Decreased total serum adiponectin and its isoforms in women with acute ischemic stroke

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Abstract

OBJECTIVE: An association between cerebral infarct risk factors and serum adiponectin levels (both total and separate isoforms) has previously been identified. The aim of this study was to assess circulating levels of all forms of adiponectin in the course of an ischemic stroke.
MATERIAL AND METHODS: Adiponectin and its isoforms (HMW, MMW and LMW) were measured in serum samples taken from 38 women in the first 24 hours of cerebral infarct and 38 controls matched for gender, body mass index (BMI) and age. In addition, biochemical parameters (glucose, insulin, lipid profile) and clinical data (blood pressure, weight, and height) were evaluated.
RESULTS: A significant reduction in serum levels of adiponectin and all examined fractions of this adipokine was observed in women suffering from acute ischemic stroke, compared with the matched controls.
CONCLUSIONS: Differences in the serum adiponectin array between stroke subjects and controls were identified and further studies are required to investigate the clinical implications of this finding.

INTRODUCTION

Ischemic stroke, a condition caused by obstruction of vessels supplying blood to the brain, belongs to a class of disorders encompassed by the term cardiovascular disease (CVD). Several medical conditions can lead to CVD, including diabetes, hypertension and serum lipid profile abnormalities. All promote atherogenesis, which is a major cause of CVD. In addition, these conditions are all directly connected with an increased amount of adipose tissue, which is found in obesity.

Many studies have revealed that adipose tissue acts not only as an energy storage organ, but is also able to produce and secrete biologically active substances. The hormone adiponectin is one such product of adipose tissue. Two specific receptors, through which adiponectin acts, have been identified: AdipoR1 and AdipoR2. The former is found mostly in skeletal muscles, whereas the latter is expressed in liver (Kadowaki *et al.* 2008). It has been reported that AdipoR2 expression also occurs in the central nervous system, especially in the hypothalamus, cortex and hippocampus (Kadowaki *et al.* 2008). In addition, both receptors are expressed in the arcuate and lateral hypothalamic nuclei as well as the human pituitary gland (Psilopanagioti *et al.* 2009). The action of

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adiponectin through AdipoR1 is associated with the AMP-dependent protein kinase (AMPK) signaling pathway, whereas AdipoR2-mediated adiponectin activity is connected with the peroxisome proliferator activator receptor (PPAR)α pathway (Yamauchi *et al.* 2007).

Interestingly, adiponectin exists in serum in three isoforms: trimers (LMW – low molecular weight), hexamers (MMW – middle molecular weight) and multimers (12–18 mers; HMW – high molecular weight) (Kadowaki *et al.* 2008).

Adiponectin influences energy homeostasis and glucose and lipid metabolism, and it also exerts a direct anti-inflammatory effect on blood vessels (Goldstein *et al.* 2009). This polypeptide is a beneficial modulator of glucose and lipid metabolism through (i) stimulation of fatty acid oxidation and reduction of plasma triglycerides, and (ii) enhancement of insulin sensitivity by stimulation of glucose utilization and fatty acid oxidation as a result of phosphorylation and activation of AMPK in muscles and liver (Yamauchi & Kadowaki, 2008). Besides its metabolic effects, adiponectin has anti-inflammatory and vaso- and cardioprotective properties and is able to suppress TNFα (tumor necrosis factor α) expression (Goldstein *et al.* 2009). It may also inhibit endothelial adhesion molecules like VCAM-1, ICAM-1 and E-selectin. In addition, it is thought to decrease transformation of macrophages to foam cells and to reduce cell proliferation (Goldstein *et al.* 2009). It may be concluded that adiponectin can mediate vasoprotective effects against the initiation and progression of atherosclerosis. Besides, adiponectin stimulates the production of nitric oxide in vascular endothelial cells through phosphatidylinositol 3-kinase-dependent pathways (Chen *et al.* 2003).

It has been reported that serum adiponectin levels are lower in obesity, type 2 diabetes, in hypertensive subjects and in those suffering from coronary artery disease (CAD) (Maury & Brichard 2010; Nakamura *et al.* 2004).

The data summarized above suggest that adiponectin plays an important role in the control of metabolism, and it might also affect the course of cerebrovascular disease.

Several studies have examined the relationship between obesity, and HMW adiponectin. It has been found that a decrease in serum HMW adiponectin levels is associated with a higher risk of insulin resistance and type 2 diabetes (Basu *et al.* 2007). It is also well documented that obesity, hypertension and diabetes are important risk factors for stroke (Goldstein *et al.* 2006). However, there have been no reports concerning the adiponectin isoform profile during the acute phase of ischemic stroke.

