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Biomarkers of Oxidative Stress, Inflammation, and Brain Damage in Mexican Women over 60 Years of Age with Obesity

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ABSTRACT

Background: Obesity and aging are risk factors for chronic degenerative diseases that favor neuroinflammation leading to cognitive and motor impairment. Mexico ranks second in obesity worldwide, being more prevalent in the female population. **Objectives:** To determine whether serum biomarkers of obesity, inflammation, oxidative stress, and brain damage vary according to age, sex, and ethnicity, we studied Mexican elderly women with obesity since this population has been historically neglected. **Methods:** A total of 156 women over 60 years of age (89 obese and 67 non-obese) were selected from the FraDySMex-2019 Cohort study samples. Serum markers of inflammation (Interleukin [IL]-6, tumor necrosis factor-α, IL-10, adiponectin, and peroxisome proliferator-activated receptor gamma [PPAR-γ]), and neurodegeneration (glial fibrillary acidic protein, brain-derived neurotrophic factor, and S100B), redox status (GSH/GSSG ratio), and protein oxidative damage were assessed. A biochemical profile was obtained and used for a factor analysis including their morphometric data. **Results:** The data from the participating elderly women clustered in relation to their obesity characteristics. The markers that were higher in obese women were GSSG, protein carbonylation, IL-6, and S100B, along with lower levels of adiponectin and PPAR-γ, suggesting they could be interesting biomarkers of neuroinflammation in obese Mexican women. **Conclusion:** Further case–control studies must be implemented to validate their prognosis value in elderly obese Mexican women with cognitive impairment. (REV INVEST CLIN. 2025;77(1):13-25)

Keywords: Obesity. S100B. Adiponectin. Redox-status. PPAR-γ. Inflammaging.

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INTRODUCTION

Life expectancy has increased globally, bringing numerous benefits but also introducing significant challenges that cannot be overlooked. These include a decline in personal autonomy, the loss of physical and mental capacities, the deterioration of family and social roles, reduced economic capacity, and the increased risk of developing incurable and progressive diseases known as chronic degenerative diseases (CDD)[1-3]. CDD creates a gap between lifespan and healthspan and represents one of the most difficult challenges faced by health systems worldwide[3]. At present, approximately 9.7% of the global population is composed of older adults, and this percentage is expected to double by 2050. Thus, finding ways to prevent CDDs and mitigate the deterioration associated with them is imperative.

Obesity is an important risk factor for developing CDD. The World Health Organization (WHO) defines obesity as an abnormal or excessive fat accumulation that poses a risk to human health. Obesity has been declared a public health emergency, as the global population of adults with obesity has nearly tripled over the last 50 years, with the highest prevalence among women[4]. Mexico ranks second globally in obesity prevalence; according to the National Health and Nutrition Survey[5], 75.2% of people over the age of 20 are overweight or obese, and the proportion is higher among women (76.8%) than men (73.5%). Obesity increased by 21.4% between 2006 and 2022, and it is remarkable that the population of adults between 40 and 60 years of age had the highest prevalence (85%)[5].

In obesity, fat accumulation leads to adipose tissue expansion, generating hypertrophic adipocytes that increase the production of various pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), whereas reducing anti-inflammatory molecules such as IL-10 and adiponectin. Adiponectin is an adipokine related to lipid metabolism, and its reduction has been linked to an increased risk of cardiovascular disease and metabolic syndrome[6]. Another molecule involved in obesity-related inflammation is peroxisome proliferator-activated receptor gamma (PPAR-γ), which regulates inflammatory responses by reducing the synthesis of pro-inflammatory mediators and enhancing the expression of antioxidant genes. In addition, PPAR-γ raises blood levels of adiponectin, improving lipid and glucose metabolism[7].

Aging also affects the immune system, triggering sterile inflammatory responses and creating a chronic state of low-grade inflammation known as "inflammaging"[8]. When aging is coupled with obesity, it creates a dual inflammatory stimulus that exacerbates systemic damage[9]. Chronic inflammation also impacts central nervous system (CNS) function by disrupting the blood–brain barrier (BBB) through structural changes in brain microvasculature and endothelial dysfunction. This results in neuroinflammation and increased oxidative stress[10]. Astrocytes, the most abundant brain cells, act as immunoreactive cells, initiating neuroinflammation in response to damage-associated molecules or pathogens, and shifting from a neuroprotective to a proinflammatory phenotype[11]. Reactive astrocytes are characterized by increased expression of glial fibrillary acidic protein (GFAP), which can cross the BBB into the serum and serve as a potential biomarker for neuroinflammation and neurodegenerative changes[12]. Similarly, the calcium-binding protein S100B, also present in astrocytes, has emerged as a serum biomarker for neuronal damage and neuroinflammation[13,14]. Another promising serum marker is brain-derived neurotrophic factor (BDNF), which plays a crucial role in the survival, proliferation, and maturation of neurons. Pro-inflammatory cytokines produced during obesity have been shown to significantly reduce BDNF expression, which has been linked to cognitive impairment[15].

Thus, both obesity and aging are risk factors that may promote neuroinflammation, leading to pathological changes associated with the decline of cognitive and motor functions[16]. As in other countries, the prevalence of obesity in Mexico is higher among women than men[17,18]. In addition, elderly Mexican women face more barriers to accessing health programs and interventions that promote healthy lifestyles, which perpetuates their involvement in the vicious cycle of obesity and declining health[19]. Serum biomarkers have been reported to vary based on age, sex, and ethnicity[20], making it important to evaluate markers of obesity, inflammation, oxidative stress, and brain damage in a population of obese older Mexican women, a group that has historically been underserved.

For this purpose, 89 women with obesity (body mass index [BMI] > 30) and 67 non-obese women (BMI < 30) over the age of 60 were selected from the FraDySMex-2019 Cohort study[21]. Serum markers of inflammatory status, neuroinflammation, and neurodegeneration were assessed, along with changes in redox state (GSH/GSSG ratio) and protein oxidative damage. A biochemical profile was obtained and used for a factorial analysis (FA) in conjunction with data from FraDySMex-2019 related to clinical variables such as BMI, fat percentage, and age.

Our analyses showed that elderly women cluster based on their obesity characteristics. A decrease in serum-reduced glutaraldehyde (GSH), adiponectin, and PPAR-γ, along with an increase in serum oxidized glutaraldehyde (GSSG), IL-6, and S100B, may be proposed as biomarkers predictive of brain CDD risk in elderly women with obesity.

METHODS

Study population

This study is a secondary cross-sectional analysis from the prospective cohort study Fragility, Dynapenia, and Sarcopenia in Mexico (FraDySMex) that was carried out in community-dwelling adults aged 50 and over living in Mexico City[21]. The study was conducted with residents of three municipalities (out of 16) in the southwest of Mexico City (Cuajimalpa, Magdalena Contreras, and Álvaro Obregón). These three municipalities comprise 12.5% of the total number of people aged 60 and over in Mexico City. Some characteristics of the FraDysMex-2019 original cohort that are important to contextualize our findings are that the mean educational level was 9.1 years, 63.8% of women were homemakers, and 78.5% of the participants had a good nutritional status. The original study included men and women, but no significant differences were found between the sexes regarding the scores in dependence on basic activities of daily living (BADL), instrumental activities of daily living (IADL), and low physical performance (PP). The mean BADL score was 98.5 suggesting near-total independence, with possibly minimal assistance or difficulty in one minor area. The IADL score of 7.6 suggests that the patients were mostly independent, with minor impairments or limitations but overall good functionality in managing daily activities. The mean PP score was 2.1, which corresponds to low PP. This cohort study has had three waves (2014, 2015, and 2019). To calculate the study size, the following formula was used to determine the sample size for a finite population:

