Biomarkers of Alzheimer Disease, Insulin Resistance, and Obesity in Childhood

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Abstract

Objective: To answer the question of whether onset of insulin resistance (IR) early in life enhances the risk of developing dementia and Alzheimer disease (AD), serum levels of 2 molecules that are likely associated with development of AD, the amyloid β-protein 42 (Aβ42) and presenilin 1 (PSEN1), were estimated in 101 preschoolers and 309 adolescents of various BMI.

Methods: Participants (215 boys; 48.8%) were normal weight (n = 176; 40%), overweight (n = 135; 30.7%), and obese (n = 129; 29.3%). The HOmeostasis Model of IR (HOMA-IR), HOMA percent β-cell function (HOMA-β) and QUantitative Insulin-sensitivity Check Index (QUICKI) were calculated.

Results: Obese adolescents had values of Aβ42 higher than overweight and normal-weight peers (190.2 ± 9.16 vs 125.9 ± 7.38 vs 129.5 ± 7.65 pg/mL; P < .0001) as well as higher levels of PSEN1 (2.34 ± 0.20 vs 1.95 ± 0.20 vs 1.65 ± 0.26 ng/mL; P < .0001). Concentrations of Aβ42 were significantly correlated with BMI (r = 0.262; P < .0001), HOMA-IR (r = 0.261; P < .0001) and QUICKI (r = −0.220; P < .0001). PSEN1 levels were correlated with BMI (r = 0.248; P < .0001), HOMA-IR (r = 0.242; P < .0001), and QUICKI (r = −0.256; P < .0001). Western blot analysis confirmed that PSEN1 assays measured the full-length protein.

Conclusion: Obese adolescents with IR present higher levels of circulating molecules that might be associated with increased risk of developing later in elderly cognitive impairment, dementia, and AD.

What's Known on This Subject: Insulin resistance plays a role in obesity. Recently it has been associated with increased risk of AD. Aβ42 and PSEN1 are molecules associated with increased risk of later AD. Patients affected by AD show elevated levels of plasma Aβ42.

What This Study Adds: Levels of Aβ42 and PSEN1 are significantly elevated in obese adolescents and correlated with the degree of both adiposity and systemic insulin resistance.
Insulin resistance (IR) is deemed a condition of chronic low-grade inflammation that is common root for most morbidities associated with obesity (ie, type 2 diabetes [T2D], elevated blood pressure, dyslipidemia, nonalcoholic fatty liver disease). In the past 2 decades, IR has come also into sight as being associated also with an increased risk of developing dementia and Alzheimer disease (AD). 

Epidemiologic studies in elderly, experimental investigations in humans and animal models consistently demonstrate that dysfunctional brain insulin signaling promotes and accelerates cognitive dysfunction and AD progression. This condition has been termed “brain insulin resistance” and seems to be a constant trait that precedes clinical symptoms of dementia and AD even for decades. Researchers refer to AD also as “type 3 diabetes” by virtue of the analogous brain glucose hypometabolism that characterizes both conditions and the high rate of their co-occurrence. Patients with T2D exhibit an increased risk of developing cognitive impairment and dementia and, vice versa, impaired fasting glucose and diabetes are highly prevalent among patients with AD. In a 9-year longitudinal study of 824 Catholic nuns, priests, and brothers, those with T2D showed, for instance, ~1.6-fold increased risk of developing AD. The Atherosclerosis Risk in Communities study, the Whitehall II study, and other studies have confirmed such an association, as reviewed by Cukierman et al. 

Pathologic features of AD include deposit of extracellular amyloid plaques that consist of aggregated amyloid β-protein (Aβ) and formation of soluble Aβ oligomers. Aβ originates from the proteolysis of the Amyloid Precursor Protein (APP) by the sequential enzymatic actions of β-site APP–cleaving enzyme 1 and γ-secretase. Presenilin 1 (PSEN1) is the most important transmembrane aspartyl protease for γ-secretase activity. According to the “amyloid cascade hypothesis,” the longer and more fibrillogenic form of Aβs, the Aβ42, deposits and forms senile plaques and then neurofibrillary tangles, leading to neuronal cell death, and ultimately dementia. There are almost 200 mutations in APP, PSEN1 and 2 genes that are known to lie behind most early-onset familial forms of AD. They all act by shifting the amyloid cascade hypothesis, originating very early in life.

