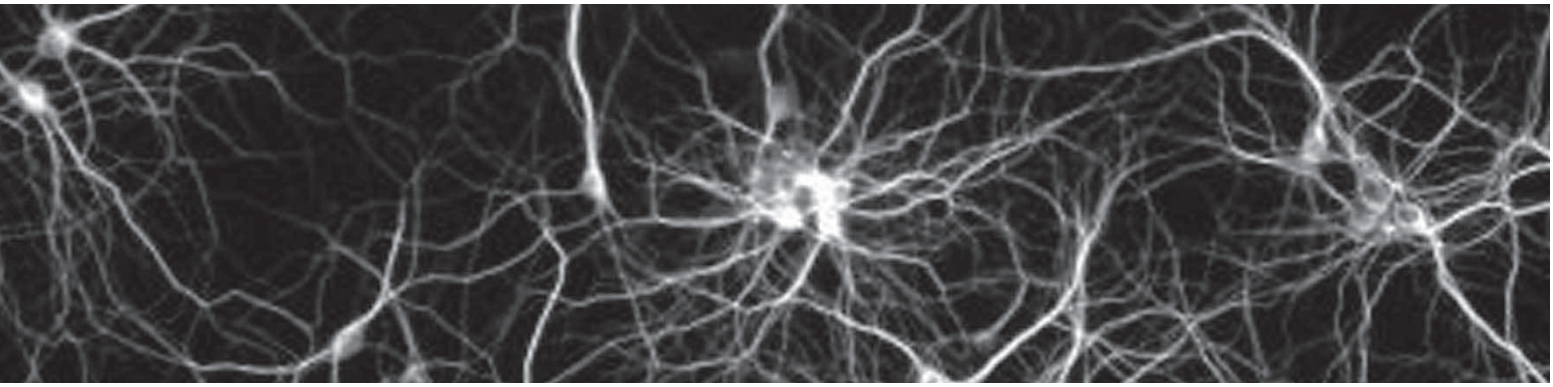

Concentrations of GH, IGF-1 and insulin in CSF of healthy people

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ABSTRACT – Growth hormone (GH), insulin-like growth factor-1 (IGF-1) and insulin are involved in brain development, and also act as neuroprotective factors. The values of these hormones in healthy population have not been published so far. We measured GH, IGF-1 and insulin concentrations in cerebrospinal fluid (CSF) of 57 healthy people (35 male and 22 female) undergoing lumbar puncture for spinal anesthesia before surgery of the knee joint. In men, the mean (SD; min-max) CSF concentrations of GH, IGF-1 and insulin were 0.67 (0.11; 0.50-0.90) μU/mL; 7.49 (0.92; 6.00-9.00) μg/L and 0.71 (0.13; 0.40-0.90) μU/mL, respectively. In women, the mean CSF concentrations of GH, IGF-1 and insulin were 0.69 (0.10; 0.50-0.90) μU/mL; 7.32 (0.95; 6.00-9.00) μg/L and 0.68 (0.13; 0.40-0.90) μU/mL, respectively. None of the study hormones showed a statistically significant sex difference. There was no significant bivariate correlation between GH, IGF-1 and insulin. The hormones did not correlate with age either. Determination of the normal range of GH, IGF-1 and insulin in CSF could help identify deviations in CSF hormone status in particular neurologic diseases. These values could prove important in the screening, diagnosis and management of various diseases involving the central nervous system.

Key words: cerebrospinal fluid, growth hormone, healthy people, insulin, insulin-like growth factor 1

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INTRODUCTION

Growth hormone (GH), insulin-like growth factor-1 (IGF-1) and insulin are involved in brain growth and development, and also act as neuroprotective factors (1-3). To the best of our knowledge, the values of these hormones in healthy population have not been published so far. Heinze *et al*. (4) have reported concentrations of GH, IGF-1, insulin-like growth factor binding protein-3 (IGFBP-3) and insulin-like growth factor binding protein-2 (IGFBP-2) in cerebrospinal fluid (CSF) of patients suffering from viral infection, leukemia, Hodgkin's disease or multiple sclerosis (MS). The authors only took samples without pathologic alterations in routine CSF analysis findings. However, their study subjects suffered from systemic diseases and could not be considered as healthy people. We measured GH, IGF-1 and insulin concentrations in CSF from 57 patients (35 male and 22 female) aged 11-71 (mean 45.56; SD 17.548) years undergoing lumbar puncture for spinal anesthesia before knee joint surgery. They had no recent history data on any other disease besides knee trauma.

