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Leptin Serum Levels are Associated With GLP-1 Receptor Agonist-Mediated Effects on Glucose Metabolism in Clozapine- or Olanzapine-Treated, Prediabetic, Schizophrenia Patients

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Background: **We previously demonstrated that the glucagon-like peptide-1 receptor agonist (GLP-1RA) liraglutide significantly reduced glucometabolic disturbances and body weight vs placebo in prediabetic, overweight, or obese schizophrenia-spectrum disorder patients treated with clozapine or olanzapine. Here, we aimed to identify potential biomarkers of prediabetes and the GLP-1RA-induced effects on glucose tolerance in schizophrenia patients treated with clozapine or olanzapine.** ***Methods:*** **Multiplexed immunoassays were used to measure 8 proteins (adiponectin, C-reactive protein, interleukin-1 receptor antagonist, leptin, macrophage migration inhibitory factor, prolactin, receptor for advanced glycation end products, and vascular endothelial growth factor [VEGF]) in fasting prediabetic and non-prediabetic patients with schizophrenia-spectrum disorder, the prediabetic patients receiving 16-week randomized treatment with liraglutide or placebo.** ***Results:*** **Serum adiponectin ($P = .004$) and VEGF ($P = .019$) levels were significantly lower in prediabetic ($n = 81$) than non-prediabetic schizophrenia-spectrum disorder patients ($n = 32$). Adiponectin levels increased significantly ($P = .022$) and leptin levels decreased significantly ($P = .017$) following treatment with liraglutide ($n = 39$) vs placebo ($n = 42$). Importantly, patients receiving liraglutide who had higher baseline leptin levels showed significantly larger reductions in the primary endpoint, the 75-g oral glucose tolerance test value, than patients with lower baseline leptin levels ($P = .009$).** ***Conclusion:*** **These results provide new evidence for metabolic alterations associated with prediabetes and GLP-1RA treatment in the context of schizophrenia. They suggest that leptin may be a valuable biomarker predicting GLP-1RA-induced improvement in glucose tolerance in overweight or obese schizophrenia-spectrum disorder patients with prediabetes treated with clozapine or olanzapine. These findings require further validation in larger numbers of individuals.**

Key words: adiponectin/biomarker/GLP-1RA/liraglutide/OGTT/prediabetes

Introduction

The well-known reduced life expectancy of about 20 years for patients with schizophrenia[1] is mainly due to an increased prevalence of cardiovascular morbidity and mortality,[2] most likely caused by genetic, lifestyle and treatment factors, including the use of antipsychotic medication.[1,3,4] Antipsychotic medication plays a

central role in the acute and maintenance treatment of schizophrenia,[5–8] but is also linked to several side effects including overweight/obesity, metabolic abnormalities, type 2 diabetes (T2D), and ultimately cardiovascular disease.[9–11] Clozapine is predominantly prescribed to treat treatment-resistant patients with schizophrenia.[12–15] Both clozapine and olanzapine are efficacious antipsychotics but also the 2 drugs associated with the greatest body weight gain and metabolic disturbances.[6,16]

Glucagon-like peptide-1 (GLP-1) is an incretin hormone, which is secreted from the small intestine in response to intake of nutrients.[17] GLP-1 increases insulin secretion from the beta cells and decreases glucagon secretion from the alpha cells of the pancreas.[17] GLP-1 also exhibits an inhibitory effect on gastric emptying, appetite, and food consumption and can reduce body weight, and by all these actions improves glycemic control in T2D.[18] Liraglutide is a GLP-1 receptor agonist (GLP-1RA) with 97% homology to naturally occurring GLP-1[18] but with a longer half-life, making it suitable for a once-daily subcutaneous injection. Recently, we demonstrated that 16 weeks of treatment with liraglutide in overweight or obese schizophrenia-spectrum disorder patients with prediabetes and on stable antipsychotic treatment with olanzapine or clozapine improved glucose tolerance and metabolic disturbances, and reduced body weight on average by 5.3 kg compared to placebo.[19] A recent, patient-level meta-analysis confirmed the efficacy of GLP-1 agonists for antipsychotic-related weight gain,[20] but the exact mechanism for these favorable effects has not been elucidated in this psychiatric patient group.

An increasing body of evidence suggests that inflammation is involved in the development of T2D, and changes in peripheral inflammatory biomarkers have been observed in prediabetic and diabetic patients as well as in overweight and obese individuals.[21–24] In addition, a substantial number of proteins are involved in the complex regulation of satiety in the general population and in antipsychotic-treated patients,[25,26] which may or may not be altered towards an anorexigenic balance by GLP-1RA treatment. Thus, we decided to measure several relevant inflammatory and appetite/satiety-regulating molecules in serum samples from schizophrenia-spectrum disorder patients treated with clozapine or olanzapine at baseline and after 16 weeks of treatment with either liraglutide or placebo.[19] We aimed to identify potential biomarkers of prediabetes, as well as the GLP-1RA-induced effects on glucose tolerance and predictors of response to GLP-1RA, in schizophrenia patients treated with clozapine or olanzapine. Measured analytes were selected based on their previously reported association with schizophrenia or prediabetes.[27,28] We hypothesized that prediabetic vs non-prediabetic patients had significantly altered levels of serum markers of inflammation and/or pro-orexigenic state, and that in prediabetic individuals liraglutide would, at least partly, normalize the levels of inflammatory and/or pro-orexigenic serum markers significantly more than placebo. We further hypothesized that baseline levels of inflammatory and/or pro-orexigenic serum markers would be significantly associated with subsequent response to liraglutide treatment.

