ORIGINAL ARTICLE

Leptin, adiponectin and ghrelin, new potential mediators of ischemic stroke

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Abstract

OBJECTIVES: Fat tissue is an important endocrine organ that produces a number of hormones and cytokines (leptin, adiponectin, resistin, plasminogen activator inhibitor-1, Tumour necrosis factor TNF α) with essential roles in regulation of many physiological functions.

METHODS: We targeted implications of adipokines in ischemic stroke patients. Patients with acute stroke were examined (n=145) and the results were compared with the control group (n=68). We have examined potential associations between leptin, adiponectin and ghrelin, and different types of stroke and traditional risk factors.

RESULTS: Significantly higher levels of leptin and lower levels of adiponectin and ghrelin were confirmed in the stroke group. The level of leptin in women with stroke was three-times higher than in men, and the leptin levels positively correlated with obesity in both sexes. Ghrelin levels correlated mildly with triglyceride levels, and were dominant in men with cardioembolic stroke. Adiponectin levels were not different between men and women with acute stroke, and correlated with atherothrombotic and lacunar stroke types in men.

CONCLUSIONS: Adipokines and ghrelin play an important role in ischemic stroke, but their function in stroke subtypes seems to be different and sex influenced. More research is required to confirm our results.

Abbreviations:

ADI - adiponectin
AH - arterial hypertension
AT - atherothrombotic stroke
BMI - body mass index
CE - cardioembolic stroke
CT - computer tomography
DM - diabetes mellitus
GHR - ghrelin
Gly - glycemia
HDL - high density lipoproteins
IL-6 - interleukin-6
IS - ischemic stroke
LAC - lacunar stroke
LEP - leptin
LDL - low density lipoproteins
MR - magnetic resonance imagine
TG - triglycerides
TNF α - tumour necrosis factor α
WC - waist circumference

INTRODUCTION

Stroke is one of the leading causes of high morbidity and mortality. To reveal risk factors is important for setting the goals for primary and secondary prevention. The impact of traditional risk factors on stroke has been studied and is more or less known. Obesity is one of the traditional risk factor. Adipose tissue, as an endocrine gland, plays a critical role by secreting specific hormones, adipokines, with paracrine, autocrine, and endocrine effects. Leptin and adiponectin appeared to be the most important for cerebrovascular signalling and central effects. Ghrelin is a novel peptide derived from gastrointestinal tract. Its role in stroke and cerebrovascular morbidity is not known yet. The aim of our study was to identify the role of adipokines in acute stroke.

METHODS

Patients and controls

145 consecutive patients (male and female) with acute ischemic stroke were enrolled for the study between the years 2006–2008. The mean age of stroke patients was 66.7 ± 12.1. During the same period 67 controls, free of acute cerebrovascular disease and matched for age and sex, were recruited. The mean age of control group was 63.06 ± 11.2. Risk factors documented in both groups included height, weight including body mass index (BMI), waist circumference (WC), glycaemia, HDL, TG levels, presence of arterial hypertension (AH), diabetes mellitus (DM), and measurement of adipokines (leptin, adiponectin and gastrointestinal hormone ghrelin, using ELISA methods).

Leptin concentrations were measured in 145 patients with ischemic stroke (80 males and 65 females) and 61 controls. Ghrelin levels were obtained from 113 stroke patients (68 males and 45 females) and 45 controls. Adiponectin levels were measured in 140 stroke patients (72 males and 68 females) and 67 controls.

All cases were examined by a neurologist and underwent neuroimaging (CT and/or MR). When clinically indicated we performed duplex ultrasound (both on extracranial carotid and vertebral arteries) or angiography (CT or MR). The stroke subtypes were determined according to TOAST criteria with the use of clinical, radiological, cardiac, and ultrasound tests. Atherothrombotic stroke (AT) was defined as >50% stenosis or occlusion of an appropriate major brain artery, with the absence of cardiac source of embolism. Lacunar (LAC), or small-vessel stroke, was defined as a relevant infarct less than 1,5 cm in a diameter, with the absence of cardiac source of embolism or large-vessel stenosis > 50%. Cardioembolic stroke (CE) was defined as the presence of atrial fibrillation or other cardiac dysfunction, with high risk source of embolism.

Statistical analysis

Differences between the groups (ischemic stroke patients and controls, men and women) were examined by Student t-test for normally distributed continuous variables or Mann-Whitney's and Kruskal-Wallis's all pairwise comparisons (Conover-Inman) tests for non-normally distributed variables. ANOVA test was performed for differentiation between leptin, ghrelin and adiponectin groups. Logistic regression models were used for determining the correlations between adipokines and stroke risk factors in every stroke subgroup.

