



Menopause Analytical Hormonal Correlate Outcome Study (MAHCOS) and the Association to Brain Electrophysiology (P300) in a Clinical Setting

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Abstract

Various studies have demonstrated that increased leptin levels and obesity are inversely related to cognitive decline in menopausal women. It is hypothesized that adiposity is inversely correlated with cognitive decline, as women with increased weight are less vulnerable to diminishing cognition. However, it is increasingly observed that menopausal women, even with increased adiposity, experience significant cognitive decline. Positron emission tomography (PET) has been used to analyze cognitive function and processing in menopausal women. Evoked potentials (P300) and neurophysiologic tests have validated brain metabolism in cognitively impaired patients. Post-hoc analyses of 796 female patients entering PATH Medical Clinic, between January 4, 2009 and February 24, 2013, were performed as part of the “Menopause Analytical Hormonal Correlate Outcome Study” (MAHCOS). Patient age range was 39–76 years (46.7 ± 0.2). P300 latency and amplitude correlated with a number of hormones: follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol, estrone, estriol, DHEA, pregnenolone, progesterone, free and total testosterone, thyroid stimulating hormone (TSH), Vitamins D 1.25 and D 25OH, leptin, and insulin-like growth factor-binding protein 3 (IGF-BP3). Corrected statistics did not reveal significant associations with P300 latency or amplitude for these hormones except for leptin plasma levels. However, factor analysis showed that FSH and LH clustered together with Vitamin D1.25 and Vitamin D25OH, P300 latency (not amplitude), and log leptin were found to be associated in the same cluster. Utilizing regression analysis, once age adjusted, leptin was the only significant predictor for latency or speed ($p = 0.03$) with an effect size of 0.23. Higher plasma leptin levels were associated with abnormal P300 speed ($OR = 0.98$). Our findings show a significant relationship of higher plasma leptin levels, potentially due to leptin resistance, and prolonged P300 latency. This suggests leptin resistance may delay electrophysiological processing of memory and attention, which appears to be the first of such an association.

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Introduction

Menopausal women become deficient in multiple hormones such as estrogen, progesterone, testosterone, and dehydroepiandrosterone (DHEA) [1–6] with increases in luteinizing hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH) [7–10]. All of these hormones have individual as well as inter-related functions in the human body, including pulmonary, cardiovascular, gastrointestinal, and immunological functions [11,12]. Aging is continually changing as life expectancy increases, and the decrease in cognition through normal aging processes is of primary clinical interest [6,13]. In fact, estrogen deficiency has been proposed as a cause of memory deficits in postmenopausal women [14]. There are studies that suggest that

LH increases after menopause with concomitant decline in cognitive performance [15]. Chorionic gonadotropin receptors and LH occur in the brain [16]. Thus, levels of LH and FSH may increase low-density lipoprotein receptor-related protein in the brain [14,17]. Levels of FSH increase dramatically in women during and after menopause and can be lowered with estrogen therapy [14,18]. Emerging evidence suggests that high TSH levels are associated with a two-fold risk of cognitive decline as well as prevalence of anomalies in musculoskeletal systems [19–21].

Menopause and Leptin

Weight gain, and associated leptin level changes during menopause have been demonstrated in numerous cases, as leptin was found to have significant association with metabolic factors

MENOPAUSE QUESTIONNAIRE

NAME: _____ **DATE OF BIRTH:** _____ **AGE:** _____ **Today's Date** _____

NOTE: This research is not limited to women currently experiencing menopause. All information disclosed here is solely for research purposes conducted by the PATH Research Foundation.

1) What is your current menopausal status?
 1) Not yet at menopause 2) Undergoing menopause 3) Through menopause

2) How long (in months or years) have you been in menopause? _____

3) Was your menopause:
 1) Natural and age-related
 2) Not age-related due to (please circle all that apply):
 A) Surgery B) Disease/injury C) Premature ovarian failure D) Chemotherapy/radiation/medication

4) Have you had any of these operations?
 1) Hysterectomy (uterus removal)
 2) Oophorectomy (ovary removal): A) One ovary B) Both ovaries
 3) None of the above

5) Are you currently using Hormone Replacement Therapy (HRT)?
 1) No—I have never taken prescription HRT
 2) No—I have taken HRT but I am currently not. **If so, how long ago did you stop using hormones?** _____
 3) Yes—estrogen only
 4) Yes—progesterone only
 5) Yes—estrogen and progesterone
 6) Yes—estrogen and other hormones

6) Are you taking any medications that affect your hormone levels? (If yes, please list them)

7) How long have you been at PATH Medical for treatment? _____

8) Please note if you have or have had any of the following conditions or diseases. If any of these conditions have increased in severity, please make a note in the margins, where applicable.
 1) Diabetes
 2) Problems with thyroid, parathyroid, adrenal glands
 3) Respiratory disease
 4) Osteoporosis
 5) Coronary artery disease
 6) Hyperlipidemia (high blood levels of lipids, including cholesterol)
 7) Breast cancer
 8) Ovarian cancer
 9) Uterine cancer
 10) Any other form of cancer? If yes, please state: _____

9) Have you gained or lost any weight within the past:
 1) GAINED/LOST: Three years? YES/NO If yes, how much? _____
 2) GAINED/LOST: Five years? YES/NO If yes, how much? _____
 3) GAINED/LOST: Ten years? YES/NO If yes, how much? _____

10) How often do you exercise?
 1) Rarely 2) 1-3 times a week 3) 4-7 times a week

HOW OFTEN DO YOU EXPERIENCE THE FOLLOWING SYMPTOMS?

	NEVER	RARELY	SOMETIMES	OFTEN	ALWAYS
	1	2	3	4	5
NEUROLOGICAL Tingling in hands and feet Dizzy spells Loss of appetite Loss of desire (to be active or do work) Headaches/Ringing in ears					
NEUROPSYCHIATRIC Nervous tension Dry nose and mouth Difficulty concentrating Depression Anxiety Memory loss					
NEUROPSYCHOLOGICAL Lack of energy/Fatigue					
Feeling sad/downhearted; tearfulness Difficulty sleeping Irritability/Agitation Panic Mood swings					
ENDOCRINE: Hot flashes Night sweats Dry/itchy eyes					
PULMONARY: Increased asthma Persistent cough					
MUSCULOSKELETAL: Joint pain or stiffness Backache Muscle tension					
GASTROINTESTINAL: Diarrhea Constipation Irritable bowel syndrome Increased reflux (heartburn) Increased gas Bloating Increased indigestion Increased nausea					
CARDIOVASCULAR: Swelling Chest pain on exertion Shortness of breath on exertion Rapid/irregular heartbeat Cold sweats					
DERMATOLOGICAL: Skin rash/irritation Dry skin Thinning skin Hair loss or thinning- head, pubic area, anywhere on body Increase in facial hair Changes in fingernails: softer, crack or break easier Increased acne					
GENITOURINARY: Discomfort passing urine Inability to control urine, especially upon sneezing, laughing Overactive bladder—frequent, sudden urges to urinate Bladder infection Leakage of feces Incidence of urinary tract infections					
IMMUNE: Increased cysts Sore throat Allergies Gum problems, increased bleeding					
GYNECOLOGICAL: Breast soreness Breast pain Vaginal discharge Dry vagina Irritation of the tissues in and around the vagina Pain in vaginal area (including during sexual intercourse) Loss of sex drive Decreased sexual response Irregular periods # of sexual events per week : _____					

Figure 1. Menopause Questionnaire.
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Table 1. A–S: Demographics of Subjects.

A: Age Demographics of Subjects With Age Range of Patients 39–76 years (46.7±0.2 years) (n = 796)		
Age Group	Count	Proportion
2: 35–39	33	0.04146
3: 40–44	217	0.27261
4: 45–49	321	0.40327
5: 50–54	207	0.26005
6: 55–59	8	0.01005
7: 60–64	8	0.01005
A 9: 71+	2	0.00251
Total	796	1
B: Dichotomized FSH Level Demographics of Subjects with 30.1±1.3 (n = 731) strongly right skewed distribution		
FSH Level Dichotomized	Count	Proportion
Pre-menopausal	428	0.5855
Post-menopausal	303	0.4145
Total	731	1
C: Demographics for Subjects with FSH Values Below and Over 40		
FSH Value over 40?	Count	Proportion
Yes	212	0.29001
No	519	0.70999
Total	731	1
D: Demographics for Subjects with FSH Values Below and Over 80		
FSH Value over 80?	Count	Proportion
Yes	92	0.12585
No	639	0.87415
Total	731	1
E: Demographics for Subjects with FSH Values Below and Over 100		
FSH Value over 100?	Count	Proportion
Yes	48	0.06566
No	683	0.93434
Total	731	1
F: Dichotomized LH Level Demographics for Subjects (19.1±0.7; n = 726; strongly right skewed distribution)		
LH Level Dichotomized	Count	Proportion
Pre-menopausal	378	0.52066
Post-menopausal	348	0.47934
Total	726	1
G: Dichotomized TSH Level Demographics for Subjects (2.0±0.2; n = 795; strongly right skewed distribution)		
TSH Level Dichotomized	Count	Proportion
Normal	728	0.91572
Abnormal	67	0.08428
Total	795	1
H: Dichotomized Leptin Level Demographics for Subjects (21.0±0.8; n = 565; strongly right skewed distribution)		
Leptin Level Dichotomized	Count	Proportion
Normal	260	0.46018
Abnormal	305	0.53982
Total	565	1
I: Dichotomized IGF-BP3 Level Demographics for Subjects (4.4±0.1; n = 306; moderately normal distribution)		
IGF-BP3 Level Dichotomized	Count	Proportion
Normal	287	0.93791
Abnormal	19	0.06209
Total	306	1

J: Dichotomized Vitamin D 25OH Level Demographics for Subjects (37.5±0.8; n=671; moderately right skewed distribution)

Vitamin D 25OH Level Dichotomized	Count	Proportion
Normal	438	0.65276
Abnormal	233	0.34724
Total	671	1

K: Dichotomized Vitamin D 1,25 Level Demographics for Subjects (61.4±1.1; n = 585; moderately right skewed distribution)

Vitamin D 1,25 Level Dichotomized	Count	Proportion
Normal	342	0.58462
Abnormal	243	0.41538
Total	585	1

L: Dichotomized % Free Testosterone Level Demographics for Subjects (0.8±0.1; n = 711; strongly right skewed distribution)

% Free Testosterone Level Dichotomized	Count	Proportion
Normal	694	0.97609
Abnormal	17	0.02391
Total	711	1

M: Dichotomized Testosterone Total Level Demographics for Subjects (30.8±1.5; n = 715; strongly right skewed distribution)

Testosterone Total Level Dichotomized	Count	Proportion
Normal	547	0.76503
Abnormal	168	0.23497
Total	715	1

N: Dichotomized Estradiol Level Demographics for Subjects (97.4±7.4; n = 720; strongly right skewed distribution)

Estradiol Level Dichotomized	Count	Proportion
Pre-menopausal	631	0.87639
Post-menopausal	89	0.12361
Total	720	1

O: Dichotomized Estrone Level Demographics for Subjects (69.7±4.2; n = 552; strongly right skewed distribution)

Estrone Level Dichotomized	Count	Proportion
Pre-menopausal	354	0.6413
Post-menopausal	198	0.3587
Total	552	1

P: Dichotomized Estriol Level Demographics for Subjects (0.2±0.0; n = 513)

Estriol Level Dichotomized	Count	Proportion
Normal	1	0.00195
Abnormal	512	0.99805
Total	513	1

Q: Dichotomized Beam Level Demographics for Subjects

Beam Level	Count	Proportion
Yes	192	0.2409
No	605	0.7591
Total	797	1

R: Dichotomized P300 Speed (Latency) Level Demographics for Subjects (319.7±1.9; n = 194) moderately normal distribution)

P300 Speed (Latency) Level Dichotomized	Count	Proportion
Normal	90	0.46392
Abnormal	104	0.53608
Total	194	1

S: Dichotomized P300 Voltage (Amplitude) Level Demographics for Subjects (4.8±0.4; n = 194; strongly right skewed distribution)

P300 Voltage (Amplitude) Level Dichotomized	Count	Proportion
Normal	5	0.02577
Abnormal	189	0.97423
Total	194	1

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Table 2. Association between FSH and P300 Speed/Voltage.

