DIURNAL VARIATION MODIFY THE PRODUCTION OF ADIPOKINES INSTEAD OF EPILEPTIC SEIZURES: A VIDEO-EEG STUDY

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ALHAINEN MIIA: DIURNAL VARIATION MODIFY THE PRODUCTION OF ADIPOKINES INSTEAD OF EPILEPTIC SEIZURES: A VIDEO-EEG STUDY

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Aiemmat tutkimukset ovat osoittaneet, että adipokiinit vaikuttavat useisiin keskushermoston sairauksiin. Adipokiinit kommunikoivat muiden sytokiinien kanssa saaden näin aikaan vasteen eri inflammatorisiin reaktioihin. Adipokiineilla onkin todettu olevan sekä inflammaatiota edistäviä että ehkäiseviä ominaisuuksia. Viimeaikaiset tutkimukset ovat myös osoittaneet, että tietyllä adipokiinilla, leptiinillä saattaa olla aivojen sähköisiä purkauksia estävä vaikutus. Tietojemme mukaan adipokiinien ja epilepsian välistä yhteyttä ei ole kuitenkaan vielä tutkittu ihmisillä. Tämän vuoksi tarkastelimme tutkimuksessamme vaikeahoitoista epilepsiaa sairastavien potilaiden seerumin adipokiinipitoisuuksien muutoksia epilepsiakohtausten jälkeen.

Tutkimukseen osallistui 51 fokaalista epilepsiaa sairastavaa potilasta Tampereen yliopistollisesta sairaalasta. Kaikkia potilaita monitoroitiin video-EEG:n avulla neljän vuorokauden ajan. Myös kohtauksia estävä lääkitys tauotettiin tarvittaessa potilailta monitoroinnin ajaksi. Plasmanäytteet kerättiin video-EEG – monitoroinnin aikana sekä päivittäin aamulla, kunnes potilaat saivat heidän ensimmäisen epileptisen kohtauksensa. Viimeisintä näytettä ennen kohtausta käytettiin määrittämään adipokiinien perustasoa plasmassa.

Ensimmäisessä tarkastelussa huomattiin leptiinipitoisuuksien nousevan epilepsiakohtausten jälkeen. Toisessa tarkastelussa otettiin kuitenkin huomioon myös leptiinierityksen normaali vuorokausivaihtelu. Todettiinkin, että plasman leptiinipitoisuudet noudattavat sille ominaista vuorokausivaihtelua epilepsiakohtauksista huolimatta. Epilepsiakohtaukset ja plasman leptiinipitoisuudet eivät siis korreloineet keskenään, vaikka ensimmäinen tarkastelu antoikin näin olettaa.

Kyseinen tutkimus osoitti, kuinka tärkeää on ottaa huomioon immunoregulatoristen hormonien vuorokausivaihtelu, kun tutkitaan niiden korrelaatiota esimerkiksi epileptisiin kohtauksiin. Tutkimustulokset olisivat olleet merkittävästi vääristyneitä ilman hormonierityksen vuorokausivaihtelun huomioon ottamista.

Sisällys

Diurnal variation modify the production of adipokines instead of epileptic seizures: A video-EEG study

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Short Title: Leptin, resistin and video-EEG monitored seizures

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Abstract

Background: There is increasing evidence that adipokines e.g. leptin, adiponectin, adipsin and resistin, are involved in diseases of the central nervous system. Leptin has displayed an anticonvulsant effect in experimental seizure models. Therefore, we examined adipokine levels in epilepsy patients after a seizure in a controlled environment taking into account diurnal variations in both seizures and adipokines.
Methods: The concentrations of leptin, adiponectin, adipsin and resistin were measured in serum in 51 patients with epilepsy [temporal lobe epilepsy (TLE, n=23), extratemporal lobe epilepsy (XLE, n=24), and idiopathic generalized epilepsy (IGE, n=4)] before and after the first verified seizure (IS; index seizure) during inpatient video-EEG monitoring.

Results: Leptin concentrations exhibited a significant increase at 3, 6, 12 and 24 h time points after the IS as compared to the baseline ($p < 0.001$; Wilcoxon signed ranks test). The levels of leptin were increased after a focal seizure in patients with impaired awareness ($p<0.001$; Friedman's test) and focal to bilateral tonic-clonic seizures ($p<0.05$; Friedman's test), but not in patients with aware seizures. Resistin concentrations were also increased 12h after the IS ($p < 0.05$, Wilcoxon signed ranks test). Further analyses demonstrated that the timing of the seizures was not randomly distributed but coincided with time points at which there were low leptin plasma levels reflecting the diurnal variation in leptin concentrations i.e. highest concentrations around midnight and lowest values between morning and noon.