To the best of our knowledge, no study has investigated this association in youth when brain and cognitive capacity are still developing. To accomplish the aim, we estimated serum levels of Aβ42 and PSEN1 in 440 young people, either preschoolers or adolescents, with BMI ranging from normal weight to overweight and overt obesity.

METHODS

Study Population

Between July 2012 and July 2013, white preschoolers (age range 2.0–5.8 years) and adolescents (age range 12.0–17.8 years) were enrolled at the “Ospedale Pediatrico Bambino Gesù” to participate in “The origin of cardiovascular disease study” and “Genetic risk profiles for complex diseases in the Italian population study,” respectively. Protocol description of “The origin study” is elsewhere described. In both studies, participants underwent evaluation of fasting glucose, insulin, lipid profile, liver function tests, white blood cell (WBC) count and C-reactive protein (CRP). Exclusion criteria were genetic or endocrine diseases, chronic illness, consumption of drugs affecting growth and carbohydrate metabolism, or acute infection disease as estimated by CRP ≥0.5 mg/dL and/or WBC ≥17 × 10⁹ μL (to avoid misdiagnosis of IR or impaired glucose metabolism owing to a condition of severe inflammation). WBC count was...
estimated in all, whereas CRP was assayed in participants with history of fever in the past month to exclude any viral or bacterial infection. Information on socioeconomic status (ie, parents’ annual income, employment status, and degree of education), child’s lifestyle habits, and medical history were collected by questionnaires.

In participants from the 2 studies whose parents provided consent to use data and biological materials for research other than that previously mentioned, circulating levels of Aβ42 and PSEN1 were assayed.

The study was approved by the Ospedale Pediatrico Bambino Gesù Ethics Committee. Written informed consent was obtained from parents and legal guardians. Patients’ data were treated to guarantee confidentiality.

Clinical Evaluation and Estimation of IR

Weight was measured with scales certified for medical use (90/384/ECE, SECA, Hamburg, Germany) with a precision of 50 g with children wearing minimal clothing and weight recorded to the nearest 100 g. Height was measured with a Holtain stadiometer and recorded to the nearest 0.5 cm. The average of 2 measurements was used.

Classifications of normal-weight/ overweight/obesity were defined according to the criteria of the International Obesity Task Force.

Systolic and diastolic blood pressure was measured on the right arm with the participant seated in an automated oscillatory system and appropriately sized arm cuffs (Dinamap; Criticon Incorporated, Tampa, FL).

The HOMeostasis Model Assessment of IR (HOMA-IR) was calculated as fasting insulin in mU/L × fasting glucose in mg/dL/405, HOMA of percent β-cell function (HOMA-β%) as (360 × fasting insulin in mU/L)/(fasting glucose in mg/dL − 63)%, and QUantitative Insulin-sensitivity Check Index (QUICKI) as 1/(log₁₀ fasting insulin in mU/L + log₁₀ fasting glucose mg/dL).²²

Blood Collection and Analysis

Blood samples were collected into serum blood collection tubes and serum immediately separated by centrifugation at 2465 g for 6 minutes at 4°C, aliquoted, and stored at −80°C until analysis. Aβ42 was evaluated by using a double antibody sandwich enzyme immunoassorbent assay (Elabscience Biotech, Ltd, Wuhan, China; minimum detectable value 9.38 pg/mL, intra-assay coefficient of variability <10%). PSEN1 was evaluated by using a sandwich enzyme immunoassay (Uscn, Life Science Inc., Wuhan, China; minimum detectable value 0.115 ng/mL, intra-assay coefficient of variability <12%). Other analytes were assayed as described elsewhere.²⁸

Western Blot Analysis of PSEN1

PSEN1 undergoes to a physiologic endoproteolytic process by an unknown protease that produces a heterogeneous ~29-kDa amino-terminal and ~18- to 20-kDa carboxyl-terminal fragments.³³

To assess whether the ELISA quantified the full-length protein or circulating fragments, Western blot analysis was performed on a set of random sera, 3 for preschoolers and 3 for adolescents (1 for each subgroup).

