Oligomerization state-dependent elevations of adiponectin in chronic daily headache

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ABSTRACT

Objective: To evaluate serum adiponectin levels in female episodic migraineurs (EMs) and chronic daily headache (CDH) sufferers.

Background: Obesity is a risk factor for headache “chronification.” Adiponectin (ADP) is an adipocytokine secreted primarily by adipose tissue. ADP and its oligomers (high-molecular-weight [HMW], middle-molecular-weight [MMW], and low-molecular-weight [LMW] ADP) have been shown to modulate several inflammatory pathways that have also been shown to be associated with migraine pathophysiology.

Methods: Age- and body mass index (BMI)-matched women participants were enrolled. Anthropometric measures (including waist-to-hip ratio [WHR] and BMI) were measured in all participants. Serum total ADP (T-ADP) levels and its oligomers were measured in EMs during headache-free periods and CDH sufferers at baseline level of pain, as compared with healthy control subjects using ELISA.

Results: Although total body obesity as estimated by BMI showed no significant association between participants, visceral obesity as estimated by WHR was significantly associated with CDH as compared with EMs and controls. WHR was also inversely related to both T-ADP (p = 0.008) and HMW-ADP (p = 0.002). After adjusting for WHR, serum T-ADP levels were higher in CDH sufferers (10.1 ± 4.0) than in both EMs (8.6 ± 3.5) and controls (7.5 ± 2.4) (p = 0.024). In addition, HMW-ADP was higher in CDH (6.1 ± 2.8) as compared with EMs (4.2 ± 1.7) and controls (3.9 ± 1.5) (p = 0.003). MMW-ADP was also higher in CDH (2.0 ± 1.2) as compared with EMs (1.5 ± 0.7) and controls (1.1 ± 0.4) (p = 0.009).

Conclusion: Serum adiponectin levels are increased in women chronic daily headache (CDH) sufferers. In addition, visceral obesity, as measured by waist-to-hip ratio, is a risk factor for CDH in women. Neurology® 2008;70:1905–1911

GLOSSARY

ADP = adiponectin; BMI = body mass index; CDH = chronic daily headache; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; EM = episodic migraineur; gADP = globular adiponectin; HC = hip circumference; HMW = high molecular weight; HMWR = ratio of HMW-ADP to T-ADP; Hx = history; ICHD-2 = International Classification of Headache Disorders, Second Edition; IL = interleukin; LMW = low molecular weight; MMW = middle molecular weight; NF = nuclear factor; PHQ-9 = Patient Health Questionnaire 9; T-ADP = total ADP; WC = waist circumference; WHO = World Health Organization; WHR = waist-to-hip ratio.

Migraine is a common and often disabling neurovascular disorder that occurs in approximately 12% of American adults and three times as many women as men.1 A subgroup of this population of episodic migraineurs (EMs) progresses to become chronic migraine. It has been estimated that 4% to 5% of the general population suffers from chronic daily headache (CDH), with half of this population being transformed or chronic migraine.2,3 CDH is also a significant clinical problem, accounting for an estimated 10% of patients who are evaluated in general neurology clinics and 40% of patients who are evaluated in headache clinics.3,4

The etiology of why some headache sufferers progress is unknown, although several risk factors for CDH have been identified, including head and neck injury, arthritis, sleep

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disorders, diabetes, and obesity. And although it is not clear whether the first neurologic event giving rise to migraine is generated from brainstem activation or cortical spreading depression, the pain of migraine is a result of neurogenic inflammation. Calcitonin gene–related protein, substance P, interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, and nuclear factor (NF)-κB have all been implicated in the pathway resulting in the neurogenic inflammation of migraine.

Recent data have shown that adipose tissue is far from being just a mere storage depot for fat. It is, in fact, an active participant in multiple physiologic and pathologic processes associated with inflammation and immunity and has been shown to secrete a variety of complement factors, growth factors, cytokines, and adipocytokines, including adiponectin (ADP). Adiponectin is primarily secreted from adipocytes in adipose tissue, although it is also secreted in low levels from cardiomyocytes, hepatocytes, and the placenta. It exhibits a sexual dimorphism, with women having higher levels than men by puberty. Human plasma ADP can exist as a full-length form; a smaller fragment of the full-length form (which is formed by cleavage of full-length ADP by proteases) termed globular adiponectin (gADP); or as one of several characteristic oligomers or multimers, including high-molecular-weight (HMW), middle-molecular-weight (MMW), or low-molecular-weight (LMW) ADP. LMW-ADP is a trimer formed via hydrophobic interactions within its globular domain. Two trimers form a disulfide-linked hexamer or MMW-ADP, which assembles into a multimeric complex of 12 to 18 monomers or HMW-ADP.

