Int J Endocrinol Metab. 2010;8(3):138-142
International Journal of
Endocrinology & Metabolism
Journal home page: www.EndoMetabol.com

# Investigation on the expression of IGF-I protein in insulin-resistant rat brain

Azita Parvaneh Tafreshi [1*], Razieh jalal [2], Shamila Darvishalipoor [1], Hoori Sepehri [3], Khosrow Adeli [4].

[1] Department of Basic Sciences in Biotechnology, The National Research Institute of Genetic Engineering and Biotechnology, Tehran, IR Iran
[2] Department of Chemistry, Ferdowsi University, Mashad, Tehran, IR Iran
[3] Department of Physiology, Faculty of Sciences, Tehran, IR Iran
[4] Department of Clinical Biochemistry, Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

*Article Type:*
Original Article

*Article history:*
Received: 5 Dec 2010
Revised: 17 Dec 2010
Accepted: 1 Jan 2011

*Keywords:*
Diabetes
Insulin resistance
Brain
IGF-I

ABSTRACT

*Background:* In insulin-resistance animal models, insulin uptake from the periphery to the brain is impaired. Although brain insulin is not involved in glucose transfer to the neurons, it is required for neuron survival and function, mediated by binding to insulin receptors. Furthermore, an insulin homologue called insulin-like growth factor (IGF-I), which is abundantly expressed in mature neurons and acts in parallel with insulin in the brain, has the ability to bind to the insulin receptor and trigger the signal transduction pathway.
*Objectives:* Although reduced levels of brain insulin and serum IGF-I have been reported during insulin resistance, no data is available on IGF-I levels in the brain. In this study, we sought to investigate if the expression of IGF-I is also altered in brains of insulin-resistant rats.
*Materials and Methods:* Wistar rats were given 10% fructose in their drinking water for up to 4 months to induce insulin resistance. The rats were then killed and perfused with PFA 4%; then, there brains were excised, sectioned, and examined for immunoreactivity of IGF-I.
*Results:* Our results showed an increased intensity of IGF-I in most brain areas of the insulin-resistant rats.
*Conclusions:* Altogether, an increased expression of IGF-I in the brain could be a compensatory mechanism and substitute for low levels or lack of insulin in the brains of insulin-resistant animals.

© 2010 *Kowsar M.P.Co.* All rights reserved.

*Implication for health policy/practice/research/medical education:*
IGF-I signaling could be regarded as a complementary pathway for insulin in brain to prevent disruption in metabolism and survival of neurons in brain. Targeting specific signaling molecules activated in response to increased level of IGF-I in insulin resistant brain would have clinical impacts on treatment of the insulin resistance.

*Please cite this paper as:*
Parvaneh Tafreshi A, Jalal R, Darvishalipour S, Sepehri H, Adeli K. Investigation on the expression of IGF-I protein in insulin-resistant rat brain. *Int J Endocriol Metab*. 2010;**8**(4):138-42

## 1. Background

Increasing evidence indicates that brain insulin plays important roles in metabolism and food uptake (1, 2). Most brain insulin is derived from the pancreas, through a receptor mediated transfer (3, 4). In insulin-resistant rats, insulin uptake and therefore its level is reduced in brain (3-5). It is generally accepted that insulin resistance

* *Corresponding author at:* Azita Parvaneh Tafreshi, The National Research Institute for Genetic Engineering and Biotechnology, Pajohesh boulevard, km 15, 14965-161, Karaj-Tehran highway, Tehran, IR,Iran. Tel: +98-2144580301-27, Fax: +98-2144580395&399
*E-mail:* aptafreshi@yahoo.com & tafreshi@nigeb.ac.ir
Copyright © 2010, IES, Published by *Kowsar M.P.Co. All rights reserved.*

is a result of altered receptor-mediated signal transduction (6). Although brain insulin is not involved in glucose uptake by neurons, it affects neuron survival and function. The effects of insulin and insulin-like growth factor (IGF-I) in the brain are receptor mediated (3, 4) . Mutant mice for insulin receptors are obese, nontolerant to glucose, and insulin resistant (7, 8). Unlike insulin which is either not synthesized or present at low levels, IGF-I is expressed in almost all areas of the adult brain (8). Altogether, with respect to the role of insulin in regulation of energy and metabolism (9) in the brain and high energy demands for formation and function of neurons and their synapses, a homologue and ubiquitous substitute for insulin is required. This substitute should fulfill autocrine and paracrine actions of insulin in energy consumption (10) as well as low levels of insulin in insulin resistance (11).