Conclusion: This study demonstrates the significance of diurnal variation in an immunoregulatory molecule, i.e. leptin, when investigating the effect of a precipitating event, e.g. a seizure

1. Introduction

In addition to specializing in lipid handling and energy balance regulation, white adipose tissue cells produce cytokines and cytokine-like hormones called adipokines. There are several adipokines e.g. leptin, adiponectin, adipsin and resistin; these have many properties including immunoregulatory functions. Many adipokines have pro- or anti-inflammatory effects and they co-operate with other cytokines in determining the overall responses to inflammatory reactions. [1] Recent findings have suggested that adipokines may play a role in the pathogenesis of certain central nervous system diseases, such as multiple sclerosis [2], Alzheimer's disease [3], [4], autism [5], and Rett syndrome [6]. On the other hand, there is evidence that resistin may contribute to the pathogenesis of brain injury and elevated resistin concentrations have been linked to a poorer outcome [7], [8], [9]. Both leptin [10], [11] and adiponectin [12], [13] have exhibited beneficial properties in animal models of epilepsy; the effects of resistin and adipsin have not been studied in these models.

In a recent study, we assayed cerebrospinal fluid (CSF) and plasma levels of leptin, adiponectin and adipsin after either provoked or unprovoked generalized tonic-clonic seizures (GTCS) or focal to bilateral tonic-clonic seizures (FBTCS) in 13 female patients and seven controls within 24 h after the seizure onset. Leptin plasma levels correlated negatively with the time of sample withdrawal, i.e. the longer the time interval between the seizure and the sample, the lower the patient's leptin levels. Interestingly, plasma adiponectin levels were significantly elevated after a seizure episode. [14]

The secretion of adipokine follows a diurnal variation. The main circadian pacemaker regulating hormone secretion is located in the suprachiasmatic nucleus of hypothalamus [15] Secondary circadian clocks are located in many peripheral organs, such as adipose tissue and liver. Sleep rhythms, meal rhythms, the amount of light and metabolic activity also affect the circadian variation of hormone secretion along with clock genes and secondary clocks [15], [16], [17].
Earlier studies have shown that the secretion of the most relevant adipokine with respect to epilepsy i.e., leptin, also follows a distinctive diurnal variation. The highest leptin concentrations of plasma have been measured at or immediately after midnight with nadir concentrations being assayed in the morning up until midday. [18]

Furthermore, the occurrence of seizures in specific types of epilepsies can follow a -specific 24-hour pattern with a non-random distribution. Circadian or sleep–wake related variations in the levels of different hormones, neuromediators and body temperature are potentially factors that could contribute to the fluctuations in neuronal excitability and the propensity for seizure occurrence. It has been recognized that

there is a 24-hour rhythmicity in seizure distribution in certain types of epilepsy, although there are rather few studies investigating this topic. [19]

This prompted us to further evaluate the seizure-induced changes of adipokines in a controlled video-EEG (VEEG) setting. The aims of this study were 1) to investigate whether these recently discovered functions of adipokines in experimental models can be detected in human epilepsy, 2) to assess changes in adipokine levels in well-characterized human focal or generalized epileptic seizures, 3) to correlate these changes with the epileptic syndrome and the localization or lateralization of the seizure focus and 4) to address the significance of the diurnal variation in epileptic seizures and concentrations of the adipokines

2. Materials and methods

2.1. The classification of patients

Fifty-one consecutive patients with refractory focal epilepsy admitted to the VEEG monitoring unit of the Tampere University Hospital were examined in this study. The study protocol was approved by the Ethics Committee of the Tampere University Hospital, and all of the patients provided written informed consent.

All patients underwent continuous VEEG monitoring lasting for four days to obtain an electroclinical characterization of their seizures as part of the routine clinical evaluation for possible epilepsy surgery. Ictal scalp recordings were collected using synchronous digital video and 24 channel standard bipolar EEG. Electrodes were placed according to the International 10-20 System with additional mastoid and anterior cheek electrodes. All patients underwent a diagnostic brain MRI examination on a 1.5 (General Electric, Sigma HD, Milwaukee,Wisconsin, USA) or 3.0 (Siemens Healthcare, Magnetom Trio A Tim system 3T, Erlangen, Germany) Tesla device. Seizures were classified according to the ILAE diagnostic criteria [20]. Anticonvulsant medication was discontinued in a stepwise fashion during the monitoring period under the supervision of the staff epileptologist.