$$n = \frac{NZ^2\,pq}{\alpha^2\,(N-1) + Z^2\,pq}$$

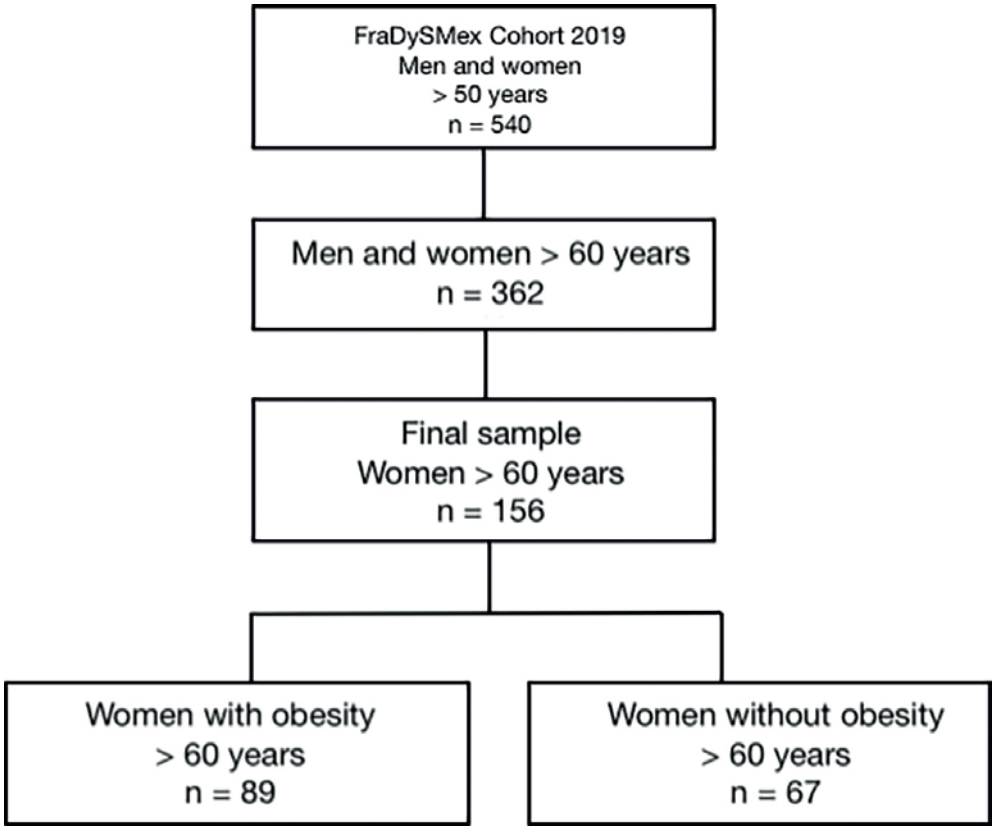
Where N = population size; Z = 95% confidence level (critical Z = 1.96); α = statistical significance level (0.05); p = percentage of older women with obesity (64%); q = percentage of older women without obesity (36%). The calculated sample size was 159 (Fig. 1). Therefore, in this study, consecutive data from the wave of 2019 (October to December) of 89 women with obesity (BMI > 30) and fat percentage > 40, and 67 non-obese women (BMI < 30) and fat percentage < 40, over 60 years of age were analyzed. The 156 people participating in the FraDysMex-2019 cohort were those who had a blood sample and completed biochemical and morphometric evaluations. The inclusion criteria for the population of women in the FraDysMex-2019 cohort were the following: (A) women able to move alone with or without assistance devices, and (B) able to answer the evaluation questionnaires alone or assisted by a caregiver. The exclusion criteria were persons living in institutions or nursing homes, those with decreased alertness, and the presence of any acute or chronic condition evaluated by a multidisciplinary team at the Functional Evaluation Research Laboratory at the National Geriatric Institute (INGER).

The FraDySMex-2019 Cohort study was approved by the INGER Ethics Committee with the registration number DI-PI-009/2019 in the official notice INGER-DG-DI-SIEG-JDEC-025-19. The assessments performed in this new part of the study were approved by the Ethics Committee of the UAMI with the resolution number CECBS23-01.

Morphometric determinations

The morphometric determinations were obtained from the FraDySMex-2019 Study[21]. Briefly, height and weight were used to obtain the BMI. In addition, body composition was determined by dual X-ray

Figure 1. Study sample calculation.



densitometry. Obesity was defined when the women presented a percentage ≥ 40% of total body fat along with BMI score.

Serum cytokine and brain markers determination

Blood samples were centrifuged at 3,500 rpm at 4°C for 15 min to obtain serum. Serum cytokine and neural markers concentration was determined by enzyme-linked immunosorbent assay as reported previously[22]. IL-6, TNF-α, IL-10, adiponectin, GFAP, S100B, and BDNF were evaluated following the manufacturer's instructions. Sera were incubated for 18 h at 4°C with PBS-Tween20 (0.05%)/0.5% BSA, washed 3 times, and incubated with the corresponding detection antibody for 2 h at RT. The bound antibodies were detected using the a-HRP (avidin-HRP/streptavidin-HRP) system using TMB/H2O2 as substrate. Optical density readings were done at 450 nm (Epoch-BioTek, VT, USA).

Redox state determination (GSH/GSSG ratio)

GSH and GSSG concentrations were determined by high-performance liquid chromatography (HPLC) following the reported protocol[22]. 50 µL of blood dissolved in 800 µL of lysis buffer (10% HCl and 10 mM bathophenanthroline+disulfonic acid) were used. The suspension was centrifuged at 13,500 rpm and 4 °C for 5 min. The supernatant was recovered and 50 µL were injected into an HPLC using a 1525 binary pump (Waters, MA) connected to a Zorbax Eclipse XDB-C18 reverse phase column (Agilent, CA; 250 × 4:6 mm i.d., particle size: 5 µm). The mobile phase consisted of 1% acetonitrile and 20 mM monobasic phosphate buffer at pH 7.0, at a flow rate of 1 mL/min. GSH and GSSG were detected in the same run at 210 nm using a 2489 UV/visible detector (Waters, MA). The area under the curve was quantified and the concentration of the analytes was detected using a standard curve of GSH and GSSG at 10, 25, 50, 50, 100, 200, and 400 µM.

Protein oxidative damage determination

Protein carbonylation was quantified[23]. Blood was centrifuged at 5000 × g for 15 min at 4°C for protein isolation. Supernatants were recovered and 20 µL of the sample were placed in a 96-well plate, along with 20 µL of dinitrophenylhydrazine (DNPH; 10 mM dissolved in 0.5 M H_3PO_4). The plate was incubated in the dark for 10 min at RT. Afterward, 10 µL of 6 M NaOH were added and incubated for an additional 10 min in the dark. Lectures were obtained at 450 nm in an H Reader-1 Elisa Reader (HLAB) microplate reader. Carbonyl concentration was determined by multiplying the absorbance value at 450 nm by the extinction factor (46.1). The value obtained was adjusted with the protein concentration in each sample.

Western blot

Blood samples were centrifuged as described above to obtain the serum and subsequently, proteins were isolated using the lysis buffer: 100 µL of 1M dithiothreitol, 100 µL of phenylmethylsulfonyl fluoride (PMSF) 0.1M, 1 tablet of mini-Complete™ (Roche, USA), 10 mL of T-PER™ (Thermo-Scientific). The homogenate was centrifuged at 13,500 rpm at 4°C for 15 min. The supernatants were reserved and stored at −80 °C. Total protein concentration was determined using the bicinchoninic acid (BCA Protein Assay Kit, Thermo-Scientific), according to the manufacturer's instructions.