Methods

Study Population

This study population has been described in detail previously.[19] In short, patients with schizophrenia-spectrum disorder (schizophrenia, schizophreniform disorder, or psychotic disorder not otherwise specified), not previously diagnosed with diabetes, were examined from May 2013 to November 2015 as part of an investigator-initiated, randomized, placebo-controlled, double-blinded clinical trial (NCT01845259).[29] The Scientific-Ethical Committee of the Capital Region of Denmark and the Danish Health Authority approved the study protocols. Informed consent was given in writing by all participants, and clinical investigations were conducted according to the Declaration of Helsinki and Good Clinical Practice. Key inclusion criteria for the patients with schizophrenia-spectrum disorders as per the International Classification of Diseases, 10th edition (ICD-10) or the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) were: (1) aged 18–65 years, (2) stable treatment with clozapine or olanzapine for ≥6 months without dose change within 30 days prior to inclusion, (3) body mass index (BMI) ≥27 kg/m^2, (4) no diabetes defined as glycated hemoglobin (HbA1c) ≥48 mmol/mol and/or receiving antidiabetic medication. For the purpose of the randomized trial and the present analyses, these non-diabetic patients were further subclassified into (1) having prediabetes, ie, fasting plasma glucose level of 6.1 to 6.9 mmol/l and/or HbA1c level of 43 to 47 mmol/mol and/or impaired glucose tolerance with a 2-hour plasma glucose level of >7.8 mmol/l during a 75-g oral glucose tolerance test (OGTT), which made them finally eligible for randomization to liraglutide vs placebo, or (2) not having prediabetes, which made them screen failures for that randomized trial, but made them eligible for the baseline comparison of prediabetic vs non-prediabetic patients in the present study.

Assessments

A detailed description of the assessments has been published previously.[19] In brief and with relevance for the current study, after ≥10 hours of fasting, potential study participants underwent blood sampling, including HbA1c and a 75-g OGTT, as well as measurements of blood pressure, height, body weight, and waist circumference. Additionally, patients were rated on the

Schizophrenia Quality of Life Scale (SQLS),[30] Clinical Global Impressions, Severity Scale (CGI-S),[31] and Global Assessment of Functioning (GAF)[32] and Alcohol Use Disorders Identification Test (AUDIT).[33]

Serum Sample Collection

Blood was sampled into serum collection tubes at week 0 (non-prediabetic patients) and at week 0 and week 16 (prediabetic patients who were randomized to liraglutide or placebo). After the blood withdrawal, samples were allowed to clot for at least 1 hour and then centrifuged. Thereafter, 0.5 ml of serum was collected and stored at −80°C until analysis in batch.

Multiplexed Immunoassays

Serum proteins were analyzed using the Luminex MAGPIX platform (Luminex Corporation). Following initial testing and optimization of 13 protein assays (adiponectin, C-reactive protein [CRP], interferon gamma [IFNγ], interleukin-1β [IL-1β], IL-1 receptor antagonist [IL-1RA], IL-6, IL-10, leptin, macrophage migration inhibitory factor [MIF], prolactin, receptor for advanced glycation end products [RAGE], tumor necrosis factor alpha [TNFα] and vascular endothelial growth factor [VEGF]; kit cat. no. LXSAHM-02, LXSAHM-04 and FCSTM09-06, R&D Systems Inc.), assays for 9 analytes showed sensitivity within the physiological protein concentration range and were used to analyze clinical samples. These included adiponectin, CRP, IL-1RA, leptin, MIF, prolactin, RAGE, TNFα and VEGF (kit cat. no. LXSAHM-02 and LXSAHM-07, R&D Systems Inc.). Analyses were performed according to the instructions provided by the manufacturer. Samples were assayed at optimized dilutions (supplementary table 1), along with 6-point standard curves and 3 quality control samples per plate. All samples were assayed in duplicate.