The localization of the seizure focus was recorded and seizures were categorized as focal aware seizures (FAS), focal impaired awareness seizures (FIAS), focal to bilateral tonic-clonic seizures (FBTCS) or generalized tonic-clonic seizures (GTCS). The first unequivocally verified seizure during the VEEG monitoring was considered as the index seizure (IS). All other seizures after the index seizure during the next 24 hours were also registered. Based on the findings in the VEEG recordings and MRI, 23 patients had temporal lobe epilepsy (TLE), 24 patients had extratemporal lobe epilepsy (XLE) (21 frontal lobe, two parietal lobe and one

multilobar epilepsy) and four patients had idiopathic generalized epilepsy (IGE). Seven patients were receiving monotherapy, 43 were being treated with polytherapy (the mean number of medications was 2.37), and one patient had no medication. In addition to antiepileptic drugs (AEDs), 4 patients were being treated with vagus nerve stimulation (VNS). One female patient with right hippocampal sclerosis was monitored twice during an interval of 23 months. Clinical data of these three groups of patients are shown in Table 1. The seizure burden was assessed by the total duration of seizures recorded during the 24h sampling period.

2.2. Plasma sampling and biochemical analyses

Plasma samples were collected at the onset of VEEG recording and daily in the morning until patients had their first verified seizure (IS; index seizure) and at 3, 6, 12 and 24 hours after the IS. The final sample collected before the IS was the baseline sample. The samples were stored at -70 ^{o}C until analyzed. Plasma concentrations of adiponectin, adipsin, leptin and resistin were determined by enzyme-linked immunosorbent assay (ELISA) with commercial reagents (DuoSet ELISA, R&D Systems Europe Ltd, Abingdon, U.K).

3. Statistical analysis

The difference between basal levels of adipokines (between study groups) was tested using non-parametric Kruskal-Wallis test or Mann-Whitney test, and the difference in adipokine levels at different time points was tested using a non-parametric related samples test (Wilcoxon signed ranks test or Friedman's test). The correlation between numeric parameters was tested using Pearson’s correlation test. All statistical analyses were performed using SPSS 17.0 software.

4. Results

4.1. Seizures

The mean number of seizures (including IS) in the TLE group was 2.6 (range: 1-9) and in the IGE group, it was 1.3 (range: 1-2). In three patients with XLE, 100 or more seizures were recorded during a 24-h period (range: 100-400). After excluding these 3 patients, the mean number of seizures in this group was 5.4 (range: 1-18). Seven patients had only brief seizures during the sampling period. The mean (seconds±SD) duration of the index seizure in TLE was 405±1491 (median 77), in XLE 57±73 (median 26) and in IGE it was

66±4 (median 66). The mean seizure burden (seconds±SD) in the TLE group was 549±1575, in the XLE group it was 1609±5429 and 81±31 in the IGE group.

4.2. The baseline levels of the adipokines

There were no –significant differences in the baseline levels of adipsin, leptin or adiponectin between different types of epilepsy (TLE, XLE and IGE). The baseline levels of resistin, however, displayed a trend towards a difference between the epilepsy syndromes ($p = 0.053$; Kruskal Wallis test) with the highest levels being found in IGE, with the TLE patients exhibiting the lowest baseline levels. The baseline levels of leptin correlated positively with BMI ($r=0.46$; $p < 0.001$; Pearson correlation), but there was no correlation between adiponectin levels and BMI ($r= -0.287$; Pearson correlation). There was no association between the baseline levels of any adipokine and the patient's age, seizure lateralization, duration of epilepsy, duration of index seizure or seizure frequency. The baseline levels of adiponectin ($p<0.01$, Mann-Whitney test) and leptin ($p<0.05$, Mann-Whitney test) were higher in female patients.

4.3. Seizure related analysis; the postictal levels of adipokines

First, the adipokine concentrations were analysed taking into consideration only the relation to seizures without accounting for diurnal variation. According to this analysis, the leptin concentrations increased in the 3h to 24 h time points after the index seizure as compared to the baseline ($p < 0.001$; Wilcoxon signed ranks test; Table 2). Leptin levels were also related to the severity of seizure types (Fig. 1); they were significantly increased after both FIAS ($p<0.001$; Friedman's test) and FBTCS ($p<0.05$; Friedman's test), but not after FAS (Fig. 1).