## 2. Objectives

In this study, we have therefore sought to examine if brain IGF-I is altered in insulin-resistant rats and can be regarded as a substitute for insulin.

## 3. Materials and methods

### *3.1. Induction of insulin resistance*

Male Wistar rats (200–250 gr) were purchased from the Pasteur institute. The animals were weighed, placed in separate cages, and divided in to control and experimental groups (n = 8). The experimental rats were fed with fructose in their drinking water (10%), and the control rats were given normal water. Both groups were fed with normal chow. To determine the insulin resistance, the levels of glucose, triglycerides, and insulin were measured every 2 weeks. Following 4 months of fructose treatment, there was a significant increase in the serum level of triglycerides ($P < 0.05$) but a decrease in the level of insulin ($P < 0.05$).

### *3.2. Measurements of serum glucose, triglyceride, and insulin levels*

Parsazmun kit was used to measure the levels of glucose and triglycerides at 496 nm, and DRG diagnostic kit for that of insulin at 450 nm. The regression equation was obtained from the absorbance values against different concentrations of the standard samples. Using the obtained equation, concentrations of the samples were calculated.

### *3.3. Perfusion*

Using 2% chloral hydrate, the animals were deeply anaesthetized and perfused by buffer phosphate followed by glutaraldehyde/paraformaldehyde (500 ml). The skulls were opened, the brains were taken out and post-fixed in the same fixative for another 24 hours. For cryosectioning, brains had to be kept for 2 weeks in sucrose (30%) with formalin (10%), and for long-term preservation, sucrose (30%) containing sodium azide (0.05%) at 4°C was used.

### *3.4. Immunohistochemistry*

Prior to cryosectioning, the brains were soaked in OCT compound, sectioned at 40 nm and mounted on poly-L-lysine–coated slides. After being completely dried, sections were soaked in PBS and incubated in sodium borohydride (0.1%) followed by periodic acid (0.1%) for 10 minutes to reduce the background. For signal intensification, sections were then incubated in citrate buffer (in a 96°C water bath) for 40 minutes. After blocking in skimmed milk for 2 hours, sections were incubated with IGF-I antibody (cell signaling, USA) overnight at 4°C. following incubations with biotinylated secondary antibody for 2 hours at room temperature, HRP-streptavidin for 1 hour at room temperature, the reaction was developed and visualized by DAB chromogen.

## 4. Results

### *4.1. Triglyceride but not glucose is increased in insulin resistant rats*

Glucose and triglyceride measurements in serum-were performed in both treated and control groups every month. The average level of serum glucose did not change significantly after 4 months (*Figure 1*; 391 ± 70 mg/dl in the fructose-fed rats compared with 140 ± 120 mg/dl in the control rats). The level of triglyceride, however, increased significantly after 4 months (*Figure 2*; 48 ± 8.5 mg/dl in the fructose-fed rats compared with 26 ± 2.2 mg/dl in the control rats; $P < 0.05$).

### *4.2. Insulin in increased in insulin resistant rats*

Serum level of insulin was assayed by using a rat-insulin Elisa kit (DRG diagnostics). The results showed asignificant increase in the level of insulin in fructose-fed animals (*Figure 3*; 0.88 ± 0.1 mg/dl in fructose fed rats compared to 0.25 ± 0.2 mg/dl in control rats; $P < 0.02$).