Adiponectin has been most often reported as having anti-inflammatory properties based on the observations that total plasma concentrations of ADP (T-ADP) have been shown to be reduced in obesity, the metabolic syndrome, and diabetes. However, recent studies have shown that ADP may also have proinflammatory properties and that several diseases associated with inflammation are associated with increased ADP levels, including arthritis, cardiovascular disease, preeclampsia, and end-stage renal disease.

The ability of ADP to exert both proinflammatory and anti-inflammatory properties seems to be determined by the form of ADP involved. Human gADP activates the proinflammatory NF-κB pathways as well as induces the secretion of nitric oxide and the proinflammatory cytokines, IL-6 and TNF-α. However, under certain conditions, gADP exerts anti-inflammatory properties. And it has been shown that gADP induces self-tolerance to re-exposure of gADP, as well as tolerance to endotoxin. In addition, the different multimers of ADP have been shown to activate different pathways and have distinct functions. Of the multimers of ADP, HMW-ADP is the only one that has been shown to activate NF-κB pathways in humans. And MMW-ADP has been shown to induce the secretion of IL-6, whereas LMW-ADP has been shown to reduce IL-6 secretion.

Finally, ADP could play an important role in the circadian periodicity and hypothalamic symptoms seen in some migraineurs, because animal studies suggest involvement of the melanocortin pathway in the central actions of ADP.

Thus, given the aforementioned data and that IL-6, TNF-α, and NF-κB have been shown to be increased during acute migraine attacks, the aim of our study was to compare and contrast the baseline serum levels of T-ADP and its characteristic oligomers, HMW-ADP, MMW-ADP, and LMW-ADP.

**METHODS** **Patients and controls.** A total of 37 age- and body mass index (BMI)-matched, normotensive, nondiabetic participants were included in the study. Because both sex and race have been shown to effect adipose tissue quantity and distribution, participation was limited to white women. Headache diagnoses were classified according to the International Classification of Headache Disorders, Second Edition (ICHD-2). According to their diagnoses, migraine participants were divided into two groups. Group 1 consisted of 12 CDH patients having an ICHD-2 code of probable medication overuse headaches or chronic migraine.
Demographic variables showed no significant differences. Although no significant difference was found in regard to those estimated to have regional or abdominal obesity based on the waist-to-hip ratio (WHR). Based on the body mass index (BMI), a significant difference was found in regard to those with an estimated total body fat level consistent with obesity as measured to the nearest 0.5 inch with a mounted stadiometer. WC was measured at the minimum circumference between the iliac crest and the rib cage. Hip circumference (HC) was measured at the maximum width over the greater trochanters. For the definition of abdominal obesity based on WC, 88 cm was used according to the recommendations for the definition of metabolic syndrome, corresponding to a BMI of 30 kg/m². It has been shown to have superior validity criterion for the diagnosis of major depressive disorder than total body obesity estimates, i.e., BMI,21-22 Thus, the anthropometric measurements for both total body obesity (BMI) and abdominal obesity (WC and WHR) were calculated for all participants.

Measurements of total body obesity. Height was measured to the nearest 0.3 inch with a mounted stadiometer. Weight was measured with a standard scale to the nearest 0.5 lb. BMI was then calculated using the formula: BMI = weight [lb]/height² * 703, and categorized on the basis of the World Health Organization (WHO) categories: <18.5 (underweight), 18.5 to 24.9 (normal weight), 25 to 29.9 (overweight), and ≥30 kg/m² (obese).27

Measurements of regional obesity. Waist and hip circumferences (centimeters) were measured with an anthropometric tape over skin or light clothing. WC was measured at the minimum circumference between the iliac crest and the rib cage. Hip circumference (HC) was measured at the maximum width over the greater trochanters. For the definition of abdominal obesity based on WC, 88 cm was used according to the recommendations for the definition of metabolic syndrome, corresponding to a BMI of 30 kg/m². Additionally, a WC of 80 to 88 cm was used to identify individuals at increased cardiovascular risk as proposed by Han et al.28-29 The WHR was calculated by dividing the WC by the HC and categorized based on the WHO recommendations of a WHR greater than 0.85 being considered increased in women.

Laboratory methods. After sampling in EDTA or serum tubes, blood was immediately chilled on ice, then centrifuged, aliquoted, and stored at −70°C until assayed.

Glucose. Glucose levels were evaluated colorimetrically in 96-well plates with the QuantiChrom glucose assay kit (BioAssay Systems, Hayward, CA). Absorbance was determined with a Spectramax 190 spectrophotometer (Molecular Devices, Sunnyvale, CA).