Proteins (30 µg) were separated on 12% SDS-PAGE and transferred to PVDF membranes (Amersham HybondTM-P); non-specific antibody binding sites were blocked with intercept (PBS) blocking buffer solution (Licor Biosciences, USA) and the membranes were washed with TBS-Tween20TM (Thermo-Scientific, USA) and incubated overnight with anti-PPAR-γ 1:1000 primary antibody (A3409A, Invitrogen), or GAPDH 1:1000 (Santa Cruz Biotechnology sc-32210) as a constitutive control. Membranes were washed 3 times with TBS-Tween and incubated with secondary goat anti-mouse infrared (IR) Dye IRDYE680 or IRDYE800 antibody (1:5000), respectively (Licor Biosciences, USA) for 1 h. After 3 consecutive washes, the blots were analyzed on a laser scanner Odyssey CLx near-infrared imaging system (Licor Biosciences, USA) at IR 680nm and IR700nm. The Odyssey system was set at 21µm resolution, high scan quality, and auto-intensity mode, and images, as well as absolute units (A.U.) values from each well, were acquired using the Licor Image Studio Software (version 3.1)[24].

Statistical analysis

Univariate statistical analyses were conducted using Prism 7.0 software (GraphPad Software, San Diego, CA, USA) to describe and compare data across the study groups. Results are reported as the mean ± standard deviation for each surveyed group. The Shapiro–Wilk test was employed to assess the normality of the data distribution.

For comparing differences between groups, independent sample *t*-tests were utilized for normally distributed data, whereas non-parametric tests, such as the Mann–Whitney *U* test, were considered for data that did not meet normality assumptions. A significance level of $p < 0.05$ was set to determine statistically significant differences between groups. Cohen's *d* values were also reported as effect size measures.

Factorial analysis

To manage the extensive number of variables in our study and elucidate underlying structures, a FA was conducted to reduce data dimensionality and consolidate the variables into a smaller number of factors. This analysis utilized the covariance matrix between variables, employing maximum likelihood as the factoring method and varimax rotation for clearer factor interpretation. A Kaiser–Meyer–Olkin (KMO) test was performed to assess sampling adequacy, ensuring the suitability of the data for FA. Variables with individual measures of sampling adequacy (MSA) ≤ 0.6 were excluded, as they did not significantly contribute to the model according to the Kaiser criterion[25]. The analysis was guided by both a scree plot and an examination of eigenvalues to determine the optimal number of factors to retain. According to Kaiser's criterion[25], we retained factors with eigenvalues > 1, resulting in the selection of three factors (Eigenvalues: factor 1 =3.0725; factor 2 = 1.4107; factor 3 = 1.0572), which collectively explained 61.56% of the total variance ($\chi^2 = 284.003$, 36 degrees of freedom, $p < 0.0001$). All graphs and the FA were executed using JMP Pro16 software.

Table 1. Morphometric variables of 156 women over 60 years of age from the FraDySMex-2019 cohort study

Variable	No obesity	Obesity	Probability
N (%)	67 (43%)	89 (57%)	
Age (years)	70.4 ± 10.69	69.22 ± 10.36	p = 0.5135
Height (cm)	151.67 ± 6.16	149.18 ± 5.91	p = 0.1205
Weight (kg)	55.15 ± 7.5	66.12 ± 9.37	p < 0.0001
BMI (kg/m^2)	23.94 ± 2.68	30.32 ± 4.46	p < 0.0001
% Fat	43.87 ± 2.83	35.27 ± 3.31	p < 0.0001

RESULTS

Morphometric data

Morphometric data were obtained from the FraDyS-Mex Study[21] by studying 156 women over 60 years of age, 89 of whom met the criteria for obesity outlined by the WHO (BMI > 30) and 67 who were not obese. Table 1 shows the main and general characteristics of the cohort; no differences in the dispersion of data by age between the two study populations, women with obesity (70.34 ± 10.69 years) and women with normal weight (69.22 ± 10.36 years). Table 1 also shows the percentage of fat, body weight, height, and BMI, for the two populations. As expected, the women in the obesity group showed a significant increase in fat, body weight, and BMI compared to those non-obese: Weight 66.12 ± 9.37 versus 55.15 ($p < 0.0001$); % body fat 43.87 ± 2.83 versus 35.27±3.31 ($p < 0.0001$); BMI 30.32 ± 4.46 versus 23.94 ± 2.68 ($p < 0.0001$).

Serum concentrations of cytokines and PPAR-γ in obese and non-obese elderly women

Figure 2 shows the concentrations of the different cytokines evaluated. Adiponectin content decreased by almost 45% in obese women regarding those non-obese ($p < 0.0001$, $d = 0.765$) (Fig. 2A), and an increase of 39 % ($p < 0.0001$, $d = -0.727$) in IL-6 concentrations (Fig. 2B). No statistically significant differences were found in TNF-α and IL-10 concentrations (Fig. 2C and D) ($p < 0.06$, $d = -0.077$).

Figure 2E shows a representative gel for PPAR-γ content, with samples from seven obese and seven non-obese women, whereas in figure 2F, the densitometry performed considering all the samples from each study population is presented. The results were first normalized against the internal loading control (GAPDH), and then, against the values obtained for non-obese women. Therefore, the results indicated that non-obese women had approximately 35% higher values of PPAR-γ than those who were obese ($p = 0.0003$, $d = 0.045$).

Obese elderly women presented increased oxidative stress

Serum-reduced glutathione concentration (GSH) (Fig. 3A) did not show differences between the groups, although there was a significant increase in oxidized glutathione (GSSG) ($p < 0.0001$, $d = 0.124$) (Fig. 3B). The same was reflected in the GSH/GSSG ratio (Fig. 3C), indicating that there is greater oxidative stress damage in women with obesity and a worse redox state. The concentration of carbonylated proteins in obese individuals was around 46 % higher ($p < 0.0001$, $d = -0.563$) (Fig. 3D).