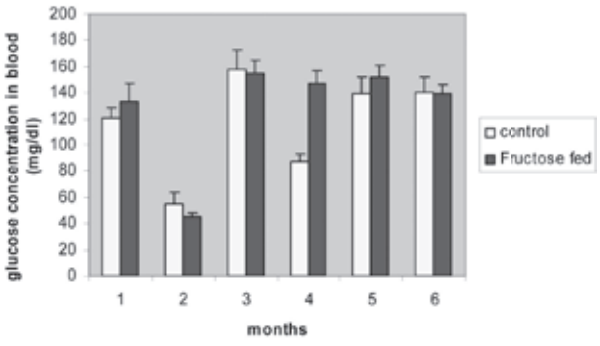
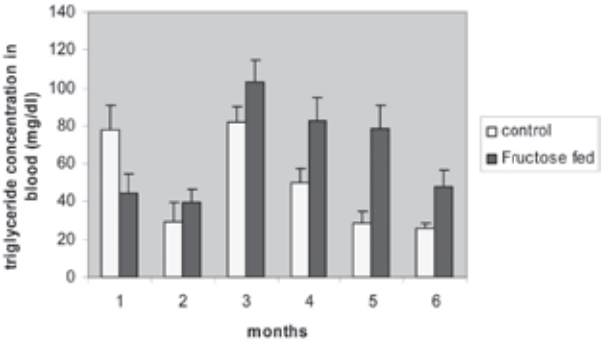



**Figure 1.** The average level of glucose in fructose fed rats was not significantly changed after 4 months compared to that in the control rats.

**Figure 2.** The average level of triglycerides in the serum was significantly increased after 4 months in the fructose-fed rats compared to that in the control rats.




*Indicates signficantly different (P<0.05).

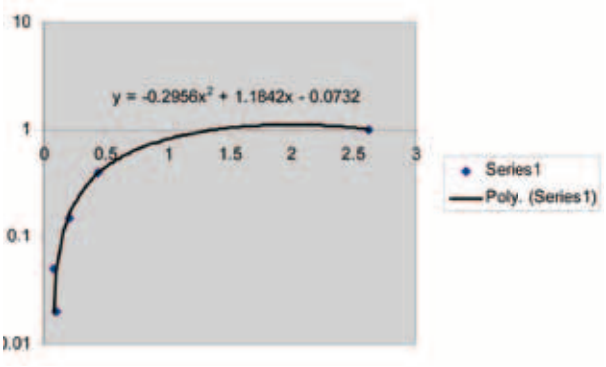



**Figure 3.** Logarithmic graph of the standard curve drawn for the absorbance values against different concentrations of insulin (μU/ml) at 450 nm.

**Table 1.** A comparison between intensities of IGF-I ir in different brain areas in the fructose-fed (F) and control rats (C).

| | Purkinje cells | | Brain stem | | Hippocampus | | Thalamus | |
|---|---|---|---|---|---|---|---|---|
| **IGF-I** (1) | F++ | C+ | F++ | C+ | F++ | C+ | F++ | C+ |
| **IGF-I** (2) | F++ | C+ | F++ | C+ | F++ | C+ | F++ | C+ |
| **IGF-I** (3) | F++ | C+ | F++ | C+ | F++ | C+ | F++ | C+ |
| **IGF-I** (4) | F++ | C+ | F++ | C+ | F+ | C+ | F+ | C+ |
| **IGF-I** (5) | F++ | C+ | F++ | C+ | F+ | C++ | F+++ | C+ |
| ***P* value** | < .05 | | < .05 | | < .05 | | < .05 | |

### *4.3. Immunoreactivity of IGF-I is increased in insulin-resistant brains*

The intensity of IGF-I immunoreactivity in different brain areas in the fructose-fed rats was compared with that in the control rats and categorized as: low (+), moderate (++) and intense (+++). Analysis of the comparisons was performed by Mann-Whitney and Kruskal-Wallis statistical method *(Table 1)*. The results show that IGF-I ir in different areas of the brain, such as the brain stem, cerebellum, hippocampus, and thalamus increased significantly ($P < 0.05$) in the fructose-fed rats *(Figure 4: a–g)* compared to the control rats *(Figure 4: b–h)*.