Test	Adjusted R2	N	F	P-Value	Chi2
Latency fitted by FSH (Bivariate)	0.02662	186	6.0595	0.0148*	-
P300 Speed fitted by FSH (Logistic)	-	-	-	0.162	1.95563
Amplitude fitted by FSH (Bivariate)	-0.00069	186	0.873	0.3513	-
P300 Voltage fitted by FSH (Logistic)	-	-	-	0.0759	3.150917
Latency fitted by Dichotomized FSH (ANOVA)	0.01976	186	4.7293	0.0309* (two-sided) 0.0155* (one-sided)	-
P300 Speed fitted by Dichotomized FSH (Contingency) Likelihood Ratio	-	-	-	0.0972	2.75
P300 Speed fitted by Dichotomized FSH (Contingency) Pearson	-	-	-	0.0977	2.742
P300 Speed fitted by Dichotomized FSH (Contingency) Fisher's Exact Test	-	-	-	-	0.1075
Amplitude fitted by Dichotomized FSH (ANOVA)	0.000838	186	1.1551	0.2839 (two-sided)	-
P300 Voltage fitted by Dichotomized FSH (Contingency) Likelihood Ratio	-	-	-	0.1895	1.721
P300 Voltage fitted by Dichotomized FSH (Contingency) Pearson	-	-	-	0.2063	1.597
P300 Voltage fitted by Dichotomized FSH (Contingency) Fisher's Exact Test	-	-	-	0.3705	-

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when compared to resistin in pre and post-menopausal women [24–33]. In particular menopausal status is linked with increased weight gain, increased central fat mass, and abnormal lipid metabolism. One case observed a significant statistical correlation with serum leptin levels and fat mass; abdominal obesity and visceral fat area were positively correlated with serum leptin level [28]. Leptin concentration was positively correlated with BMI and weight gain in healthy menopausal women [86]. There is indeed a definitive correlation with plasma leptin levels in peri- and postmenopausal women and weight gain, which suggests that there is a dynamic interaction between plasma leptin and body composition. These changes may further indicate that a significant association with leptin levels and metabolic factors could impact other hormonal composition changes that occur during menopause.

One important question that concerns menopausal women is weight gain and early cognitive decline [22]. In some studies, researchers have found that obesity-related waist circumference had a positive correlation with global cognition [23]. There have been controversial reports indicating that naturally occurring estrogen may have a beneficial effect on cognition in women [33–37]. There is a strong relationship between the hormone leptin and obesity especially in females [36–39]; however, leptin's role in cognition has been studied with mixed results [28–30] and there is a notable lack of published data on P300 utilization and its relationship with plasma leptin levels, despite studies indicating the association between leptin and cognitive genes. The P300 evoked potential measures electrical brain waves and their function in particular regions of the brain. It is a diagnostic tool used to identify neurological deficits or abnormalities, such as cognitive decline and neurotransmitter deficiencies.

Moreover delayed P300 latency and obesity are both associated with dementia. Leptin resistance may delay electrophysiological processing of memory and attention. [87].

The Clinical Significance of P300 Testing

When used in a clinical setting, the P300 serves as an invaluable resource towards early diagnosis and treatment of poor cognition and loss of brain vitality due to menopausal transition. Analysis of the P300 and standard neuropsychological variables to assess patients with mild cognitive impairment (MCI) and Alzheimer's disease can provide valuable information for the detection of MCI and AD [80]. A number of studies determine that there is justification and efficacy in employing more resources unto converting P300 as an acceptable biological marker for AD [81–84]. P300 is responsive to the deterioration of language, memory, and executive functions, thereby acting as a useful tool in evaluating cognitive function [85]. The P300 was therefore a suitable tool in evaluating the variations in cognitive function and quality in subjects.

We hypothesized that women presenting with complaints related to menopause would have a number of associated hormonal changes relating specifically to both somatic and neurological symptoms. We also hypothesized that there would be an inverse relationship between plasma leptin levels and P300 speed and voltage based on literature suggesting that as women transition into menopause, they are more likely to gain weight due to associated changes leptin levels, which have been associated with a decrease in cognitive functioning [40–43]. In this paper, we focus on the role of leptin plasma levels and brain electrophysiology as measured by P300 amplitude (voltage) and latency.

Methods

Subjects

All female patients who presented to a private clinic, at or approaching the typical age of menopause onset (age 39–59), were examined. We also extended this study to include a small number of women (n = 33) between 35 to 39 years, as well as 10 women ≥ 60 years. The descriptive distribution summary of each measured variable collected from January 4, 2009 to February 24, 2013 in

Table 3. Association between Categorized FSH and P300 Speed/Voltage.

Test	Adjusted R2	N	F	t	P-Value	Chi2
Latency fitted by FSH over 40 (ANOVA)	0.019772	186	4.7316	2.17523	0.0309* (two sided) 0.0154* (one-sided)	-
P300 Speed fitted by FSH over 40 (Contingency) Likelihood Ratio	-	-	-	-	0.2678	1.228
P300 Speed fitted by FSH over 40 (Contingency) Pearson	-	-	-	-	0.2689	1.223
P300 Speed fitted by FSH over 40 (Contingency) Fisher's Exact Test	-	-	-	-	0.2785	-
Amplitude fitted by FSH over 40 (ANOVA)	0.00413	186	1.7671	-	0.1854 (two-sided)	-
P300 Voltage fitted by FSH over 40 (Contingency) Likelihood Ratio	-	-	-	-	0.0444*	4.041
P300 Voltage fitted by FSH over 40 (Contingency) Pearson	-	-	-	-	0.1133	2.507
P300 Voltage fitted by FSH over 40 (Contingency) Fisher's Exact Test	-	-	-	-	0.174	-
Latency fitted by FSH over 80 (ANOVA)	0.007259	186	2.3527	-	0.1268 (two-sided)	-
P300 Speed fitted by FSH over 80 (Contingency) Likelihood Ratio	-	-	-	-	0.6516	0.204
P300 Speed fitted by FSH over 80 (Contingency) Pearson	-	-	-	-	0.6522	0.203
P300 Speed fitted by FSH over 80 (Contingency) Fisher's Exact Test	-	-	-	-	0.6862	-
Amplitude fitted by FSH over 80 (ANOVA)	-0.00327	186	0.3977	-	0.5291 (two-sided)	-
P300 Voltage fitted by FSH over 80 (Contingency) Likelihood Ratio	-	-	-	-	0.1982	1.656
P300 Voltage fitted by FSH over 80 (Contingency) Pearson	-	-	-	-	0.34	0.911
Latency fitted by FSH over 100 (ANOVA)	0.031206	186	6.9591	2.638016	0.0091* (two-sided) 0.0045* (one-sided)	-
P300 Speed fitted by FSH over 100 (Contingency) Likelihood Ratio	-	-	-	-	0.1258	2.343
P300 Speed fitted by FSH over 100 (Contingency) Pearson	-	-	-	-	0.1323	2.266
P300 Speed fitted by FSH over 100 (Contingency) Fisher's Exact test	-	-	-	-	0.2015	-
Amplitude fitted by FSH over 100 (ANOVA)	-0.0054	186	0.0061	-	0.9376 (two-sided)	-
P300 Voltage fitted by FSH over 100 (Contingency) Likelihood Ratio	-	-	-	-	0.3241	0.972
P300 Voltage fitted by FSH over 100 (Contingency) Pearson	-	-	-	-	0.4722	0.517

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Table 4. Association between FSH and P300 Speed/Voltage for FSH under 80.

Test	Adjusted R2	N	F	P-value	Chi2
Latency fitted by FSH (bivariate)	0.015967	158	3.5476	0.0615	-
P300 Speed fitted by FSH (logistic)	-	-	-	0.1212	2.402083
Amplitude fitted by FSH (bivariate)	0.000377	158	1.0592	0.305	-
P300 Voltage fitted by FSH (logistic)	-	-	-	0.2116	1.560134

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Table 5. Association between FSH and P300 Speed/Voltage for FSH over 80.

Test	Adjusted R2	N	F	P-value	Chi2
Latency fitted by FSH (bivariate)	-0.00906	28	0.7575	0.3921	-
P300 Speed fitted by FSH (logistic)	-	-	-	0.3595	0.839701
Amplitude fitted by FSH (bivariate)	0.063924	28	2.8438	0.1037	-

doi:10.1371/journal.pone.0105048.t005

this retrospective study is as follows: The initial sample size was $n = 796$ female patients. Missing values are common in this dataset, and certain variables were dichotomized based on the cutoff chart with the normal ranges given. Each patient filled out an approved PATH Foundation NY IRB (PFNYIRB) informed consent form, and the PFNYIRB committee approved the study [Registration # IRB00002334; Protocol #: LEXMEN001].

All subjects underwent a thorough medical evaluation including a full screen for hormones including DHEA sulfate, estradiol, estrone, FSH, LH, pregnenolone, progesterone, free and total testosterone, and TSH obtained from BioReference Laboratories, Inc. Hormone blood levels were drawn between 9:30 am and 3:00 pm. A detailed medical history was obtained including information on the stage of menopause (pre, undergoing, or post), origin of menopause, and history of hormone replacement therapy as well as anthropometric characteristics such as weight, height, BMI, body fat percentage, muscle, and bone density recorded via dual-energy x-ray absorptiometry (DXA). These anthropometric characteristics were not included in the study's final data analysis, but may be considered for future studies. A Menopause Questionnaire (Figure 1) was given to all women ($n = 796$) following a preliminary screening. The quantitative section of the Menopause Questionnaire consisted of 64 questions related to symptoms of menopause. Each symptom was rated on a Likert scale of frequency from 1 (never) to 5 (always). The total number of endorsed symptoms was calculated as a gross indicator of menopausal symptomatology. Mean values of the Likert ratings also were calculated within each of 12 domains of symptoms: neurological, neuropsychiatric, neuropsychological, endocrine,

pulmonary, musculoskeletal, gastrointestinal, cardiovascular, immune, dermatological, genitourinary, and gynecological. A grand mean of the Likert rating across all domains also was calculated. All reported blood levels and preliminary questionnaires were recorded within 3 weeks of each other.