RESULTS

Leptin levels were significantly increased in stroke patients compared to controls. Both ghrelin and adiponectin levels were significantly decreased in the stroke group in comparison with the control group. Data are shown in Table 1.

The differences between men and women with acute stroke were evident for leptin and ghrelin levels, but were not found in adiponectin concentrations. In patients with ischemic stroke, women have shown three times higher levels of leptin than men and ghrelin levels were significantly increased in men in comparison with women. Results are shown in Table 2.

Determination of stroke subtypes was based on clinical, radiological and ultrasonography test results. The distribution of stroke subtypes was as follows: AT stroke was the most frequent, 45 out of the men (56.2%) and 31 out of the women (45.5%). LAC stroke was diagnosed in 24 men (30.0%) and 24 women (35.3%). CE stroke was found in 11 men (13.8%) and 13 women (19.21%). Results are in Figure 1.

Adipocytokines and ghrelin levels have shown differences between various stroke types in men. GHR and ADI levels were significantly higher in the group of patients with cardioembolic stroke than in AT and LAC types of stroke, and it was the highest of all the groups with acute stroke. On the other hand, GHR and ADI were the lowest in AT and LAC stroke subtype groups.

Tab. 1. Leptin, ghrelin and adiponectin levels in stroke patients and controls.

	LEP (ng/ml)	GHR (pg/ml)	ADI (ng/ml)
Stroke patients	17.59 ± 19.5	827.2 ± 574.4	9.58 ± 16.24
Controls	7.97 ± 7.52	1297.2 ± 493.4	12.76 ± 17.4
p-value	**0.0002**	**<0.0001**	**0.001**

LEP = leptin, GHR = ghrelin, ADI = adiponectin,
p=0.05 statistical significance

Tab. 2. Sex differences of leptin, ghrelin and adiponectin levels in stroke group.

	LEP (ng/ml)	GHR (pg/ml)	ADI (ng/ml)
Men IS	8.91 ± 6.31 (n = 80)	914.2 ± 773.7 (n = 68)	9.4 ± 5.15 (n = 72)
Women IS	28.2 ± 17.8 (n = 65)	695.7 ± 544.8 (n = 45)	9.7 ± 4.4 (n = 68)
p-value	**<0.0001**	**0.04**	0.57

IS = ischemic stroke, LEP = leptin, GHR = ghrelin, ADI = adiponectin
p=0.05 statistical significance, Man-Whitney statistical test.

Tab. 3. Distribution of leptin, ghrelin and adiponectin levels according to stroke types in men.

Men	I. AT IS	II. LAC IS	III: CE IS	*p*-value
LEP (ng/ml)	9.9 med 6.28 (n = 45)	8.3 med 6.8 (n = 24)	6.0 med 5.7 (n = 11)	0.59
GHR (pg/ml)	868.2 med 576.0* (n = 41)	854.1 med 672.3* (n = 20)	**1355.1 med 1497.7** (n = 7)	**0.05***
ADI (ng/ml)	7.35 med 1.73** (n = 37)	10.25 med 4.8* (n = 22)	**13.9 med 10.2** (n = 13)	**0.003**** **0.05***

AT = atherothrombotic, LAC = lacunar, CE = cardioembolic stroke,
IS = ischemic stroke, LEP = leptin, GHR = ghrelin, ADI = adiponectin
p=0.05 statistical significance; *,** = differences among subgroups
Kruskal-Wallis all pairwise comparisons (Conover-Inman) statistical test; med = median

Leptin levels and medians remained stable and similar in all the subgroups. The differences were not statistically significant. The result are shown in Table 3.

We have not found any differences in adipocytokines and ghrelin concentrations in stroke subtypes in women. The results are shown in Table 4.

The concentrations of LEP, GHR and ADI were adjusted to age. In men, LEP reflected the obesity parameters and the amount of body fat. The strongest positive association of LEP were obtained with WC and BMI, as shown in Table 5 and graph 2. GHR levels negatively correlated in trend with TG level.

In women, LEP concentrations were significantly associated with obesity parameters, similar to men. Significant correlations were found between LEP and WC, as well as LEP and BMI index. The correlation between GHR and WC showed a trend towards a negative correlation but it was not significant. The results are in Table 6 and Figure 2.