Data Processing

Raw immunoassay data were processed using the xPONENT software 4.1 (Luminex Corporation). Mean value of the replicate samples was used for analysis. Protein concentration was calculated from a 5-parameter logistic standard curve, after subtracting background fluorescence. Supplementary table 1 shows a summary of the immunoassay data, including between- and within-plate coefficients of variation (CVs), lower and upper limits of quantitation, and number of missing and out-of-range values. Within-plate CVs were calculated as an average of the CVs derived for each assay plate from the triplicate quality control sample. Between-plate CVs were calculated as an average of the CVs derived for each of the triplicate quality control samples from across the plates. All assays showed CVs below the industry standard of 15%,[34] with CVs within plates ranging from 0.6% for adiponectin to 6.9% for RAGE, and CVs between plates ranging from 1.1% for CRP to 11.5% for VEGF. All concentration values for TNFα were below the quantitation limit and were excluded from further analysis. For the remaining analytes, values below the lower limit of quantitation (0.9% of all data points) were replaced with half the value of the lowest standard[35] and values above the upper limit of quantitation (ie, 20% of CRP values) were extrapolated for analysis as more representative than truncated or substituted data.[36]

Statistical Analysis

Statistical analysis was conducted in R software v.3.4.2.[37] Demographic and clinical characteristics were compared between groups (prediabetic vs non-prediabetic, liraglutide vs placebo, and low vs high biomarker levels) using t-test for continuous variables and Fisher's exact test/Pearson's chi-squared test for categorical variables (R "tableone" package). Protein levels were $\log_{10}$-transformed for analysis to normalize distribution. Normality was assessed using Shapiro-Wilk's test. Patients with missing outcome, covariate or biomarker level values ($n = 0$ to $n = 4$; for missing values refer to table 1 and supplementary table 1) were excluded from the analysis. Protein levels were compared between the prediabetic and non-prediabetic patient groups using fixed-effects analysis of covariance adjusted for the single covariate differing significantly between the groups (other than the group-defining 2-h, 75-g OGTT variable), ie, waist circumference. Concentrations before and after treatment with liraglutide or placebo were compared using mixed effects analysis of covariance (R "nlme" package) adjusted for the single significant covariate, ie, antipsychotic treatment with clozapine or olanzapine. Association of the primary and secondary clinical endpoints with baseline levels of selected biomarkers in the liraglutide treatment group was assessed using fixed effects analysis of (co)variance adjusted for significant covariates, ie, sex, age, duration of diagnosis, and body weight, when comparing patients with low and high baseline leptin levels (the model was not adjusted for the 2 remaining significant covariates, BMI and waist circumference, which were correlated with body weight; $P < .001$), and without covariates when comparing patients with low and high baseline adiponectin levels. Patients from the liraglutide treatment group were assigned to the "low" and "high" groups using a median biomarker concentration cutoff. The median-split approach is frequently used in biomarker research,[38–43] in particular when analyzing data with irregular distribution or where a clinically meaningful biomarker-based categorization

Table 1. Demographic and Clinical Characteristics of Study Participants at Baseline

Characteristic	Diabetes Status				Treatment Group			
	Non-prediabetic (n = 32)	Prediabetic (n = 81)	P value	Missing (%)	Placebo (n = 42)	Liraglutide (n = 39)	P value	Missing (%)
Sex, No. (%)								
Male	17 (53.1)	50 (61.7)	.531	0	25 (59.5)	25 (64.1)	.845	0
Female	15 (46.9)	31 (38.3)			17 (40.5)	14 (35.9)		
Age, mean (SD), y	41.2 (10.9)	42.7 (10.9)	.509	0	42.5 (10.7)	42.9 (11.3)	.872	0
Duration of diagnosis, mean (SD), y	16.7 (9.4)	15.1 (8.9)	.459	15.9	14.8 (8.5)	15.5 (9.4)	.755	14.8
Treatment, No. (%)								
Clozapine	21 (67.7)	56 (69.1)	.301	0.9	33 (78.6)	23 (59.0)	.014*	0
Olanzapine	10 (32.3)	20 (24.7)			5 (11.9)	15 (38.5)		
Combined	0 (0.0)	5 (6.2)			4 (9.5)	1 (2.6)		
Dose, mean (SD), mg								
Clozapine	376.2 (195.5)	329.1 (167.4)	.290	0	347.3 (180.2)	301.0 (144.7)	.296	0
Olanzapine	17.8 (9.9)	20.2 (17.3)	.676	0	23.6 (26.0)	18.3 (10.5)	.474	0
Clinical characteristics, mean (SD)								
Body weight, kg	99.3 (19.7)	102.4 (20.3)	.464	0	102.0 (23.8)	102.8 (16.0)	.865	0
BMI, kg/m^2	32.4 (4.8)	33.5 (5.7)	.344	0	33.6 (6.5)	33.5 (4.8)	.931	0
Waist circumference, cm	110.2 (14.5)	115.9 (13.1)	.048*	1.8	115.0 (14.6)	116.8 (11.4)	.537	1.2
Systolic blood pressure, mm Hg	125.1 (14.4)	125.2 (12.2)	.953	0	124.7 (13.9)	125.8 (10.2)	.686	0
Diastolic blood pressure, mm Hg	84.0 (8.0)	84.4 (8.8)	.829	0	84.5 (8.1)	84.3 (9.6)	.921	0
2-h, 75-g OGTT value, mmol/l	6.4 (1.0)	9.8 (1.9)	<.001***	0	9.8 (2.0)	9.7 (1.8)	.734	0
Alcohol consumption, mean (SD)								
AUDIT score	5.2 (6.3)	4.8 (4.9)	.747	0	4.8 (5.0)	4.9 (4.9)	.973	0
Drinks/week	4.9 (11.7)	4.3 (10.0)	.787	0	4.7 (11.9)	3.9 (7.5)	.727	0
Rating scales, mean (SD), score								
SQLS								
Psychosocial	39.0 (22.7)	38.6 (25.3)	.936	0	38.1 (26.5)	39.2 (24.3)	.849	0
Motivation and energy	29.5 (25.6)	34.5 (26.0)	.356	0	37.7 (27.2)	31.0 (24.4)	.242	0
Adverse effects	23.3 (24.3)	26.4 (21.0)	.491	0	25.4 (20.7)	27.5 (21.5)	.654	0
GAF	49.6 (9.3)	49.2 (8.8)	.843	1.8	48.1 (7.9)	50.5 (9.5)	.224	1.2
CGI-S	3.7 (0.6)	3.6 (0.6)	.524	1.8	3.7 (0.6)	3.5 (0.6)	.152	1.2