There was also an increase in postictal plasma levels of resistin; this elevation was statistically significant at the 12h time point ($p < 0.05$, Wilcoxon signed ranks test); it was statistically significant only in patients with TLE ($p< 0.01$; Wilcoxon signed ranks test), but not in those with XLE. Adipsin and adiponectin concentrations remained unchanged at the different time points (Table 2). There was no association between postictal levels of adipsin, adiponectin or resistin and the seizure type. Plasma concentrations of adipsin, adiponectin or leptin were not found to be related to the epilepsy syndrome (TLE, XLE or IGE) or lateralization

4.4. Diurnal variation related analysis; seizure and epilepsy types

When classified according to seizure types, out of 51 index seizures, 5 were FAS, 36 FIAS and 10 TCS. When classified according to epilepsy types, 4 patients had IGE, 23 had TLE with the remaining 24 having XLE. There was a diurnal variation with regard to both the different seizure and epilepsy types. The patients with

TCS had 20% of the index seizures in the morning, 40% in the late afternoon/evening and 40% during the night. In contrast, patients with impaired awareness seizures experienced 53 % of their seizures in the morning, 33% in the late afternoon/evening and only 14% during the night. (Table 3)

The baseline sample was the blood sample taken when patients were admitted to the VEEG unit on the day of arrival which occurred for practical reasons during the afternoon, if the patient did not experience a seizure during the first day, the second blood sample was taken in the morning of the second day. The sample taken before the index seizure was defined as the baseline sample. Most of the baseline samples were collected between 08:00 and 12:00. The second largest numbers of samples were collected between 12:00 and 15:00. The patients with FIAS and FAS had more seizures during the day of arrival leading to a difference in timing of the baseline samples: FAS 20% morning and 80% afternoon, FIAS 71% morning and 19% afternoon, TCS 80% morning and 20% afternoon. (Table 3)

4.5. Diurnal variation related analysis; adipokine concentrations

Absolute values of leptin vary considerably according to gender and BMI, therefore the analysis was conducted by calculating the relative percentage change of leptin levels in comparison to the determined baseline level. This enables the results to be evaluated regardless of gender or BMI.

When leptin concentrations were measured after the seizures, the highest concentrations were observed around midnight with the lowest values detected between morning and midday. Even after a seizure, relative leptin concentrations followed their diurnal variation almost without exception. (Fig. 2)

Patients were categorized by the time of the index seizure into three different groups when analyzing the collected data of leptin concentrations during the day. Group 1 index seizure was at 06:00-14.00, group 2 between 14:00-22:00 and group 3 between 22:00-06:00. Group 1 had their postictal sample collection during evening and midnight. An increase in the relative leptin concentrations could be detected around midnight with group 1. Group 2 sample collection was conducted during evening and morning. They exhibited an initial small increase in leptin levels, but then they started to decline. Group 3 sample collection started after midnight: towards the morning hours, a strong decrease could be observed followed by a strong increase towards the next midnight. (Fig. 2)

Even TCS follows this diurnal variation i.e. when examined separately in patients who were suffering from TCS, the changes of leptin concentrations could be seen to follow the normal diurnal variation. Even if gender and BMI were taken into account, the results still remained the same. The lowest leptin concentrations occurred around 10:00 in the morning with the highest levels being detected around

midnight. Leptin concentrations were not influenced by gender, BMI and the type of seizure, instead the variation in the leptin concentration mainly followed a diurnal pattern. (Fig. 3)

5. Discussion

The major finding emerging from our study is that there is a diurnal variation in both leptin concentrations and seizure types when assessing the influence of epileptic events on adipokine responses. We have previously reported the effects of epileptic seizures on various biomarkers reflecting neuronal plasticity without taking acoount of major diurnal patterns [21]. If analysed only in relation to seizure occurrences, then the results would be totally misleading. Our recent results emphasize the importance of taking into account all possible confounding factors when analyzing the impact of a seizure on a given biomarker.

When adipokine changes were only evaluated in relation to seizures, it appeared that there was a significant elevation in the leptin concentrations after the baseline seizure. There was some change in resistin concentrations, but the effect was only marginal but levels of neither adiponectin nor adipsin were affected. There seemed to be even a logical difference in terms of seizure severity; the response was fastest in TCS with a change of similar magnitude but more delayed after impaired awareness seizures. With the mildest seizure types (FAS), there were only small and transient changes. However after taking into consideration the timing of the index seizure, then all these effects disappeared, highlighting the major influence of diurnal variation in determining the leptin concentrations.

