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RESEARCH ARTICLE

Levels of Alzheimer's disease blood biomarkers are altered after food intake—A pilot intervention study in healthy adults

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Abstract

INTRODUCTION: Blood biomarkers accurately identify Alzheimer's disease (AD) pathophysiology and axonal injury. We investigated the influence of food intake on AD-related biomarkers in cognitively healthy, obese adults at high metabolic risk.

METHODS: One-hundred eleven participants underwent repeated blood sampling during 3 h after a standardized meal (postprandial group, PG). For comparison, blood was sampled from a fasting subgroup over 3 h (fasting group, FG). Plasma neurofilament light (NfL), glial fibrillary acidic protein (GFAP), amyloid-beta (Aβ) 42/40, phosphorylated tau (p-tau) 181 and 231, and total-tau were measured via single molecule array assays.

Henrik Zetterberg, Marie-Christine Simon these authors contributed equally.

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RESULTS: Significant differences were found for NfL, GFAP, Aβ42/40, p-tau181, and p-tau231 between FG and PG. The greatest change to baseline occurred for GFAP and p-tau181 (120 min postprandially, $p < 0.0001$).

CONCLUSION: Our data suggest that AD-related biomarkers are altered by food intake. Further studies are needed to verify whether blood biomarker sampling should be performed in the fasting state.

KEYWORDS
amyloid beta, biomarkers, blood, diurnal variations, fasting, food intake, glial fibrillary acidic protein, neurofilament light, obesity, phosphorylated tau, plasma, postprandial, pre-analytical standardization, total tau

Highlights

- Acute food intake alters plasma biomarkers of Alzheimer's disease in obese, otherwise healthy adults.
- We also found dynamic fluctuations in plasma biomarkers concentration in the fasting state suggesting physiological diurnal variations.
- Further investigations are highly needed to verify if biomarker measurements should be performed in the fasting state and at a standardized time of day to improve the diagnostic accuracy.

1 | BACKGROUND

Alzheimer's disease (AD) is associated with pathophysiological events caused by protein aggregation in the cerebral cortex. The accumulation of amyloid-β (Aβ) peptides in amyloid plaques in the extracellular neural tissue and hyper-phosphorylated tau aggregates as intracellular neurofibrillary tangles and dystrophic neurites surrounding the amyloid plaques are considered the core signs of AD pathology,[1–4] together with a progressive degeneration of neurons and their synapses. It is long known that there is a continuum between 'normal aging' and AD dementia,[5,6] which is also accompanied by the fact that AD can be characterized by a long asymptomatic phase.[2,7,8] The gold-standard methods to measure the proteinopathies in AD pathology are positron emission tomography (PET) imaging of Aβ and tau pathologies, and cerebrospinal fluid (CSF) analyses of Aβ and tau proteins.[9] Since imaging methods are expensive and time-consuming, and CSF sampling via lumbar puncture is sometimes regarded as an invasive procedure, more accessible approaches such as analyses of blood-based biomarkers would unburden diagnosis and monitoring of the disease. In this context, primary targets include biomarkers of Aβ pathology, tau pathology, neurodegeneration, and markers of glial reactivity.[10] Recent studies validated a high performance of plasma concentrations of Aβ40 and Aβ42,[11–14] phosphorylated tau (p-tau) 181,[14–19] p-tau231,[20] neurofilament light (NfL),[21] and glial fibrillary acidic protein (GFAP)[22,23] in confirming AD pathologies, axonal injury, and astrocytic activation. Some but not all of these biomarkers are increased in preclinical AD,[24,25] and are associated with disease progression. Since early detection of cognitive impairment and neurodegenerative diseases with high sensitivity and specificity, especially in the preclinical phase, is of great societal and scientific interest, the discovery of blood-based biomarkers reflecting AD pathology has been a milestone in AD research. Until today, however, a standardized procedure for sample handling and protein measurements could not be established due to uncertainties with respect to unknown confounders.

In recent years, intensified effort has, thus, been put into determining the influence of pre-analytical procedures,[26,27] storage stability,[28] and freeze-thaw cycles[29,30] on biomarker analysis to recommend a standardized procedure for sample handling and protein measurement. Furthermore, light was shed on the question if physiological factors including circadian rhythm and the quality and quantity of food intake influence the actual concentrations of plasma AD biomarkers; while one study showed no effect of food intake on plasma levels of Aβ40 or Aβ42 in samples taken 3 weeks apart in the same healthy individuals,[31] no conclusive answer has been reached to date.[32,33] Therefore, the aim of the present study was to investigate the effect of acute food intake on plasma biomarkers of Aβ and tau pathology, neurodegeneration, and glial reactivity in cognitively normal adults.

