Insulin Resistance Is Associated With Enhanced Brain Glucose Uptake During Euglycemic Hyperinsulinemia: A Large-Scale PET Cohort

OBJECTIVE
Whereas insulin resistance is expressed as reduced glucose uptake in peripheral tissues, the relationship between insulin resistance and brain glucose metabolism remains controversial. Our aim was to examine the association of insulin resistance and brain glucose uptake (BGU) during a euglycemic hyperinsulinemic clamp in a large sample of study participants across a wide range of age and insulin sensitivity.

RESEARCH DESIGN AND METHODS
[18F]-fluorodeoxyglucose positron emission tomography (PET) data from 194 participants scanned under clamp conditions were compiled from a single-center cohort. BGU was quantified by the fractional uptake rate. We examined the association of age, sex, M value from the clamp, steady-state insulin and free fatty acid levels, C-reactive protein levels, HbA1c, and presence of type 2 diabetes with BGU using Bayesian hierarchical modeling.

RESULTS
Insulin sensitivity, indexed by the M value, was associated negatively with BGU in all brain regions, confirming that in insulin-resistant participants BGU was enhanced during euglycemic hyperinsulinemia. In addition, the presence of type 2 diabetes was associated with additional increase in BGU. On the contrary, age was negatively related to BGU. Steady-state insulin levels, C-reactive protein and free fatty acid levels, sex, and HbA1c were not associated with BGU.

CONCLUSIONS
In this large cohort of participants of either sex across a wide range of age and insulin sensitivity, insulin sensitivity was the best predictor of BGU.

The incidence and prevalence of obesity and type 2 diabetes (T2D) have increased continuously during the past decades and have reached epidemic dimensions (1,2). Both obesity and T2D have been linked to an increased risk of several neurodegenerative disorders, including Alzheimer disease (AD) (3,4). Thus, there is a concern that the incidence of AD could increase substantially in the future with the epidemics of obesity and T2D. This association between neurologic and metabolic disorders, as well as the incomplete understanding of the pathophysiology of obesity and insulin resistance (5), has led to an increased interest in how insulin resistance affects brain metabolism.
Positron emission tomography (PET) with $^{18}$F-fluorodeoxyglucose ([$^{18}$F-FDG]) is the gold standard technique for the in vivo quantification of brain glucose uptake (BGU) and, indirectly, of brain glucose metabolism. [$^{18}$F-FDG-PET has been used widely to study AD. It is now well established that AD is characterized by regionally specific glucose-uptake reductions in parietotemporal areas (6), posterior cingulate cortex (7), and medial temporal lobe (8). As the disease progresses, frontal cortices become also involved. BGU is associated with clinical disabilities in dementia (9), and clinical AD symptoms do not occur without decreases in BGU, the extent of which is related to the severity of cognitive impairment (10).

Whereas glucose uptake in the brain is mediated by the insulin-independent glucose transporters GLUT1 and GLUT3, insulin receptors are widely expressed in the brain. Regions with high density of insulin receptors are typically affected by amyloid plaque deposition in AD, and patients with mild cognitive impairment (MCI; a prodromal state of AD) have high rather than low brain glucose metabolism (11). On the basis of these findings, it has been suggested that patients in whom AD is prone to develop exhibit, at least temporarily, brain hypermetabolism to preserve cognitive function.

Insulin-resistant states such as obesity and T2D have been linked to an increased risk of AD, and central insulin resistance has been demonstrated in AD in ex vivo studies (12). Accordingly, studying the effect of systemic insulin resistance on BGU is of paramount importance for understanding metabolic and neurologic disorders. The existing data, however, do not provide a clear-cut answer to this question.