The demographics for the study are outlined in Table 1 (subsections A–S). The age range of the patients was 39–76 years (46.7 ± 0.2 years, $n = 796$) with $\sim 93.6\%$ of patients between ages 40 and 54 ($n = 745$); FSH levels were 30.1 ± 1.3 mIU/mL ($n = 731$) with strong right skewed distribution; LH levels were 19.1 ± 0.7 mIU/mL ($n = 726$) with strong right skewed distribution; TSH levels were 2.0 ± 0.2 μ IU/mL ($n = 795$) with strong right distribution; leptin levels were 21.0 ± 0.8 ng/mL ($n = 565$) with strong right skewed distribution; IGF-BP3 levels were 4.4 ± 0.1 μ g/mL ($n = 306$) with moderately normal distribution; Vitamin D 25OH levels were 37.5 ± 0.8 ng/mL ($n = 671$) with moderately right skewed distribution; Vitamin D 1,25 levels were 61.4 ± 1.1 pg/mL ($n = 585$) with moderately right skewed distribution; % Free Testosterone, Testosterone Free, and Testosterone Total levels were $2.9 \pm 0.4\%$ ($n = 26$), 0.8 ± 0.1 pg/mL ($n = 711$), and 30.8 ± 1.5 ng/dL ($n = 715$), respectively, Testosterone Free and Testosterone Total with strong right skewed distributions; estradiol levels were 97.4 ± 7.4 pg/mL ($n = 740$) with strong right skewed distribution; estrone levels were 69.7 ± 4.2 pg/mL ($n = 522$) with strong right skewed distribution; estriol levels were 0.2 ± 0.0 ng/mL ($n = 513$); $\sim 24\%$ of patients underwent Brain Electrical Activity Mapping (BEAM) ($n = 796$); P300 latency levels were 319.7 ± 1.9 ms ($n = 194$) with moderately normal distribu-

Table 6. Association between FSH and P300 Speed/Voltage for FSH under 100.

Test	Adjusted R2	N	F	P-value	Chi2
Latency fitted by FSH (bivariate)	0.000685	169	1.1151	0.2925	-
P300 Speed fitted by FSH (logistic)	-	-	-	0.5632	0.33424
Amplitude fitted by FSH (bivariate)	0.005156	169	1.8706	0.1732	-
P300 Voltage fitted by FSH (logistic)	-	-	-	0.1379	2.200685

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Table 7. Association between FSH and P300 Speed/Voltage for FSH over 100.

Test	Adjusted R2	N	F	P-value	Chi2
Latency fitted by FSH (bivariate)	-0.04976	17	0.2416	0.6302	-
P300 Speed fitted by FSH (logistic)	-	-	-	0.6857	0.163758
Amplitude fitted by FSH (bivariate)	-0.03501	17	0.4587	0.5085	-

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Table 8. Association between FSH over 40 and Estradiol, Estrone, Estriol.

Test	Adjusted R2	N	F	t	P-value	Average	Std. Error	Lower 95%	Upper 95%	Chi2
Estradiol fitted by FSH over 40 (ANOVA)	0.04977	713	38.2927	-6.18811	<.0001* (two-sided) <.0001* (one-sided)	-	-	-	-	-
Estradiol fitted by FSH over 40	-	206	-	-	-	27.092	13.48	0.63	53.56	-
Estradiol fitted by FSH under 40	-	507	-	-	-	126.01	8.592	109.14	142.88	-
Dichotomized Estradiol fitted by FSH over 40 (Contingency) Likelihood Ratio	-	-	-	-	<.0001*	-	-	-	-	100.562
Dichotomized Estradiol fitted by FSH over 40 (Contingency) Pearson	-	-	-	-	<.0001*	-	-	-	-	111.681
Dichotomized Estradiol fitted by FSH over 40 (Contingency) Fisher's Exact Test	-	-	-	-	<.0001*	-	-	-	-	-
Estrone fitted by FSH over 40 (ANOVA)	0.036116	546	21.4206	-4.62824	<.0001* (two-sided) <.0001* (one-sided)	-	-	-	-	-
Estrone fitted by FSH over 40	-	168	-	-	-	41.0796	7.5164	26.315	55.844	-
Estrone fitted by FSH under 40	-	378	-	-	-	82.8889	5.0109	73.046	92.732	-
Dichotomized Estrone fitted by FSH over 40 (Contingency) Likelihood Ratio	-	-	-	-	<.0001*	-	-	-	-	51.146
Dichotomized Estrone fitted by FSH over 40 (Contingency) Pearson	-	-	-	-	<.0001*	-	-	-	-	52.243
Dichotomized Estrone fitted by FSH over 40 (Contingency) Fisher's Exact Test	-	-	-	-	<.0001*	-	-	-	-	-
Estriol fitted by FSH over 40 (ANOVA)	0.005501	507	3.7991	1.949129	0.0518 (two-sided) 0.0259* (one-sided)	-	-	-	-	-
Estriol fitted by FSH over 40	-	152	-	-	-	0.19625	0.00776	0.181	0.2115	-
Estriol fitted by FSH under 40	-	355	-	-	-	0.178169	0.00508	0.16819	0.18815	-
Dichotomized Estriol fitted by FSH over 40 (Contingency) Likelihood Ratio	-	-	-	-	0.1203	-	-	-	-	2.414
Dichotomized Estriol fitted by FSH over 40 (Contingency) Pearson	-	-	-	-	0.1261	-	-	-	-	2.34
Dichotomized Estriol fitted by FSH over 40 (Contingency) Fisher's Exact Test	-	-	-	-	0.2998	-	-	-	-	-

doi:10.1371/journal.pone.0105048.t008

Table 9. Association between FSH and Estradiol, Estrone, Estriol for FSH under 40.

Test	Adjusted R2	N	F	P-value	Chi2
Estradiol fitted by FSH (bivariate)	0.013439	507	7.8927	0.0052*	-
Dichotomized Estradiol fitted by FSH (logistic)	-	-	-	0.57	0.322627
Estrone fitted by FSH (bivariate)	0.008586	378	4.2649	0.0396*	-
Dichotomized Estrone fitted by FSH (logistic)	-	-	-	0.0283*	4.812526
Estriol fitted by FSH (bivariate)	-0.00209	355	0.2614	0.6094	-

doi:10.1371/journal.pone.0105048.t009

Table 10. Association between FSH and Estradiol, Estrone, Estriol for FSH over 40.

Test	Adjusted R2	N	F	P-value	Chi2
Estradiol fitted by FSH (bivariate)	0.13044	206	31.7514	<.0001*	-
Dichotomized Estradiol fitted by FSH (logistic)	-	-	-	<.0001*	30.51419
Estrone fitted by FSH (bivariate)	0.019264	168	4.2803	0.0401*	-
Dichotomized Estrone fitted by FSH (logistic)	-	-	-	0.0035*	8.534631
Estriol fitted by FSH (bivariate)	-0.00661	152	0.009	0.9245	-
Dichotomized Estriol fitted by FSH (logistic)	-	-	-	0.9758	0.000924

doi:10.1371/journal.pone.0105048.t010

Table 11. Association between Leptin and P300 Speed/Voltage.

Test	Adjusted R2	N	F	P-value	Chi2
Latency fitted by Leptin (bivariate)	0.048107	159	8.985	0.0032*	-
P300 Speed fitted by Leptin (logistic)	-	-	-	0.0083*	6.972178
Amplitude fitted by Leptin (bivariate)	-0.00036	159	0.9426	0.3331	-
P300 Voltage fitted by Leptin (logistic)	-	-	-	0.7599	0.09338
Latency fitted by Dichotomized Leptin (ANOVA)	0.000685	159	1.1083	0.2941 (two-sided)	-
P300 Speed fitted by Dichotomized Leptin (Contingency) Likelihood Ratio	-	-	-	0.1014	2.683
P300 Speed fitted by Dichotomized Leptin (Contingency) Pearson	-	-	-	0.1018	2.677
P300 Speed fitted by Dichotomized Leptin (Contingency) Fisher's Exact Test	-	-	-	0.1131	-
Amplitude fitted by Dichotomized Leptin (ANOVA)	-0.00364	159	0.4273	0.5143 (two-sided)	-
P300 Voltage fitted by Dichotomized Leptin (Contingency) Likelihood Ratio	-	-	-	0.8087	0.059
P300 Voltage fitted by Dichotomized Leptin (Contingency) Pearson	-	-	-	0.8094	0.058

doi:10.1371/journal.pone.0105048.t011

Table 12. Latency Variables predicted by Leptin with Age (ANOVA).

Test	R2	Adjusted R2	N	F	P-value
Latency predicted by Leptin with Age (ANOVA)	0.1573	0.140884	158	9.582	<.0001*

doi:10.1371/journal.pone.0105048.t012

Table 13. Regression Analysis of Latency predicted by Leptin with Age (ANOVA).

Parameter Estimates Term	Estimate	Std.Error	t	P-value	Lower 95%	Upper 95%
Intercept	322.37092	4.244617	75.95	<.0001*	313.98573	330.75611
Leptin	0.2306531	0.10853	2.13	0.0352*	0.0162537	0.4450526
Age Group: 2&3&4&5-6&7&9	-12.66445	3.382663	-3.74	0.0003*	-19.34686	-5.982038
Age Group: 2&3-4&5	-5.758462	2.11586	-2.72	0.0072*	-9.938319	-1.578605

doi:10.1371/journal.pone.0105048.t013

Table 14. P300 Speed Variables predicted by Leptin with Age (logistic).

Test	N	Chi2	P-value
P300 Speed predicted by Leptin with Age(logistic)	158	18.57717	<.0001*

doi:10.1371/journal.pone.0105048.t014

Table 15. Regression Analysis of P300 Speed predicted by Leptin with Age (logistic).

Parameter Estimates Term	Estimate	Std.Error	t	P-value	Lower 95%	Upper 95%
Intercept	-0.5940827	0.2620591	5.14	0.0234*	-1.1230387	-0.0913201
Leptin	0.02216648	0.0100457	4.87	0.0273*	0.00320555	0.04286365
Age Group: 2&3-4&5&6&7&9	-0.6206882	0.1834263	11.45	0.0007*	-0.9905093	-0.2683189

doi:10.1371/journal.pone.0105048.t015

Table 16. P300 Speed Odds Ratio predicted by Leptin with Age (logistic).