Adiponectin. Serum levels of total, HMW, and combined MMW-ADP were determined by multimeric enzyme immunoassay (ALPCO, Salem, NH) with an effective range between 0.39 and 24.7 μg/mL. The MMW-ADP concentration was calculated by subtracting the HMW-ADP concentration from the combined MMW-ADP + HMW-ADP concentration, and the LMW-
ADP concentration was calculated by subtracting the combined HMW-ADP + MMW-ADP concentration from the T-ADP concentration. All assays were performed in duplicate according to the manufacturer’s instructions.

Recent data have suggested that HMW-ADP may be more relevant in the prediction of insulin resistance and that the ratio of HMW-ADP to T-ADP (HMWR) may be a more significant predictor of disease than T-ADP. Thus, HMWR was calculated for all participants.28

**Statistical analysis.** Statistical analyses were carried out using SPSS, version 15 (SPSS Inc., Chicago, IL). All data are presented as mean ± SD where appropriate. For demographic variables, analysis of variance or independent sample t tests were calculated for quantitative variables. Nominal and categorical data were analyzed by χ² test. For outcome variables, analysis of variance was calculated for T-ADP, its isomers, and the HMWR for each group. Bivariate correlations were performed to test relationships between controls and outcome variables. Linear multiple regression analyses were conducted to model the strength of various measures as correlates of T-ADP, its isomers, and the HMWR, including the WHR and serum glucose. Given the problems that conservative Bonferroni-type α level corrections pose for the statistical power of small sample sizes, the issue of multiple comparisons is handled through an additive method, whereby the sum of the p values for all claimed effects must be less than the α level.

**RESULTS** All patients and controls completed the study. The concentration of ADP was above the detection level in all blood samples.

The demographic characteristics and serum glucose levels showed no significant difference between CDH sufferers, EMs, and controls (table 1). There were no significant differences between groups for self-reported history of depression or in those with a PHQ-9 score positive for major depression. BMI and WC were not significantly associated with serum ADP levels or any of the ADP isomers. However, WHR was correlated with both T-ADP (p = 0.008) and HMW-ADP (p = 0.002; table 2). Mean estimates of total body fat (BMI) and mean abdominal obesity (WHR) were not significant between groups. In addition, based on the WHO recommendations, the percentage of participants with a BMI consistent with obesity (i.e., BMI ≥ 30 kg/m²) was not significant between groups. However, the percentage of CDH sufferers with abdominal obesity (i.e., WHR > 0.85) was greater as compared with EMs and controls (p = 0.049; table 1).

After adjusting for WHR, serum T-ADP levels were higher in CDH sufferers (10.1 ± 4.0 µg/mL) than in both EMs (8.6 ± 3.5 µg/mL) and controls (7.5 ± 2.4 µg/mL) (p = 0.024; figure). In addition, HMW-ADP was higher in CDH (6.1 ± 2.8 µg/mL) as compared with EMs (4.2 ± 1.7 µg/mL) and controls (3.9 ± 1.5 µg/mL) (p = 0.003). And MMW-ADP was also higher in CDH (2.0 ± 1.2 µg/mL) as compared with EMs (1.5 ± 0.7 µg/mL) and controls (1.1 ± 0.4 µg/mL) (p = 0.009). No significant difference was found for LMW-ADP levels or the HMWR, although a trend toward higher levels of LMW-ADP in CDH (2.9 ± 2.1 µg/mL) as compared with EMs (2.4 ± 1.3 µg/mL) and controls (2.2 ± 1.0 µg/mL) (p < 0.22) was seen. When groups were separated as migraine with aura and migraine without aura, no significant differences in T-ADP or any of its multimers were seen (data available on request).

**DISCUSSION** Adipose tissue is an important source of cytokines and adipocytokines. Adiponectin is an adipocytokine, primarily secreted from adipose tissue that has both proinflammatory and anti-inflammatory properties. Our current cross-sectional study has three significant findings in regard to the associations between migraine, adipose tissue, and ADP. The first is that interictal serum T-ADP levels were increased in women CDH sufferers as compared with matched EMs and controls in our study. This
finding supports the possible proinflammatory role of ADP in the neurogenic inflammatory cascade resulting in migraine “chronification.”

The majority of studies evaluating ADP levels in humans have reported reduced concentrations of T-ADP levels in metabolic disorders such as obesity and diabetes. In addition, previous studies have reported that HMW-ADP and the ratio of HMW to T-ADP are better predictors of metabolic markers and insulin sensitivity than T-ADP. However, the role of ADP in inflammatory and immunologic diseases seems to be somewhat different than in metabolic diseases. As with our current study in CDH sufferers, increased levels of ADP have been reported in several inflammatory disorders, including arthritis, cardiovascular disease, and end-stage renal disease.