Whereas early PET studies reported no association between BGU and insulin sensitivity (13), others have reported that insulin resistance (assessed with HOMA-IR) and prediabetes/early-onset diabetes associate with cerebral hypometabolism under fasting conditions in key brain areas that are affected in AD (14,15). On the contrary, we have shown that during euglycemic hyperinsulinemia, obese individuals and patients with impaired glucose tolerance have higher BGU as compared with lean and normal glucose tolerant individuals, respectively (16,17), and this finding has been confirmed by us and others in humans and animals (18–20). Thus, the mixed findings, to some extent, may be attributable to the different metabolic conditions in which brain glucose metabolism was studied (fasting vs. clamp conditions) and to differences in the study populations.

In recent years, the statistical power of neuroimaging studies has been questioned, and there is consensus that larger samples and data pooling are needed to guard from false-positive and -negative findings (21). In this study, we applied Bayesian hierarchical modeling to estimate the effect of insulin sensitivity on BGU during an insulin clamp in a large sample of participants across different degrees of glucose tolerance. We also analyzed the effect of other anthropometric and biochemical parameters (sex, age, BMI, T2D, steady-state insulin and free fatty acid (FFA) levels, HbA1c, and C-reactive protein levels) to gain additional insight into the factors that may associate with BGU.

**RESEARCH DESIGN AND METHODS**

**Study Population**

We pooled and reanalyzed all studies that had brain [$^{18}$F-FDG PET scans carried out during a euglycemic hyperinsulinemic clamp. All included studies were performed at Turku PET Centre, Turku, Finland, during 2005–2020. Altogether, 194 participants were included. Of them 14% had T2D, 45% had hypertension, and 35% had dyslipidemia. Twelve percent were morbidly obese patients studied before they underwent bariatric surgery. Forty percent were healthy control participants (i.e., normal BMI; absence of T2D, dyslipidemia, or hypertension; and normal biochemical results, including renal function and transaminases). None had a clinical diagnosis of neurologic disease. Patients with T2D used either metformin (1–3 g daily), or a combination of metformin and dipeptidyl peptidase-4 inhibitors. Patients receiving insulin treatment were excluded. All participants underwent a screening visit before inclusion in the study. Metformin was withheld 24–72 h and dipeptidyl peptidase-4 inhibitors 24 h before the metabolic study.

Prior to inclusion, each participant gave written consent. Each protocol included in this study was approved by the Ethics Committee of the Hospital District of Southwest Finland (Turku, Finland) and conducted in accordance with the Declaration of Helsinki. The anthropometric and metabolic characteristics of all study participants are listed by study in Supplementary Table 1.

**Euglycemic Hyperinsulinemic Clamp [$^{18}$F-FDG Studies**

In this cohort, BGU was quantified only during a euglycemic hyperinsulinemic clamp. The euglycemic hyperinsulinemic clamp was performed as previously described (22). In brief, a primed, continuous infusion of insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was given at a rate of 40 mU·m$^{-2}$·min$^{-1}$. During the clamp, a variable rate 20% glucose solution was infused to maintain euglycemia at ~5 mmol/L. Plasma glucose levels were measured every 5–10 min throughout the clamp. At 100 ± 10 min into the clamp, $^{18}$F-FDG (187 ± 9 MBq) was injected intravenously over 15 s and the acquisition of brain radioactivity started either immediately afterward ($n = 62$) or ~1 h after [$^{18}$F-FDG injection ($n = 133$). During the clamp, samples for plasma insulin and serum FFA measurement were taken at baseline and at 30 and 60 min, respectively, thereafter.

**Quantification of Brain Glucose Uptake**

Although compartmental modeling and graphical Gjedde-Patlak analysis could have been used for the “early” data (as originally planned for these experiments (16,17)), we used fractional uptake rate (FUR) for all data to homogenize the mathematical modeling across the whole data set. Of note, the Gjedde-Patlak analysis and FUR strongly correlate with each other (23). Thus, BGU (in μmol·100 g$^{-1}$·min$^{-1}$) was calculated at the voxel level as fractional uptake rate multiplied by the average plasma glucose concentration from the injection until the end of the brain scan, divided by the lumped constant for the brain (set at 0.65) (24). For the early scans, the FUR calculation was restricted between 30 and 40 min. For the late scans, all frames were included. To account for possible differences between early and late studies, we derived a regularization parameter from an ad hoc experiment, as described in the Supplementary Material.