Odd Ratios Term	Odds Ratio	Lower 95%	Upper 95%	Reciprocal
Leptin	1.022414	1.003211	1.043796	0.9780774
Age Group: 2&3-4&5&6&7&9	0.537574	0.371387	0.764664	1.8602078

doi:10.1371/journal.pone.0105048.t016

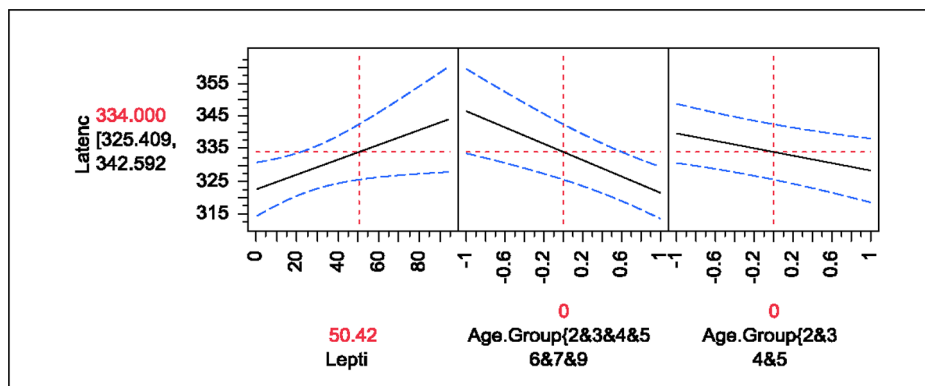


Figure 2. Prediction Profiler for Latency predicted by Leptin with Age (ANOVA).

doi:10.1371/journal.pone.0105048.g002

Table 17. Principal Component Analysis in the Raw Scale Correlations Estimated by Pairwise Method.

	Age	FSH	LH	TSH	Leptin	IGF-BP3	Vitamin D 25OH	Vitamin D 1,25	Testosterone Free	Testosterone Total	Estradiol	Estrone	Estriol	P300 Latency	P300 Amplitude
Age	1	0.4089	0.3833	0.0212	0.0491	-0.1876	0.0285	0.0185	0.0456	0.0471	-0.0623	-0.0243	0.0475	0.3349	-0.0062
FSH	0.4089	1	0.855	0.0188	-0.0065	0.0676	0.0051	0.0013	-0.0282	-0.0848	-0.2378	-0.2036	0.0685	0.1786	-0.0687
LH	0.3833	0.855	1	0.0456	0.0531	0.0337	0.0213	-0.0252	0.0135	-0.0431	-0.159	-0.1531	0.0635	0.2161	-0.0605
TSH	0.0212	0.0188	0.0456	1	0.0861	-0.1212	-0.0426	-0.1096	-0.0099	-0.0109	-0.0168	-0.0229	-0.0505	0.0286	0.0314
Leptin	0.0491	-0.0065	0.0531	0.0861	1	0.0089	-0.3006	0.1707	0.0141	0.1042	-0.0135	0.0942	0.1898	0.2327	0.0773
IGF-BP3	-0.1876	0.0676	0.0337	-0.1212	0.0089	1	0.0375	0.1707	0.0553	0.0029	-0.1208	-0.0747	0.0538	-0.1793	0.1971
Vitamin D 25OH	0.0285	0.0051	0.0213	-0.0426	-0.3006	0.0375	1	0.4501	0.0149	0.0463	0.0211	-0.0437	-0.1422	-0.0048	0.0313
Vitamin D 1,25	0.0185	0.0013	-0.0252	-0.1096	-0.2316	0.1707	0.4501	1	0.0278	0.0514	0.1066	0.1357	0.0964	-0.0093	-0.0129
Testosterone Free	0.0456	-0.0282	0.0135	-0.0099	0.0141	0.0553	0.0149	0.0278	1	0.3713	0.0535	0.0678	0.1010	0.067	0.0027
Testosterone Total	0.0471	-0.0848	-0.0431	-0.0109	0.1042	0.0029	0.0463	0.0514	0.3713	1	0.3284	0.4243	0.1873	0.1572	-0.015
Estradiol	-0.0623	-0.2378	-0.159	-0.0168	-0.0135	-0.1208	0.0211	0.1066	0.0535	0.3284	1	0.8814	0.0845	-0.0129	0.1637
Estrone	-0.0243	-0.2036	-0.1531	-0.0229	0.0942	-0.0747	-0.0437	0.1357	0.0678	0.4243	0.8814	1	0.1776	0.0289	0.1819
Estriol	0.0475	0.0685	0.0635	-0.0505	0.1898	0.0538	-0.1422	0.0964	0.0102	0.1873	0.0845	0.1776	1	0.1752	0.1386
P300 Latency	0.3349	0.1786	0.2161	0.0286	0.2327	-0.1793	-0.0048	-0.0093	0.067	0.1572	-0.0129	0.0289	0.1752	1	-0.0019
P300 Amplitude	-0.0062	-0.0687	-0.0605	0.0314	0.0773	0.1971	0.0313	-0.0129	0.0027	-0.015	0.1637	0.1819	0.1386	-0.0019	1

doi:10.1371/journal.pone.0105048.t017

Table 18. Principal Component Analysis in the Raw Scale Eigenvalues.

Number	Eigenvalue	Percent	Cumulative Percent	Chi2	DF	P-value
1	2.592	17.28	17.28	3675.13	104.201	<.0001*
2	2.1618	14.412	31.692	3088.95	94.006	<.0001*
3	1.7409	11.606	43.298	2585.9	83.647	<.0001*
4	1.345	8.967	52.265	2203.61	73.1	<.0001*
5	1.1998	7.999	60.264	1968.51	62.613	<.0001*
6	1.0451	6.967	67.231	1754.61	52.88	<.0001*
7	1.0085	6.724	73.955	1575.81	43.82	<.0001*
8	0.8587	5.725	79.679	1353.07	35.394	<.0001*
9	0.7376	4.918	84.597	1164.67	27.732	<.0001*
10	0.5945	3.963	88.56	998.46	20.907	<.0001*
11	0.5553	3.702	92.262	886.619	14.907	<.0001*
12	0.5431	3.621	95.883	736.788	9.894	<.0001*
13	0.3825	2.55	98.433	420.531	5.908	<.0001*
14	0.1394	0.93	99.363	27.714	2.852	<.0001*
15	0.0956	0.637	100	-	.	-

doi:10.1371/journal.pone.0105048.t018

Table 19. Principal Component Analysis in the Raw Scale Variable Clustering Summary.

Cluster	Number of Members	Most Representative Variable	Proportion of Variation Explained
1	5	LH	0.453
2	5	Estrone	0.443
3	5	Vitamin D 25OH	0.342

doi:10.1371/journal.pone.0105048.t019

Table 20. Principal Component Analysis in the Raw Scale Cluster Members.

Cluster	Members	R2 with Own Cluster	R2 with Next Closest	1-R2 Ratio
1	LH	0.779	0.021	0.226
	FSH	0.776	0.047	0.235
	Age	0.478	0	0.522
	Latency	0.223	0.015	0.789
	IGF-BP3	0.01	0.009	0.999
2	Estrone	0.856	0.019	0.147
	Estradiol	0.795	0.029	0.212
	Testosterone Total	0.42	0	0.58
	Testosterone Free	0.084	0	0.917
	Amplitude	0.063	0.004	0.941
3	Vitamin D 25OH	0.64	0	0.36
	Vitamin D 1,25	0.53	0.014	0.477
	Leptin	0.44	0.007	0.564
	TSH	0.051	0.002	0.951
	Estriol	0.05	0.032	0.982

doi:10.1371/journal.pone.0105048.t020

Table 21. Principal Component Analysis in the Raw Scale Standardized Components.

Variable	Cluster 1	Cluster 2	Cluster 3
	Coefficients	Coefficients	Coefficients
Age	0.4593895	0	0
FSH	0.5852787	0	0
LH	0.5863316	0	0
TSH	0	0	-0.172013
Leptin	0	0	-0.506993
IGF-BP3	-0.065645	0	0
Vitamin D 25OH	0	0	0.6119382
Vitamin D 1,25	0	0	0.5566771
Testosterone Free	0	0.194114	0
Testosterone Total	0	0.4353115	0
Estradiol	0	0.598654	0
Estrone	0	0.6213278	0
Estriol	0	0	-0.170329
Latency	0.3135539	0	0
Amplitude	0	0.1684897	0

doi:10.1371/journal.pone.0105048.t021

tion; and, P300 amplitude levels were $4.8 \pm 0.4 \mu\text{v}$ ($n = 194$) with strong right skewed distribution.

Statistical Analysis

In order to examine the relationships among the variables, each and every pair of response and explanatory variables were examined using contingency analysis, bivariate scatter plots, simple linear regressions, as well as one-way analysis of variance (ANOVA) or Welch's t-test for classification variables. When the test of multiple means turned out to be statistically significant at 5% level, all pairs of means were compared using Tukey-Kramer's honestly significant difference as a post-hoc method.

Pearson product-moment correlations were calculated between hormone levels and the 12 mean domain scores, the total number of endorsed systems, and the grand mean across all 64 questions of the Menopause Questionnaire. A one-way ANOVA was performed for the origin of menopause variable for each of the 12 symptom domains, with a Bonferroni correction of $p < .004$ required for significance. Given the large number of domains and likely high inter-correlations between them, a factor analysis with principal components extraction and varimax rotation was performed on the 12 mean domain scores. Factor scores were generated for each patient and entered into the correlation analysis with the hormone levels. A similar factor analysis was performed on the hormone levels to reduce redundancy of highly inter-correlated values. Regression analyses were performed to predict the symptom domain score factors from the 15 hormone levels and again using the hormone level factors.

EEG, P300, and Evoked Potentials Data

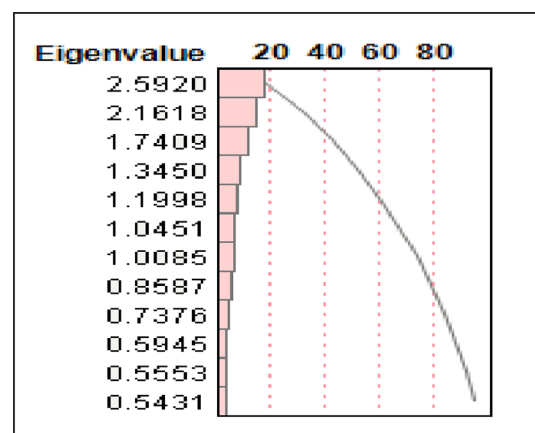
Lexicor and Cognitrace were used to determine P300 potential. A total of twenty electrodes were utilized: 3 along the central sulcus, 3 in the parietal region, 2 in the parietal/temporal region, 3 in the occipital region, 2 in the frontal/temporal region, as well as 5 in the frontal region. Calibration of the two machines was completed by repeat scans. Auditory stimuli of high and low beeps are utilized by Lexicor and Cognitrace with an output of latency

and amplitude, dependent on age-based preprogrammed base-lines. Listed in the patients' charts and determined through computer algorithm, latency (in milliseconds) and amplitude (in microvolts) from the waveform were selected for analysis.