The aim of this study was to assess the serum concentration of different adiponectin isoforms – especially HMW adiponectin – in women with risk factors of cardiovascular disease who suffer from acute ischemic stroke.

MATERIAL AND METHODS

Subjects

The study population was comprised of 76 Caucasian women: 38 with the first episode of acute ischemic stroke (aged 60–85 years, mean 76.2) and 38 controls without stroke in their medical history (aged 60–85 years, mean 75.29), matched head to head for age and body mass index (BMI). Patients with acute ischemic stroke were hospitalized in the Neurology Clinic of Warsaw Medical University. Women from the control group were recruited as volunteers and were admitted to the Outpatient Clinic. The study concentrated on women to avoid gender differences in the adipokine profile.

All subjects in the stroke patient group suffered from at least one of the following diseases: hypertension, type 2 diabetes or lipid disturbances. The diagnosis of these conditions was made 6 or more months prior to their admission to the neurology unit and the women were receiving appropriate treatment.

The incidence of hypertension was 92% (n=35), type 2 diabetes – 55% (n=21) and obesity (BMI >30 kg/m^2) – 32% (n=12).

Some women from the control group have a history of hypertension (n=29; 76%) or diabetes (n=3; 8%), and 75% of them were receiving lipid-lowering treatment. In addition, 47% of controls (n=18) were overweight (BMI 25–29.9 kg/m^2) and 26% of them (n=10) were obese (BMI ≥30 kg/m^2).

Stroke was defined according to the NIHSS (National Institutes of Health Stroke Scale) criteria. Sudden disturbances of brain function with symptoms persisted for at least 24 hours. The initial diagnosis was confirmed using brain CT or MRI scans for each patient.

Hypertension was defined in accordance with ESH and ESC criteria. A diagnosis of type 2 diabetes was established when fasting plasma glucose was ≥126 mg/dl, or the plasma glucose concentration was ≥200 mg/dl two hours after a 75 g oral glucose load (oral glucose, or when the recorded plasma glucose level was ever ≥200 mg/dl.

The criteria for exclusion from the study were (i) acute or chronic renal or hepatic disease, (ii) chronic circulatory failure, (iii) a history of neoplasm or acute inflammatory processes, and (iv) a history of excessive alcohol consumption and/or smoking.

Data including blood pressure, height, weight and BMI were collected.

The study protocol was approved by the Local Ethics Committee in the Medical Centre for Postgraduate Education in Warsaw. All subjects and/or their caregivers were informed of the purpose of the study and written consent was obtained.

Adiponectin measurements and assessment of lipid and glucose profiles.

Blood samples were taken from all subjects in the morning after overnight (12 h) fasting. In the group of

women with stroke, this was done within 24 hours of the first symptoms of stroke.

Immediately after collection, the specimens were centrifuged at 4 °C and the serum was frozen at –70 °C for later analysis. The levels of total adiponectin and its HMW, MMW and LMW isoforms were measured using an EIA test (ALPCO). Insulin concentration was established by IRMA methods (BioSource) and insulin resistance was estimated using the homeostasis model assessment method (HOMA-IR). Serum lipid and glucose profiles were characterized using standard laboratory procedures.

The intra- and inter-assay coefficients of variation were <10% for all investigated parameters.

Statistical analyses

Statistical analyses were performed using STATISTICA version 7.1PL software.

The normality of distribution was investigated using the Shapiro-Wilk test and the Kolmonogorow-Smirnov test with the Lillefors correction.

The differences between groups were calculated using the Kruskall-Wallis rank test, followed by the Mann-Whitney U-test. The Spearman test was applied to calculate the correlation coefficients between levels of adiponectin and its isoforms, and the anthropometric data and biochemical parameters.

Statistical significance was accepted at $p \leq 0.05$.

RESULTS

The results of the clinical examination and biochemical parameters are presented in Tables 1 and 2, respectively.

A comparison between individuals with acute stroke and the matched controls revealed no significant differences in age, BMI or diastolic blood pressure. Higher values for systolic blood pressure were recorded in women with acute ischemic stroke.

Unexpectedly, the controls exhibited significantly higher values for total cholesterol and triglycerides as well as higher levels of HDL cholesterol than the acute stroke patients.

The parameters of insulin resistance were comparable between the two groups, except the glucose level, which was higher in women with acute stroke.