**Calculation of Insulin-Stimulated Glucose Disposal (M Value)**

The M value was calculated as a measure of whole-body insulin sensitivity,
as previously described (25), and expressed per kilogram of fat-free mass (\(\mu\text{mol} \cdot \text{kg}_{\text{FFM}}^{-1} \cdot \text{min}^{-1}\)), because this normalization minimizes differences due to sex, age, and body weight (26).

**Neurosynth Data Set**

To test whether regional effects of insulin-stimulated BGU colocalize with broad domains of cognition, we used meta-analytic functional MRI activation patterns for attention, language, executive function, and working memory retrieved from the Neurosynth database (https://www.neurosynth.org). Meta-analytic uniformity maps of the four selected key cognitive domains were downloaded. Next, the meta-analytic activation maps (from NeuroSynth) and the t value map of the association between BGU and the M value of our data set \((n = 194)\) were correlated at the voxel level. This approach examines the extent to which M-value–dependent BGU effects correspond with cerebral localization of different cognitive functions.

**Statistical Modeling**

We explored variables influencing BGU using Bayesian hierarchical modeling. The models were estimated with the R package **BRMS** that uses the Markov chain Monte Carlo sampling tools of **RStan** (https://mc-stan.org/users/interfaces/rstan). We estimated varying intercepts and slopes for each brain lobe and varying intercepts for the participants. To capture project-specific variation unrelated to variables of interest (e.g., effects of scanners, scan durations, source of input function, early vs. late scans), we also estimated varying intercepts for the projects. The following variables were included in the model: insulin sensitivity (as indexed by the M value), age, sex, steady-state insulin level, and presence of T2D. BMI was not included in the model, because of its high collinearity with the M value (Supplementary Fig. 1). Including all these predictors in the same model allowed us to identify the unique contribution of each of these variables while adjusting for the others. BGU values were log transformed because posterior predictive checking indicated that log transformation significantly improves model fit. For regularizing purposes, we used the standard normal distribution as the prior distribution for regression coefficients. We also provided an informative prior for the difference between early and late scans (see Supplementary Material for more details on priors and the statistical modeling). Otherwise, we used the default prior distributions of the **BRMS** package. In post hoc analyses, we also estimated the effects of FFAs \((n = 187)\), C-reactive protein \((n = 92)\), and HbA\(_1c\) \((n = 150)\). These effects were estimated by adding each of these three variables, in turn, to the main model to use maximal amount of data for each variable while adjusting for all the variables included in the main model.

**Statistical Parametric Mapping Analysis**

Linear regressions were performed in statistical parametric mapping (SPM12 toolbox for Matlab) to evaluate correlations between BGU and single regressors (M value, age, T2D, sex). The clustering threshold was set at \(P < 0.05\), and only statistically significant clusters (false discovery rate corrected \(P < 0.05\)) are reported.

**RESULTS**

**Overall Characteristics of the Euglycemic Hyperinsulinemic Clamp Studies**

The data set comprised of 194 participants. Data on the anthropometric and metabolic characteristics of all study participants are reported as means and ranges in Table 1. During the euglycemic hyperinsulinemic clamp, median serum insulin levels were 72 (interquartile range, 23) \(\mu\text{mol/L}\) at steady-state. Plasma glucose levels were maintained throughout the studies at 5.0 ± 0.3 mmol/L, FFAs were suppressed to a mean steady-state FFA value of 0.05 (interquartile range, 0.05) mmol/L. Insulin sensitivity, indexed by the M value, was reciprocally related to both steady-state insulin levels \((r = -0.27)\) and steady-state FFA levels \((r = -0.48)\), whereas there was no correlation between the M value and steady-state plasma glucose levels during the clamp \((r = 0.03)\) (Fig. 1).