## 5. Discussion

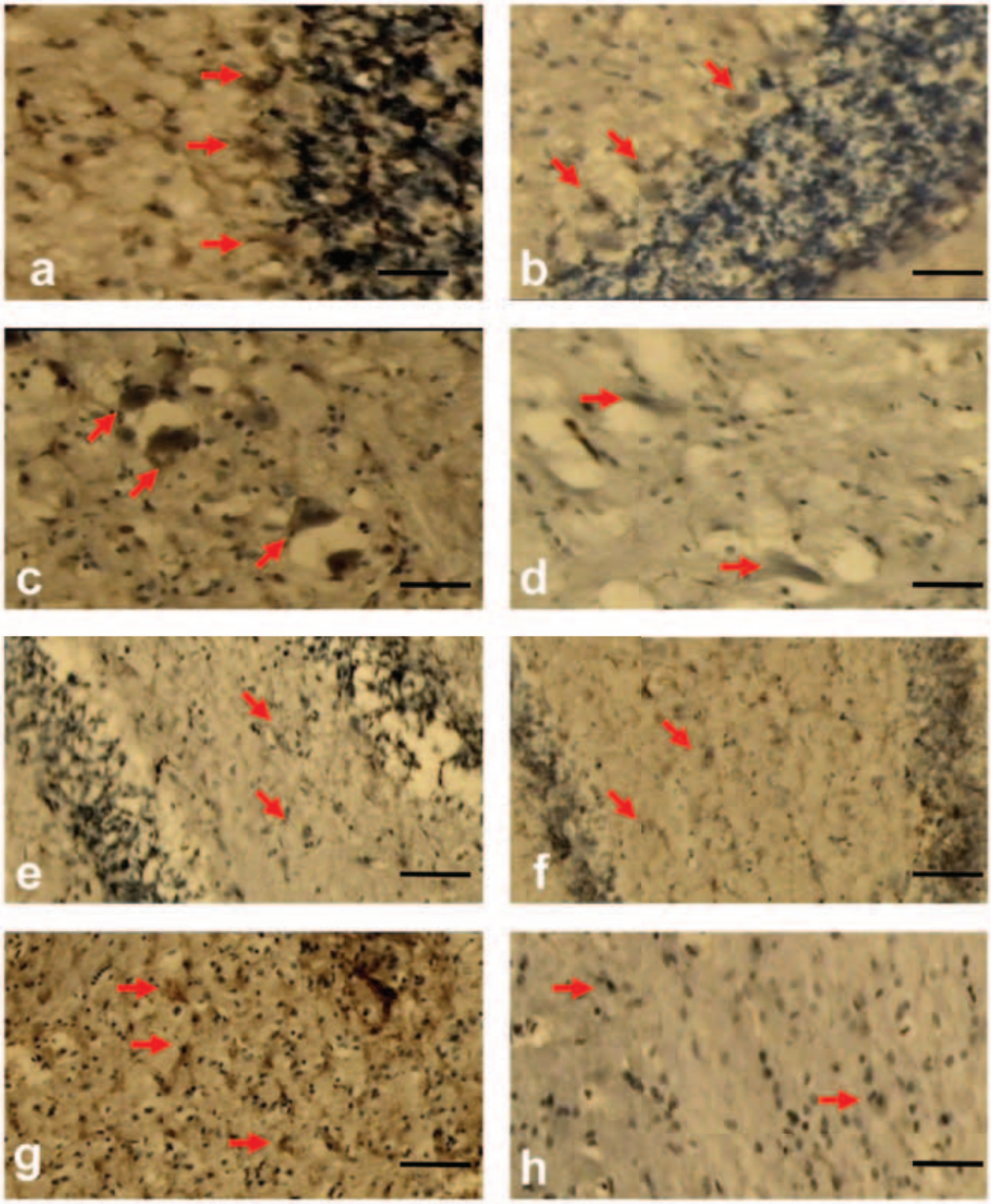
### *5.1. Fructose diet induces insulin resistance*