In previous studies, the lowest concentrations of plasma leptin were measured between 08.00-10.00 and the highest concentrations between 01.00-.04.00 i.e. there is a distinct circadian variation [18]. In our study, the highest concentrations were found around midnight with the lowest concentrations detected during morning and around midday (Fig. 3). We conclude that the leptin concentrations adhere to the normal diurnal variation even in the presence of epileptic seizures.

The second confounding factor is related to differences in diurnal variation of different seizure and epilepsy types. In our study, the patients with TCS experienced more events in the night-time or late afternoon/evening and differed in this respect from individuals with impaired awareness seizures. There was even a difference in their baseline levels, reflecting the number of seizures in the different seizure types. The patients with FIAS and FAS suffered more seizures during the day of arrival, leading to a bias in the timing of baseline samples as compared with TCS. If the baseline sample was drawn in the afternoon, the possibility of determining a higher leptin concentration was greater as compared with a sample taken in the morning, leading to a smaller change between baseline and seizure related samples.

Prior studies have shown that some epileptic seizure types can follow distinctive circadian variation. In a VEEG study, a non-uniform seizure distribution was observed in seizures arising from the temporal lobe (mesial temporal lobe and neocortical temporal lobe), with two peaks observed in both 3- and 4-hour bins: 10:00–13:00/16:00–19:00 and 08:00–12:00/16:00–20:00 respectively. In contrast, no specific 24-hour pattern was identified in seizures with an extratemporal location. [19]

There are some experimental and clinical studies suggesting a possible role for the adipokines in seizure-related neuronal plasticity. For example, leptin has displayed an anticonvulsant effect in different experimental seizure models [10], [11]. Adiponectin has been demonstrated to possess anti-inflammatory properties and also to be neuroprotective in animal models of seizures [12], [13]. In experimental studies, there is the possibility of directly measuring the adipokine concentrations in the brain whereas in humans, most often only plasma samples will be available for analysis, such as in our case with VEEG monitoring. However, in our present study, the results revealed that it was the diurnal variation, not the presence of the epileptic seizures, which explained the changes of leptin concentrations in plasma.

In conclusion, the changes in the leptin concentrations during the day are not affected by seizures, but instead adhere to a diurnal rhythm. This study emphasizes the importance of taking into account the diurnal variation of immunoregulatory molecules, when studying their correlation with some pathological process, for example with epileptic seizures, which also occur in a non-random temporal manner depending on the specific seizure type.