Results

Preliminary Analyses

The detailed results of these preliminary analyses are not shown here due to the length, but they were used in constructing more elaborate multiple regression models and interpreting the results in the Corrected Analysis Section. In particular, the following tables summarize the statistical association of certain variables of interest.

**Figure 3.** Principal Component Analysis in the Raw Scale Eigenvalue Plot.

doi:10.1371/journal.pone.0105048.g003

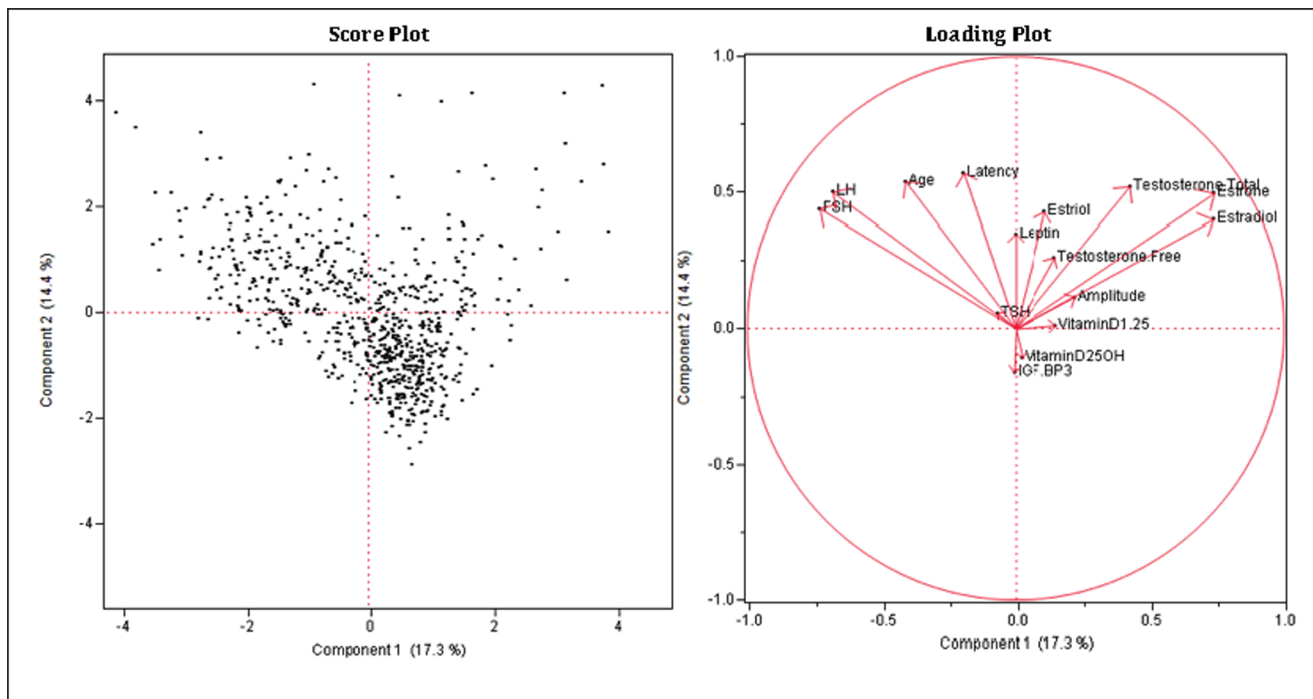


Figure 4. Principal Component Analysis in the Raw Scale Score and Loading Plot.
doi:10.1371/journal.pone.0105048.g004

Association between Dichotomized FSH and P300 Speed/Voltage

Table 2 outlines the association between FSH and P300 speed/voltage. Latency was correlated with FSH ($p = .02$), but the fit was very poor ($R^2 = .02$). P300 speed (dichotomized latency) is shown to be uncorrelated with FSH ($p = .10$). Amplitude or P300 voltage was also uncorrelated with FSH ($p = .19$).

Association between Categorized FSH and P300 Speed/Voltage

Table 3 outlines the association between categorized FSH range and P300 latency/amplitude (i.e., speed/voltage). P300 speed was uncorrelated with FSH over 40 mIU/mL (or under 40 mIU/mL) ($p = .27$). Amplitude or P300 voltage also was uncorrelated with FSH over 40 mIU/mL (or under 40 mIU/mL) ($p = .18$). P300 speed was uncorrelated with FSH over 80 mIU/mL (or under 80 mIU/mL) ($p = .65$). P300 voltage also was uncorrelated with FSH over 80 mIU/mL (or under 80 mIU/mL) ($p = .20$). P300 speed was uncorrelated with FSH over 100 mIU/mL (or under 100 mIU/mL) ($p = .13$). P300 voltage also was uncorrelated with FSH over 100 mIU/mL (or under 100 mIU/mL) ($p = .32$).

Association between FSH and P300 Speed/Voltage for FSH under/over 80

Tables 4 and 5 outline the associations between FSH range and P300 speed/voltage for FSH under/over 80 mIU/mL. Again, P300 speed was uncorrelated with FSH for the group with FSH under 80 mIU/mL ($p = .12$). P300 Voltage also was uncorrelated with FSH for the group with FSH under 80 mIU/mL ($p = .21$). Similarly, P300 speed was uncorrelated with FSH for the group with FSH over 80 mIU/mL ($p = .36$). Amplitude also was uncorrelated with FSH for the group with FSH over 80 mIU/mL ($p = .10$).

Association between FSH and P300 Speed/Voltage for FSH under/over 100

Tables 6 and 7 outline the associations between FSH range and P300 speed/voltage for FSH under/over 100 mIU/mL. Again, P300 speed was uncorrelated with FSH for the group with FSH under 100 mIU/mL ($p = .29$). P300 voltage also was uncorrelated with FSH for the group with FSH under 100 mIU/mL ($p = .13$). Similarly, P300 speed was uncorrelated with FSH for the group with FSH over 100 mIU/mL ($p = .63$). Amplitude also was uncorrelated with FSH for the group with FSH over 100 mIU/mL ($p = .51$).

Association between FSH over 40 and Estradiol, Estrone, and Estriol

Table 8 outlines the associations between FSH over 40 mIU/mL and estradiol, estrone and estriol. There was a strong association between estradiol and FSH over 40 mIU/mL (or under 40 mIU/mL) ($p < .0001$). More explicitly, estradiol level was significantly higher for the group with FSH under 40 mIU/mL (126.0 ± 8.6 pg/mL) than for the group with FSH over 40 mIU/mL (27.1 ± 13.5 pg/mL) ($p < .0001$). There was a higher percentage of patients with the pre-menopausal level of estradiol in the group with FSH under 40 mIU/mL than in the group with FSH over 40 mIU/mL ($p < .0001$).

Similarly, there was a strong association between estrone and FSH over 40 mIU/mL (or under 40 mIU/mL) ($p < .0001$). More explicitly, estrone level was significantly higher for the group with FSH under 40 mIU/mL (82.9 ± 5.0 pg/mL) than for the group with FSH over 40 mIU/mL (41.1 ± 7.5 pg/mL) ($p < .0001$). There was a higher percentage of patients with the pre-menopausal level of estrone in the group with FSH under 40 mIU/mL than in the group with FSH over 40 mIU/mL ($p < .0001$).

There was a weak association between estriol and FSH over 40 mIU/mL (or under 40 mIU/mL) ($p = .03$). Estriol level

Table 22. Principal Component Analysis in the Log Scale Correlations Estimated by Pairwise Method.

	Age	log FSH	log LH	log TSH	log Leptin	IGF-BP3	log Vitamin D 25OH	log Vitamin D 1,25	log Testosterone Free	log Testosterone Total	log Estradiol	log Estrone	log Estril	P300 Latency	log P300 Amplitude
Age	1	0.4881	0.409	-0.0145	0.0703	-0.1876	0.0427	0.0276	0.0577	0.0153	-0.2272	-0.1179	0.0348	0.3349	-0.0551
log FSH	0.4881	1	0.9034	-0.0002	0.0654	0.0219	-0.0343	-0.0615	-0.0069	-0.0804	-0.5312	-0.3933	0.0385	0.1488	-0.1109
log LH	0.409	0.9034	1	0.0128	0.1233	0.0134	-0.0507	-0.1047	0.0008	-0.0281	-0.2988	-0.2568	0.0559	0.1629	-0.086
log TSH	-0.0145	-0.0002	0.0128	1	0.0563	-0.1445	-0.0747	-0.0622	-0.0087	-0.1286	-0.0267	-0.0712	-0.038	-0.0535	0.0254
log Leptin	0.0703	0.0654	0.1233	0.0563	1	0.0368	-0.372	-0.2557	0.0666	0.1137	-0.0967	0.1198	0.2249	0.2878	-0.124
IGF-BP3	-0.1876	0.0219	0.0134	-0.1445	0.0368	1	0.0179	0.188	0.1104	0.0185	-0.1823	-0.0768	0.0919	-0.1793	0.256
log Vitamin D 25OH	0.0427	-0.0343	-0.0507	-0.0747	-0.372	0.0179	1	0.445	-0.0519	-0.0004	0.0534	-0.0968	-0.1206	0.0314	0.0362
log Vitamin D 1,25	0.0276	-0.0615	-0.1047	-0.0622	-0.2557	0.188	0.445	1	0.037	0.0988	0.0252	0.0454	0.1083	0.0168	0.0939
log Testosterone Free	0.0577	-0.0069	0.0008	-0.0087	0.0666	0.1104	-0.0519	0.037	1	0.4632	0.1064	0.1742	0.1082	0.0966	0.0816
log Testosterone Total	0.0153	-0.0804	-0.0281	-0.1286	0.1137	0.0185	-0.0004	0.4632	0.4632	1	0.2736	0.3963	0.2832	0.1846	0.0303
log Estradiol	-0.2272	-0.5312	-0.2988	-0.0267	-0.0967	-0.1823	0.0534	0.0252	0.1064	0.2736	1	0.6419	0.0133	-0.0557	0.0696
log Estrone	-0.1179	-0.3933	-0.2568	-0.0712	0.1198	-0.0768	-0.0968	0.0454	0.1742	0.3963	0.6419	1	0.3282	0.0728	0.0222
log Estril	0.0348	0.0385	0.0559	-0.038	0.2249	0.0919	-0.1206	0.1083	0.1082	0.2832	0.0133	0.3282	1	0.1453	0.1198
P300 Latency	0.3349	0.1488	0.1629	-0.0535	0.2878	-0.1793	0.0314	0.0168	0.0966	0.1846	-0.0557	0.0728	0.1453	1	-0.1278
log P300 Amplitude	-0.0551	-0.1109	-0.086	0.0254	-0.124	0.256	0.0362	0.0939	0.0816	0.0303	0.0696	0.0222	0.1198	-0.1278	1

doi:10.1371/journal.pone.0105048.t022

Table 23. Principal Component Analysis in the Log Scale Eigenvalues.