**Predictors of BGU During Euglycemic Hyperinsulinemia**

Posterior intervals (80% and 95%) for each of the parameters of interest in relation to BGU are shown in Fig. 2. BGU was negatively associated with M value and age. For M value, the effect was similar across all the brain lobes. This finding was also confirmed in the statistical parametric mapping analysis (Fig. 3). For age, however, there was regional variation: the effect was strongest in limbic and temporal lobes, whereas the frontal and parietal lobes only showed a negative trend. We could not find evidence for age dependency of BGU in the occipital lobe. Sex did not affect BGU. The data also suggest that T2D, adjusting for insulin sensitivity, is associated with elevated BGU. There is, however, uncertainty about the magnitude of the effect (as indicated by the wide posterior intervals). Steady-state insulin levels did not make an independent contribution to BGU. Similarly, the post hoc analyses revealed that C-reactive protein levels, steady-state FFA levels, and HbA\(_1c\) made no unique contributions to BGU (80% posterior intervals include zero; data not shown).

**Colocalization Between M Value–Dependent BGU and Cognitive Functions**

Localization of M value–dependent BGU \((n = 194)\) was associated with localization of all the tested cognitive functions derived from NeuroSynth (Fig. 4). The strongest association was with working memory \((r = 0.13)\), whereas the association was almost nonexistent with the language-related areas \((r = 0.01)\).

### Table 1—Anthropometric and biochemical characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 63)</th>
<th>Women (n = 131)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>56 (11)</td>
<td>56 (14)</td>
</tr>
<tr>
<td><strong>BMI (kg \cdot m(^{-2})</strong></td>
<td>29 (6)</td>
<td>30 (7)</td>
</tr>
<tr>
<td><strong>HbA(_1c) (%)</strong></td>
<td>5.6 (0.3)</td>
<td>5.6 (0.4)</td>
</tr>
<tr>
<td><strong>Insulin sensitivity (mmol/mol)</strong></td>
<td>38 (4)</td>
<td>38 (8)</td>
</tr>
<tr>
<td><strong>M value ((\mu\text{mol} \cdot \text{kg}_{\text{FFM}}^{-1} \cdot \text{min}^{-1}))</strong></td>
<td>40.2 (24.5)</td>
<td>49.1 (25.3)</td>
</tr>
<tr>
<td><strong>Type 2 diabetes, n (%)</strong></td>
<td>7 (11)</td>
<td>20 (15)</td>
</tr>
</tbody>
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Euglycemic Hyperinsulinemic Clamp

- BMI: body mass index
- HbA\(_1c\): hemoglobin A1c
- M value: metabolic rate
- FFMs: fat-free mass
- FFA: free fatty acids
The underlying mechanisms for this characteristic of brain metabolism are not known. Some authors have speculated that insulin resistance does not have an effect on the expression of GLUT transporters in the brain, whereas their expression is markedly reduced in skeletal muscle in insulin resistance (19). In line with this, findings of a preclinical study indicated that whereas fasting and diabetes markedly decreased GLUT4 expression in adipose tissue, brain GLUT4 expression was only marginally affected by the same conditions (27). On the basis of recent evidence that the \[^{18}F\]-FDG uptake in the brain is driven by astrocytes (28) and that a high-fat diet leads to astrocyte proliferation and activation (called astrogliosis) (29), we hypothesize that the increased BGU in insulin resistance is driven by brain inflammation. We are investigating this hypothesis in a clinical trial (Clinical trial reg. no. NCT04343469, clinicaltrials.gov). However, if astrogliosis is one part of the picture, hyperinsulinemia is a prerequisite for the higher BGU in the context of insulin resistance, because in the fasting conditions, neither we, studying humans (16), nor Bahri et al. (19), studying minipigs, found any association between BGU and insulin resistance. In turn, systemic hyperinsulinemia may either activate central circuits directly or this effect could be mediated by the periphery through retrograde signaling to the brain. Of note, it has been shown that insulin stimulates glucose uptake in cultured glial cells from brain tissue (30) and that human astrocytes, upon insulin stimulation, synthesize glycogen and proliferate (31). All in all, astrocytes represent optimal candidate cells to explain this peculiar brain characteristic regarding BGU during insulin stimulation, but more research is warranted to reveal the underlying cellular mechanisms. Even though the relevance of our findings under clamp conditions may be criticized because of their experimental nature, systemic insulin levels achieved during euglycemic hyperinsulinemic clamps were those typically seen in the postprandial state. Information about brain glucose metabolism in more physiologic conditions is scanty, but Daniele et al. (32) found that after bariatric surgery, BGU during the oral glucose tolerance test decreased, a finding which is solidly in line with our current findings.