Tab 4. Distribution of leptin, ghrelin and adiponectin according to stroke types in women.

Women	I. AT IS	II. LAC IS	III: CE IS	*p*-value
LEP (ng/ml)	27.9 med 25.06 (n = 31)	26.6 med 20.1 (n = 24)	22.3 med 13.5 (n = 10)	0.9
GHR (pg/ml)	716.3 med 577.5 (n = 21)	624.6 med 367.4 (n = 16)	783.7 med 698.7 (n = 8)	0.67
ADI (ng/ml)	11.01 med 4.66 (n = 27)	8.29 med 3.68 (n = 28)	10.2 med 7.9 (n = 13)	0.9

AT = atherothrombotic, LAC = lacunar, CE = cardioembolic stroke,
IS = ischemic stroke, LEP = leptin, GHR = ghrelin, ADI = adiponectin,
p=0.05 statistical significance; Kruskal-Wallis all pairwise comparisons (Conover-Inman) statistical test; med = median

Tab. 5. Correlation of leptin, ghrelin and adiponectin with risk factors in men.

Men stroke	LEP (ng/ml) (n = 80)	GHR (pg/ml) (n = 68)	ADI (ng/ml) (n = 72)
WC (cm)	*p*<0.0001, r=0.55	*p*=0.42	*p*=0.75
AH (%)	*p*=0.33	*p*=0.64	*p*=0.44
Gly (mmol/l)	*p*=0.31	*p*=0.55	*p*=0.69
HDL (mmol/l)	*p*=0.64	*p*=0.31	*p*=0.32
TG (mmol/l)	*p*=0.93	*p*=0.07, r=–0.22	*p*=0.92

WC = waist circumference, AH = arterial hypertension,
Gly = glycemia, HDL = high density lipoproteins, TG = triglycerids,
LEP = leptin, GHR = ghrelin, ADI = adiponectin
p=0.05 statistical significance, multiple linear regression test

Tab. 6. Correlation of leptin, ghrelin and adiponectin with risk factors in women.

Women stroke	LEP (ng/ml) (n = 65)	GHR (pg/ml) (n = 45)	ADI (ng/ml) (n = 68)
WC (cm)	*p*=0.002, r=0.37	*p*=0.09, r=–0.27	*p*=0.28
AH (%)	*p*=0.56	*p*=0.29	*p*=0.83
Gly (mmol/l)	*p*=0.6	*p*=0.31	*p*=0.72
HDL (mmol/l)	*p*=0.33	*p*=0.66	*p*=0.85
TG (mmol/l)	*p*=0.52	*p*=0.48	*p*=0.87

WC = waist circumference, AH = arterial hypertension,
Gly = glycemia, HDL = high density lipoproteins, TG = triglycerids,
LEP = leptin, GHR = ghrelin, ADI = adiponectin
p=0.05 statistical significance, multiple linear regression test

DISCUSSION

Leptin (LEP) is an adipocyte-derived hormone and cytokine that regulates energy expenditure via central hypothalamic receptors. The concentrations of leptin correlate positively with the body fat mass stored. When fasting, a fall in leptin initiates a complex body response in order to safe energy stores (Hrnčiar 2007; Konturek *et al.* 2004; Levine 2006). We have confirmed a positive correlation between the amount of body fat and the leptin levels in men and women with acute

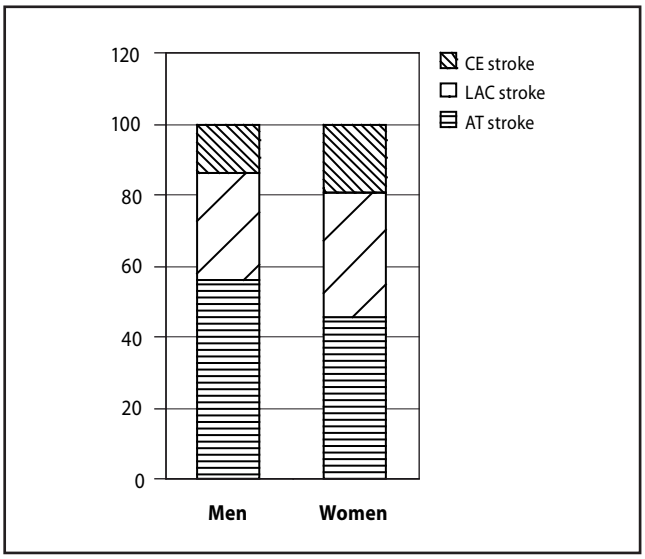

Fig. 1. Stroke type distribution in men and women. AT = atherothrombotic stroke, LAC = lacunar stroke, CE = cardioembolic stroke