Fructose syrups, as the most commonly used sweetener in food products, result in increased uptake of daily carbohydrates and therefore increase the chance of insulin resistance and metabolic disturbances (12, 13). Based on both animal and human studies (12-15), we used fructose 10% in drinking water of adult rats to induce an animal model for insulin resistance. Our measurements of triglyceride and insulin levels indicated that the levels of both increased significantly after 4 months. This time point should be considered as a critical point to reduce both the time and efforts in triglyceride and insulin measur ments. The latency of 4 months also has been documented elsewhere (14, 16). Moroz and colleagues who have used high fat diet in obese mice, introduced measurments of insulin concentration as a reliable indicator which increased with a latency of 16 weeks(16).

### *5.2. Increased expression of IGF-I in insulin-resistant brain as a compensatory mechanism*

Reports have shown that despite the essential role of brain insulin in controlling peripheral metabolism, food intake, and body weight, it is not involved in glucose uptake by neurons. In mutant mice lacking insulin receptors in the brain, studies found that control of food intake and metabolism was disturbed and resulted in insulin resistance (1, 2). Insulin resistance witch disturbs insulin transfer from the liver to the brain, impairs neuron function and survival and therefore memory and learning (16). IGF-I as a homologue for insulin has been shown to act in parallel with insulin (9). In this study therefore, we sought to examine if IGF-I level has also been changed in insulin resistant brain. According to our findings, the level of IGF-I significantly increased in different brain areas of rats fed with fructose in their drinking water 10% for at least 4 months. Similarly, Moroz and colleagues

**Figure 4.** Photomicrographs of IGF-I immunostained sections from the fructose-fed rats (a = Purkinje cells of cerebellum, c = brain stem, e = hippocampus, g = thalamus) and the control rats (b = Purkinje cells of cerebllum, d = brain stem, f = hippocampus, h = thalamus). A significant increase was observed in the IGF-I ir in different areas of the insulin-resistant brains. 1 cm bar = 90 μm



(16) have also shown that in mice fed with high fat food, IGF-I mRNA is significantly increased in brain. Since IGF-I acts through insulin receptors and triggers the intracellular pathways, one may conclude that the increased brain IGF-I compensates for the reduced insulin levels in insulin-resistant brain. Furthermore, the increased IGF-I may also compensate for the reduced level of IGF-I in serum and peripheral tissues in diabetes type II(17, 18). Indeed, the regulatory feedback mechanism driven by peripheral IGF-I to control brain IGF-I level has also been documented by Torres alleman (19). Altogether, IGF-I signaling could be regarded as a complementary pathway for insulin in the brain to prevent disruption in metabolism and survival of neurons in the brain. In patients with diabetes type III or Alzheimer's disease, the IGF-I signaling pathway is impaired even at the early stages of the disease (20, 21), resulting in dramatic changes in the brain. Future studies on signaling molecules activated in response to the increased levels of IGF-I in insulin-resistant brains would lead to a better understanding of the complex dynamic of insulin resistance.

## Financial support

This project was supported by a grant from the National Research Institute of Genetic Engineering and Biotechnology of Iran.

## Conflict of interest

There are no conflicts of interest.

## Acknowledgments

Technical assistance in generating the animal model was kindly provided by the animal facility in the Departmen of Clinical Biochemistry, Hospital for Sick Kids, University of Toronto, Toronto, Canada.