2 | METHODS

2.1 | Study protocol

In this cross-sectional study, 111 cognitively unimpaired, obese adults ingested a standardized test meal wherein blood samples were collected at six intervals over a duration of 180 min. Anthropometric

measurements and collection of fasting (time point 0 min) and postprandial (the period after food intake) blood samples (15, 30, 45, 60, 120, and 180 min after meal ingestion) were performed in all participants (postprandial group, PG). As control, a subgroup of the PG ($n = 26$) was monitored in a second visit over the same time period of 180 min without consuming any test meal or fluids (fasting group, FG). As with the PG, fasting venous blood samples in the FG were collected via venous catheter at 0, 15, 30, 45, 60, 120, and 180 min. In all subjects and both PG and FG, the first blood collection was performed in the morning between 7:30 a.m. and 9:00 a.m.

The study protocol was designed in accordance with the Declaration of Helsinki, approved by the local ethics committee, and registered at German Clinical Trials Register (DRKS; http://www.drks.de) under identifier DRKS00015861. Informed written consent was obtained from all participants.

2.2 | Participants

Overweight or obese men and women (body mass index (BMI) ≥27.0/<40.0 kg/m^2) aged 45–70 years with no history of neurodegenerative diseases were recruited at the Institute of Nutrition and Food Sciences, University Bonn, Germany, through announcements in local newspapers, on the radio, or via flyers. The exclusion criteria were smoking; diabetes mellitus type 2; chronic neurological, liver, kidney, gastrointestinal, thyroid, or inflammatory diseases; prior cardiovascular events; acute infections; recent surgeries; pregnancy or breastfeeding; alcohol, drug, or medicine abuse; chronic intake of dietary supplements (i.e., n-3 fatty acids, vitamin E and magnesium); and participation in a weight loss program or in other clinical studies.

2.3 | Standardized meal test

After 12 h of overnight fasting and a fasting blood collection, all participants in the PG ingested 300 ml of a protein chocolate drink within 3 min (303.8 kcal; carbohydrates, 41.8 g (19 g sugar); protein, 19.0 g; fat, 7.6 g; Boost High Protein, Nestlé Health Science, Vevey, Switzerland). The entire test meal was supervised by the study personnel.

2.4 | Measurements

2.4.1 | Anthropometric and blood pressure measurement

Anthropometric measurements were performed according to standard operation procedures as published earlier.[34] In brief, body weight and body composition (fat mass and fat-free mass) were measured by air displacement plethysmography using the BodPod body composition system (Cosmed, Fridolfing, Germany). Body height was determined to the nearest 0.1 cm using a stadiometer (Seca scale 704, Seca GmbH and Co. KG, Hamburg, Germany). Waist circumference as measure of visceral fat distribution was determined midway between the lowest rib and the iliac crest at maximal exhalation to the nearest 0.1 cm. Blood pressure was measured twice after a 30-min rest period in the sitting position using a semiautomatic blood pressure measurement device (Boso Carat professional, Bosch + Son GmBH and Co. KG, Jungingen, Germany).

RESEARCH IN CONTEXT

1. **Systematic Review**: We reviewed the available scientific literature on PubMed for articles and conference abstracts examining the impact of food intake and diurnal variations on plasma biomarkers in Alzheimer's disease (AD), specifically neurofilament light, glial fibrillary acidic protein, amyloid beta, total tau, and phosphorylated tau 181 and 231 measured by single molecule array, but also using other methods.
2. **Interpretation**: Our findings indicate that food intake alters the concentration of AD-related plasma biomarkers in healthy adults with a maximum divergence of 30%. Furthermore, we showed that biomarkers fluctuate dynamically in the fasting state. Further studies need to verify if biomarker measurements should be performed in the fasting state and at a standardized time of day to improve the diagnostic accuracy.
3. **Future Directions**: We investigated the impact of food intake in obese but otherwise healthy adults. Whether the results are of relevance to non-obese individuals and AD patients needs to be examined in future studies.