Number	Eigenvalue	Percent	Cumulative Percent	Chi2	DF	P-value
1	2.9257	19.505	19.505	4057.56	104.433	<.0001*
2	2.182	14.547	34.051	3265.1	94.786	<.0001*
3	1.743	11.62	45.672	2709.8	84.338	<.0001*
4	1.4633	9.755	55.427	2284.04	73.744	<.0001*
5	1.0488	6.992	62.419	1925.5	63.375	<.0001*
6	1.0103	6.736	69.154	1760.28	53.245	<.0001*
7	0.9309	6.206	75.36	1565.27	43.927	<.0001*
8	0.8221	5.481	80.841	1358.58	35.545	<.0001*
9	0.654	4.36	85.201	1160.88	27.874	<.0001*
10	0.5703	3.802	89.003	1035.24	21.008	<.0001*
11	0.464	3.093	92.097	924.225	14.962	<.0001*
12	0.4505	3.003	95.1	854.797	9.884	<.0001*
13	0.42	2.8	97.9	734.788	5.813	<.0001*
14	0.2629	1.753	99.653	464.47	2.814	<.0001*
15	0.0521	0.347	100	0	0.03	0.3172

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appeared to be slightly higher for the group with FSH over 40 mIU/mL (0.20 ± 0.01 ng/mL) than for the group with FSH under 40 mIU/mL (0.18 ± 0.01 ng/mL). After dichotomization, however, menopausal level of estriol was found to be uncorrelated with FSH over 40 mIU/mL (or under 40 mIU/mL) ($p = .12$).

Tables 9 and 10 outline the association between FSH and estradiol, estrone, and estriol for FSH under and over 40 mIU/mL. Estriol was uncorrelated with FSH for the group with FSH under 40 mIU/mL ($p = .61$). Estriol was uncorrelated with FSH for the group with FSH over 40 mIU/mL either ($p = .92$). Estradiol was strongly associated with FSH for the group with FSH over 40 mIU/mL ($p < .0001$). Estrone also was associated with FSH for the group with FSH over 40 mIU/mL ($p < .01$).

Association between Leptin and P300 Speed/Voltage

Table 11 outlines the association between leptin and P300 speed/voltage. High leptin levels were determined as >5 ng/mL and high P300 latencies were determined by a patient's age + 300 ms (i.e., a patient age 50 should have a P300 latency of 350, so any value greater than 350, shows a delay in latency). P300 speed appeared to be correlated with leptin ($p = .01$), with higher leptin level associated with abnormal P300 speed (OR = 0.97). However, once dichotomized, this association was insignificant ($p = .10$). Amplitude or P300 voltage was uncorrelated with leptin ($p > .30$). Furthermore, higher leptin levels are indicative of obesity and its risks and delayed latency shows slowed brain speed and attention.

Table 24. Principal Component Analysis in the Log Scale Cluster Members.

Cluster	Members	R2 with Own Cluster	R2 with Next Closest	1-R2 Ratio
1	log FSH	0.924	0.028	0.079
	log LH	0.775	0.032	0.233
	Age	0.419	0.064	0.62
	log Estradiol	0.379	0.153	0.734
	log Testosterone Total	0.673	0.035	0.339
2	log Testosterone Free	0.381	0.015	0.629
	log Estrone	0.484	0.186	0.634
	log Estriol	0.34	0.053	0.697
	log TSH	0.04	0.006	0.967
	log Vitamin D 1,25	0.723	0.032	0.287
3	log Vitamin D 25OH	0.723	0.045	0.291
	Latency	0.644	0.046	0.373
	log Leptin	0.644	0.136	0.412
	IGF-BP3	0.628	0.015	0.378
	Estriol	0.05	0.032	0.982

doi:10.1371/journal.pone.0105048.t024

Table 25. Principal Component Analysis in the Log Scale Standardized Components.

Variable	Cluster 1 Coefficients	Cluster 2 Coefficients	Cluster 3 Coefficients
Age	0.4098452	0	0
log FSH	0.6082957	0	0
log LH	0.5570797	0	0
log TSH	0	-0.14372	0
log Leptin	0	0	0
IGF-BP3	0	0	0
log Vitamin D 25OH	0	0	0.7071068
log Vitamin D 1,25	0	0	0.7071068
log Testosterone Free	0	0.4456279	0
log Testosterone Total	0	0.5923475	0
log Estradiol	-0.389443	0	0
log Estrone	0	0.502436	0
log Estriol	0	0.4212397	0
Latency	0	0	0

doi:10.1371/journal.pone.0105048.t025

Regression Analysis

In order to assess the pattern of each hormone level on P300 speed/voltage accounting for any (additive) effect of covariate measured in this study such as age, multiple regression models were constructed with P300 speed/voltage as response variables. Final models were then built by reducing the full models through a stepwise regression method. Only the main effects were considered in the model, and dependency among the response variables was not considered for an individual examination of each response variable. The following tables and figures summarize the results of the reduced model fit, ANOVA, and effect estimates, along with prediction profiler of main effects in the reduced model.

Latency predicted by Leptin with Age (ANOVA) and P300 Speed predicted by Leptin with Age (Logistic)

Once age adjusted, leptin was found to be the only statistically significant predictor for latency or P300 amplitude ($p = .03$), with the effect size of 0.23. Hence, the higher the leptin level, the longer the latency. After age adjustment, higher leptin level was more

associated with abnormal P300 latency (OR = 0.98). Age alone also was a statistically significant factor for latency or P300 speed ($p < .01$): the older the patient, the longer was the latency. However, even after age adjustment, amplitude and P300 voltage were found to be uncorrelated with leptin ($p > .25$). Also, once age adjusted, none of FSH, LH, or TSH were found to be statistically significant predictors of the P300 measures ($p > .25$) (see Tables 12–16 and Figure 2).

Principal Component Analysis

The principal component analysis (factor analysis) was performed to derive a small number of independent linear combinations (viz., principal components) of a set of variables that capture as much of the variability in the original variables as possible. Reducing the dimensionalities of this multivariate data, the most important components were used to visualize the structure of the data. Each principal component was calculated by taking a linear combination of an eigenvector of the correlation matrix with a variable. The corresponding eigenvalues were the variance of each component.

Principal Component Analysis in the Raw Scale

The proportion of variation explained by clustering was 0.413. The first two principal components accounted for 31.7% of the variability while the first three components accounted for 43.3% of the variability. Based on these components, clustering of the variables was done producing three clusters, each with five variables. There was no distinct or definite association found among the variables (at least the ones being examined). One of the causes was the incompleteness of the data, with many missing values, along with the retrospective aspect of the study. Although some variables tended to have a sizable R2, those strongly right skewed variables from the raw data in a continuous scale were log-transformed to re-perform the principal component analysis to determine possible additional results (see Tables 17–21; Figures 3, 4).

Principal Component Analysis in the Log Scale

The first two principal components accounted for 34.1% of the variability, while the first three components accounted for 45.7%

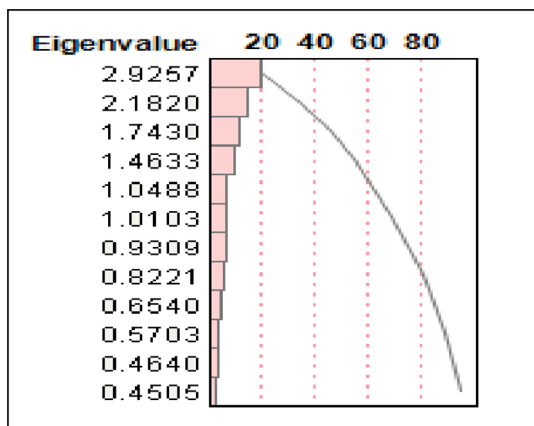


Figure 5. Principal Component Analysis in the Log Scale Eigenvalue Plot.

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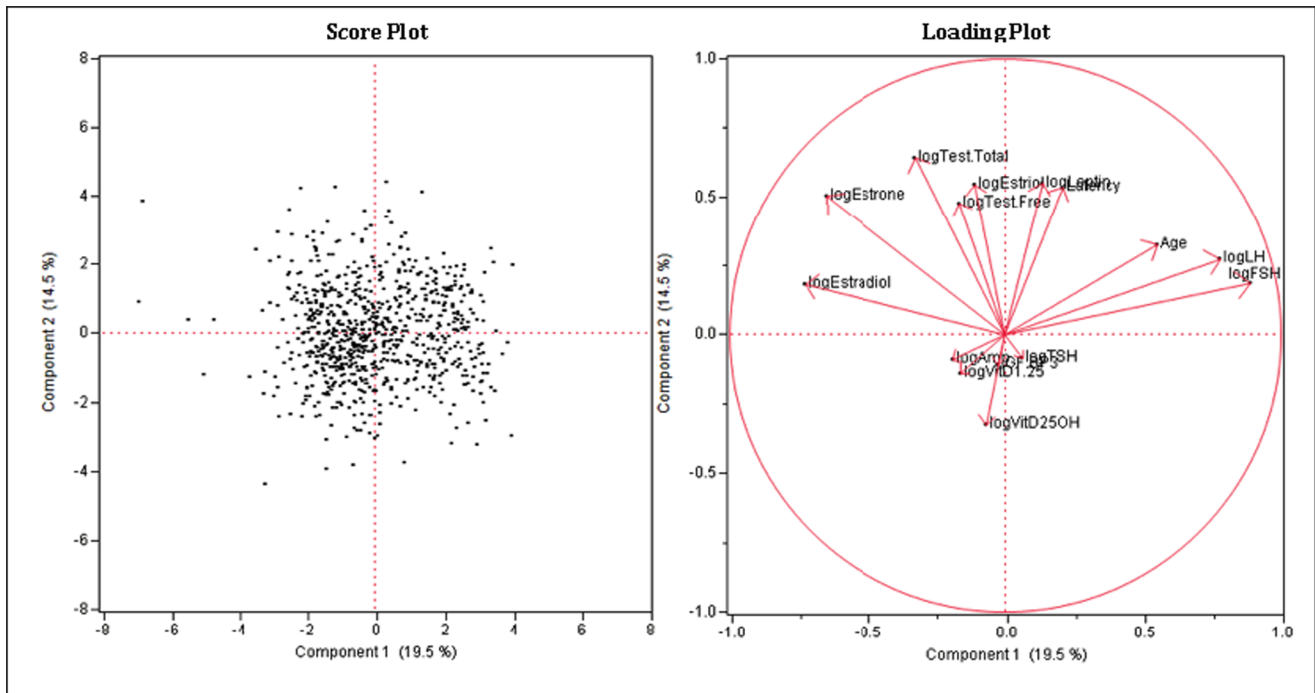


Figure 6. Principal Component Analysis in the Log Scale Score and Loading Plot.
doi:10.1371/journal.pone.0105048.g006

of the variability, slightly better than the previous result in the raw scale. Based on these components, clustering of the variables was done producing five clusters, each with a varying number of variables. With an increased number of clusters, each cluster had even fewer associated variables. This confirmed the findings of no clear associations among the variables (among those being examined) and the results of regression analyses. Even in the log scale, some variables were still clustered together. FSH and LH were found to be associated while Vitamin D 1,25 and Vitamin D 25 OH tended to be together. Also, P300 latency and log leptin were found to be associated in the same cluster (see Tables 22–25; Figures 5, 6).