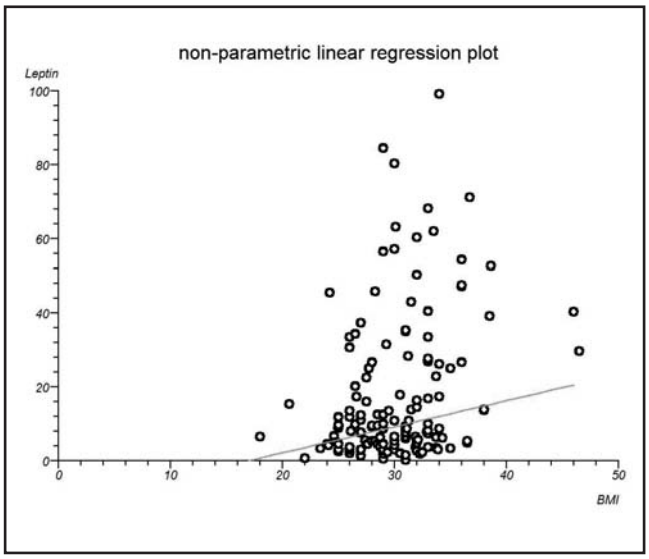

Fig. 2. LEP and BMI correlation. BMI = body mass index, LEP = leptin, p=0.0009

stroke. These levels were three times higher in women than in men, but still higher than in controls. Recent studies have found the phenomenon of leptin resistance. It is a situation when large amounts of circulating leptin do not signal enough to maintain healthy weight and energy equilibrium. The mechanisms of leptin resistance include leptin self-regulation (leptin induced down-regulation of its receptors) based on permanently higher energy intake; presence of cellular and circulating inhibitory molecules; or rare genetic mutations of leptin receptor (Martin *et al.* 2008). Leptin resistance is considered to be a mechanism increasing LEP concentrations in patients with ischemic stroke. Central leptin resistance causes obesity, and obesity-induced leptin resistance affects numerous tissues and organs. LEP with evolving leptin resistance leads to alteration of immune functions, over-expression of tumour necrosis factor α (TNF α), proinflammatory cytokines, interleukin-6 (IL-6), and components of renin-angiotensin-aldosterone system (Levine 2006). Atherosclerosis is considered to be an inflammatory disease, driven by lipoproteins, hemodynamic stress, and the activity of immune cells. In leptin resistance, LEP promotes atherosclerosis and endothelial dysfunction (Levine 2006; Martin *et al.* 2008; Norata *et al.* 2007). We have not found differences in LEP levels between the different stroke groups. Its higher levels can lead to atherosclerosis and proinflammatory potency in all types of ischemic stroke. Measuring the leptin/adiponectine ratio would bring more information about atherosclerotic process. The ratio appears to be a better predictor of intima-media thickness, as was noted by Norrata et al (2007). Leptin-mediated central sympathetic activation, that originates in hypothalamus, and renal renin-angiotensin-aldosteron mechanisms are both involved in hypertension response of the organism, and the development of arterial hypertension. Leptin-induced insulin resistance is associated with hyperinsulinemia, which is independent of BMI (Martin *et al.* 2008). Normally leptin decreases insulin secretion by directly influencing pancreatic B-cell receptors, enhancing skeletal muscle glucose uptake, increases wholebody glucose utilization and suppressing hepatic glucose production (Levine 2006; Martin 2008). Positive relationship was not found between LEP, AH and glycaemia levels in our study.

Adiponectin (ADI) is a hormone and cytokine with opposite function to leptin. ADI is synthesized in adipose tissue, but blood levels of adiponectin are inversely correlated to body fat mass. ADI plays role in controlling the body weight and energy homeostasis by increasing energy expenditure. Peripheral effects of ADI are known to depend on activation of c AMP kinase. ADI does not cross the blood-brain-barrier and is not detectable in cerebrospinal fluid (Spranger *et al.* 2006). ADI receptors were found to be expressed on brain endothelial cells. The direct mechanism of central activity of ADI is still not known, but a protective effect of ADI to vessel wall was confirmed (Levine 2006; Nishimura *et al.* 2008).