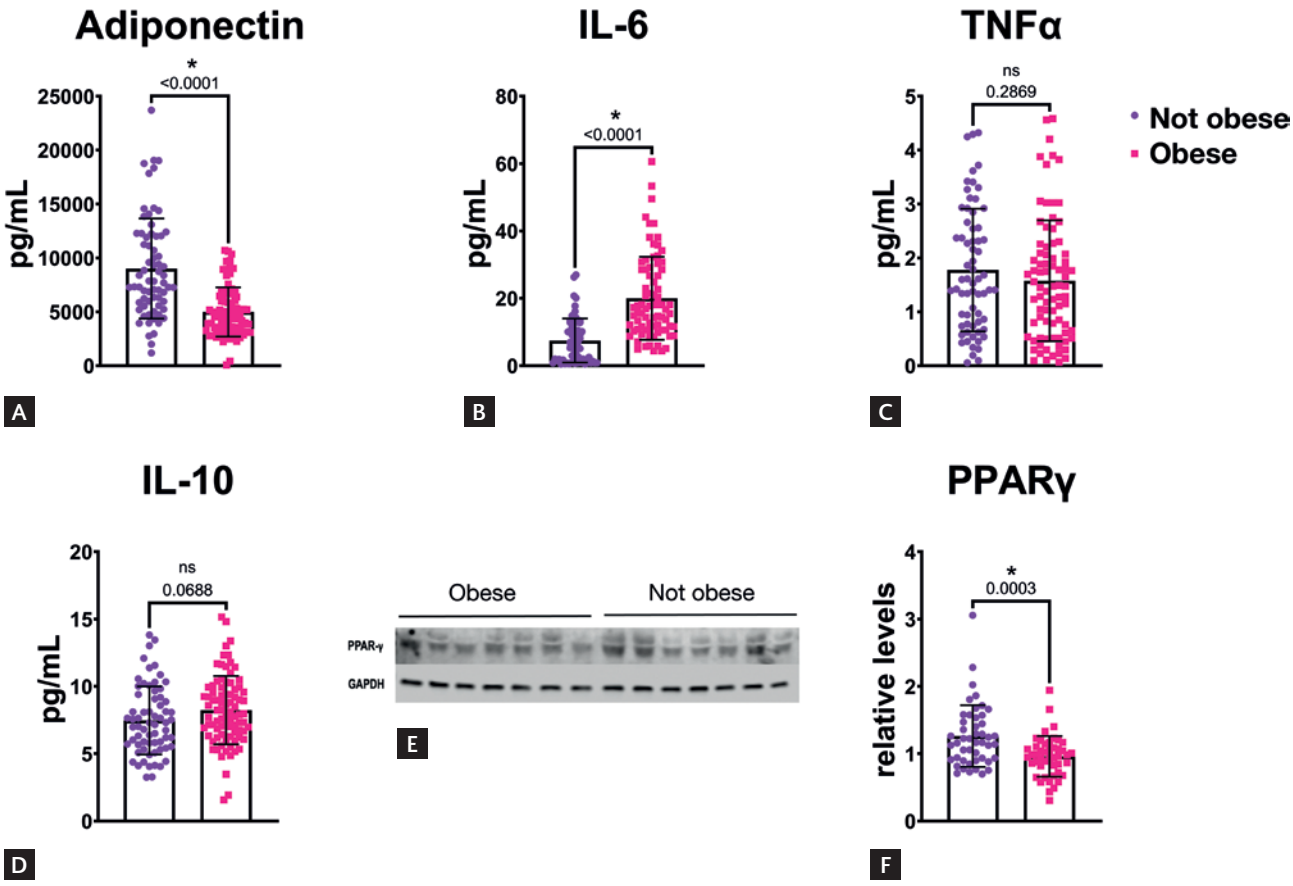
S100B gliosis marker increased in the serum of obese elderly women

No significant differences were found in BDNF nor GFAP content (Fig. 4A and B). Conversely, a significant increase in the S100B gliosis marker was found ($p < 0.01$) (Fig. 4C) ($p < 0.01$, $d = -0.303$).

Factorial analysis

FA was performed to identify latent structures within our dataset, simplifying interrelated variables into distinct factors. This approach revealed key relationships

Figure 2. Circulating cytokine and PPAR-γ levels in elderly obese and non-obese women. **A:** adiponectin; **B:** TNF-α; **C:** IL-6; **D:** IL-10; **E:** representative blot for PPAR-γ; **F:** PPAR-γ densitometry. For **A, B, C & D:** each point in the study population represents one woman over 60 years of age. * p < 0.001, Mann–Whitney test for unpaired samples. For **E** & **F:** PPAR-γ protein was assessed using the Western blot technique described in the Methods section. Each bar represents the average densitometry of the results normalized with the GPDH loading control and then against those with obesity as a value of 1.0. * p < 0.05, Mann–Whitney test for unpaired samples. Mean ± Standard error is represented.



between metabolic, oxidative, and inflammatory markers, differentiating obese from non-obese groups and shedding light on pathways related to cognitive impairment risks. FA is well-suited for complex, multidimensional data like ours, where high inter-variable correlations are expected. We reduced variables to the minimum number of factors that explained at least 60% of the phenomenon. Three factors explained 61.56% of the total variance. In the X and Y axes of figure 5A and B, factor 1 explains 34.1%, factor 2 explains 15.7%, and factor 3 explains 11.7% of the total variance. Variables such as BDNF, TNFα, IL-10, GSH, GSSG, the GSH/GSSG ratio, and age were excluded due to low individual MSA (< 0.6), leaving carbonylated proteins, body weight, BMI, fat percentage, GFAP, PPARγ, adiponectin, IL-6, and S100B in the final FA model. Figure 5 illustrates the variable data of each woman plotted without categorical variables for a blinded analysis. Eigenvalue-based vectorial scores were plotted across three factors (figure 5B), with positive numbers indicating positive associations, negative numbers indicating negative associations, and near-zero scores suggesting minimal associations. Black dots represent non-obese women, whereas gray squares denote obese women. The graphs of factor 1 versus factor 2 (upper quadrant) and factor 2 versus factor 3 (lower right quadrant) in Fig. 5A shows distinct clustering of the two groups, suggesting different associations with the variables. No clear separation was found between groups in factor 1 versus factor 3, indicating a shared association with those variables.

Figure 3. Redox state analysis of elderly obese and non-obese women. **A, B:** level of circulating GSH and GSSG in serum; **C:** evaluation of redox state (GSH/GSSG ratio); **D:** carbonyl protein levels in the blood. Each point in the study population represents one woman over 60 years of age. * p < 0.001, using the Mann–Whitney test for unpaired samples. Mean ± Standard error is represented.