In our previous study, we measured the concentrations of GH, IGF-1 and insulin in CSF and serum of healthy people serving as a control group for comparison with the values recorded in patients suffering from amyotrophic lateral sclerosis (ALS). We found ALS patients to have significantly lower CSF levels of GH, IGF-1 and insulin than healthy people (5).

The stimulatory action of GH on the proliferation of cerebrocortical brain cells could be mediated by an antiapoptotic action of GH that promotes cell survival (6). It is unclear whether GH is synthesized within the nervous tissue or it is solely taken up from the circulation (4). The high density of GH receptors in the choroid plexus (CP) suggests a possible receptor-mediated transcytosis transport (7-9). IGF-1 acts as a neuroprotective survival factor under pathologic conditions such as stroke, brain trauma, MS, or Alzheimer's disease (AD) (10-13). It is also effective in slowing the progression of motor neuron degeneration in wobbler mice (14,15). IGF-1 may reduce myelin breakdown and promote myelin regeneration in demyelinating diseases (16,17). The inhibitory effect on apoptosis is associated with the prevention of bcl-2 reduction by a mechanism resulting in the phosphorylation of the apoptotic BAD protein (18). Insulin in brain does not stimulate glucose metabolism in neurons; it stimulates glucose uptake in rat brain glial cells and human glioblastoma and enhances glycogen accumulation in astroglia-rich primary cultures from neonatal rat brain (19-22).

Since examinations of these substances in CSF have only recently been introduced and as yet experimentally used in clinical evaluation, relevant data on their normal values are still lacking. The aim of this study was to measure the concentrations of these factors in healthy people in order to enable recognition of abnormal values in particular neurologic diseases, having in mind how important role they play in physiologic neuroprotective processes.

PATIENTS AND METHODS

The study included 57 patients that underwent lumbar puncture for spinal anesthesia before knee joint surgery. They had no recent history data on any other disease except for knee trauma. The group consisted of 35 males and 22 females (mean age 45.56, SD 17.548 years). The patients were examined at University Department of Neurology, Zagreb University Hospital Center in Zagreb, Croatia. The approval for this study was granted by the Ethics Committee of the School of Medicine, University of Zagreb and Zagreb University Hospital Center, Zagreb, Croatia. Providing anonymity and informed consent obtained, the approval permitted CSF sampling when it could be safely obtained during lumbar puncture performed for anesthesia in knee joint surgery. An informed consent was obtained from each study participant.

CSF samples were obtained by lumbar puncture between 8.00 AM and 10.00 AM considering the well-known circadian rhythm of GH and IGF-1 concentrations in serum. All samples were immediately centrifuged, frozen in small aliquots and stored at -80 ^{o}C until analysis. To exclude the possible CSF contamination with blood constituents, only samples with less than 15 erythrocytes/μL were analyzed. The CSF leukocyte content was within the normal limits (<5 cells/μL). The CSF GH level was determined using the polyclonal immunoradiometric assay (IRMA) kit (BioSource hGH-IRMA, KIP1081, Nivelles, Belgium) according to the manufacturer's instructions (23). The detection level was 0.2 μU/mL. The CSF IGF-1 level was determined using the two-site immunoenzymometric assay (IEMA) according to the manufacturer's instructions (AC-27PL-GB; OCTEIA IDS Inc., Fountain Hills, AZ, USA), with sensitivity defined as the concentration corresponding to the

mean plus 2 SD of 20 replicates of the zero Calibrator (1.9 μg/L). The CSF insulin level was measured by using the microparticle enzyme immunoassay (MEIA) following the manufacturer's instructions (Abbott IMX system, 2A10, Abbott Laboratories, Wiesbaden, Germany); the sensitivity was 1.0 μU/mL.