For Western blot analysis, the same amount of total serum protein for each sample was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis under reducing conditions, transferred, and blotted using specific antibody anti-PSEN1 (Abcam, Cambridge UK).

Statistical Analysis

Continuous data are reported as means and SE, with categorical data as counts and percentages. Normal distribution was tested using the Kolmogorov-Smirnov test and all the variables except BMI and fasting glucose had skewed distribution.

Skewed variables were logarithmically transformed before plotting in figures. For clarity of interpretation, results are expressed as untransformed values in the text and tables.

Between-group comparison was performed by using the χ² test for categorical variables; the Mann-Whitney U test, and the Kruskal-Wallis test with the Dunn multiple comparison test. Correlations were sought by using the Spearman test, simple and multiple linear regressions as appropriate.

The GraphPad Prism software version number 5 (San Diego, CA) was used for statistical analysis. A result with P < .05 was considered statistically significant.

RESULTS

Table 1 summarizes anthropometric and laboratory parameters of the sample population. In the whole population, BMI was significantly correlated with fasting glucose (r = 0.241; P < .0001), fasting insulin (r = 0.621; P < .0001), high-density lipoprotein cholesterol (r = −0.164; P = .01), triglycerides (r = 0.270; P < .0001), uric acid (r = 0.148; P = .035), HOMA-IR (r = 0.598; P < .0001), HOMA-β% (r = 0.203; P < .0001), and QUICKI (r = −0.583; P < .0001).

No significant difference was found between genders in age, obesity status, HOMA-IR, QUICKI, and circulating levels of Aβ42 and PSEN1; HOMA-β was significantly higher in girls than in boys (32.49 ± 31.43 vs 252.2 ± 30.92; P < .0001).

Aβ42

Serum concentrations of Aβ42 were significantly lower in preschoolers than in adolescents (Table 1). No difference was observed between normal-weight and overweight or obese preschoolers (Fig 1A), whereas obese adolescents had the highest values of Aβ42 in comparison with overweight and
normal-weight peers (190.2 ± 9.16 vs 125.9 ± 7.38 vs 129.5 ± 7.65 µg/mL; P < .0001) (Fig 1B).

In the whole sample, serum levels of Aβ42 were significantly correlated with age (ρ = 0.267; P = .0002); BMI (Fig 2A; ρ = 0.262; P < .0001); uric acid (ρ = 0.143; P = .04); fasting insulin (ρ = 0.212; P < .0001); HOMA-IR (ρ = 0.261; P < .0001) (Fig 2B); HOMA-% (ρ = 0.175; P < .0001); and QUICKI (ρ = -0.220; P < .0001). No correlation was found with fasting glucose or lipid profile (data not shown).

HOMA-IR (β = 7.097) and not BMI z-score (β = 3.244) predicted significant serum Aβ42 in a stepwise regression model (R = 0.182; P = .002).

**PSEN1**

Serum concentrations of PSEN1 were significantly lower in preschoolers than in adolescents (Table 1) with no significant difference among preschoolers with different BMIs (Fig 1C).

Obese adolescents exhibited statistically significantly (P < .0001) higher levels (2.34 ± 0.20 ng/mL) of PSEN1 than normal-weight (1.65 ± 0.26 ng/mL) and overweight (1.95 ± 0.20 ng/mL) peers (Fig 1D).

Circulating levels of PSEN1 were significantly correlated with Aβ42 (ρ = 0.298; P < .0001); age (ρ = 0.359; P < .0001); BMI (Fig 2C; ρ = 0.248; P < .0001); fasting insulin (ρ = 0.261; P < .0001); triglycerides (ρ = 0.209; P = .01); WBCs (ρ = 0.160; P = .001); HOMA-IR (ρ = 0.242; P < .0001) (Fig 2D); HOMA-% (ρ = 0.259; P < .001); and QUICKI (ρ = -0.256; P < .0001). Levels of PSEN1 did not correlate with fasting glucose or lipids (data not shown).