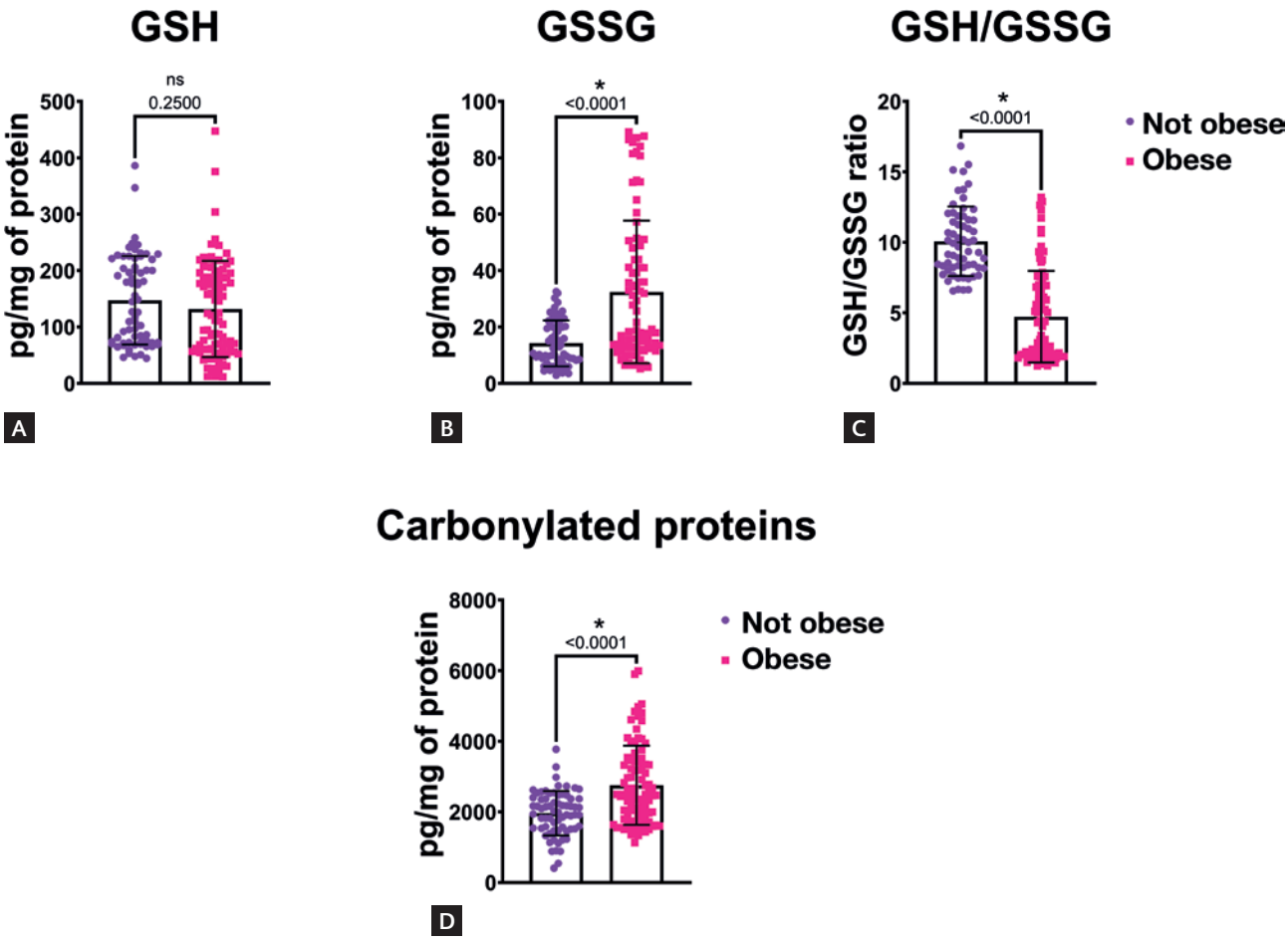


Figure 4. Circulating brain damage markers in elderly obese and non-obese women. **A:** BDNF; **B:** GFAP; **C:** S100B. Each point in the study population represents one woman over 60 years of age. * p < 0.01, Mann–Whitney test for unpaired samples. Mean± Standard error is represented.

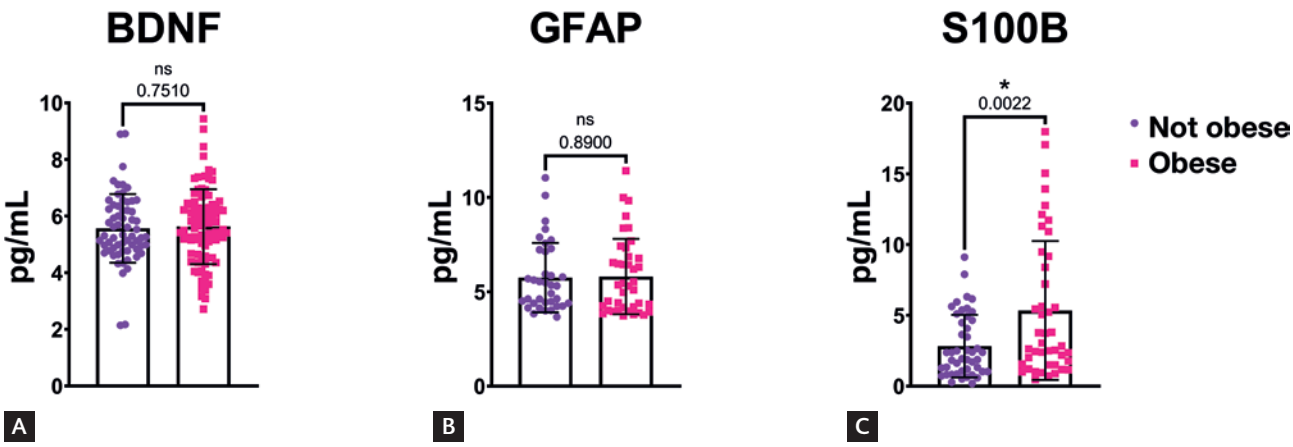
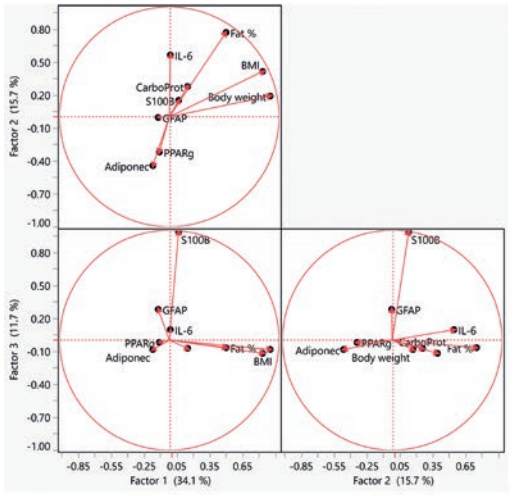
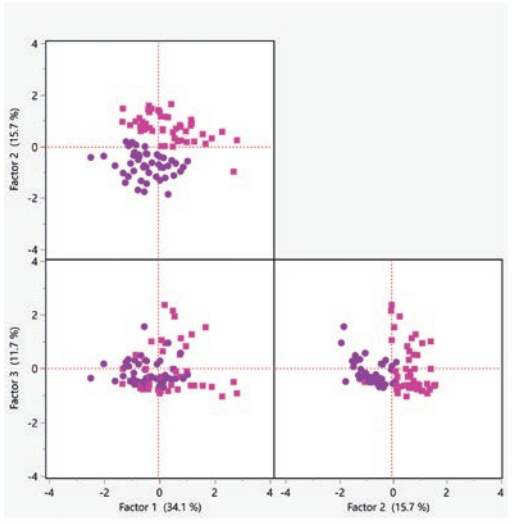


Figure 5. Factorial analysis. **A:** biplot; **B:** loading plot; **C:** score plot. Each point in the study population represents one woman over 60 years of age. Black circles represent non-obese women and gray squares represent obese women; red arrows represent graphically each variable vectorial load; each variable total load is represented as a black dot on the head of arrows. Red circles represent the confidence ellipses for each plot.



A



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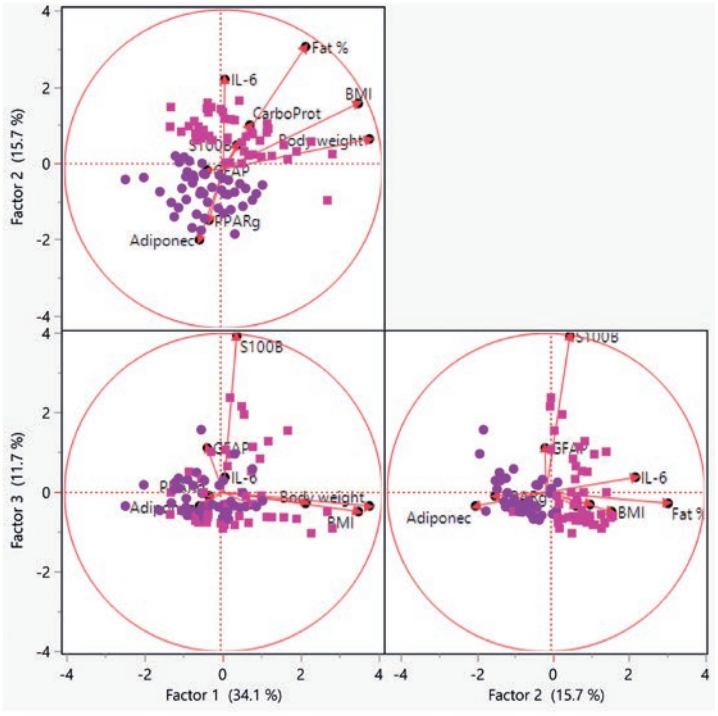

C

Figure 5B shows the strength and direction of variable associations with each factor, represented by arrow length and direction. The longer the arrow, the stronger the association with the corresponding factor. Figure 5C overlays figures 5A and B, confirming the model's validity. It reveals strong associations between obesity-related variables and obese individuals, whereas S100B showed a milder association. Non-obese women were associated with adiponectin and PPAR-γ, markers of healthy metabolic function. In the lower right plot (factor 2 vs. factor 3), obese women clustered on the right and non-obese on the left, mirroring the first plot's findings. Obese individuals were strongly associated with S100B, IL-6, and mildly with GFAP, whereas non-obese women were linked to adiponectin and PPAR-γ. Overall, non-obese women were associated with markers of healthy metabolic function, whereas obese women correlated with markers of oxidative stress, inflammation, and high-fat mass.