The software package SPSS 17.0 (SPSS inc, Chicago, IL, USA) was used throughout data analysis. As all data showed normal distribution, the results were presented as mean ± SD. Due to the descriptive aim of the study, the minimal and maximal values were reported, irrespective of the normality of data distribution. Differences were tested using Student's t-test and correlations using Pearson's correlation test. $P<0.05$ was considered statistically significant.

RESULTS

The mean (SD; min-max) CSF concentration of GH was 0.68 (0.10, 0.50-0.90) μU/mL for the study group as a whole, 0.67 (0.11, 0.50-0.90) μU/mL for men, and 0.69 (0.10, 0.50-0.90) μU/mL for women (Fig. 1a, Table 1). The values showed normal distribution (skewness=0.222). The mean CSF concentration of IGF-1 was 7.42 (0.93, 6.00-9.00) μg/L for the study group, 7.49 (0.92, 6.00-9.00) μg/L for men, and 7.32 (0.95, 6.00-9.00) μg/L for women (Fig. 1b, Table 1). IGF-1 concentration also showed normal distribution (skewness=0.029). The mean CSF concentration of insulin was 0.70 (0.13, 0.40-0.90) μU/mL for the study group, 0.71 (0.13, 0.40-0.90) μU/mL for men, and 0.68 (0.13, 0.40-0.90) μU/mL for women (Fig. 1c, Table 1). Insulin concentration also showed normal distribution (skewness=-0.356). CSF concentrations of GH, IGF-1 and insulin are presented in Fig. 1a-c, respectively. Results according to sex subgroups are shown in Table 1. There were no sex differences in CSF concentrations of GH (t=-0.625; P=0.534), IGF-1 (t=0.662; P=0.510) or insulin (t=0.744; P=0.460). CSF concentration of GH did not correlate significantly either with IGF-1 (r=0.054; P=0.691) or with insulin (r=0.140; P=0.298) (Fig. 2a,b). There was no significant correlation between CSF concentrations of IGF-1 and insulin either (r=0.036; P=0.793)

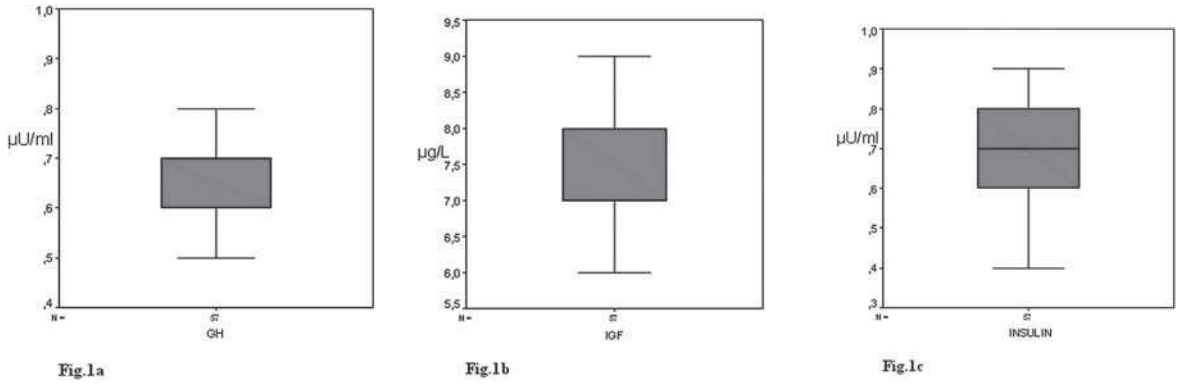

Fig. 1. *The mean CSF concentration of GH, IGF-1 and insulin in healthy people (N=57): (a) GH (μU/mL); (b) IGF-11 (μg/L); (c) insulin (μU/mL).*

Table 1. *Gender differences in CSF concentrations of GH, IGF-1 and insulin in healthy people.*

	Gender	n	Mean	SD	Min	Max	t	*P*
GH μU/mL	m	35	0.67	0.11	0.50	0.90	-0.625	0.534
	f	22	0.69	0.10	0.50	0.90		
IGF-1 μg/L	m	35	7.49	0.92	6.00	9.00	0.662	0.510
	f	22	7.32	0.95	6.00	9.00		
Insulin μU/mL	m	35	0.71	0.13	0.40	0.90	0.744	0.460
	f	22	0.68	0.13	0.40	0.90		