2.5 | Laboratory analysis

2.5.1 | Blood samples

Blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA), fluoride, or coagulation activator (Sarstedt, Nümbrecht, Germany). Fasting plasma glucose concentration was measured by the enzymatic reference method with hexokinase and VIS photometry (Cobas 8000 modular analyzer series, Roche Diagnostics, Basel, Switzerland). Hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (HPLC) and ion-exchange method (D-100, BioRad, Munich, Germany). Serum concentrations of creatinine, uric acid, and urea were measured using VIS photometry (Cobas 8000 modular analyzer series, Roche Diagnostics, Basel, Switzerland). Serum concentration of high-sensitive C-reactive protein (hsCRP) was measured using a turbidimetric immunoassay (Cobas 8000 modular analyzer series, Roche Diagnostics, Basel, Switzerland). All analyses were performed at the Central Laboratory of the Institute of Clinical Chemistry and Clinical Pharmacology at the University Hospital

Bonn, Germany within 4 h after blood sampling. Method specifications are available online (https://www.ukbonn.de/ikckp/zentrallabor/leistungsverzeichnis/).

For AD-related plasma protein concentration measurements, EDTA samples were centrifuged for 15 min at 3000 g and 8°C and plasma supernatants were immediately frozen in cryovials at −80°C. All samples underwent one freeze-thaw cycle before analysis, except for total-tau, where two cycles were applied. After thawing, plasma samples were centrifuged at 4000 g for 10 min at room temperature and immediately prepared for measurement. Plasma protein concentrations were measured employing the HD-X ultra-sensitive single molecule array (Simoa) platform (Quanterix, Billerica, Massachusetts, USA). NfL, GFAP, and Aβ 42/40 concentrations were measured using the Neurology 4-Plex E kit (Lot-503212, Quanterix, Billerica, Massachusetts, USA). Total-tau was measured using the Tau Advantage kit (Lot-503307, Quanterix, Billerica, Massachusetts, USA). Reagents from a single lot were used for the analysis of all samples. P-tau181 and p-tau231 were measured using in-house assays as previous described.[18,20] Plasma samples were diluted four-fold for Neurology 4-Plex and total-tau and two-fold for in-house p-tau measurements. All measurements were performed at the Clinical Neurochemistry Laboratory at the University of Gothenburg, Sweden. The inter-assay/intra-assay variability was 5%/6% for NfL, 8%/8% for GFAP, 7%/7% for Aβ40, 5%/5% for Aβ42, 9%/14% for p-tau181, 5/7% for p-tau231, and 5%/11% for total-tau, respectively.

2.6 | Statistical analysis

All statistical analyses were performed using SPSS (version 28.0, IBM Corp., Chicago, IL, USA) and RStudio (version 4.1.2, Boston, MA, USA).

To analyze the time course of protein concentration changes, we used a polynomial spline of degree 3 for time and included an interaction term (time *x* group) to test for a difference in parameter progression over time between PG and FG. In all tests, the residuals were checked for relevant deviations from normal distribution and homoscedasticity. All variables, except for Aβ40 and Aβ42, were log-transformed prior to analysis. All models were adjusted for age, sex, and BMI.

Postprandial metabolites during the 3-h test period in FG and PG were summarized by the total area under the curve (AUC_{180min}). In the postprandial setting, the AUC is the definite integral of the concentration of a biomarker in blood plasma as a function of time and represents the total biomarker concentration across time. The trapezoid rule was used to estimate the AUC based on the single measurement at the described time points. Two-sided *t* tests were used to check for differences of AUC between PG and FG.

Statistical analyses of biomarker concentrations were corrected for multiple testing using the Bonferroni-Holm correction.[35] For all analyses, the significant level was set as $p < 0.05$. Unless otherwise described, data are presented as the arithmetic mean ± standard deviation (SD).

TABLE 1 Participant characteristics.[a]

	PG $n = 111$	FG $n = 26$	*p*-Value[b]
Sex,[c] m/f	47/64	13/13	0.585
Age, years	60 ± 7	61 ± 6	0.311
BMI, kg/m^2	31 ± 4	30 ± 3	0.058
Waist circumference, cm	105.9 ± 9.5	104.0 ± 9.2	0.344
Fat mass, %	39.8 ± 7.1	41.0 ± 7.9	0.476
BP systolic, mmHg	137.4 ± 16.6	138.4 ± 15.6	0.761
BP diastolic, mmHg	87.5 ± 10.4	88.9 ± 11.5	0.552
Creatinine, μmol/l	70.7 ± 15.8	71.2 ± 14.7	0.886
Uric acid, μmol/l	322.6 ± 63.8	320.4 ± 66.5	0.872
Urea, mmol/l	5.0 ± 1.3	5.1 ± 1.3	0.678
Plasma glucose, mmol/l	5.2 ± 0.6	5.3 ± 0.6	0.786
HbA1c, %	5.4 ± 0.4	5.4 ± 0.4	0.648
hsCRP, mg/l	3.1 ± 2.7	2.8 ± 2.1	0.632