Second, in our study, we found that HMW-ADP and MMW-ADP were increased and not the LWM-ADP in CDH sufferers. HMW-ADP has been shown to activate the NF-κB pathway as well as to increase IL-6 secretion, whereas LMW-ADP has been shown to decrease IL-6 secretion. In addition, a previous study by Perini et al. found higher levels of TNF-α, IL-1β, and IL-10 in EM participants. And more recent studies by Sarchielli et al. found increased plasma levels of TNF, IL-6, and NF-κB during acute migraine attacks in seven EM patients. Thus, our findings support that it is the multimeric distribution of ADP which is critical in determining whether ADP functions in either a proinflammatory or an anti-inflammatory role in CDH sufferers. In addition, given the results of our current study, it is possible that ADP could serve as a biomarker for CDH. It is also possible that T-ADP, HMW-ADP, and MMW-ADP could be increased in EM as well. Additional work is needed to evaluate the levels of ADP and its multimers interictally as compared with ictally in migraineurs to determine whether ADP is a biomarker for CDH or migraine in general.

The third significant finding from our study is that the distribution of adipose tissue seems to play a significant role in the association between adipose tissue and migraine. In our study, participants were matched based on age and sex as well as BMI, which is an estimation of total body fat—when height and weight are directly measured. We found that although total body obesity as defined by a BMI ≥ 30 kg/m² was not significant in our participants, abdominal obesity as estimated by the WHR was significantly greater in CDH sufferers as compared with EMs and controls.

The connection between abdominal obesity and migraine may be through ADP’s modulation of IL-6 (a proinflammatory cytokine and one of the chief hepatic inducers of C-reactive protein), because approximately 30% of circulating IL-6 is estimated to be secreted by adipocytes. Furthermore, IL-6 has been reported to be more significantly secreted from the visceral or abdominal adipose tissue; and visceral adipose tissue has been shown to be significantly associated with IL-6 concentrations in obese individuals even after adjustment for BMI.

There are several limitations of our study. The first is our small sample size. Larger sample sizes are needed to determine whether ADP plays a significant role in EMs in addition to CDH sufferers. Second, the generalizability of this study to all CDH sufferers is limited by the inclusion of only white women. This limitation was deemed necessary to limit the sex- and race-related influences seen with ADP levels and adipose tissue distribution in previous studies. In addition, our study is limited by not identifying the phase in the menstrual cycle that the study participants were in at the time the ADP levels were drawn, because sex hormones, including both estrogen and testosterone, have been shown to decrease ADP levels.

It is interesting to note that adult women have higher T-ADP and HMW-ADP levels than men, with these levels reached at puberty. Furthermore, both T-ADP and HMW-ADP decrease through puberty in males. Similarly, before puberty, migraine occurs in slightly more boys than girls. However, in parallel with the puberty-related increases in T-ADP and HMW-ADP, migraine occurs in three times as many women as men after puberty. In addition, menstrually related migraine has been shown to occur most frequently 2 days before to 3 days after the onset of menses, at the time in the menstrual cycle when estrogen levels are significantly declining. Because estrogen has been shown to suppress ADP, it would suggest that it is at this time that ADP may significantly increase and contribute to neurogenic inflammation through stimulation of NF-κB pathways and release of cytokines. However, testosterone modulates ADP levels as well. And testosterone replacement (which would cause a decrease in serum ADP levels) in refractory, male cluster headache sufferers has been shown to effectively resolve headache. Thus, future studies will need to control for sex hormones in both sexes.

Despite these limitations, the present study demonstrates for the first time that T-ADP serum
levels are increased in female CDH sufferers inter-
ictally. In addition, because the individual iso-
mers of ADP that were shown to be increased 
were the HMW-ADP and MMW-ADP isomers, 
our data support that HMW-ADP and MMW-
ADP are the isomers responsible for the increase 
of T-ADP in female CDH sufferers.

It has been suggested that further evaluation of 
neuroendocrine peptides such as orexin may pro-
vide a link between the behavioral manifesta-
tions and the triggering of migraine.42 Our current 
work with ADP similarly falls into this category 
and underscores the importance of adipose tissue 
as a dynamic neuroendocrine organ with multiple 
possible links within the pain system. Further re-
search in regard to the role of ADP as well as 
other obesity-related neuroendocrine peptides in 
the neuronal system is warranted.

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