HOMA-IR (β = 0.168) and not BMI z-score (β = 0.007) predicted significant serum PSEN1 in a stepwise regression model (R = 0.208; P < .0001)

The Western blot analysis demonstrated that PSEN1 assays estimated the 52-kDa full-length protein (Fig 3).

**DISCUSSION**

This is the first report that demonstrates the association between systemic IR as estimated by the HOMA-IR and serum concentrations of Aβ42 and PSEN1 in young individuals. This association was independent of the BMI z-score. Interestingly, concentrations of both Aβ42 and PSEN1 were significantly higher in overweight and obese adolescents than in normal-weight peers but not yet in overweight and obese preschoolers as compared with normal-weight preschoolers.

Circulating levels of the 2 molecules increased linearly with aging from infancy to adolescence, but levels of Aβ42 tended to differ between normal-weight preschoolers and adolescents (P = .06), suggesting that overweight and obesity accelerate release of serum Aβ42.

Two studies reported an association between BMI and plasma Aβ42 in healthy adults without dementia and aging individuals. Balakrishnan et al found plasma Aβ42 correlating with BMI in 18 healthy individuals aged 23 to 64 years (r = 0.547; P = .02). On the contrary, Mayeux et al reported an inverse and poor correlation between plasma Aβ42 and BMI (r = -0.1; P = .05) in a larger sample of 530 individuals older than 65 years.

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<th>TABLE 1 Anthropometrics and Laboratory Parameters of the Sample as a Whole and in the 2 Age Groups</th>
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Results are expressed as mean ± SE or absolute number (percentage). Statistical significance at the comparison between preschoolers and adolescents: * P < .0001; ** P < .01; † P = .02.

d Thirteen data excluded.

a P < .0001 boys versus girls.
In our series, and PSEN1 levels were correlated with BMI but also with age in keeping with the findings of Meyeux et al, who observed a significant correlation with age \( (r = 0.15; P < .001) \).

Evidence from longitudinal studies in adults and elderly individuals consistently supports the notion that high levels of plasma Aβ42 work to predict the risk of developing cognitive decline and overt AD decades before the onset of the disease. In those who develop the disease, plasma levels of Aβ42 decrease at the time of the diagnosis, being inversely correlated to the cognitive decline rate and remaining, however, higher in patients than in healthy age-matched controls. However, no longitudinal study has verified yet the accuracy of Aβ42 to predict incident dementia and AD in elderly individuals, and consensus is lacking.

In our series, concentrations of Aβ42 and PSEN1 were correlated significantly with fasting insulin and IR. Balakrishnan et al observed a positive trend between levels of Aβ42 and fasting insulin \( (r = 0.422; P = .08) \) in healthy adults. In obesity, IR and low-grade inflammation may cause enhanced release even from organs other than the brain of Aβ42 (ie, the pancreas) that contributes to the impairment of cognitive capacity. Increased circulating levels of Aβ peptides may be due to the increased APP gene expression peripherally in adipocytes. Lee et al demonstrated that the APP expression is upregulated in adipocytes from obese humans and correlate with the degree of IR as estimated by the HOMA-IR, hyperinsulinemia, and expression of proinflammatory genes. Other mechanisms of increased APP expression may be related directly to the condition of hyperinsulinemia. Insulin can influence APP metabolism by accelerating APP trafficking to the plasma membrane from the trans-Golgi network, where it is generated. Therefore, hyperinsulinemia can promote APP secretion and formation of Aβ. Insulin can interfere with brain Aβ metabolism, causing increased oxidative stress, mitochondrial dysfunction, and production of advanced glycation end products. Insulin competitively inhibits the degradation of Aβs by binding the
insulin-degrading enzyme, which is responsible for the degradation of both insulin and Aβ. Competitive inhibition of Aβ degradation causes the enhanced deposit of Aβ into the neuronal cells. To this regard, patients with T2D exhibit reduced insulin-degrading enzyme activity that leads to increased levels of circulating and brain Aβ. In keeping with this notion, patients with a positive history of mild cognitive impairment showed levels of circulating Aβ42 that were correlated with fasting insulin.