Abbreviations: BMI, body mass index; BP, blood pressure; f, female; FG, fasting group; HbA1c, hemoglobin A1c; hsCRP, high sensitive C-reactive protein; m, male; PG, postprandial group.
[a]Data are shown as mean ± SD.
[b]Compared using unpaired two-sided *t*-test.
[c]Compared using Pearson's chi-squared test.

3 | RESULTS

3.1 | Participants

The participant characteristics are listed in Table 1. All 111 participants were overweight (BMI ≥27 kg/m^2) or obese (BMI ≥30 kg/m^2) and under high metabolic risk, emphasized by a visceral fat distribution (waist circumference ≥80 cm in women, ≥94 cm in men), elevated fat mass (>36% in women, >25% in men), present prehypertension (systolic blood pressure ≥120 mmHg and diastolic blood pressure ≥80 mmHg), and systemic low-grade inflammation (hsCRP ≥2.0 mg/l). No significant differences were found at baseline in age, sex, and anthropometric and metabolic data between PG and FG.

All 111 participants consumed the entire test drink within the given time frame, no intolerances were reported. The FG (control group) and a random subgroup of 26 participants out of the 111 participants from the PG, completed the second visit as planned, monitoring and blood sampling were performed in a comparable setting, but without any food or fluid intake. All participants finished the study, and their respective per-protocol data were included in the analysis.

3.2 | Postprandial changes in plasma biomarker concentrations

In the PG, all biomarkers changed significantly over time after test meal ingestion (time effect, all *p* values for time-effect <0.0001). Plasma NfL