Note: Statistical tests included t-test for continuous variables and chi-squared test for categorical variables. Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; BMI, body mass index; CGI-S, Clinical Global Impressions Scale; GAF, Global Assessment of Functioning; OGTT, oral glucose tolerance test; SD, standard deviation; SQLS, Schizophrenia Quality of Life Scale; y – years.
*P < .05, ***P < .001 (2-sided).

is missing.[44] Sensitivity analyses were conducted by excluding covariates (n = 0 to n = 4) from the analysis of covariance models. All tests were 2-sided and Bonferroni correction was used to adjust P values for multiple hypothesis testing. In a follow-up analysis of the liraglutide treatment group, association between changes in leptin levels and changes in body weight was assessed using fixed effects regression unadjusted for covariates, as none of the baseline demographic or clinical variables was significantly associated with changes in body weight (P > .075). Figures were prepared in R software, Adobe Illustrator CC 2017 v.21.0.0 (Adobe Systems Incorporated) and Inkscape v.0.92.4. Plots show mean ± standard error.

Results

Patient Characteristics

We analyzed levels of 8 serum proteins previously linked to schizophrenia and prediabetes in 32 non-prediabetic and 81 prediabetic patients with schizophrenia-spectrum disorder. We used the 2-h, 75-g OGTT value as the primary endpoint in lieu of the 4-h value used as the primary endpoint in the original investigation,[19] as the latter was not available for the non-prediabetic group that was not randomized. Not surprisingly, patients with prediabetes had significantly higher 2-h OGTT value compared to non-prediabetic patients (one of the criteria for prediabetes; 9.8 ± 1.9 vs 6.4 ±

1.0 mmol/l, $P < .001$; table 1). Except for a greater waist circumference in patients with prediabetes (115.9 ± 13.1 vs 110.2 ± 14.5 cm, $P = .048$), no other characteristics differed significantly between the prediabetes and non-prediabetes groups.

Patients with prediabetes ($n = 81$) were randomized to 16 weeks of adjunctive treatment with the GLP-1RA liraglutide ($n = 39$) or placebo ($n = 42$).[19] The liraglutide and placebo treatment groups were matched for all characteristics, except for the antipsychotic treatment distribution ($P = .014$; table 1). Variables that differed significantly between the groups were adjusted for in subsequent group comparison analyses.

Biomarkers of Prediabetes

Levels of 2 serum proteins, adiponectin, and VEGF, were significantly different between the non-prediabetic and prediabetic patient groups after adjusting for waist circumference. Compared to patients in the non-prediabetic group, patients in the prediabetic group had significantly lower levels of adiponectin (mean difference in $\log_{10}$-transformed protein concentration in pg/mL of -0.17, with 95% confidence intervals [CI] from −0.28 to −0.06, $P = .004$; figure 1 and supplementary table 2) and VEGF (−0.16 $\log_{10}$ (pg/ml), 95% CI −0.29 to −0.03, $P = .019$). Differences between the groups in adiponectin and VEGF levels were also significant without adjustment for the covariate ($P = .002$ and .045, respectively; supplementary table 3) and the difference in adiponectin levels remained significant after Bonferroni correction (adjusted $P = .030$; supplementary table 2).

Effects of Liraglutide

After 16 weeks of treatment, levels of 2 serum proteins—leptin and adiponectin—changed significantly in the liraglutide group compared to the placebo group, after adjusting for treatment with clozapine or olanzapine. Levels of leptin decreased more in the liraglutide treatment group compared to the placebo group, with mean difference between the groups of −0.081 $\log_{10}$ (pg/ml) (95% CI −0.147 to −0.015, $P = .017$; figure 2 and supplementary table 4). In addition, concentrations of adiponectin decreased after treatment with placebo and increased after treatment with liraglutide, with a mean between-group difference of 0.140 $\log_{10}$ (pg/ml) (95% CI 0.021 to 0.259, $P = .022$; figure 2 and supplementary table 4). Differences between the groups in leptin and adiponectin levels were significant also without adjustment for the covariate ($P = .017$ and .023, respectively; supplementary table 5), but not after Bonferroni correction (supplementary table 4). Baseline levels of leptin and adiponectin were not different between the liraglutide and the placebo groups ($P = .842$ and .400, respectively, adjusted for specific antipsychotic drug treatment).