DISCUSSION

Mexico ranks second worldwide in obesity prevalence, and since this condition is recognized as a major risk factor for CDD, significant efforts have recently been made in the country to understand, evaluate, and address this public health problem. In the last years, numerous studies have been conducted to identify markers for diabetes, metabolic syndrome, and other obesity-related conditions[26,27]. Significant progress has been made in identifying gender-specific markers and single-nucleotide polymorphisms associated with metabolic syndrome and diabetes[28,29]. However, most obesity-related studies have relied on traditional biochemical markers such as glucose, insulin, triglycerides, and HDL cholesterol[30]. To date, no studies have investigated inflammatory and brain damage markers alongside oxidative stress in obese elderly Mexican women. Despite global efforts to identify health markers for older adults, results remain inconsistent due to variations in age, ethnicity, and sex. This is particularly relevant for obese elderly women, who are at higher risk. Therefore, studying this specific population is imperative, as obesity in aging can lead to physical frailty and cognitive impairment, reducing the ability to perform daily activities, diminishing quality of life, and increasing the risk of premature death[31].

To address this gap, we obtained experimental data on inflammatory molecules, brain damage markers, and oxidative stress. We conducted a FA to explore their relationship with obesity and propose biomarkers that could serve as predictors of damage and neuroinflammation associated with obesity in elderly Mexican women.

The most commonly used blood and serum markers of oxidative stress in women with obesity are malondialdehyde and thiobarbituric acid-reactive substances, which are indicators of lipid oxidation[32]. These are often analyzed alongside total antioxidant capacity and antioxidant enzymes such as superoxide dismutase[33]. Interestingly, very few studies report the GSH/GSSG ratio or carbonyl levels. Therefore, it was interesting to find that in our study, the GSH/GSSG ratio was lower, and oxidized carbonyl levels were higher in obese women compared to lean women, reinforcing the validity of our findings.

Regarding the relationship between cognitive decline and acute inflammation markers (TNF-α, IL-6, IL-1β, IFN-γ, IL-2, IL-4, IL-10, and hs-CRP), only limited results have been reported, concluding that these markers alone are insufficient for predicting cognitive impairment, and therefore suggesting the need for additional determinants to improve the understanding of the phenomenon and its clinical implications34. Here we found a significant increase in IL-6 levels in the obese population compared to the lean population, whereas no differences were observed for IL-10 and TNF-α. However, a positive association between elevated IL-6 levels, cognitive impairment, and obesity still needs to be evaluated. Decreased serum adiponectin levels in obesity have been related to cardiovascular disease, diabetes, osteoporosis, cancer, and other CDD. More recently, adiponectin levels have also been used as a marker in severe cases of dementia and Alzheimer's disease, although their predictive value in mild cognitive decline remains controversial[35]. A positive association between higher serum adiponectin levels and improved cognitive function in obese postmenopausal women has been reported[36]. Moreover, obese or overweight postmenopausal Mexican women showed a lower expression of adiponectin and a higher *ADIPOR1* expression in breast cancer tissue when compared with women with a normal BMI[37]. However, there are basically no studies that evaluate this adipokine alongside brain damage markers such as GFAP or S100B.

Conversely, PPAR-γ has been much less explored as a biomarker of cognitive decline in obesity and aging. Despite this, it has been studied in Alzheimer's disease, where PPAR-γ agonists have been shown to improve cognitive and functional outcomes by reducing the inflammatory response[38].

Recent studies have reported that low molecular weight adiponectin can cross the BBB and bind to specific receptors, AdipoR1 and AdipoR2, which are found in several brain regions, including the hippocampus and cortex. AdipoR2 activates the PPAR-γ pathway, leading to the reduction of inflammatory molecules such as IL-6, inhibiting oxidative stress, and producing neuroprotective effects. This mechanism helps restore microglial function and mitigates the cognitive deficits caused by a high-fat diet[39]. In line with these findings, here we found lower levels of adiponectin and PPAR-γ in the population of obese

women, and both molecules were significantly represented in our FA. This suggests that they could be promising candidates for further investigation as potential biomarkers of cognitive decline related to obesity in women. Numerous studies have reported a negative correlation between serum BDNF levels, obesity markers, inflammation, and cognitive decline in animal models[22]. However, in humans, findings are contradictory, with some studies showing improvements whereas others report worsening memory and cognition in relation to changes in BDNF concentration[15,40,41]. Our results showed no significant differences in BDNF levels and this neurotrophic factor did not play a key role in the FA. S100B and GFAP have not been extensively studied in relation to oxidative stress, with most research focusing on their role in inflammation. Their significance in obesity remains controversial[42]. Still, they are common markers that are often used interchangeably or together but have different origins and therefore different implications. GFAP is a protein primarily of astrocytic origin, forming part of the astrocyte's cytoarchitecture, hence it should not be found in the cerebrospinal fluid or the systemic circulation, and its presence indicates a loss of cellular (mainly glial) integrity and damage[43]. On the other hand, S100B is a cytoplasmic protein actively secreted by cells, serving various extracellular functions. In the CNS, it is primarily produced by astrocytes and oligodendrocytes, with its effects being concentration-dependent. At nanomolar concentrations, S100B has neurotrophic effects, whereas micromolar concentrations can lead to cell death and neurotoxicity[14]. As a result, circulating levels of these two proteins have been used as markers of brain damage and neurodegeneration. GFAP has shown promise as a marker for traumatic brain injury (TBI), stroke, and mood disorders such as major depressive disorder. However, its role as a biomarker for cognitive function or health-related quality of life remains under debate[44,45]. The case of S100B is more complex. While it is widely recognized as a biomarker for assessing brain damage in neurodegenerative diseases, depression, TBI, and BBB integrity, the fact that S100B can be produced outside the CNS raises questions about its direct role in the brain[13,14,46]. S100B is produced by various non-neural cells, most notably adipocytes[42], leading to the suggestion that it may act as an inflammatory adipokine within the CNS[14,47]. Recently it has been proposed that S100B could help relate and explain the association between obesity and cognitive impairment leading to dementia and could be used as a therapeutic target for different diseases of inflammatory origin[13,47]. In our study, the significant elevation of S100B levels in obese women, along with its prominent role in the FA, is encouraging for further investigation of this molecule as a prognostic biomarker. While we did not find direct differences in GFAP levels between groups, it also played a role in the FA, suggesting that its evaluation in future studies should not be dismissed.

Our study was not designed as a case–control investigation of elderly women with cognitive impairment, but rather focused on obesity. Therefore, the next step should be to specifically select obese women with cognitive decline to evaluate the markers proposed here. An interesting and unexpected outcome of this study, as we searched for information on biomarkers used to predict cognitive impairment in obese elderly women, was the variability of reported results. Depending on various conditions, biomarkers were reported to increase, decrease, or remain the same, highlighting the challenge of identifying reliable biomarkers for accurately diagnosing and predicting pathological outcomes.