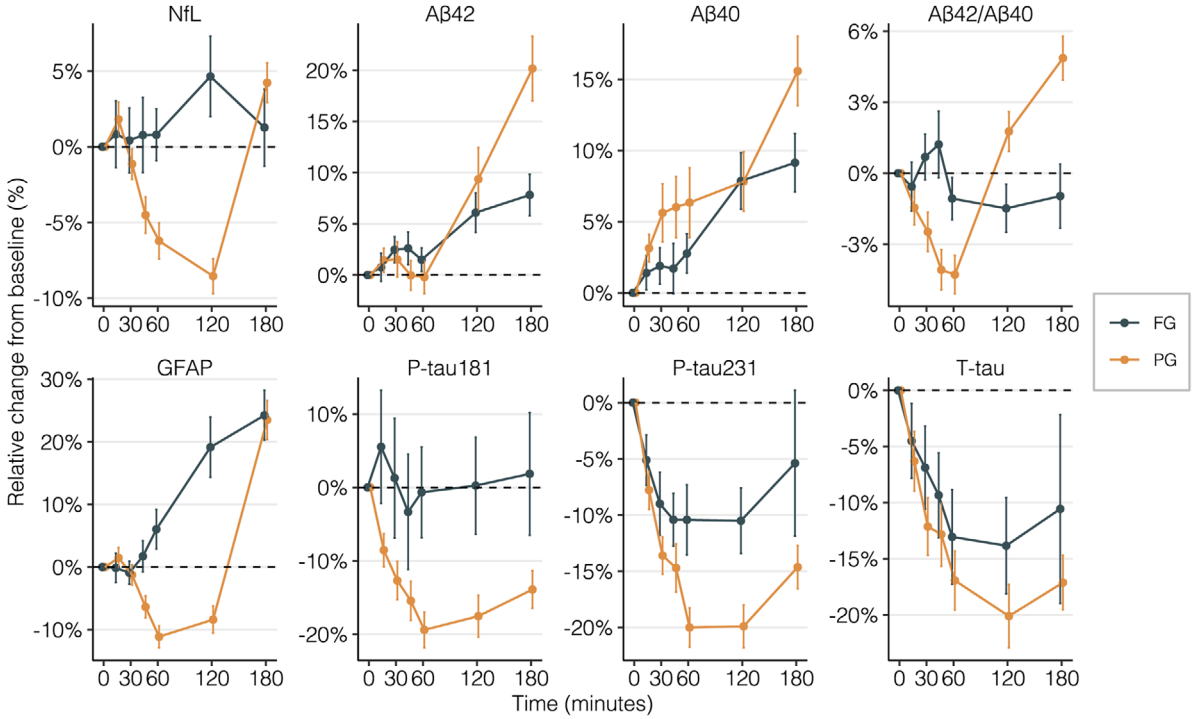

FIGURE 1 Relative changes in biomarker concentrations from baseline after test meal ingestion compared to a fasting group. Data are shown as mean ± SD; $n = 111$ in the PG, $n = 26$ in the FG. Aβ, amyloid-beta; FG, fasting group; GFAP; glial fibrillary acidic protein, NFL, neurofilament light; PG, postprandial group; P-tau, phosphorylated tau; T-tau, total-tau.

and Aβ42 showed a slight but significant increase in the first 15 min after test drink ingestion, then a subsequent decrease was followed by an increase at the 2 h time point. For Aβ40, there was persistent increase which was more pronounced in the late postprandial phase. For GFAP and Aβ42/40 ratio, there were decreases in the early and increases in the later postprandial phase. For p-tau181, p-tau231 and total-tau levels, there were sustained decreases over 3 h, which were greater in the early postprandial phase (Table S1).

In the FG, we found significant changes in GFAP ($p < 0.0001$), Aβ40 and Aβ42 (both $p < 0.0001$), p-tau231 ($p = 0.0021$), and total-tau ($p = 0.0014$) levels over the 3-hour test period, but not for NfL, Aβ42/40 ratio, or p-tau181. However, when comparing the time course of protein concentration changes between the PG and the FG, we found significant differences for NfL, GFAP, Aβ42, Aβ42/40 ratio, p-tau181, and p-tau231 (all $p < 0.05$), but not for Aβ40 and total-tau (Table S1). These results remained unchanged when adjusting for age, sex, and BMI (Table S1). Our findings imply that the dynamics in protein concentrations were significantly greater after food intake than in the fasting state.

Furthermore, we calculated the relative difference to baseline (0 min) for each of the following time points and each biomarker in both groups (Figure 1, for the subset analysis see Figure S1). For NfL, and total-tau, the greatest differences to baseline occurred 120 min after food intake (−9%, and −20%, respectively), while for Aβ40, Aβ42, and GFAP, the greatest difference to baseline was observed at 180 min postprandially (+16%, +20%, and +24%, respectively). For Aβ42/40 ratio, p-tau181 and p-tau231, the biggest difference to baseline occurred in the earlier postprandial phase 60 min after drink ingestion (−5%, −20%, and −20% respectively).

To consider the observed protein fluctuations in the fasting state, we analyzed the difference between changes to baseline between PG and FG study participants. The largest distance between the curves representing the change from baseline (Figure 1) was observed for GFAP and p-tau181. At time point 120 min, the concentration changes from baseline differed by 30% and 18% between PG and FG (18% in GFAP and 24% in p-tau181 levels in the subset, respectively, Figure S1). Also, for NfL, the maximum distance between PG and FG was observed after 2 h (14%), while for Aβ40, Aβ42, Aβ42/40 ratio, and total-tau, it was after 3 h (13%, 13%, 6%, and 7%, respectively). For p-tau231, the change to baseline varied the most between PG and FG at time point 60 min (10%).

Moreover, the integrated protein concentrations over the 3-h period (AUC_{180min}) of NfL and GFAP were significantly smaller after test meal ingestion than in the fasting state (Figure 2, Table S2)

4 | DISCUSSION

To our knowledge, this is the first human intervention study investigating the impact of food intake on a broad panel of AD-related plasma proteins in cognitively healthy adults. All measured AD biomarkers showed a significant change over time after ingestion of a single test meal. These effects were significantly more distinct than the concentration changes also observed in the fasting state for GFAP, NfL,