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References

1. Tilg H, Moschen AR. Adipocytokines: Mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006;6:772-783.
2. Hietaharju A, Kuusisto H, Nieminen R, Vuolteenaho K, Elovaara I, Moilanen E. Elevated cerebrospinal fluid adiponectin and adipsin levels in patients with multiple sclerosis: A Finnish co-twin study. Eur J Neurol. 2010;17:332-334.
3. Lee EB. Obesity, leptin, and Alzheimer's disease. Ann N Y Acad Sci. 2011;1243:15-29.
4. Lieb W, Beiser AS, Vasan RS, Tan ZS, Au R, Harris TB, Roubenoff R, Auerbach S, DeCarli C, Wolf PA, Seshadri S. Association of plasma leptin levels with incident alzheimer disease and MRI measures of brain aging. JAMA. 2009;302:2565-2572.
5. Ashwood P, Kwong C, Hansen R, Hertz-Picciotto I, Croen L, Krakowiak P, Walker W, Pessah IN, Van de Water J. Brief report: Plasma leptin levels are elevated in autism: Association with early onset phenotype? J Autism Dev Disord. 2008;38:169-175.
6. Blardi P, de Lalla A, D'Ambrogio T, Vonella G, Ceccatelli L, Auteri A, Hayek J. Long-term plasma levels of leptin and adiponectin in Rett syndrome. Clinical endocrinology. 2009;70:706-709.
7. Dong XQ, Hu YY, Yu WH, Zhang ZY. High concentrations of resistin in the peripheral blood of patients with acute basal ganglia hemorrhage are associated with poor outcome. J Crit Care 2010a;25:243-247.
8. Efstathiou SP, Tsiakou AG, Tsioulos DI, Panagiotou TN, Pefanis AV, Achimastos AD, Mountokalakis TD. Prognostic significance of plasma resistin levels in patients with atherothrombotic ischemic stroke. Clin Chim Acta. 2007;378:78-85.
9. Dong XQ, Yang SB, Zhu FL, Lv QW, Zhang GH, Huang HB. Resistin is associated with mortality in patients with traumatic brain injury. Crit Care. 2010b;14:R190.
10. Xu L, Rensing N, Yang XF, Zhang HX, Thio LL, Rothman SM, Weisenfeld AE, Wong M, Yamada KA. Leptin inhibits 4-aminopyridine- and pentylenetetrazole-induced seizures and AMPAR-mediated synaptic transmission in rodents. J ClinInvest. 2008;118:272-280.
11. Erbayat-Altay E, Yamada KA, Wong M, Thio LL. Increased severity of pentylenetetrazol induced seizures in leptin deficient ob/ob mice. Neurosci Lett. 2008;433:82-86.
12. Jeon BT, Shin HJ, Kim JB, Kim YK, Lee DH, Kim KH, Kim HJ, Kang SS, Cho GJ, Choi WS, Roh GS. Adiponectin protects hippocampal neurons against kainic acid-induced excitotoxicity. Brain Res Rev 2009;61:81-88
13. Lee EB, Warmann G, Dhir R, Ahima RS. Metabolic dysfunction associated with adiponectin deficiency enhances kainic acid-induced seizure severity. J Neurosci. 2011;31:14361-14366.
14. Palmio J, Vuolteenaho K, Lehtimäki K, Nieminen R, Peltola J, Moilanen E. CSF and plasma adipokines after tonic–clonic seizures. Seizure. 2016;39:10-12.
15. Challet E: Circadian aspects of adipokine regulation in rodents. Best Pract Res Clin Endocrinol Metab. 2017;31(6):573-582.
16. Tsanq AH, Astiz M, Friedrichs M, Oster H: Endocrine regulation of circadian physiology. J Endocrinol. 2016;230(1):R1-R11.
17. Kiehn JT, Tsanq AH, Heyde I, Leinweber B, Kolbe I, Leliavski A, Oster H: Circadian rhythms in adipose tissue physiology. Compr Physiol. 2017;16;7(2):383-427
18. Sinha MK, Ohannesian JP, Heiman ML, et al. Nocturnal rise of leptin in lean, obese, and non-insulin-dependent diabetes mellitus subjects.J Clin Invest. 1996;97:1344–1347
19. Nzwalo H, Menezes Cordeiro I, Santos AC, Peralta R, Paiva T, Bentes C. 24-hour rhythmicity of seizures in refractory focal epilepsy. Epilepsy & Behavior. 2016;55:75-78.
20. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017;58:522-530.
21. Liimatainen S, Lehtimäki K, Palmio J, Alapirtti T, Peltola J. Immunological perspectives of temporal lobe seizures. J Neuroimmunol. 2013;15;263(1-2):1-7.

Appendix

Table 1. Clinical characteristics of patients in video-EEG.

	Temporal lobe epilepsy	Extratemporal lobe epilepsy	Generalized epilepsy
No. of patients	23	24	4
Male/female	12/11	11/13	0/4
Age, mean (range)	40.0 (20-58)	30.0 (16-52)	32 (27-45)
BMI, mean (±S.D.)	25.9±3.96	25.0±4.39	26.2±3.51
Mean duration of epilepsy, years (range)	23.2 (2-56)	19.3 (1-52)	18.3 (6-32)
Mean seizure frequency/month[a] (range)	8.5 (0.5-30)	56.4 (0.5-240)	1.9 (9.5-4)
Mean number of AEDs	2.17	2.58	2.25
Patient on mono-/polytherapy	4/19	2/21	1/3
MRI findings			
Normal	4	12	3
HS	13	-	
Cortical dysplasia	3	6	
Other	3	6	1
Index seizure type			
FAS	1	4	
FIAS	19	17	
FBTCS	3	3	
GTCS	-	-	4
Lateralization			
Right	4	11	
Left	18	6	
Right and left or unknown	1	7	
VNS/ earlier epilepsy surgery	2/0	2/1	-/-

AED, Antiepileptic drug; BMI, Body mass index =weight kg/height m^2; FAS, Focal aware seizure; FBTCS, Focal to bilateral tonic-clonic seizure; FIAS, Focal impaired awareness seizure; GTCS, Generalized tonic-clonic seizure; HS, Hippocampal sclerosis; VNS, Vagus nerve stimulator [a] During the last year

Table 2. Concentrations of four adipokines in 51 patients at baseline, and repeated measurements at 3, 6, 12, and 24 hours after index seizure, values are median ± SD.