SD = standard deviation; GH = growth hormone; IGF-1 = insulin-like growth factor-1; m = male; f = female; Min = minimum; Max = maximum; n = number

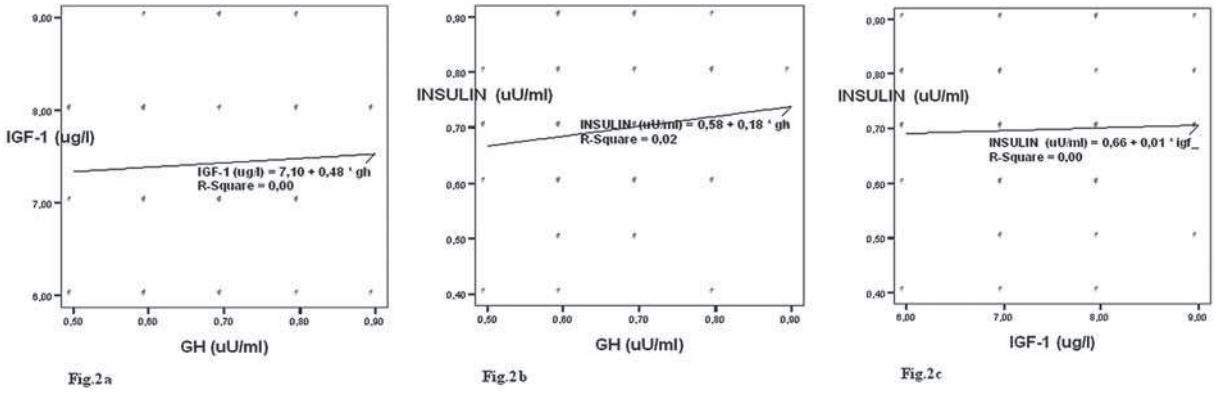

Fig. 2. *Relation between GH, IGF-1 and insulin in CSF of healthy people (n=57), linear regression: (a) GH (μU/mL) vs. IGF-1 (μg/L); (b) insulin (μU/mL) vs. GH (μU/mL); (c) insulin (μU/mL) vs. IGF-1 (μg/L).*

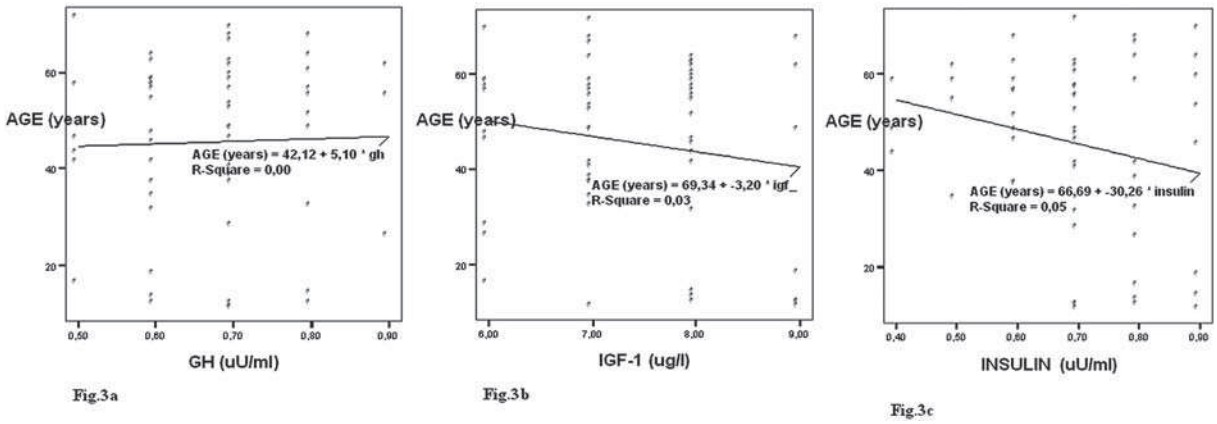

Fig. 3. *Relation between GH, IGF-1, insulin and age (years) in CSF of healthy people (N=57), linear regression: (a) GH (μU/mL) and age (years); (b) IGF-1 (μg/L) and age (years); (c) insulin (μU/mL) and age (years).*

(Fig. 2c). There was no significant correlation between age and CSF concentrations of GH (r=0.030; *P*=0.824), IGF-1 (r=-0.169; *P*=0.209) and insulin (r=-0.277; *P*=0.090) (Fig. 3a-c).