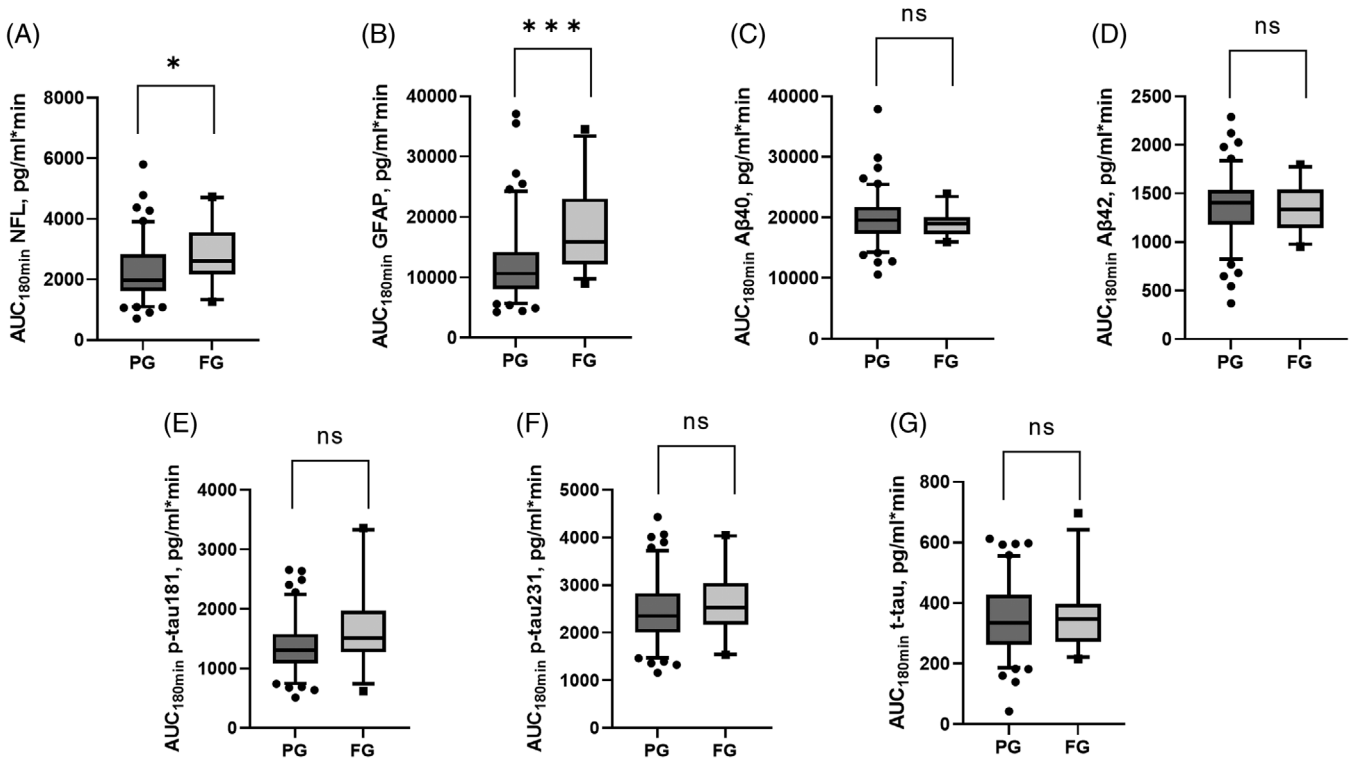

FIGURE 2 Areas under the curve of (A) NFL, (B) GFAP, (C) Aβ40, (D) Aβ42, (E) p-tau181, (F) p-tau231, and (G) t-tau concentrations after food intake (PG) and fasting (FG) over the 180-min test duration. The box and whiskers plot the 5th to the 95th percentile, the line is plotted at the median; PG versus FG comparison; ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$; $n = 111$ in the PG group, $n = 26$ in the FG group. Aβ, amyloid-β; AUC, area under the curve; FG, fasting group; GFAP, glial fibrillary acidic protein; NFL, neurofilament light; PG, postprandial group; p-tau, phosphorylated tau; t-tau, total-tau.

Aβ42/40 ratio, Aβ42, p-tau231, and p-tau 181. Since the consideration of a fasting cohort validates our results against diurnal variation or temporal dynamics; we assume that the observed differences in biomarker concentrations between PG and FG are caused by acute food intake and related metabolic events. Our results, especially the observed effect sizes, indicate clinically relevant variations in the fasting versus the non-fasting state.

