

Insulin and total protein dosage in human aqueous humor and serum

Carlos Jorge Simal Rodrigues, MD * & Viriato Luiz de Magalhães Ferreira, MD **

INTRODUCTION

Little has been written about insulin in human aqueous humor. Immunoreactive insulin was demonstrated in human aqueous humor (1, 2, 3). A fall in aqueous humor insulin concentration has been shown with age, attributed to vascular sclerosis in normoglycemic patients (2, 3). Low molecular weight is considered responsible for the high aqueous humor insulin concentration when compared to other proteins (3).

Our purpose is to study aqueous humor and serum insulin and total protein concentrations, changes with age, and the influence of the molecular weight on diffusion through the blood-aqueous barrier.

MATERIAL AND METHODS

— Material: the aqueous humor and serum of 33 volunteers were studied as follows: **Group A:** 9 patients with refractive and/or strabismic ambliopia aged 8 to 36 years (mean 22.9 years).

Group S: 24 patients with senile cataract, aged between 51 and 77 years (mean 68.4 years).

Patients did not present other ocular or systemic changes, and were not using medicines.

— Methods: material was obtained under surgical conditions with fasting patients. Three drops of proparacaine were used prior to a small corneal incision with a fragmented blade, close to the limbus. Aqueous humor was aspirated with a thin canule. Following this procedure, blood was obtained from the medial cubital vein.

Aqueous humor was kept in poliethylene tubes at — 20°C for posterior immunoreactive insulin and total protein dosage. Serum was separated in two parts one for immediate glucose and total protein and the other was kept at — 20°C for posterior immunoreactive insulin dosage.

Insulin was measured using radioimmunoassay (Biodata, code 1624, Hypolab SA, Switzerland). Aqueous humor total proteins

were measured using Lowry's et alii (1951)⁴ method, and serum total proteins with the biuret reaction. Serum glucose was measured in the Technicon auto-analyser, by the terricianide reduction method.

RESULTS

Mean serum glucose concentrations were: Group A — 85.75 ± 7.29 mg% and Group S — 95.78 ± 18.76 mg%. The difference is not statistically significant.

Mean total protein serum concentrations were: Group A — 6.7 ± 0.5 g% and Group S — 6.7 ± 0.7 g%. The difference is not statistically significant.

Tables I to V show mean values (\bar{x}) obtained for both groups, sample size (n), standard deviation (s) and Student's *t* test values and their significance (p).

TABLE I
Aqueous humor mean total proteins concentration in groups A and S

Group	n	\bar{x}	s
A	9	21.63	8.431
S	24	35.59	2.928

$t = 2.980$
 $p < 0.005$

TABLE II
Serum mean insulin concentration in groups A and S (μ IU/ml)

Group	n	\bar{x}	s
A	9	8.82	2.996
S	24	13.11	7.248

$t = 1.708$
 $p < 0.05$

TABLE III
Aqueous humor mean insulin concentration in groups A and S (μ IU/ml)

Group	n	\bar{x}	s
A	9	5.23	1.653
S	24	4.74	1.347

$t = 0.872$
 $p = \text{non significant}$

* Ophthalmologist of the Hospital Sarah Kubitschek, Belo Horizonte, MG.

** Head of the Central Laboratory of the Hospital das Clínicas, Federal University Minas Gerais.

Reprints should be requested to Dr. SIMAL RODRIGUES

Rua Tomás Brandão, 291, 30000 — Belo Horizonte — MG — Brasil.

Research was done at the Hospital São Geraldo — Federal University of Minas Gerais and at the Central Laboratory of the Hospital das Clínicas — Federal University of Minas Gerais.