Total adiponectin (TA) as well as levels of the HMW, MMW and LMW adiponectin isoforms were significantly lower in the acute stroke patients in comparison with the controls. However, there was no difference in the HMW/TA ratio between the two groups (Table 3).

A number of correlations were identified in women suffering from acute stroke:

The total adiponectin concentration correlated negatively with diastolic blood pressure and positively with HDL cholesterol. The level of HMW adiponectin correlated positively with HDL cholesterol. All correlations are presented in detail in Table 4.

Tab. 1. Clinical data for patients with acute ischemic stroke (AIS) and matched healthy control subjects (HC).

	AIS n=38	HC n=38	*p*-value
Age (yrs)	76.2±6.32	75.29±5.27	ns
BMI (kg/m^2)	28.9±4.21	27.63±3.82	ns
SBP (mmHg)	146.58±25.04	133±13.22	0.01
DBP (mmHg)	87.76±12.66	81±10.19	ns
NIHSS	6.95±5.62	X	

Data are presented as mean ±SD
BMI – body mass index
SBP – systolic blood pressure
DBP – diastolic blood pressure
NIHSS – National Institutes of Health Stroke Scale

Tab. 2. Biochemical data for patients with acute ischemic stroke (AIS) and matched healthy control subjects (HC).

	AIS n=38	HC n=38	*p*-value
Insulin (μIU/ml)	11.04±9.57	10.92±7.58	ns
Glucose (mmol/l)	6.9±2.6	5.67±1.02	<0.01
HOMA-IR	3.41±3.30	2.87±2.4	ns
Total cholesterol (mg/dl)	189.08±52.87	213.65±44.3	<0.05
LDL cholesterol (mg/dl)	112.88±44.68	119.78±39.061	ns
HDL cholesterol (mg/dl)	52.69±14.47	61.67±9.24	<0.001
Triglycerides (mg/dl)	113.84±56.18	161.24±62.63	<0.001

Data are presented as mean ±SD
HOMA-IR – homeostasis model assessment of insulin resistance

Tab. 3. Serum concentrations of total adiponectin and isoforms of adiponectin (HMW, MMW, LMW) in patients with acute ischemic stroke (AIS) and matched healthy control subjects (HC).

	AIS n=38	HC n=38	*p*-value
Total adiponectin (TA) (μg/ml)	6.33±3.35	8.97±2.74	<0.001
HMW adiponectin (μg/ml)	3.31±2.03	4.52±1.88	<0.01
MMW adiponectin (μg/ml)	1.59±1.11	2.38±1.19	<0.01
LMW adiponectin (μg/ml)	1.43±0.79	2.06±1.14	<0.01
HMW/TA	0.51±0.11	0.50±0.11	ns

Data are presented as mean ±SD
HMW – high molecular weight adiponectin
MMW – medium molecular weight adiponectin
LMW – low molecular weight adiponectin

Tab. 4. Correlations between total adiponectin, HMW adiponectin and clinical and biochemical parameters in patients with acute ischemic stroke.

Parameter A	Parameter B	R	*p*-value
Total adiponectin	HDL cholesterol	0.39	<0.05
HMW adiponectin	HDL cholesterol	0.36	<0.05
Total adiponectin	Diastolic BP	–0.21	<0.05

DISCUSSION

Adiponectin belongs to a group of substances, produced by the adipose tissue, called adipocytokines. The level of circulating adiponectin is inversely correlated with the fat mass. It has been clearly demonstrated that this polypeptide has antiatherogenic and anti-inflammatory activity. However, the results of previous studies on the role of adiponectin in the course of CVD are equivocal. It is still an open question whether high circulating levels of adiponectin are beneficial or not. On the one hand, higher values for total adiponectin were recorded in a group of extremely old individuals and their descendants (Atzmon *et al.* 2008; Bik *et al.* 2006), but on the other, it has been found that increased circulating adiponectin is a predictor of mortality in heart failure (Dekker *et al.* 2008).