Only a few earlier studies have investigated the influence of acute food intake on the concentration of AD-related CSF and plasma proteins. Two studies in 9 to 10 healthy individuals showed no differences in fasting versus non-fasting Aβ40 and Aβ42,[31,33] and NR1 tau and NfL[33] plasma concentrations. It is worth mentioning that in the study of Mengel et al., the non-fasting conditions as the time and type of the last food intake were not further specified. Since we were able to show that protein concentrations changed already in the very early postprandial phase, it is likely that the acute postprandial phase was not reflected. In contrast to this and in line with our findings, in a pilot study, serum NfL levels decreased after food intake and increased again after 120 min.[36] Also CSF levels of metabolites associated with brain-related diseases were reported to be putatively affected by food intake.[37] Moreover, the intake of a protein-rich beverage[38] and an oral glucose load[39] led to increases in amyloid precursor protein (APP), Aβ40, and Aβ42 plasma levels in healthy individuals. Here, the authors additionally found that the postprandial plasma Aβ40 and Aβ42 changes were significantly different between AD and non-AD.[39] In line with this, another human intervention study showed that the postprandial response upon two differently composed test meals in Aβ40 and Aβ42 concentrations differed between participants with and without mild cognitive impairment (MCI).[32] These results indicate that the postprandial response of AD-related biomarkers varied in different stages of cognitive impairment highlighting that further investigations are needed to validate if there are clinically relevant effects on the AD blood biomarkers, including fasting and non-fasting participants (optimally with both variants of samples from the same individuals) of different ages as well as patients with AD-type pathology. Following from this, we speculate that recommendations on whether samples should be taken in the fasting or non-fasting state can be derived. Moreover, it is of great interest to explore the impact of meal composition and dietary macronutrients on postprandial AD biomarker levels. However, in one pilot study in obese adults, two differently composed high-caloric breakfast meals led to similar postprandial decreases in NfL levels.[38] Further studies are highly needed.

We saw a rapid change in protein concentrations immediately after food intake which suggests an involvement of mechanisms beyond amyloid and tau pathology and neuronal damage and degeneration. It has been speculated that, in response to acute food intake, accumulated Aβ in tissues might be released and possibly mediated by elevated blood glucose and insulin levels.[39] Since AD is also

importantly characterized by brain glucose metabolism dysfunction,[40] it is reasonable to assume that postprandial variations in AD biomarkers are also related to postprandial glucose regulation. Moreover, it was reported that blood triglyceride levels, which increase physiologically after food intake, were associated with plasma Aβ42 concentrations.[32] Also, the once reported immediate, periodic increase in APP after food intake was found to be related to the simultaneous increase in Aβ42, possibly by saturating the turnover pathways and thus facilitating the accumulation of Aβ42.[38]

Our study investigated the impact of food intake on AD biomarkers in overweight and obese individuals. After adjusting all analyses for BMI, the postprandial changes in all biomarkers remained similar, suggesting that a BMI ranging from 27.0–39.9 kg/m^2 did not have a decisive influence on the observed food intake-related dynamics. However, it would be of great interest to investigate the impact of food intake in lean and overweight/obese individuals in a direct comparison, as previous studies found that obesity in middle-aged individuals was associated with an increased risk of cognitive impairment and AD.[41–43]

A meta-analysis of independent datasets showed that the plasma Aβ42/40 ratio in Aβ PET-positive and Aβ PET-negative groups of participants differed by ~10%.[44] Considering that we observed maximum differences between the FG and PG of 6% for the Aβ42/40 ratio, it seems clear that blood samples for amyloid analysis should be taken in the fasting state to be able to use the test as an Aβ pathology marker. Despite the fact that p-tau levels can vary up to 100% between cognitively impaired and unimpaired individuals[45] and that the food-induced concentration changes compared to the baseline levels were ~20% in p-tau181 and p-tau231, these differences might still lead to misclassification and misdiagnosis, especially in the preclinical phase where fold changes in protein levels are smaller than in manifest AD,[24] in particular in plasma p-tau231 which was recently described as superior biomarker in the preclinical stage of AD, since it reached abnormal levels with the lowest Aβ burden and was associated with longitudinal increases in Aβ PET uptake in individuals without Aβ pathology at baseline.[24,45] Moreover, it has recently been reported that measures of renal function including creatinine were positively correlated with p-tau217[46] and NfL.[46,47] We did not find significant differences in renal function between the study groups at baseline. In addition, the blood-brain barrier (BBB) has been described to be crucial for maintaining the normal metabolism of Aβ, including clearance from the brain to the blood[48,49]; BBB changes could not be investigated in our participants.