These discrepancies may be attributed to several factors, including sex differences, ethnic variations, molecular polymorphisms and isoforms, and epigenetic differences. Lifestyle factors such as diet, exercise, and habits also play a crucial role[42,48,49]. Furthermore, recent studies suggest that genetic factors may increase the propensity for obesity and obesity-related morbidities, but the extent to which these are modifiable by lifestyle factors remains unclear[50].

It has been reported that factors such as age, sex, urban location, marital status, socioeconomic status, and education level influence obesity, especially in middle- and low-income countries[51]. In addition, obese women tend to have different nutrition, low physical activity, and many other factors.

In Mexico, researchers such as Quezada and Lozada-Tequeanes[52], and Levasseur[53], emphasize that poor women in urban areas are disproportionately affected by obesity, with marriage being another contributing factor. Therefore, incorporating these variables into future studies would be highly beneficial. Another critical issue is the general lack of knowledge about

healthy lifestyles. Interestingly, women who received care at family medicine clinics and self-reported having a healthy lifestyle had a 12% lower BMI compared to those who did not. However, it is unclear whether individuals with a high BMI truly understand what constitutes a healthy lifestyle. This highlights the need to improve education and awareness surrounding healthy habits. Therefore, in addition to quantitative analyses, it would be desirable to conduct qualitative studies that explore individuals' life trajectories to improve the understanding of the different outcomes.

To our knowledge, this is the first study to evaluate molecular biomarkers in obese Mexican women over 60 years of age. Hence, it is essential to continue with a case–control cohort study involving obese women with cognitive decline. The most attractive molecules for further evaluation are IL-6, S100B, GFAP, adiponectin, PPAR-γ, and GSSG. In addition, it would be desirable to perform longitudinal studies with this type of cohort to strengthen the results. Being able to find biomarkers to predict the risk of developing cognitive impairment in elderly Mexican women is very important for early detection and to generate a possible comprehensive intervention. Nevertheless, it is important to recognize and make visible a national health problem that is not fully acknowledged.

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SUPPLEMENTARY DATA

Supplementary data are available at DOI: 10.24875/RIC.24000207. These data are provided by the corresponding author and published online for the benefit of the reader. The contents of supplementary data are the sole responsibility of the authors.

REFERENCES

1. Muradás Troitiño MC. Panorama demográfico de México, 2019. In: La Situación Demográfica en México. Hamburgo: Consejo Nacional de Población; 2019. p. 15-33.
2. Ham-Chande R. El envejecimiento: una nueva dimensión de la salud en México. Salud Publ Mex. 1996;38:409-18.
3. Instituto Nacional de Estadística y Geografía. Censo de Población y Vivienda. México; 2020. Información Demográfica y Social. Available from: https://www.inegi.org.mx/programas/ccpv/2020/#resultados_generales
4. World Health Organization. Obesity; 2023. Available from https://www.who.int/health-topics/obesity#tab=tab_1
5. Secretaría de Salud INdSP, Instituto Nacional de Estadística y Geografía,. Encuesta Nacional de Salud y Nutrición (ENSANUT). Presentación de Resultados; 2022. Available from: https://www.insp.mx/informacion-relevante/la-salud-de-los-mexicanos-en-cifras-resultados-de-la-ensanut-2022
6. Dragoumani K, Troumbis A, Bacopoulou F, Chrousos G. Childhood and adolescent obesity with somatic indicators of stress, inflammation, and dysmetabolism before and after intervention: a meta-analysis. J Pers Med. 2023;13:1322.
7. Duszka K, Gregor A, Guillou H, König J, Wahli W. Peroxisome proliferator-activated receptors and caloric restriction-common pathways affecting metabolism, health, and longevity. Cells. 2020;9:1708.
8. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000;908:244-54.
9. Koelman L, Pivovarova-Ramich O, Pfeiffer AF, Grune T, Aleksandrova K. Cytokines for evaluation of chronic inflammatory status in ageing research: reliability and phenotypic characterisation. Immun Ageing. 2019;16:11.
10. Castro A, Macedo-De la Concha L, Pantoja-Meléndez C. Low-grade inflammation and its relation to obesity and chronic degenerative diseases. Rev Méd Hosp Gen Méx. 2017;80:101-5.
11. Bobermin LD, De Souza Almeida RR, Weber FB, Medeiros LS, Medeiros L, Wyse AT, et al. Lipopolysaccharide induces gliotoxicity in hippocampal astrocytes from aged rats: insights about the glioprotective roles of resveratrol. Mol Neurobiol. 2022;59:1419-39.
12. Sanchez E, Wilkinson T, Coughlan G, Mirza S, Baril AA, Ramirez J, et al. Association of plasma biomarkers with cognition, cognitive decline, and daily function across and within neurodegenerative diseases: results from the ontario neurodegenerative disease research initiative. Alzheimers Dement. 2024;20:1753-70.
13. Michetti F, Clementi ME, Di Liddo R, Valeriani F, Ria F, Rende M, et al. The S100B protein: a multifaceted pathogenic factor more than a biomarker. Int J Mol Sci. 2023;24:9605.
14. Gayger-Dias V, Vizuete AF, Rodrigues L, Wartchow KM, Bobermin L, Leite MC, et al. How S100B crosses brain barriers and why it is considered a peripheral marker of brain injury. Exp Biol Med (Maywood). 2023;248:2109-19.
15. Raharjo S, Pranoto A, Rejeki PS, Harisman AS, Pamungkas YP, Andiana O. Negative correlation between serum brain-derived neurotrophic factor levels and obesity predictor markers and inflammation levels in females with obesity. Maced J Med Sci. 2021;9:1021-6.
16. Nguyen JC, Killcross AS, Jenkins TA. Obesity and cognitive decline: role of inflammation and vascular changes. Front Neurosci. 2014;8:375.
17. Castro-Porras L, Rojas-Russell M, Aguilar-Rodríguez MA, Giraldo-Rodríguez L, Agudelo-Botero M. Sociodemographic and clinical factors associated with severe obesity in adults. Arch Med Res. 2022;53:196-204.
18. Cervera SB, Barrera LH, Valdivia BT, Shamah-Levy T, Nonato IC, Dommarco JA. Obesidad en México, prevalencia y tendencias en adultos. Ensanut 2018-19. Salud Publ Mex. 2020;62:682-92.
19. Zavala GA, Ainscough TS, Jimenez-Moreno AC. Barriers to a healthy diet and physical activity in Mexican adults: results from the Mexican health and nutrition survey. Nutr Bull. 2022;47:298-306.
20. Gonzales MM, Vela G, Philip V, Trevino H, LaRoche A, Wang CP, et al. Demographic and clinical characteristics associated with serum GFAP levels in an ethnically diverse cohort. Neurology 2023;101:e1531-41.
21. Martínez-Hernández BM, Rosas-Carrasco O, López-Teros M, González-Rocha A, Muñoz-Aguirre P, Palazuelos-González R, et al. Association between physical activity and physical and functional performance in non-institutionalized Mexican older adults: a cohort study. BMC Geriatr. 2022;22:388.
22. Salas-Venegas V, Santín-Márquez R, Ramírez-Carreto RJ, Rodríguez-Cortés YM, Cano-Martínez A, Luna-López A, et al. Chronic consumption of a hypercaloric diet increases neuroinflammation and brain senescence, promoting cognitive decline in middle-aged female Wistar rats. Front Aging Neurosci. 2023;15:1162747.