	Baseline	3h	6h	12h	24h
Leptin (ng/ml)	14.5±21.1	19.2±24.3***	17.8±25.3***	18.3±25.9***	15.8±25.9***
Resistin (ng/ml)	4.8±1.9	4.8±1.9	5.0±2.0	5.3±2.1*	5.0±2.0
Adiponectin (µg/ml)	2.5±1.1	2.6±1.1	2.6±1.1	2.5±1.1	2.5±1.1
Adipsin (µg/ml)	1.0±0.3	1.0±0.3	1.0±0.3	1.1±0.3	1.0±0.3

*** P<0.001, *p<0.05 Wilcoxon Signed Ranks Test (compared to baseline)

Table 3. The number of seizure types and epilepsy types classified according to the time of baseline sample and by the time of index seizure. Baseline samples are missing from two (2) XLE. TCS (tonic-clonic seizures) includes both GTCS and FBTCS.

	Time of baseline sample			Time of index seizure		
Seizure type	08-10	10-12	12-15	06-14	12-22	22-06
FAS	1	0	4	1	2	2
FIAS	20	4	10	19	12	5
TCS	8	0	2	2	4	4
Epilepsy type						
IGE	4	0	0	0	3	1
TLE	17	1	5	10	10	3
XLE	8	3	11	12	5	7

Figure 1. The mean change in leptin levels (Δ leptin) is presented according to the different seizure types. Error bars represent standard error of mean (SEM). Abbreviations: FAS: focal aware seizures; FIAS: focal impaired awareness seizures; TCS: tonic-clonic seizures (includes FBTCS: focal to bilateral tonic-clonic seizures and GTCS: generalized tonic-clonic seizures). The absolute levels of leptin were significantly elevated after FIAS ($p < 0,001$; Friedman's test) and TCS ($p < 0,05$; Friedman's test), but not after FAS.

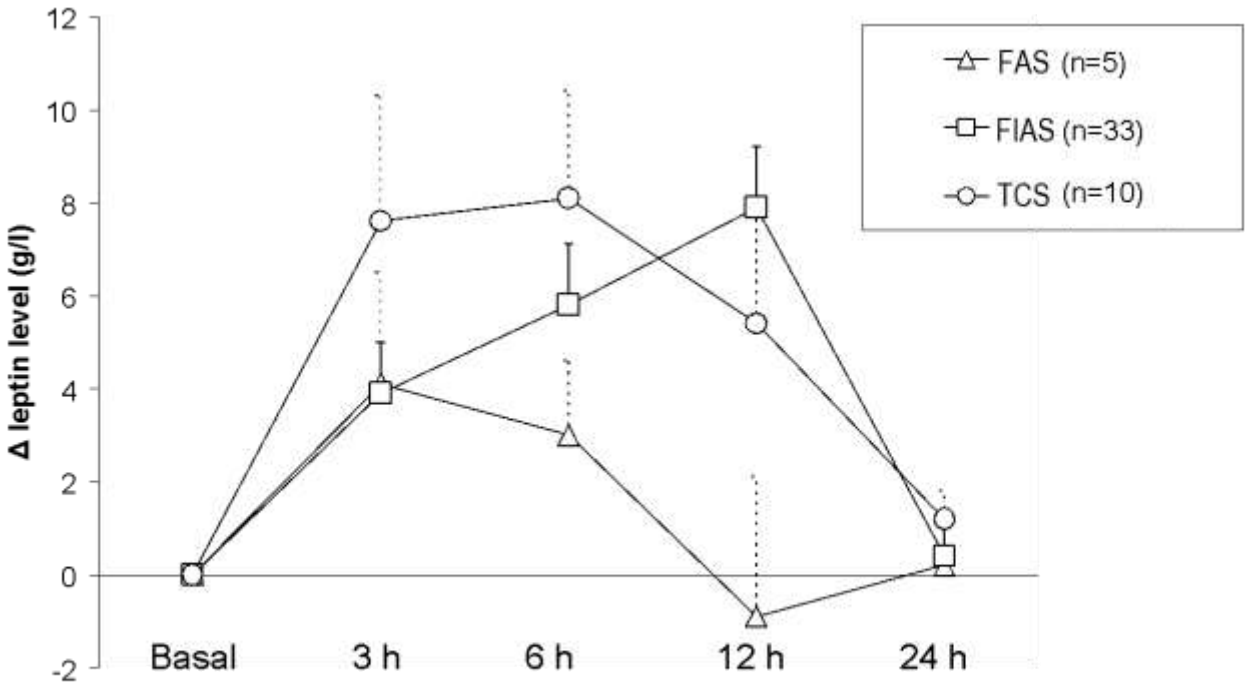

Figure 2. The change percentage of leptin according to the time of the index seizure as determined in the 3-hour, 6-hour, 12-hour and 24-hour samples.