One of the biggest concentration differences between fasting and non-fasting groups was observed in plasma GFAP. It was recently proposed that the gut microbiota regulates astrocyte number, activation, and function in the brain via immune, neurotransmission, and neuroendocrine pathways.[50] In the light of dietary content determining intestinal microenvironment, even in the very short-term,[51] it can be speculated that the gut microbiota might be involved in postprandial changes in GFAP-expression. Also blood volume was described as a confounding factor for plasma/serum NfL and GFAP measurements.[52,53] However, large enough blood volume changes to explain the changes observed in our current study are unlikely to occur due to the exposures of the study participants. Furthermore, blood volume changes should result in similar changes for most of the biomarkers, which was not observed here.

In the control FG, significant divergences within the 3 h sampling period were found for GFAP, Aβ40, Aβ42, and p-tau231, even if the observed dynamics were significantly greater after food intake. Evidence on how plasma AD biomarkers are influenced by circadian rhythm or underly inter-day variations is scarce. In contrast to our findings, two studies with 10 to 12 healthy volunteers reported that Aβ42, GFAP, NT1 and total tau, and NfL did not change over a multi-day interval[33,54] or in the morning versus evening.[33] Research on the effect of circadian rhythm on plasma AD biomarkers is highly warranted.

A particular strength of this study was the use of standardized protocols for the application of the meal test, the pre-analytical handling and concentration measurements to minimize laboratory analytical influences and validated our results against a control FG. Furthermore, we included a broad panel of AD-related proteins in our analyses. It could be convincingly described that the food-induced concentration changes in AD-related biomarkers come close to pathological changes. Additionally, we observed that some biomarkers, for example, GFAP, changed over time also in fasting individuals, suggesting that a standardized time of day, next to the fasting state, for blood sampling should be considered. The major weakness of our study includes the smaller number of the individuals that attended both the FG and the PG as well as the restricted sampling period of 3 h so that a limited image of intra-day and intra-individual variations of AD biomarker concentrations has been presented. We believe that our results in obese, cognitively normal individuals with high metabolic risk have a high clinical relevance, since a large proportion of MCI or AD patients have a high average age often resulting in metabolic concomitant diseases. However, whether the results are of relevance to non-obese individuals needs to be examined in future studies.

5 | CONCLUSION

Our results showed that food intake altered the concentration of AD-related biomarkers in obese but otherwise healthy adults. Furthermore, we were able to show that biomarkers also fluctuate dynamically in the fasting state, which, however, was exceeded by the postprandial effect. The maximum divergence of biomarker concentrations between PG and FG was 30%, which has a high clinical relevance for the application of biomarkers for the diagnosis and monitoring of AD pathology. We conclude that these differences must be considered when using these biomarkers for diagnosis and monitoring of disease progression and drug response. Further studies are needed to verify whether AD blood biomarker sampling should be performed in the fasting state and at a standardized time of day to improve the diagnostic accuracy.

AUTHOR CONTRIBUTIONS

Hanna Huber and Alina Schieren conducted the clinical trial. Hanna Huber, Laia Montoliu-Gaya, Guglielmo Di Molfetta, Juan Lantero-Rodriguez, Lana Grötschel, and Birgit Stoffel-Wagner performed the laboratory analysis; Hanna Huber and Leonie Weinhold performed

the statistical analysis. Nicholas J. Ashton, Martin Coenen, Matthias Schmid, Kaj Blennow, Peter Stehle, Henrik Zetterberg, and Marie-Christine Simon supervised the trail and the laboratory and data analysis. Hanna Huber prepared the first draft of the manuscript, which was subsequently finalized in close collaboration with Nicholas J. Ashton, Laia Montoliu-Gaya, Peter Stehle, Henrik Zetterberg, and Marie-Christine Simon All authors provided substantial content contributions and edited the manuscript. Hanna Huber and Wagner S. Brum created and edited the figures. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

N.J.A. has given lectures in symposia sponsored by Eli-lily, Roche, and Quanterix. K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Ono Pharma, Pharmatrophix, Prothena, Roche Diagnostics and Siemens Healthineers, and is a cofounder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. K.B. has served as a consultant, at advisory boards, or at data monitoring committees for Acumen, ALZPath, BioArctic, Biogen, Eisai, Julius Clinical, Lilly, Novartis, Ono Pharma, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. H.Z. has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work). Author disclosures are available in the supporting information.

CONSENT STATEMENT

Informed written consent was obtained from all participants.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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