Previous studies in patients prone to AD under fasting conditions have reported that insulin resistance associates with brain hypometabolism in key brain areas that are affected in AD (14,15). Regarding the insulin effect, seminal work by Talbot et al. (12) showed normal activation of the insulin-signaling pathway in ex vivo studies in cognitively normal brains and brains with MCI under normal and supraphysiologic insulin levels, and of insulin resistance in AD brain slices. Accumulating evidence supports the notion that AD may be considered a metabolic disease of the brain, in which brain glucose use is impaired and, whereas early brain glucose hypermetabolism (i.e., MCI) may be considered a compensatory phase to the initial neurodegenerative insult, this compensation may eventually accelerate the degenerative process and ultimately lead to brain hypometabolism (33). Similar temporal paradoxical patterns have been described in other neuroimaging studies, in which memory-related functional MRI showed hyperactivation in less-impaired patients with MCI and hypoactivation in more-impaired patients with MCI (34). More research is definitely warranted to clarify the complex pathophysiology that links systemic metabolic and central disorders. In this context, we think a cross-sectional and longitudinal comparison of brain glucose metabolism in conditions of euglycemic clamp between BMI and age-matched individuals with normal cognition, MCI, and AD could aid understanding of the present findings.

BGU decreased with advancing age, and this effect was especially evident in the limbic lobe, in line with previous studies showing that fasting BGU decreases with aging (35). Thus, we extend this finding to the insulin-stimulated state. Several other parameters were tested for their contribution to BGU. Presence of T2D seemed to further increase BGU, although there was uncertainty about the magnitude of this effect, as indicated by the wide posterior intervals. This finding is in line with the established notion that worse metabolic control is associated with more severe insulin resistance. FFAs cross the blood-brain barrier, and we have previously shown that obese patients have increased brain FFA uptake as compared with lean individuals (36). On the basis of previous studies showing that hypothalamic sensing of circulating FFAs is important in the control of nutrient intake and energy balance (37), we hypothesized...
that FFAs could be key players in the cross-talk between brain and peripheral tissues in the context of insulin resistance. However, our data showed that when accounting for insulin resistance, steady-state FFA levels were not an independent predictor of BGU. Likewise, we did not find any evidence for an association between plasma insulin levels and BGU. In clamp experiments, a feature of patients with insulin resistance is higher plasma insulin levels compared with insulin-sensitive patients, as also seen in our study, despite similar rates of exogenous insulin infusion. Despite being higher in patients with insulin resistance, plasma insulin levels did not correlate with BGU. In a previous study, researchers showed that whereas obese patients had increased plasma insulin levels, they had relative lower central nervous system insulin levels compared with lean individuals (38), suggesting that the central effects of insulin cannot be predicted by the peripheral plasma insulin levels.

BGU in the insulin-stimulated state was not significantly affected by sex. Previous studies regarding the effect of sex on BGU have yielded mixed results (39,40). In our data, men tended to have lower BGU across all brain regions examined. However, as shown in Fig. 2, there was wide uncertainty about the effect size of sex differences in BGU, which could be the reason for the conflicting results reported in the literature.