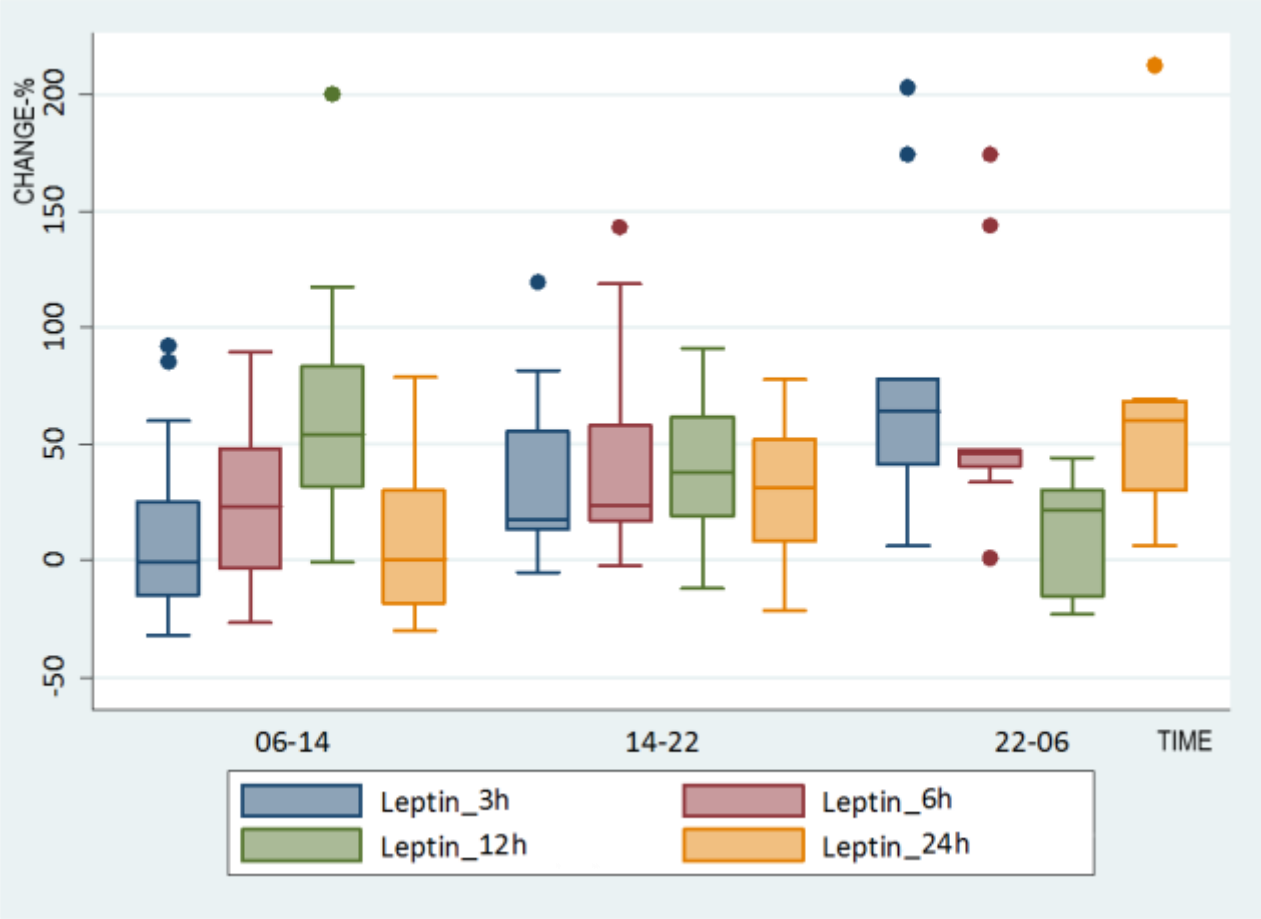

Figure 3. Development of leptin concentration in the TCS-group expressed as a percentage change. Three-hour, 6-hour, 12-hour and 24-hour samples are proportioned to the baseline-sample. Grey colour indicates women and black men. The dashed line indicates those patients who had BMI ≥ 25. The continuous line indicates those patients who had BMI < 25.