K-Means Clustering Analysis

As an alternative clustering analysis, the K-means clustering analysis with $K=2$ was performed in the log scale of selected variables, which were strongly right skewed. Initially, the hierarchical clustering was performed to produce a tree-like classification structure, but no distinctive branches could be located to cluster the data in an effective way. In the K-means clustering, many different numbers of clusters were tried, but the two-means seemed to produce the most reasonable outcome, given the incompleteness of the dataset. The variables were scaled individually, and the within-cluster standard deviations were used producing cubic clustering criterion = -4.9793 . Each cluster

contained 31 and 45 points, respectively. The most representative variable separating these two clusters was log FSH, followed by log LH. The data resolution was found to be weak over other variables; see the scatterplot matrix below. With aforementioned reasons, nevertheless, the clustering was not highly successful with this dataset as the points were scattered without distinctive associations. Although the data were forced to cluster into two here, the parallel coordinate plots showed that the variable patterns were quite similar between the two groups, comparable to the overall cluster means, which resulted in low resolution of clustering (see Tables 26–30; Figures 7–10).

Discussion

Although our data did not support a significant association between sex hormones and the electrophysiological parameters examined, most importantly, we did find a significant association between plasma increased leptin levels and P300 latency. Leptin was found to be the only statistically significant predictor for latency or P300 speed. Thus, the higher the leptin level was, the longer was the latency. After age adjustment, higher leptin level was associated more with abnormal P300 speed. While we found that age alone was a statistically significant factor for latency or P300 speed, we inferred that the older the patient was, the longer was the latency. However, even after age adjustment, amplitude

Table 26. K-Means Clustering Analysis Cluster Summary.

Cluster	Count
1	31
2	45

doi:10.1371/journal.pone.0105048.t026

Table 27. K-Means Clustering Analysis Cluster Averages by Variable (Age+Specified Hormones).

Cluster	Age	log FSH	log LH	log TSH	log Leptin	IGF-BP3	log Vitamin D 25OH	log Vitamin D 1,25
1	50.4193548	3.84692834	3.33115457	0.07086968	2.62396293	4.40645161	3.37464635	3.83294885
2	44.4444444	1.60898738	1.40340109	0.37980236	2.14941452	4.52888889	3.55641941	4.05081038

doi:10.1371/journal.pone.0105048.t027

Table 28. K-Means Clustering Analysis Cluster Averages by Variable (Specified Hormones+P300).

Cluster	log Testosterone Free	log Testosterone Total	log Estradiol	log Estrone	log Estriol	P300 Latency	log P300 Amplitude
1	-0.432203	2.6949935	3.08398269	3.52383408	-2.1642274	318.480645	1.28841661
2	-0.4176911	2.84516834	4.33986953	3.86399908	-2.3025851	309.948889	1.37825673

doi:10.1371/journal.pone.0105048.t028

Table 29. K-Means Clustering Analysis Cluster Standard Deviations by Variable (Age+Specified Hormones).

Cluster	Age	log FSH	log LH	log TSH	log Leptin	IGF-BP3	log Vitamin D 25OH	log Vitamin D 1,25
1	5.13541903	0.81303646	0.67265226	1.2872815	1.09259949	1.06587401	0.50428182	0.45624656
2	3.49320328	0.53294581	1.08362395	0.63573686	1.20339793	0.68268171	0.53741888	0.43474834

doi:10.1371/journal.pone.0105048.t029

Table 30. K-Means Clustering Analysis Cluster Standard Deviations by Variable (Specified Hormones+P300).

Cluster	log Testosterone Free	log Testosterone Total	log Estradiol	log Estrone	log Estriol	P300 Latency	log P300 Amplitude
1	0.21883302	0.87241656	1.24017776	0.6873002	0.47147219	31.8035443	0.64528174
2	0.28383649	0.85136274	0.87015612	0.56004314	1.33E-15	21.0254938	0.38876941

doi:10.1371/journal.pone.0105048.t030

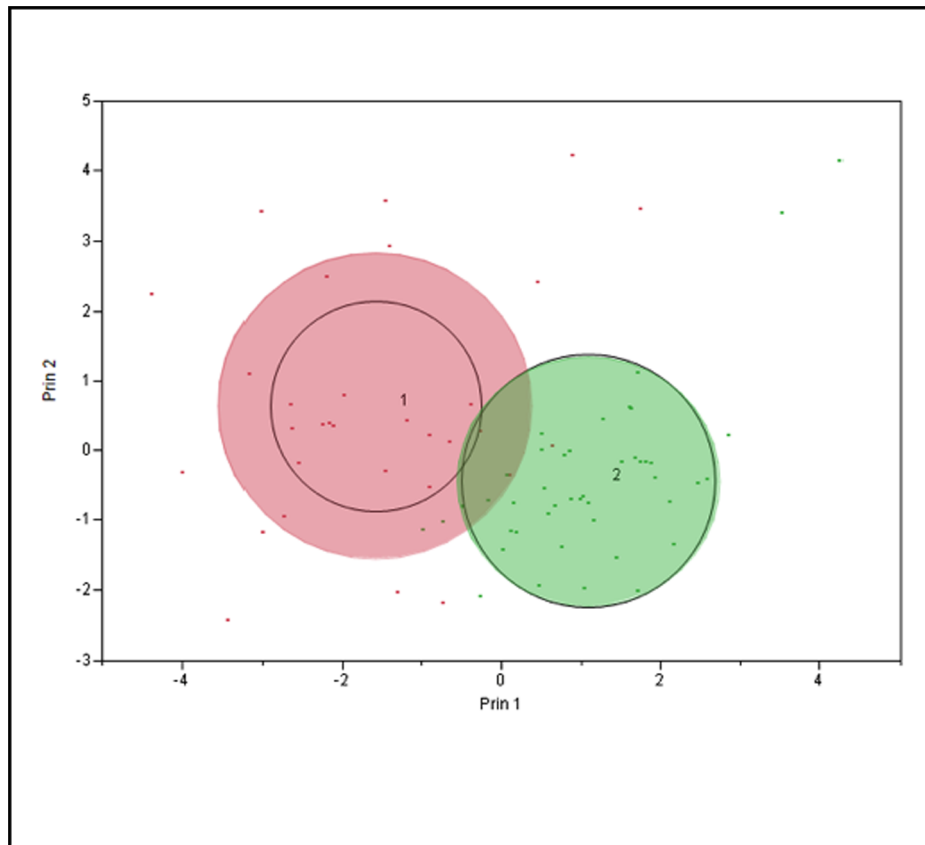


Figure 7. K-Means Clustering Analysis Cluster Bi-Plot.
doi:10.1371/journal.pone.0105048.g007

and P300 voltage were found to be uncorrelated with leptin. Also, once age was adjusted, none of the FSH, LH, and TSH levels were found to be statistically significant predictors for P300 speed and voltage despite some research suggesting otherwise. In particular, Davis *et al.* [19] found that triiodothyronine (T_3) levels were much lower in advanced AD Braak stages V–VI. Short *et al.* [15] also found significantly increased levels of LH and GRH in estrogen-free AD patients. In spite of our results, there is a historical positive correlation between the fluctuation in specific sex hormones (e.g. T_3 , LH, GRH, etc.) and advanced stages of AD, which should be further investigated in future studies elucidating such effects.

There is also significant evidence showing that P300 evoked potentials and neurophysiological tests validate PET measures of brain metabolism in cognitively impaired patients [73]. P300 has important clinical utility in that its measurements are representative of cognitive decline, especially those patients who are already suffering from progressive cognitive impairments or dementia. Reduced P300 amplitudes, delayed latencies, and neuropsychological deficits show positive correlations with PET brain hypometabolism; thus, the P300 serves as a cost-effective indicator that is essential in primary care settings and may help determine early abnormalities in cognition.

These findings may be useful in determining the role of leptin in future studies focusing on obesity and cognition. Certainly there is enough evidence to link leptin plasma levels as a function of cellular resistance and obesity [44], especially in menopausal women [45]. While this is fairly clear, less is known concerning the role of leptin and cognition.

There have been studies suggesting that obesity and excessive weight in middle aged adults is a risk factor for cognitive decline later in life [46–48]; congruently, observational studies of Alzheimer's Disease (AD) patients provides potential links between adipose tissue metabolism and cognition, weight loss occurring some years prior to the onset of clinical AD symptoms [49,50]. The adipokine leptin, a cytokine secreted by adipose tissue, serves as an appetite suppressor and helps to regulate the body's energy expenditure—increased plasma levels of leptin associated with excessive body fat [51].

Mouse models have linked disrupted leptin receptors as potential sources for long-term potentiation impairments, synaptic plasticity issues, and deficiencies in spatial learning [52]. Furthermore, hippocampal viral vector introduction of leptin in rodents induced neuronal precursor proliferation and reduced neurodegeneration; in addition, it suppressed memory consolidation and reduced the spatial awareness for food of administered rodents [53,59]. The creation and removal of beta amyloid ($A\beta$) in rodents occurs through leptin modulation [54,55], $A\beta$ hippocampal removal was related to advanced object recognition [46]. In addition, following ischemic challenge, neuronal survival in the hippocampus for Gerbils treated with leptin was greater than for gerbils without leptin treatment [56]. Senescence-accelerated prone mice (SAMP8) with impaired learning and memory showed improved retention after hippocampal leptin injections [57]. Through such rodent modeling, much has been learnt about leptin and cognition.