23. Hernández-Arciga U, Hernández-Álvarez D, López-Cervantes SP, López-Díazguerrero NE, Alarcón-Aguilar A, Luna-López A, et al. Effect of long-term moderate-exercise combined with metformin-treatment on antioxidant enzymes activity and expression in the gastrocnemius of old female Wistar rats. Biogerontology. 2020;21:787-805.
24. Santín-Márquez R, Ramírez-Cordero B, Toledo-Pérez R, Luna-López A, López-Diazguerrero NE, Hernández-Arciga U, et al. Sensory and memory processing in old female and male Wistar rat brain, and its relationship with the cortical and hippocampal redox state. Geroscience. 2021;43:1899-920.
25. Kaiser HF. An index of factorial simplicity. Psychometrika. 1974; 39:31-6.
26. Pérez-Tepayo S, Rodríguez-Ramírez S, Unar-Munguía M, Shamah-Levy T. Association of diet quality and body mass index in Mexican adults: a pseudo-panel analysis. Nutr Hosp. 2024; 41:636-48.
27. Elizaled-Barrera CI, Estrada-García T, López-Saucedo C, Rubio-Guerra AF, Ramírez-Velsaco DE. Gender differences in the predictive value of obesity indices for insulin resistance in adult Mexican individuals. Endocr Metab Immune Disord Drug Targets. 2024;24:1640-50.
28. Contreras-Hernández IF, Vargas-De-León C, García-Cortes LR, Flores-Miranda A, Romero-Nava R, Ocharán-Hernández ME. Comparison of ten surrogate insulin resistance and obesity markers to identify metabolic syndrome in Mexican adults. Metabolites. 2024;14:358.
29. Velazquez-Roman J, Angulo-Zamudio UA, Leon-Sicairos N, Flores-Villaseñor H, Benitez-Baez M, Espinoza-Salomón A, et al. Association of PCSK1 and PPARG1 allelic variants with obesity and metabolic syndrome in Mexican adults. Genes (Basel). 2023;14:1775.
30. Salazar-García S, Ibáñez-Salazar A, Lares-Villaseñor E, Gaytan Pacheco N, Uresti-Rivera E, Portales-Pérez DP, et al. Analysis of SIRT1 genetic variants in young Mexican individuals: relationships with overweight and obesity. Front Genet. 2024;15: 1278201.
31. Nascimento MM, Gouveia ÉR, Gouveia BR, Marques A, Marconcin P, França C, et al. The role of cognitive performance and physical functions in the association between age and gait speed: a mediation study. Geriatrics (Basel). 2022;7:73.
32. Černiauskas L, Mažeikienė A, Mazgelytė E, Petrylaitė E, Linkevičiūtė-Dumčė A, Burokienė N, et al. Malondialdehyde, antioxidant defense system components and their relationship with anthropometric measures and lipid metabolism biomarkers in apparently healthy women. Biomedicines. 2023;11:2450.
33. Choromańska B, Myśliwiec P, Dadan J, Maleckas A, Zalewska A, Maciejczyk M. Effects of age and gender on the redox homeostasis of morbidly obese people. Free Radic Biol Med. 2021; 175:108-20.
34. Tegeler C, O'Sullivan JL, Bucholtz N, Goldeck D, Pawelec G, Steinhagen-Thiessen E, et al. The inflammatory markers CRP, IL-6, and IL-10 are associated with cognitive function--data from the berlin aging study II. Neurobiol Aging. 2016;38:112-7.
35. Rizzo MR, Fasano R, Paolisso G. Adiponectin and cognitive decline. Int J Mol Sci. 2020;21:2010.
36. De Franciscis P, Barbieri M, Leo S, Dalise AM, Sardu C, Marfella R, et al. Serum adiponectin levels are associated with worse cognitive function in postmenopausal women. PLoS One. 2017; 12:e0186205.
37. Orozco-Arguelles L, De Los Santos S, Tenorio-Torres A, Méndez JP, Leal-García M, Coral-Vázquez R, et al. Adiponectin and adiponectin receptor 1 expression proteins levels are modified in breast cancer in postmenopausal women with obesity. J Clin Pathol. 2021;74:571-6.
38. Zhong H, Geng R, Zhang Y, Ding J, Liu M, Deng S, et al. Effects of peroxisome proliferator-activated receptor-gamma agonists on cognitive function: a systematic review and meta-analysis. Biomedicines. 2023;11:246.
39. Gómez-Romero P, Alarcón-Sotelo A, Rodríguez-Weber F, Díaz-Greene E. La adiponectina como blanco terapéutico. Med Interna Méx. 2017;33:770-7.
40. Daďová K, Petr M, Tufano JJ, Sontáková L, Krauzová E, Štěpán M, et al. Calanus oil supplementation does not further improve short-term memory or brain-derived neurotrophic factor in older women who underwent exercise training. Clin Inter Aging. 2023;17:1227-36.
41. Gravesteijn E, Adam JJ, Mensink RP, Winkens B, Plat J. Effects of the egg protein hydrolysate NWT-03 on cognitive function in men and women with the metabolic syndrome: a randomized, double-blind, placebo-controlled study. Nutr Neurosci. 2023; 26:1212-21.
42. Riuzzi F, Chiappalupi S, Arcuri C, Giambanco I, Sorci G, Donato R. S100 proteins in obesity: liaisons dangereuses. Cell Mol Life Sci. 2020;77:129-47.
43. Oeckl P, Halbgebauer S, Anderl-Straub S, Steinacker P, Huss AM, Neugebauer H, et al. Glial fibrillary acidic protein in serum is increased in Alzheimer's disease and correlates with cognitive impairment. J Alzheimers Dis. 2019;67:481-8.
44. Rebelos E, Rissanen E, Bucci M, Jääskeläinen O, Honka MJ, Nummenmaa L, et al. Circulating neurofilament is linked with morbid obesity, renal function, and brain density. Sci Rep. 2022;12:7841.
45. Vrettou CS, Vassiliou AG, Keskinidou C, Mourelatos P, Asimakos A, Spetsioti S, et al. A prospective study on neural biomarkers in patients with long-COVID symptoms. J Pers Med. 2024; 14:313.
46. Steiner J, Myint AM, Schiltz K, Westphal S, Bernstein HG, Walter M, et al. S100B serum levels in schizophrenia are presumably related to visceral obesity and insulin resistance. Cardiovasc Psychiatry Neurol. 2010;2010:480707.
47. Michetti F, Di Sante G, Clementi ME, Valeriani F, Mandarano M, Ria F, et al. The multifaceted S100B protein: a role in obesity and diabetes? Int J Mol Sci. 2024;25:776.
48. Bernardi S, Toffoli B, Tonon F, Francica M, Campagnolo E, Ferretti T, et al. Sex differences in proatherogenic cytokine levels. Int J Mol Sci. 2020;21:3861.
49. Kocagoncu E, Nesbitt D, Emery T, Hughes LE, Henson RN, Rowe JB, et al. Neurophysiological and brain structural markers of cognitive frailty differ from Alzheimer's disease. J Neurosci. 2022;42:1362-73.
50. Kim MS, Shim I, Fahed AC, Do R, Park WY, Natarajan P, et al. Association of genetic risk, lifestyle, and their interaction with obesity and obesity-related morbidities. Cell Metab. 2024;36: 1494-503.e3.
51. Emamian MH, Fateh M, Hosseinpoor AR, Alami A, Fotouhi A. Obesity and its socioeconomic determinants in Iran. Econ Hum Biol. 2017;26:144-50.
52. Quezada AD, Lozada-Tequeanes AL. Time trends and sex differences in associations between socioeconomic status indicators and overweight-obesity in Mexico (2006-2012). BMC Public Health. 2015;15:1244.
53. Levasseur P. Causal effects of socioeconomic status on central adiposity risks: evidence using panel data from urban Mexico. Soc Sci Med. 2015;136-7:165-74.