Low-grade systemic inflammation with overproduction of inflammatory cytokines in individuals with IR can contribute to cognitive decline and dementia. Interleukin (IL)-1β and IL-6 have been found to disrupt neural circuits involved in cognition and memory, and elevated plasma levels of IL-6 and IL-12 levels associated with impaired processing speed and executive function at the Stroop Interference and digit symbol testing in a group of elderly participants. A recent meta-analysis confirmed the association between raised levels of CRP and IL-6 and increased risk of dementia. Peripheral cytokines can act on the brain also to cause local release of cytokines, but their effect at this stage on the prefrontal cortex is yet to be investigated.

On the contrary, no data are available from literature on circulating levels of PSEN1 in humans. Western blot analysis in our study confirmed that higher levels of PSEN1 in overweight and obese adolescents were due to raised circulating levels of the full-length protein (Fig 3), but the clinical meaning of this observation is not clear and deserves further studies. Concentrations of PSEN1 have been measured in cerebrospinal fluid and brains from patients with dementia and AD, with inconsistent results.

Phosphorylation of PSEN1 seems to increase the profibrillogenic Aβ42/40 ratio and it has been found enhanced in AD brains. However, PSEN1 phosphorylation also promotes cleavage of brain insulin receptors. The insulin receptor domain generated by γ-secretase leads to the downregulation of the activity of the glycogen-synthase kinase 3-β via transactivation of AKT. PSEN1 is an unprimed substrate of the glycogen synthase and, thus, the cleavage of the insulin receptor by the γ-secretase can in the long run inhibit PSEN1 phosphorylation and reduce the formation of Aβ42. This mechanism would, in turn, favor brain IR.

The strength of the current study is the young age of the patients and the relatively short exposure to modestly increased adiposity and reduced insulin sensitivity, particularly for preschoolers who were enrolled soon after their BMI switched from normality to overweight. All participants in the study were sufficiently “naïve” to constitute a model to study the first line of events triggered by increased adiposity that can promote cognitive impairment later in elderly individuals. Robust evidence demonstrates that obesity in childhood is associated with a reduced cognitive performance, but there is no consensus of whether the latter results from a deprived social status or is a consequence of the obesity status per se. If the social status favors unhealthy lifestyle and consequent body weight gain, results from this investigation support the concept that increased adiposity and IR may pathogenetically contribute to impaired cognitive function. In early childhood, myelination is a rapid and massive phenomenon and the developing brain is highly sensitive to any metabolic insult, including IR.

On the other hand, we are aware of methodological caveats of the current study, such as cross-sectional design of the study, lack of school-age children, no pubertal staging, no scoring of the participants’ cognitive performance, and estimation of the IR by proxy indexes instead of gold-standard measurements. With regard to the lack of information on the pubertal stage of teens and considering that, on average, girls begin puberty at ages 10 to 11 (according to the inclusion criteria, no girl was of this age) and boys at 11 to 12 years, we report results of Aβ42, PSEN1, and estimates of insulin metabolism in boys between 12 and 13 and >13 years (Supplemental Table 2). One-third of the adolescent boys were younger than 13 years whereas the older two-thirds were very probably pubertal. We observed a significant difference in the mean values of HOMA-IR between overweight individuals younger or older than 13 years as expected based on the literature, but no difference in mean values of Aβ42 and PSEN1, suggesting that the pubertal development does not exert a major effect on circulating concentrations of these molecules.

In conclusion, for the first time, we observed that overweight and obese adolescents exhibit higher levels of circulating Aβ42 and PSEN1 than normal-weight peers and their concentrations are significantly correlated with both adiposity and IR. The clinical meaning of this observation is unclear, and longitudinal studies may be helpful to this regard.

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REFERENCES


30. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide:


33. Oh YS, Turner RJ. Protease digestion indicates that endogenous presenilin 1 is present in at least two physical forms. *Biochem Biophys Res Commun*. 2006;348(1):330–334


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