DISCUSSION

This is a report on the concentrations of GH, IGF-1 and insulin in CSF of healthy people. The mean CSF GH was 0.68 (0.10) μU/mL, IGF-1 7.42 (0.93) μg/L and insulin 0.70 (0.13) μU/mL. The values of all three study parameters showed normal distribution. None of the study hormones showed any statistically significant sex difference. There was no significant bivariate correlation between GH, IGF-1 and insulin. The hormones did not correlate with age either.

Early studies of the brain glucose metabolism established the axiom that insulin is not required for glucose utilization by the central nervous system (CNS). A corollary to this concept was the belief that circulating insulin is incapable of crossing the blood-brain barrier (BBB). While the first of these tenets remains unchallenged, the second has been subjected to detailed scrutiny, following the identification of both insulin and its receptor in the adult mammalian brain (24-26). Some brain areas like olfactory bulb and hypothalamus have immunoreactive insulin concentrations that are two- to three-fold those of other brain regions (27-29). Insulin receptors appear to be concentrated on the CSF-facing surface of choroidal epithelium (24,30), and it is possible that insulin receptors may contribute to CSF insulin removal (24,30,31). In contrast to its action in peripheral tissues, insulin does not stimulate glucose metabolism in neurons. Glucose transport, glucose oxidation, and glycogen synthesis in cultured fetal chick neurons are not altered by insulin (20,24). Evidence suggests a regionally specific effect of insulin on brain glucose metabolism (32). Insulin does not seem to influence basal cerebral glucose metabolism or transport of glucose into the brain (32,33). *In vitro* studies showed that insulin regulated glucose uptake by glial cells, but did not influence neuronal glucose uptake (32,34). Insulin receptor mRNA concentrations in

the rat CNS are maximal at birth and decline to minimal levels in adult brain (35). Studies in human and animal models have shown that an increase in brain insulin has a cognition-enhancing effect (34). Cognitive dysfunction and dementia have recently been proven to be common and underrecognized complications of diabetes mellitus (DM) (36). Patients suffering from AD and ALS were found to have lower than normal CSF levels of insulin (5,36). Too much insulin in the brain may be associated with reduced amyloid-β clearance due to competition for their common and main depurative mechanism, the Insulin Degrading Enzyme (36). On the other hand, hyperglycemia by producing hyperinsulinemia may lead to an increased production of Reactive Oxygen Species (ROS), protein glycation and oxidative stress, some of the processes important even for physiologic brain aging. Neurons have been shown to share more similarities with the insulin-producing pancreatic islet cells than with any other cell type. The root of this similarity is uncertain, but it may lie in the islets' evolution from an ancestral insulin-producing neuron (37). Not more than 10 years ago, the brain was described in medical textbooks as "an insulin insensitive organ". Evidence for the presence of insulin and its receptors in the CNS has challenged this notion in recent years (38-40).