In that context, our stroke patients showed evidently lower levels of ADI than controls, and the lowest levels were found in men with AT stroke and in women with LAC stroke. These findings are supported by report-listed authors who inform of correlation between symptomatic intracranial atherosclerosis and lower ADI levels. Patients with more severe metabolic abnormalities were more likely to have lower ADI

(Bang *et al.* 2007; Lawlor *et al.* 2005; Matsubara *et al.* 2003). ADI enhances endothelial production of nitric oxide, promotes growth of new blood vessel, improves insulin sensitivity, and has anti-inflammatory effects. ADI suppresses the production of proinflammatory cytokines: TNF α, IL-6 and others (Lawlor *et al.* 2005; Levine 2006). Accumulation of ADI in the subendothelial space has been reported after arterial injuries. This process was followed by ADI-modulated endothelial adhesion molecules suppression (Levine 2006). ADI has a potent protective effect on endothelial dysfunction and improves vascular impairment (Bang *et al.* 2007; Hrnčiar 2007; Lawlor 2005). Hypoadiponectinemia can serve as an independent predictor of mortality after stroke (Efstathiou *et al.* 2005). Stroke patients in our group had hypoadiponectinemia but we did not confirm any associations with other risk factors.

Ghrelin is a peptide hormone, with growth-hormone-releasing and appetite-inducing effects, with predominant expression in gastric mucosa and in lesser extent in other body parts –such as heart, pulmonary tissue (Kola *et al.* 2005; Konturek *et al.* 2004). It has been suggested that ghrelin favours lipid stores and carbohydrate-derived fuel by stimulating specific centres in hypothalamus. Central effects of ghrelin are similar to cannabinoids (Kola *et al.* 2005). Ghrelin levels are high during fasting and with low BMI. Ghrelin suppresses production of proinflammatory cytokines and improves proinflammatory milieu by inhibiting T lymphocytes and monocytes (Vestergaard *et al.* 2008, St-Pierre *et al.* 2003). Ghrelin demonstrates potent vasodilator properties based on increased endothelial nitric oxide expression, that is endothelium and growth hormone independent (Nishimura *et al.* 2008; Zaloga 2005). Ghrelin has been shown to have beneficial effect in the ischemic heart disease via reducing cardiac afterload, increasing cardiac output without increasing heart rate (Zaloga 2005).

In our study we have found decreased levels of GHR in the stroke group compared to the controls. We suppose that these findings reflect metabolic dysregulation and loss of protective effect of GHR to arterial wall during ischemic stroke. We have not found any other published information about a role of a ghrelin in stroke.

We have found that the lowest levels of GHR present in LAC and AT types of stroke. Ghrelin appeared at the highest concentration only in men with CE-type stroke. It is known that GHR and its receptor are also synthesized in cardiac tissue and have beneficial cardiovascular effect. More than 20-fold increase of right ventricular production of GHR has been reported to be connected with right ventricular hypertrophy (Zaloga 2005). As patients with cardioembolic stroke have serious cardiac symptoms prior to stroke, ghrelin production may be regarded as a cardiac response to overload, and the effort to tackle the disadvantage.

CONCLUSIONS

Adipokines are supposed to play a specific role in the process of atherogenesis and in imflammatory processes generally. Bivalent activity of leptin and adiponectin is normally balanced, resulting in a healthy and steady state. Accumulation of leptin levels, started to be dangerous in situation of inadequate metabolic compensation and in impaired defensive role of adiponectin. Our results confirmed differences of leptin and adiponectin levels between the stroke and the control groups. We have found significantly higher levels of leptin and lower levels of adiponectin in stroke patients. Ghrelin's function in etiopathogenesis of stroke is not known fully. The ghrelin positive role was found on cardiovascular functions (e.g. vasodilatatory effects on coronary arteries), but its task on brain vascular system is not proven. We have confirmed the highest levels of ghrelin in the cardioembolic stroke subgroup. Obesity and adipokines can be targeted for aggressive intervention and therapy due to higher prevalence of adipose hormones and ghrelin impairment among individuals with stroke. Leptin lowering therapy is based on weight reduction, insulin sensitivity reactivation, and lipid profile improvement. Except the above-mentioned positive effect, fenofibrate was published to depress leptin levels (Damci *et al.* 2003). Thiazolidindions, inhibitors of angiotensin-converted enzyme, and angiotenzin II. blockers may serve as potent stimulators of adiponectin production and they reinforce positive vascular effects (Efstathiou *et al.* 2005). How to artificially influence grelin is not yet known.

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