Subgroup Analyses

Subgroup analyses were conducted in the liraglutide treatment group to investigate the association between baseline levels of biomarkers that were significantly associated with liraglutide treatment (figure 2) and clinical outcomes at week 16. Patients were assigned to "low" and "high" groups using median biomarker concentration cutoff, ie, 18.8 ng/ml for leptin and 2.4 µg/ml for adiponectin. Patients in "low" and "high" leptin groups differed significantly in sex, age, duration of diagnosis, body weight, BMI, and waist circumference (supplementary table 6), which were adjusted for in subsequent subgroup analysis, excluding collinear covariates. There were no significant differences in patient characteristics between the "low" and "high" adiponectin groups. After 16 weeks of treatment with liraglutide, patients who had higher leptin levels at baseline showed significantly larger reduction in the primary endpoint, 2-h OGTT value, than patients with lower baseline leptin levels (mean difference between the groups −2.62 mmol/l, 95% CI −4.54 to −0.70, $P = .009$; figure 3 and supplementary table 7). This association remained significant without adjustment for covariates ($P = .030$; supplementary table 8), when additionally adjusted for baseline OGTT value to account for the potential "regression to the mean" effect ($P = .040$) and remained significant after adjusting for multiple comparisons of the primary endpoint (adjusted $P = .018$; supplementary table 7). Additional analyses showed that baseline leptin levels in participants treated with liraglutide did not predict change in body weight ($P > .42$; supplementary tables 7 and 8), although changes in leptin levels were positively correlated with changes in body weight ($P = .021$).

Discussion

In the present study, we measured serum levels of 8 proteins relevant to metabolic disturbances (adiponectin, CRP, IL-1RA, leptin, MIF, prolactin, RAGE, and VEGF). When comparing baseline protein levels between prediabetic and non-prediabetic patients, significantly lower levels of adiponectin and VEGF were found in prediabetic patients. No other significant differences were observed.

Adiponectin is a hormone mainly secreted from adipose tissue. It is involved in glucose regulation by improving insulin sensitivity, but also exerts anti-inflammatory and anti-atherogenic properties associated with reduced risk for cardiovascular disease.[45] A large body of evidence suggests an inverse relationship between adiponectin levels and the presence of metabolic syndrome, obesity, and T2D.[45] Our result is in accordance