## References

1. Gerozissis K. Brain insulin: regulation, mechanisms of action and functions. *Cell Mol Neurobiol*;**23**(1):1-25.
2. Porte D, Jr., Baskin DG, Schwartz MW. Insulin signaling in the central nervous system: a critical role in metabolic homeostasis and disease from C. elegans to humans. *Diabetes*. 2005;**54**(5):1264-76.
3. Banks WA. The source of cerebral insulin. *ejp*. 2004;**490**(1-3):5-12.
4. Banks WA, Jaspan JB, Kastin AJ. Effect of diabetes mellitus on the permeability of the blood-brain barrier to insulin. *Peptides*. 1997;**18**(10):1577-84.
5. Baskin DG, Stein LJ, Ikeda H, Woods SC, Figlewicz DP, Porte D, Jr., et al. Genetically obese Zucker rats have abnormally low brain insulin content. *Life sciences*. 1985;**36**(7):627-33.
6. Mielke JG, Taghibiglou C, Liu L, Zhang Y, Jia Z, Adeli K, et al. A biochemical and functional characterization of diet-induced brain insulin resistance. *JNC*. 2005;**93**(6):1568-78.
7. Bruning JC, Gautam D, Burks DJ, Gillette J ,Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. *Science (New York, NY*. 2000;**289**(5487):2122-5.
8. Rotwein P, Hall LJ. Evolution of insulin-like growth factor II: characterization of the mouse IGF-II gene and identification of two pseudo-exons. *DNA and cell biology*. 1990;**9**(10):725-35.
9. Cheng CM, Reinhardt RR, Lee WH, Joncas G, Patel SC, Bondy CA. Insulin-like growth factor 1 regulates developing brain glucose metabolism. *Proc Nat1 Acad Sci USA*. 2000;**97**(18):10236-41.
10. Bondy CA, Cheng CM. Insulin-like growth factor-1 promotes neuronal glucose utilization during brain development and repair processes. *Inrrev Neurobiol*. 2002;51:189-217.
11. Tritos NA, Mantzoros CS. Clinical review 97: Syndromes of severe insulin resistance. *jcEM*. 1998;**83**(9):3025-30.
12. Bezerra RM, Ueno M, Silva MS, Tavares DQ , Carvalho CR, Saad MJ. A high fructose diet affects the early steps of insulin action in muscle and liver of rats. *jN*. 2000;**130**(6):1531-5.
13. Elliott SS, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. *ajcn*. 2002;**76**(5):911-22.
14. Harati M, Ani, M. and Messripour, M. Effect of Vanadyl Sulfate on Fructose-Induced Insulin Resistance Rat. *IBj* 2003;**7**(4):179-82.
15. Huang BW, Chiang MT, Yao HT, Chiang W. The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. *Diabetes Obes Metab*. 2004;**6**(2):120-6.
16. Moroz N, Tong M, Longato L, Xu H, de la Monte SM. Limited Alzheimer-type neurodegeneration in experimental obesity and type 2 diabetes mellitus. *J Alzheimers Dis*. 2008;**15**(1):29-44.
17. Haluzik M, Yakar S, Gavrilova O, Setser J, Boisclair Y, LeRoith D. Insulin resistance in the liver-specific IGF-I gene-deleted mouse is abrogated by deletion of the acid-labile subunit of the IGF-binding protein-3 complex: relative roles of growth hormone and IGF-1 in insulin resistance. *Diabetes*. 2003;**52**(10):2483-9.
18. Janssen JA, Lamberts SW. The role of IGF-I in the development of cardiovascular disease in type 2 diabetes mellitus: is prevention possible? *Eur J Endocrinol*. 2002;**146**(4):467-77.
19. Torres-Aleman I. Toward a comprehensive neurobiology of IGF-I. *Dev Neurobiol*. 2010;**70**(5):384-96.
20. Rivera EJ, Goldin A, Fulmer N, Tavares R, Wands JR, de la Monte SM. Insulin and insulin-like growth factor expression and function deteriorate with progression of Alzheimerɔs disease: link to brain reductions in acetylcholine. *J Alzheimers Dis*. 2005;**8**(3):247-68.
21. Steen E, Terry BM, Rivera EJ, Cannon JL, Neely TR, Tavares R, et al. Impaired insulin and insulin-like growth factor expression and signaling mechanisms in Alzheimerɔs disease--is this type 3 diabetes? *J Alzheimers Dis*. 2005;**7**(1):63-80.