasa, S. L. (1981): *Mendeliana*, 5, 39-48.
20. Tarrés, M. C., Martínez, S. M., Liborio, M. M., Picena, J. C. and Rabasa, S. L. (1986): *Medicina*, 46, 429-434.
21. Tarrés, M. C., Martínez, S. M., Montenegro, S. M., Picena, J. C., Naves, A., Figueroa, N. and Rabasa, S. L. (1990): *Medicina*, 50, 235-243.
22. Tarrés, M. C., Martínez, S. M., Montenegro, S. M., Llorens, A, Picena, J. C. and Naves, A (1992): *Am. J. Pathol.*, 141, 761-763.