Controversies concerning the level of adiponectin in ischemic stroke patients also exist. The results of the present study, examining circulating adiponectin and its isoforms within 24 hours of ischemic stroke, revealed that levels of all the measured forms were significantly lower than those found in healthy controls. The lower level of total adiponectin following ischemic stroke is in agreement with the findings of Marousi and co-workers (Marousi *et al.* 2010). These authors not only noted an acute decrease in circulating adiponectin after an ischemic stroke, but also demonstrated long-lasting suppression of this adipokine, with reduced levels still present 6 months after the stroke. Data from another investigation also confirmed the suppression of total adiponectin in the course of stroke (Sasaki *et al.* 2010). In addition, another study revealed that serum adiponectin concentrations were even more severely reduced 14 days after cerebral infarction, but then returned to the basal level (Sasaki *et al.* 2007). However, results from an animal model of ischemic brain infarction suggest that ischemia causes a dual phase change in total adiponectin values. During the acute phase, a short-term rise in adiponectin levels was observed, but it subsequently diminished. Moreover, an accumulation of adiponectin was found in the vessels of the damaged brain and this phenomenon may explain the decrease in circulating levels of this polypeptide (Yatomi *et al.* 2009).

Interestingly, another study conducted by Marousi and co-workers showed that the early post stroke change in serum adiponectin did not have an impact on the infarct severity, progression or long-term outcome in humans (Marousi *et al.* 2009).

Adiponectin appears to be a promising target for treatments aimed at preventing cerebral infarction because it possesses anti-atherogenic, anti-diabetic, anti-inflammatory and neuroprotective properties. The mechanism of neuroprotection afforded by adiponectin is heterogeneous. This polypeptide was found to be able to regulate NO synthase (Nishimura *et al.* 2008). By influencing endothelial cells, it may increase production of NO and decrease ROS (reactive oxygen species) (Chen *et al.* 2003). In addition, adiponectin may suppress cerebral expression of myeloperoxidase and pro-inflammatory cytokines such as IL-1, IL-8 and TNFα (Chen *et al.* 2009). Moreover, by lowering levels of TNFα, adiponectin inhibits TNFα-dependent expression of cell adhesion molecules (Ouchi *et al.* 1999). Adiponectin is also thought to modulate the activity of the potent proinflammatory factor NF-kappaB by inhibiting its translocation from the cytoplasm into the nucleus (Chen *et al.* 2009). However, despite its diverse activities, the hypothesis that adiponectin may be a protective agent against ischemic stroke is contradicted by the results of several studies. A Japanese nested case-control study failed to find any significant difference in the odds of stroke between the quartiles exhibiting the lowest and highest levels of adiponectin (Matsumoto *et al.* 2008). Moreover, no linear trend towards a reduced risk of stroke was found at higher adiponectin levels (Matsumoto *et al.* 2008). In addition, no association between total adiponectin and risk of stroke was found in a study of the Swedish population (Söderberg *et al.* 2004). However, data from a nested case-control study within the PROSPER (Prospective Study on Pravastatin in the Elderly) project indicated that elevated plasma adiponectin was associated with a lower risk of ischemic stroke, but only on univariate analysis, as total adiponectin did not contribute in the multivariate models (Stott *et al.* 2009). Furthermore, Hegener and co-workers identified an association between two adiponectin gene polymorphisms and increased risk of ischemic stroke (Hegener *et al.* 2006). Finally, the results of Efstathiou and colleagues indicated that low levels of this adipocytokine were associated with increased mortality rate in the five years following ischemic stroke (Efstathiou *et al.* 2005).

Although serum HMW adiponectin is thought to be negatively correlated with impaired glucose and lipid parameters, obesity and hypertension, no association between HMW adiponectin levels and the incidence of ischemic stroke was found in postmenopausal women investigated in the WHI (Women's Health Initiative) Study (Ogorodnikova *et al.* 2010). Data concerning plasma HMW adiponectin in the course of ischemic stroke are very limited. Sasaki and co-workers failed to find any significant difference in HMW adiponectin levels between subjects with cerebral insult and healthy controls (Sasaki *et al.* 2010). This contrasts with the results of the present study where HMW adiponectin values were significantly decreased in the acute phase of ischemic stroke.

MMW and LMW adiponectin levels were also assessed in the present study and significantly lower values were recorded in the stroke group compared with the controls. There is a dearth of data in the literature concerning these isoforms of adiponectin and, to our knowledge, this is the first time that the whole array of adiponectin isoforms has been examined in acute cerebral infarction.

Despite the novel findings of the present study, we recognize that it has several limitations, one of which is the small size of the group of subjects suffering from acute stroke. Also, no follow up was performed at the time of the study. In future studies designed to corroborate and extend the findings presented here we will enlarge the studied group and a post-stroke follow-up will be included. In addition, the genetic factors responsible for adiponectin polymorphisms will be investigated.

In conclusion, we have found that total serum adiponectin and its isoforms are decreased in the first 24 hours of acute cerebral infarction. Further investigations are required to determine the mechanism of these reductions and their clinical consequences.

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