In humans, there remains controversy regarding the role of leptin in cognition. Lieb *et al.* indicated that the presence of

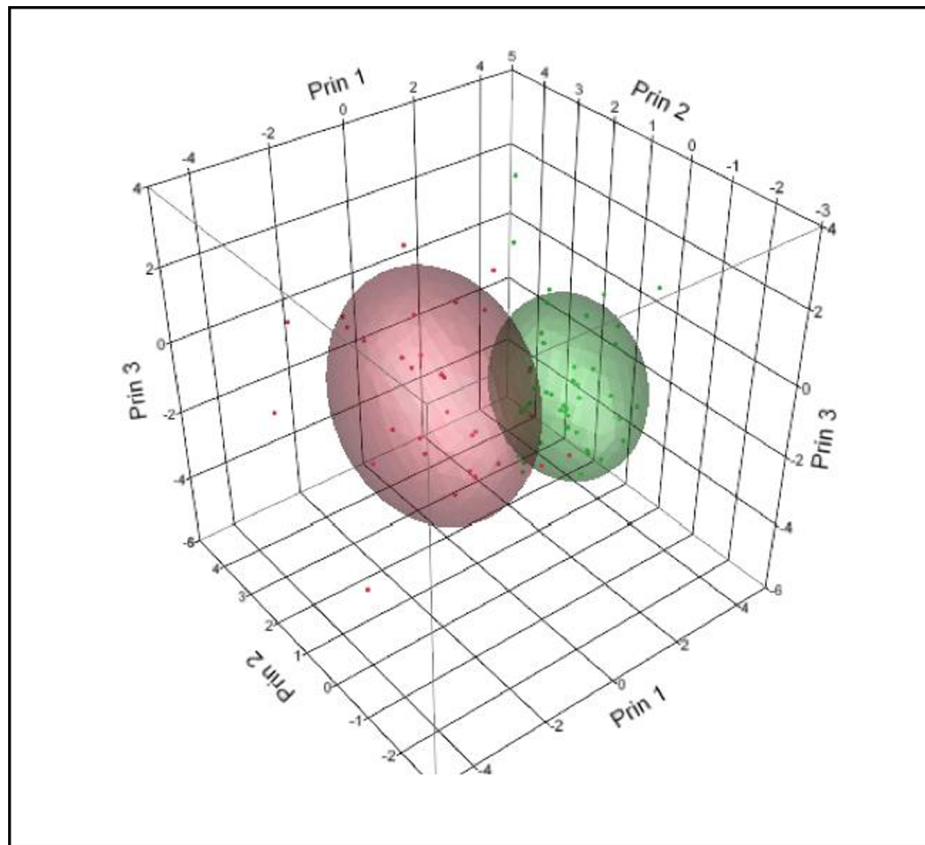


Figure 8. K-Means Clustering Analysis Cluster Bi-Plot in 3D.
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increased hippocampal and whole brain volume were associated with higher plasma levels of leptin, suggesting that these higher plasma leptin levels associated with reduced incidence of AD [60]. Controversially, dementia and AD development in older adults has been linked to low plasma leptin levels [61]. Some studies have proved inconclusive regarding a difference in leptin between AD patients and controls, unless an ApoE4 allele was present [62].

Clinical attention to leptin has indicated that there is a statistical trend for increased cognitive scores to be associated with lower plasma leptin levels [58]. Interestingly, when stratified by race and gender, excessive leptin in white men was associated with higher

cognitive scores, as assessed by the Montreal Cognitive Assessment, while the opposite trend persisted amongst black men [58]. AD patients with excessive low weight and decreased leptin plasma levels, implicates a hypothalamic level malfunction [63]. Using the Modified Mini Mental State Exam, there is shown to be a protective quality of cellular leptin combating cognitive decline [64,65].

Finally, the increased gender difference regarding the incidence of AD in women can potentially be linked to the increased relative loss of adipokines in women than in men. Further gender differences in leptin occur regarding C-reactive protein (CRP)

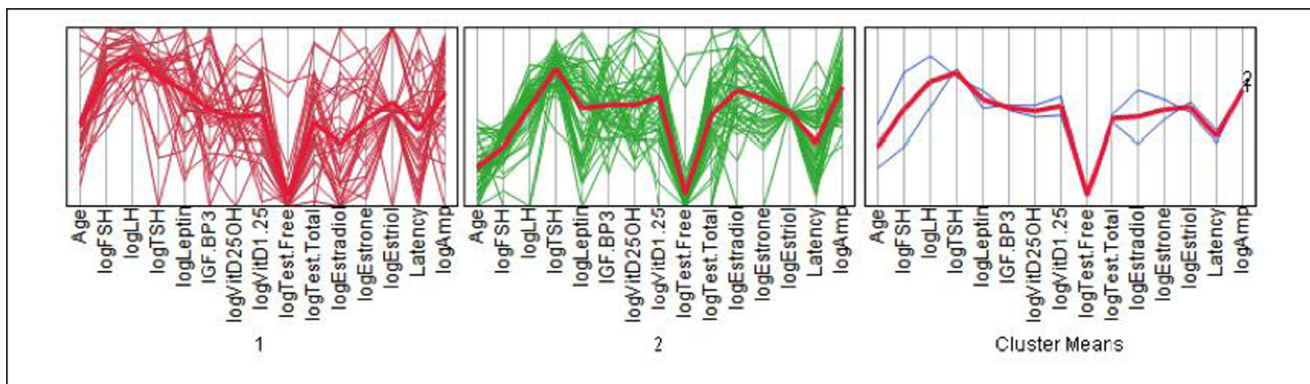


Figure 9. K-Means Clustering Analysis Parallel Coordinate Plot.
doi:10.1371/journal.pone.0105048.g009

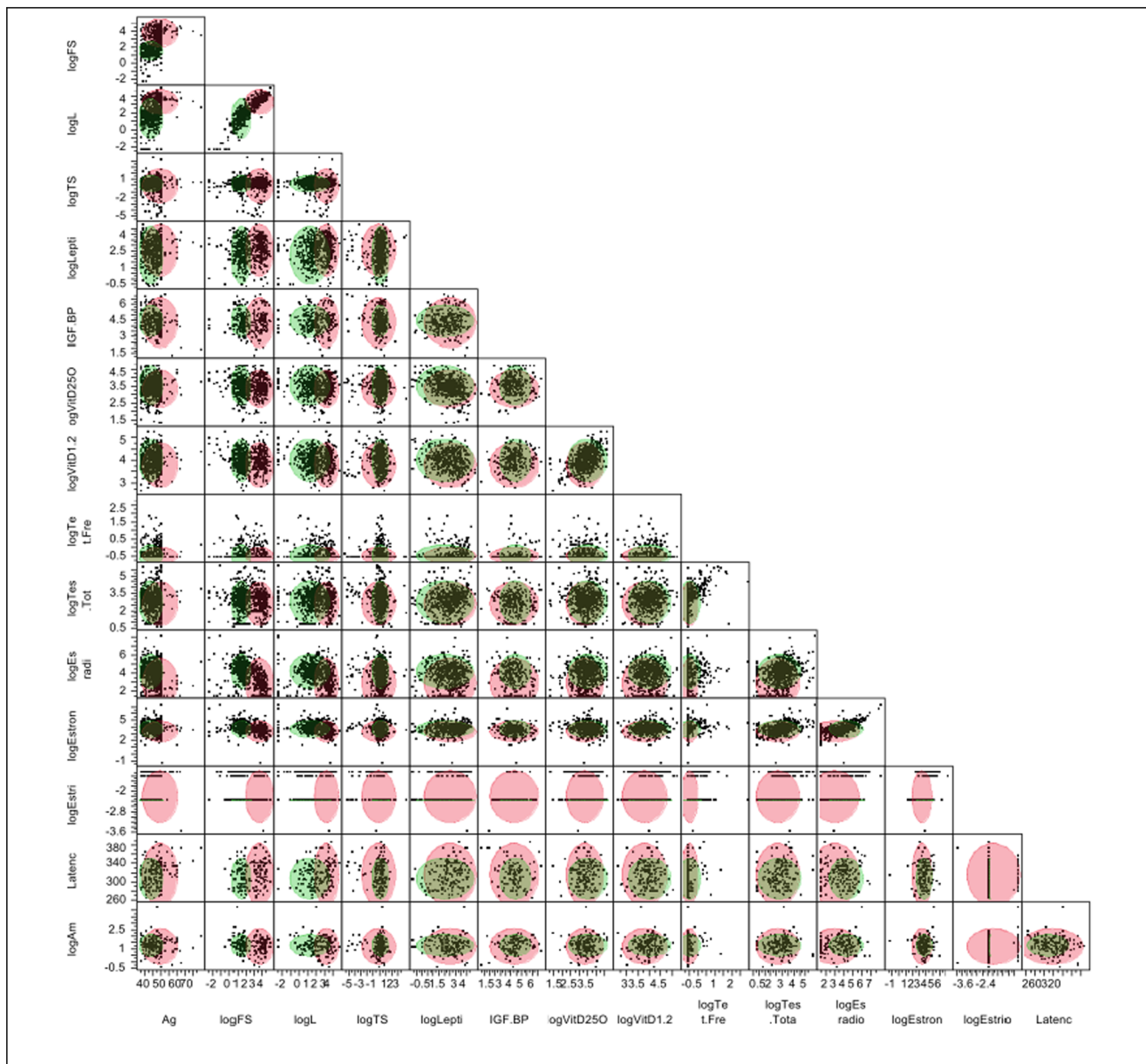


Figure 10. K-Means Clustering Analysis Scatter Plot Matrix.
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levels, highly correlated to leptin levels in women but not in men, which potentially links increased CRP levels to the increased relative adipose load for women [66]. Increased abdominal fat in Japanese men has been associated with mild cognitive impairment, the association inconclusive for Japanese women [67].

We are cognizant of the extensive literature related to the effects of a number of hormones that we studied in this article and their respective roles of influencing leptin function: LH [68,69]; FSH [70–72]; estradiol [61,63,72].

Limitations

Although we started with a cohort of 796, after sub analysis, the lowest N was 194 due to missing values. Therefore, we caution any definitive interpretation of these data. Of the remaining 194 subjects, only 159 subjects had leptin values available for statistical

analysis against P300 values. While we are encouraging more research in this area of important investigation, we suspect that leptin resistance may have a negative influence on global cognition in women based on the limited cohort of 159 women that was investigated.

We are cognizant that P300 latency may change as a function of pharmaceutical treatments, anxiety, and other physiological and behavioral factors. Thus, these factors must be considered seriously, and we propose caution in interpreting these data. Subsequently, in future studies we will also include Mini-Mental State Examination status, Wechsler Memory Scale tests, and other neurocognitive measures [73] along with electrophysiological measurements.

In pre- and post-menopausal women, we attempted to determine the relationship between plasma leptin levels and brain functionality as measured by P300. However, a PubMed literature

search on 10-27-2013 revealed no articles on P300 measures and plasma leptin levels, in spite of many references associating leptin levels and genes with cognition [74–79].

Specific demographics and an investigation of correlated BMI levels with leptin should be further expanded in future studies. We were unable to include additional demographic data such as body weight, height, and BMI among other fields. We are aware that these additional parameters may add to this study and better evaluate the changes in body composition among menopausal women in addition to cognitive function. A specialized study of this nature would be a significant follow-up to our current observations.

Conclusions

We are proposing that since leptin has been considered to be a cognitive enhancer, whereby cellular leptin acts on hippocampal cells (as one example) and improves performance in object recognition; leptin resistance and subsequent higher plasma levels

should induce cognitive decline. As we expected, higher plasma leptin levels significantly and positively associated with a prolonged latency, which is a determinant for cognitive function.

Thus, our findings show significant association of higher plasma leptin levels, potentially due to leptin resistance, and prolonged P300 latency appears to be the first of such an association. Leptin resistance may delay electrophysiological processing of memory and attention.

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Author Contributions

Conceived and designed the experiments: EB MOB TK KB. Performed the experiments: DH KB KD ML CT DS FK. Analyzed the data: DH KB KD. Contributed reagents/materials/analysis tools: EB KB DH. Wrote the paper: KB KD FK ML.

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