TABLE IV

Relation aqueous humor total proteins/serum total proteins in groups A and S ($\times 10^{-3}$)

Group	n	\bar{x}	s
A	9	3.24	1.270
S	24	5.44	2.341
			$t = 2.649$
			$p < 0.01$

TABLE V

Relation aqueous humor insulin/serum insulin in groups A and S

Group	n	\bar{x}	s
A	9	0.63	0.221
S	24	0.46	0.259
			$t = 1.749$
			$p < 0.05$

COMMENTS

Serum glucose and total protein concentration are within normal limits in both groups (5). A clear rise in aqueous humor total protein concentration with age with age was observed (table I), which is in agreement with others authors (6, 7, 8).

The relation between filtrate and filtrant solute concentration shows to a certain extent the degree of permeability of a membrane to a given solute (9).

We found that the permeability rate of the blood — aqueous barrier to proteins increases with age (table IV).

Insulin is a protein hormone. The conversion from international insulin units to protein weight is made by the correspondence $24 \mu \text{IU} \sim 1 \text{ mg}$ (10).

Although an increase in serum insulin concentration with age was observed, (table II), there was no significant difference between aqueous humor levels in both groups (table III). The permeability rate of the blood — aqueous barrier to insulin thus decreases with age (table V).

Toselli et alii 1970 (2) and Greco et alii 1973 (3) suggest that the fall in aqueous humor insulin concentration with age is a consequence of vascular sclerotic changes, in normoglycemic patients. However, it is difficult to conceive a type of vascular sclerosis that reduces the permeability to insulin and increases the passage of other larger proteins. In fact, the main restriction site to macromolecule passage in the blood — aqueous barrier is the zonula occludens of the non pigmented ciliary epithelial cell layer, and not the ciliary vessels (11, 12, 13).

Assuming that there is an increase in the blood — aqueous barrier permeability to proteins (large molecules) simultaneous to a reduction of permeability to insulin (small molecule), then, the molecular weight of this hormone is not the main factor res-

ponsible for the high aqueous humor insulin concentration when compared to total protein concentration. It is reasonable to conceive a facilitating mechanism for insulin passage through the blood-aqueous barrier which becomes less efficient with age.

The role of insulin in the lens metabolism is controversial. Some studies suggest a direct or indirect action of insulin on the lens glucose metabolism (14, 15, 16). Other studies discard this idea (17). The action of insulin on protein metabolism is partly known (18). In chicken embryos, a δ — crystallin protein synthesis stimulating action is seen when insulin is introduced (19). Functional insulin receptors have been identified in vitro in lens epithelial cells of rabbits (20).

This data and the high insulin concentration relative to total proteins suggests the existence of a physiologic role for this hormone in lens metabolism. Possibly, the fall in aqueous humor insulin with age effects this metabolism, giving rise to cataract.

SUMMARY

Insulin and total protein serum and aqueous humor concentrations were studied. An increase in aqueous humor protein concentration was observed with age, although this did not occur with aqueous insulin concentration. The relation aqueous humor/serum concentration increased with age for total proteins, and decreased with age for insulin. Possible explanations and implications are discussed.

ACKNOWLEDGEMENTS

The authors wish to thank Biochemist Angela M. F. Ribeiro for technical assistance, and Dr. Paulo Gustavo Galvão for his suggestions.