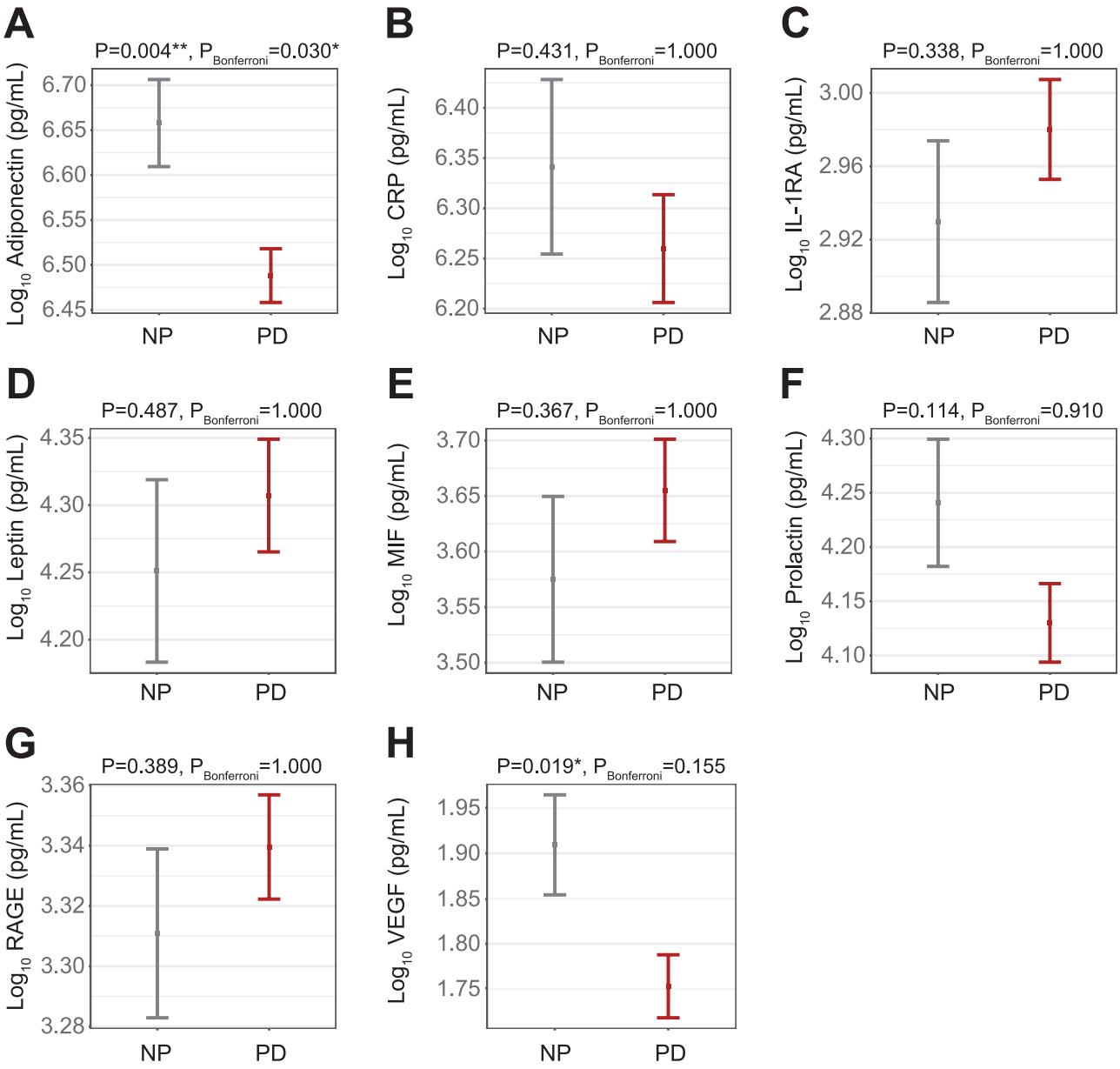

Fig. 1. Baseline differences in biomarker levels between non-prediabetic (NP; $n = 32$) and prediabetic (PD; $n = 81$) schizophrenia patients. Values represent mean $\log_{10}$-transformed protein concentrations (in pg/ml) and standard error for (A) adiponectin, (B) C-reactive protein (CRP), (C) interleukin-1 receptor antagonist (IL-1RA), (D) leptin, (E) macrophage migration inhibitory factor (MIF), (F) prolactin, (G) receptor for advanced glycation endproducts (RAGE), and (H) vascular endothelial growth factor (VEGF). All values were derived from the fixed effects analysis of covariance adjusted for waist circumference (supplementary table 2). $P_{\text{Bonferroni}}$ – Bonferroni-adjusted P value. *$P < .05$, **$P < .01$.

with the literature and suggests that the same inverse relationship is observed in schizophrenia-spectrum disorder patients on stable antipsychotic treatment that have their own metabolic effects.[9]

VEGF is mainly produced in the kidney, and higher circulating levels of VEGF are observed in patients with metabolic syndrome, hyperglycemia, but not with obesity.[46] In schizophrenia, circulating VEGF levels are unaltered in antipsychotic drug-naïve and first-episode psychosis patients, and increased in medicated patients.[47] Surprisingly, we found lower serum levels of VEGF in prediabetic patients compared to non-prediabetic patients, despite similar antipsychotic treatment regime. The reason for this discrepancy remains unclear and requires further study.

Next, we investigated the prediabetic patient group for changes in protein levels following 16 weeks of treatment with liraglutide vs placebo.[19] Since only prediabetic patients were included in the trial, we could not evaluate treatment effects in non-prediabetic patients. We