Strengths of our study are its large size across a wide range of insulin sensitivity and age; the application of Bayesian hierarchical model for the investigation of the effects not only of insulin sensitivity but also of other potential effectors of brain glucose uptake; and the application of gold standard techniques (i.e., PET and the euglycemic hyperinsulinemic clamp). Our study has also limitations.
First, the current analysis documents associations but does not explain the mechanisms underlying the observed increase in BGU in the context of insulin resistance. Second, we combined data from several projects that originally focused on different research questions, and the data, therefore, are not optimally balanced across different covariates. However, we used a large sample, analyzed all data with the same approach, and accounted for differences in the projects using statistical modeling. Whereas graphical analysis is a more accurate method of quantification, it could not be performed for the "late" studies; thus, we chose to quantify BGU using the FUR. Even though the FUR is considered a less accurate method, it correlates very well with Patlak (23) and is a valid alternative of quantification of PET data, which could be applied more often in research settings. The individuals included in the current data set had apparent normal cognitive function, but cognitive function testing was not performed. Still, we used a meta-analytic approach that showed that BGU clusters with domains of cognitive function. Unfortunately, this type of analysis does not allow an evaluation of the brain areas involved. This further underlines the need for studies to investigate how BGU is linked to cognitive function and whether an increased BGU at baseline can predict cognitive decline in the long term. Finally, due to the physics of the PET, small brain areas such as the hypothalamus cannot be examined. Even though the study of the hypothalamus is of special interest in metabolic investigation, our results demonstrate that the interplay between insulin resistance and BGU is present at the whole-brain level.

In conclusion, in a large sample of participants across a wide range of age and insulin sensitivity, we have shown that insulin-stimulated BGU correlates negatively with the degree of insulin sensitivity. Presence of T2D was also associated with enhanced BGU and, as expected, age was a negative independent predictor of BGU. As the incidence of metabolic and neurodegenerative disorders increases, there is a compelling need to identify the common pathophysiologic pathways of these conditions, which may eventually lead to efficient treatments and prevention.

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Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. P.I., K.A.V., I.H., M.L., P.N. conceived the study design. E.R., M.B., T.K., J.C.H., A.L.-R., J.H., R.P., and L.N. analyzed data and literature and drafted the manuscript. J.C.H. conducted the clinical positron emission tomography studies. M.B., T.K., V.O., and L.N. analyzed the compartmental data analyses. A.B., I.H., M.L., E.F., P.I., L.N., and P.N. reviewed the manuscript. All authors approved the final version of the manuscript. P.N. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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AUTHOR QUERIES

PLEASE ANSWER ALL QUERIES

Q1: AQ: In the sentence beginning “At 100 ± 10 min into the clamp” and elsewhere in the article, please identify if the ± data (e.g., 100 ± 10 min, 187 ± 9 MBq, 5.0 ± 0.3 mmol/L) refer to simply “more or less,” standard deviation, standard error, or another measure.

Q2: AQ: In the sentence beginning “The cluster-forming threshold was set at $P < 0.05$,,” please confirm the abbreviation FDR was defined correctly.

Q3: AQ: In the sentence beginning “Data on the anthropometric and metabolic characteristics,” and throughout the article, “average” was changed to “mean”; is this edit OK?

Q4: AQ: In the sentences beginning “During the euglycemic hyperinsulinemic clamp, median serum insulin levels” and “FFAs were suppressed,” data are identified as interquartile ranges, but only one value is reported, not a range. Please review.

Q5: AQ: As a reminder of the previous query in the Abstract, in the sentence beginning “Plasma glucose levels were maintained” and elsewhere, where “±” data are reported (e.g., 5.0 ± 0.3 mmol/L in this sentence), please specify if they refer to standard deviation, standard error, or something else.

Q6: AQ: In the sentence that now begins “It appears to be a consistent finding that during euglycemic hyperinsulinemia,” the term “consolidated,” which was used in the original version of the sentence, was changed to “consistent.” Does the edit preserve your intended meaning?

Q7: AQ: In the Prior Presentation statement, please review the added information on meeting locations and dates and confirm they are correct.

Q8: Check that the conflict of interest information for each author is presented in full in the Duality of Interest section.