REFERENCES

1. TOSELLI, C.; BERTONI, G.; GRECO, A. V. & FEDELLI, G. — L'insuline dans l'humeur aqueuse chez l'homme; déterminations radioimmunologiques. *Bull. Soc. Belge Ophthalmol.*, 153: 671-19, 1959.
2. TOSELLI, C.; BERTONI, G.; GRECO, A. V. & GHIRLANDA, G. — L'insuline dans l'humeur aqueuse chez l'homme; déterminations radioimmunologiques dans la cataracte et dans la rétinopathie diabétique. *Bull. Soc. Belge Ophthalmol.*, 155: 496-500, 1970.
3. GRECO, A. V.; GHIRLANDA, G.; FEDELLI, G.; FENICIO, R.; BERTONI, G. & GAMBASSI, G. — Immunoassay of insulin in the aqueous humour of diabetics and non-diabetics with or without cataract. *J. Med. (Westbury)*, 4: 197-201, 1973.
4. LOWRY, O. H.; ROSEYROUGH, N. J.; LEWIS FARR, A. & RANDALL, R. J. — Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 256-75, 1951.
5. BORGES, D. R.; BORGES FILHO, D. R.; FERMAN, M.; GUERRA, C. C. C.; KAZNIACKOWSKI, M. R.; MACHADO, A. B. B. & CHIORBOLI, E. — Parâmetros considerados "normais" nos exames de laboratório. 3 ed., s. 1., Programa Nacional de Atualização Médica Fontoura-Wyeth, S.D.
6. KRAUSE, V. & RAUNIO, V. — Protein content of normal human aqueous humor in vivo. *Acta Ophthalmol.*, 47: 215-21, 1969.
7. EESSIERE, Ed.; CROCKETT, R.; Le REBELLE, M. J.; MAURAIN, C. & GRENIER, D. — Méthodes chimiques de dosage de l'humeur aqueuse normale. *Albrecht von Graefes Arch. Klin. exp. Ophthalmol.*, 187: 273-88, 1973.

8. GRABNER, G.; ZEHETBAUER, G.; BETTELHEIM, H.; HÖNIGSMANN, C. & DORDA, W. — The blood-aqueous barrier and its permeability for proteins of different molecular weight. *Albrecht von Graefes arch. Klin. exp. Ophthalmol.*, 207: 137-48, 1978.
9. LANDIS, E. M. & PAPPENHEIMER, J. R. — Exchange of substances through the capillary walls. In: HAMILTON, W. F. & DOW, P., ed. *Handbook of Physiology*. Washington, American Physiological Society, 1963. Section 2, v. 2, chap. 29, p. 961-1034.
10. DOCUMENTA GEIGY — Patrones biológicos internacionales y preparaciones biológicas internacionales de referencia. In —. *Tablas Científicas*. 6 ed., Brasilea, 1971, p. 748.
11. SMITH, R. S. & RUDT, L. A. — Ultrastructural studies of the blood-aqueous barrier; II. The barrier to horseradish peroxidase in primates. *Am. J. Ophthalmol.*, 76: 937-47, 1973.
12. BILL, A. — Blood circulation and fluid dynamics in the eye. *Physiol. Rev.*, 55: 383-417, 1975.
13. TÖNJUN, A. M. & PEDERSEN, O. O. — The permeability of the human ciliary and iridal epithelium to horseradish peroxidase; an in vitro study. *Acta Ophthalmol.*, 55: 781-8, 1977.
14. LEVARI, R.; KORNBLUETH, W. & WERTHEIMER, E. — The effect of insulin on the uptake of monosaccharides by the rat lens. *J. Endocrinol.*, 22: 361-9, 1961.
15. FARKAS, T. G. & MAHAJAN, Y. L. — In vitro insulin effect on glucose utilization of isolated rat lenses. *Ophthalmic. Res.*, 1: 72-8, 1970.
16. FARKAS, T. G. & WEESE, W. C. — The role of aqueous humor, insulin and trivalent chromium in glucose utilization of rat lenses. *Exp. Eye Res.*, 9: 132-6, 1970.
17. ELERINK, J. & BHILER, I. — Characteristic of the membrane transport of sugars in the lens of the eye. *Biochim. Biophys. Acta*, 282: 337-51, 1972.
18. JEFFERSON, L. S. — Role of insulin in the regulation of protein synthesis. *Diabetes*, 29: 487-93, 1980.
19. MILSTONE, L. M. & PIATIGORSKI, J. — δ -crystallin gene expression in embryonic chick lens epithelia cultured in the presence of insulin. *Exp. Cell Res.*, 105: 9-14, 1977.
20. HOLLENBERG, M. D. — Receptors for insulin and epidermal growth factor: relation to synthesis of DNA in cultured rabbit lens epithelium. *Arch Biochem Biophys*, 171: 371-7, 1975.