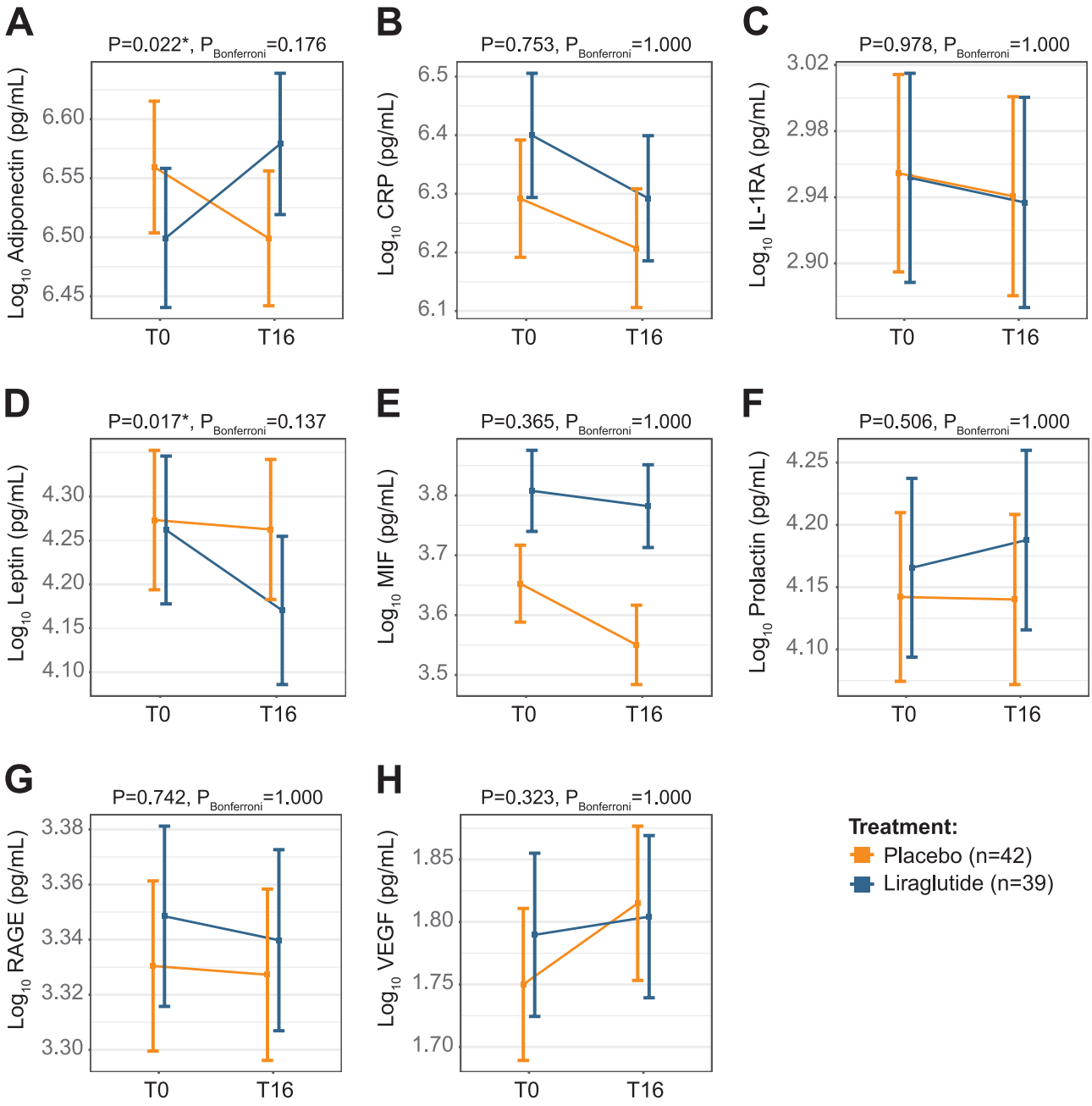

Fig. 2. Change in biomarker levels from baseline (T0) to week 16 (T16) in the placebo (n = 42) and liraglutide (n = 39) treatment groups. Values represent mean $\log_{10}$-transformed protein concentrations (in pg/ml) and standard error for (A) adiponectin, (B) CRP, (C) IL-1RA, (D) leptin, (E) MIF, (F) prolactin, (G) RAGE, and (H) VEGF. All values were derived from the mixed effects analysis of covariance adjusted for antipsychotic treatment (olanzapine, clozapine, or combined; supplementary table 4). P values refer to the estimated liraglutide vs placebo treatment difference. $P_{\text{Bonferroni}}$ – Bonferroni-adjusted P value. *$P < .05$.

observed a significant effect of liraglutide on serum levels of adiponectin and leptin compared to placebo. Adiponectin serum levels increased with liraglutide, which is in concordance with preclinical data[48] and data from a clinical study where adiponectin was increased in Chinese patients with T2D following treatment with liraglutide.[49] However, no significant difference was observed in Spanish patients with T2D treated with liraglutide.[50] This discrepancy may be due to different patient population, ie, prediabetic patients with schizophrenia in the present study as compared to patients with T2D previously treated with metformin.[50]

Leptin serum levels in the liraglutide group decreased over the 16 weeks treatment period compared to placebo. Leptin is produced in adipose tissue and mediates regulation of energy balance, suppresses food intake and is thereby associated with weight loss.[51] Leptin binds to the leptin receptor, which exists either as membrane-bound