The insulin-like growth factors (IGFs) first appeared early in phylogeny about 600 million years ago and have increased in number through gene duplication (41). In mammalians, IGFs are expressed in all tissues and are found in many biological fluids (42,43). IGF-1 promotes differentiation, proliferation and prevents apoptosis of brain-derived cells, and helps in myelinization, dendrite growth and cytoskeleton protection (44). The mechanism by which IGF-1 prevents cells from entering a death program has not been completely defined, but the phosphatidylinositol kinase pathway is implicated and it seems that neuroprotective action of IGF-1 is linked to the Bcl family (45,46). Furthermore, IGF-1 is thought to have a neuromodulatory function affecting glutamic acid decarboxylase and choline acetyltransferase activities as well as dopamine uptake (47,48). The levels of IGF-1 and its binding proteins may be altered in CSF in various neurologic disorders like autism, ALS, AD and MS (5,42,49,50). In rats, IGF is secreted by the choroid plexus (51). During embryonic development, IGF-1 mRNA is detectable in many brain regions, its expression being particularly high in the midbrain and cerebral cortex (42,52). IGF-1 is also expressed in the leptomeninges and choroid plexus, enabling growth factors to diffuse to their sites of activity (42,53). In most neurons, IGF-1 transcription decreases significantly postnatally, correlating with the degree of cell maturation and reaching low levels in adults (54). Systemic IGF-1 is not readily transported through BBB, and therefore the local production of IGF-1 is the main and primary source of this neural growth factor for the brain (42). IGF in the CSF can pass through the ependymal layer and reach the brain parenchyma (55). Choroid plexus-derived IGF might be important for cellular survival and recovery shortly after injury, before the neural tissue starts to produce its own IGF. Neurotrophic factor is therefore secreted into the brain ventricles and conveyed by CSF bulk flow to various regions of the brain and spinal chord, bringing many neurons in contact with this valuable molecule necessary for physiologic functioning of neural tissue (56). For example, the exercise in physiologic conditions causes increase in circulating IGF-1 levels, which is proven to be neuroprotective in the cases of various brain injuries, and when IGF-1 uptake into the CSF is blocked, the neuroprotective effect is lost (57).

GH was isolated in 1944 and used for therapeutic purposes in the 1960s (58). The DNA encoding GH was cloned in 1979 and recombinant GH was approved for clinical use in 1985 (59). Favorable effects of GH substitution on the metabolism, cardiovascular system and body composition have been described, but during the past two decades, the effects of somatotropic axis on the CNS have come into the focus of interest. In fact, the stimulatory action of GH on the proliferation of cerebrocortical brain cells could perhaps be mediated by an antiapoptotic action of GH that promotes cell survival. GH is present in the brain of human embryos during the 8th week of development, prior to its appearance in the pituitary gland at the end of the first trimester (60). It remains unclear whether these effects are mediated directly by GH or by its mediator, IGF-1 (61). In human brain, GH receptor can be found in many brain areas. They are detected in highest concentrations in choroid plexus, like those for IGF-1 and insulin, but also in the hippocampus, basal ganglia and hypothalamus. A reduction in GH binding sites in the brain is seen with increasing age (62). The somatotropic axis plays a central role in the development and growth of the CNS. Distinction between GH and IGF-1 mediated effects is often difficult, but transgenic mouse models have shown that overproduction of GH induces an increase in body size and motoneuron size, whereas overproduction of IGF-1 induces increase in body size only (6,61).

The possible roles of insulin, GH and IGF-1 in the pathogenesis of neurodegenerative diseases (Parkinson's disease, AD, ALS and Huntington's disease) have already been investigated (63,65). It is also necessary to clarify the role of these neuroprotective and neurotrophic factors in neurologic diseases and mechanisms involved in cell death (apoptotic or other).

Considering the pain, side effects and risks associated with the procedure of lumbar puncture, it may be difficult to compose an adequate control group for investigations of GH, IGF-1 and insulin in humans. We decided to measure CSF concentrations of insulin, GH and IGF-1 in healthy subjects to help in future investigations focused on their concentrations and roles in various neurologic diseases and to try to establish their standard values. Determination of these neural growth factors in CSF of healthy people could be important for distinction of their pathologic values in certain neurologic and other diseases (66,67). These parameters could possibly serve as markers of disease activity, or as a screening method, which is impossible without the knowledge of their normal levels.

All the neural growth factors measured, GH, IGF-1 and insulin, are involved in neural development and they are probably an important component in the physiologic neuroprotective processes as well as in brain aging. It is surprising that despite all their known important roles in brain development, aging and possible rejuvenation, they have shown modest therapeutic effects in the treatment of neurodegenerative diseases. It seems likely that GH, insulin and IGF-1 do not cross BBB efficiently enough for therapeutic goals and therefore should be administered intrathecally in various therapeutic strategies.

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