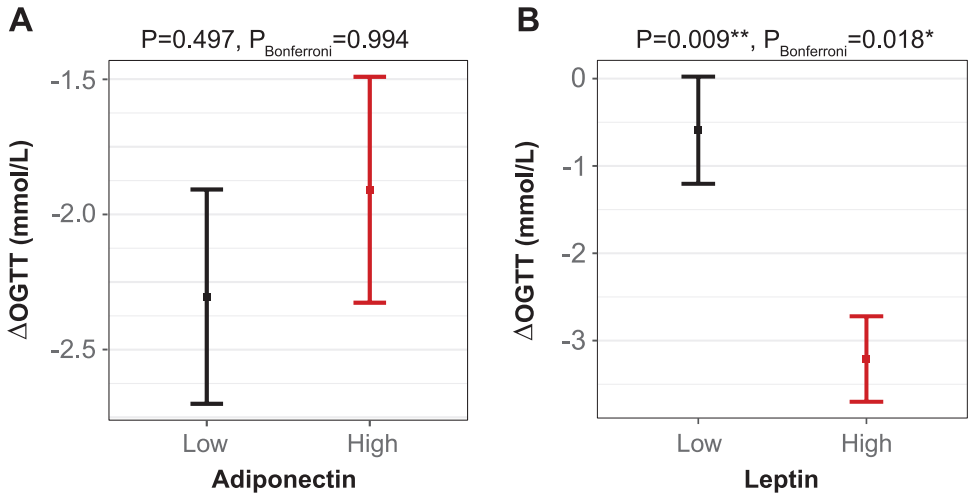

Fig. 3. Association of the change from baseline to week 16 in the primary clinical endpoint, 2-h 75-g oral glucose tolerance test (ΔOGTT), with baseline (A) adiponectin and (B) leptin levels in the liraglutide treatment group. Baseline protein concentrations were categorized as "low" or "high" by median split. All values were derived from the fixed effects analysis of variance (adiponectin) or covariance (leptin; adjusted for sex, age, duration of diagnosis, and body weight; supplementary table 7). Shown are adjusted means ± standard error. $P_{\text{Bonferroni}}$ – Bonferroni-adjusted P value. $^{*}P < .05$, $^{**}P < .01$.

isoforms or a circulating soluble form, the latter believed to be the main leptin-binding entity in human blood.[51] When leptin or its receptor is dysregulated, obesity and T2D are a common consequence.[51,52] At baseline, we did not observe significantly higher serum leptin levels in prediabetic patients compared to non-prediabetic patients, which is consistent with comparable BMI and body weight between the 2 groups. However, 16 weeks of liraglutide treatment did reduce serum leptin levels compared to placebo in our study, which could be explained by the significantly greater weight reduction with liraglutide vs placebo,[19] which results in lower leptin levels due to decreased adipocytes. A similar effect was observed also prior to changes in body weight in a study in patients with T2D; however, it was not significant after adjusting for covariates.[53] This may be due to different patient populations, ie, prediabetic patients with schizophrenia (present study) versus patients with T2D as well as fewer participants and shorter duration of intervention in the referenced study.[53]

In the third part of the experiment, we further investigated serum levels of adiponectin and leptin—the 2 biomarkers significantly affected by GLP-1RA treatment—for their association with the clinical endpoints in the liraglutide arm of the trial.[19] Patients were divided by median split into groups with low or high baseline biomarker levels. Interestingly, we found that high baseline levels of serum leptin were associated with significantly better response in our primary endpoint, OGTT. This has, to our knowledge, not been observed earlier in schizophrenia or general patient population. We did not find evidence that this is an epiphenomenon of weight reduction, given that baseline leptin levels were unrelated to changes in body weight; however, such possibility requires further evaluation. Our results suggest that serum leptin could be a potential predictive biomarker of improvement in glucose tolerance in prediabetic, antipsychotic treated, overweight, or obese patients with schizophrenia. The mechanism behind this association is at present unknown. A possibility would be that GLP-1 and GLP-1RA act as "leptin-sensitizing agents"[54] explaining why patients with high serum leptin levels respond better to GLP-1RA treatment than patients with low serum leptin levels. This hypothesis is in line with results from studies in animal models that show synergistic interactions between leptin and a GLP-1RA exendin-4 in reducing food intake[55] and restoration of leptin responsiveness by a co-agonist of the GLP-1 and glucagon receptors.[56] Interestingly, it has been demonstrated that another hormone, amylin, primarily secreted from pancreatic beta cells to the blood stream,[57] acts as a "leptin-sensitizer" enhancing leptin signaling.[58] However, such mechanism of action has, to our knowledge, not previously been reported for GLP-1.

The results of this study must be interpreted within its limitations. First, these are post hoc analyses from a study that had a different primary aim, ie, testing the effect of liraglutide vs placebo in prediabetic patients with schizophrenia-spectrum disorders on stable treatment with clozapine or olanzapine. Second, the sample size was still modest, especially in the non-prediabetic patients and in the subgroup analyses of the liraglutide-treated sample, reducing the power of the analyses. Third, only 8 of the initially selected and assessed 13 putative biomarkers were within the analytical range of the assays and could be analyzed, removing most inflammatory markers from the study. Furthermore, some of the measured values were substituted or extrapolated prior to analysis due to being

outside of the linear range of the assays and might not represent true concentrations in the serum. Fourth, not all statistically significant results survived Bonferroni correction, making these results more hypothesis-generating than hypothesis-testing. Finally, many additional putative and potentially relevant biomarkers could have been examined but were unavailable to us. Nevertheless, despite these limitations, to our knowledge, this is the first study to investigate markers of satiety and energy homeostasis in relationship to prediabetes status, liraglutide treatment and biomarker distribution in patients with schizophrenia-spectrum disorders.

In summary, results from this study indicate the role for adiponectin and VEGF in prediabetes, and the involvement of adiponectin and leptin in response to treatment with GLP-1RA in prediabetic, overweight or obese schizophrenia-spectrum disorder patients treated with clozapine or olanzapine. Moreover, they suggest that leptin might be a predictive biomarker of GLP-1RA treatment efficacy in improving glucose tolerance in this patient population. Further studies are required to validate these findings, evaluate their potential for personalized management of prediabetes in the context of schizophrenia and investigate their application to other patient groups, eg, prediabetic and diabetic patients without a diagnosis of schizophrenia.

Supplementary Material

Supplementary data are available at *Schizophrenia Bulletin Open* online.

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