The link between lymphocyte subpopulations in peripheral blood and metabolic variables in patients with severe obesity

Tania Rivera-Carranza[1], Oralia Nájera-Medina[2], Rafael Bojalil[3], Carmen Paulina Rodríguez-López[2], Eduardo Zúñiga-León[4], Angélica León-Téllez Girón[5], Alejandro Azaola-Espinosa[4]

[1] Doctorado Ciencias Biológicas y de la Salud, DCBS, Universidad Autónoma Metropolitana, Coyoacán, México DF, México

[2] Departamento de Atención a la Salud, Laboratory H-104. DCBS, Universidad Autónoma Metropolitana, Coyoacán, México DF, México

[3] Departamento de Atención a la Salud, Laboratory H-103 DCBS, Universidad Autónoma Metropolitana, Coyoacán, México DF, México

[4] Departamento de Sistemas Biológicos, Laboratory N-104 DCBS, Universidad Autónoma Metropolitana, Coyoacán, México DF, México

[5] División de Nutriología Clínica, Hospital General Dr. Manuel GEA González, Tlalpan, México DF, México

Corresponding Author:

Oralia Nájera-Medina onajera@correo.xoc.uam.mx, Alejandro Azaola-Espinosa azaola@correo.xoc.uam.mx

Laboratories H-104 and N-104. DCBS, Universidad Autónoma Metropolitana, México DF, 04960, México

Abstract

Background. Obesity, a public health problem, is a state of metainflammation that influences the development of chronic degenerative diseases, particularly in patients with severe obesity.

Objective. The objective of this study was to evidence immunometabolic differences in patients with different degrees of obesity, including severe obesity, by determining correlations between lymphocyte subpopulations and metabolic, body composition, and clinical variables.

Methods. Peripheral blood immune cells (CD4+, CD8+ memory and effector T lymphocytes) were analyzed, and measures of body composition, blood pressure, and biochemical composition (glucose, glycated hemoglobin [HbA1c], insulin, C-reactive protein [CRP], and the lipid profile) were carried out in patients with different degrees of obesity.

Results. The patients were classified according to total body fat (TBF) percentage as normal body fat, class 1 and 2 obesity, class 3 obesity, and class 4 obesity. The greater the TBF percentage, the more pronounced the differences in body composition (such as a decrease in the fat free mass (FFM) that is defined as sarcopenic obesity) and the immunometabolic profile. There was an increase ofCD3+ T lymphocytes (mainly CD4+, CD4+CD62-, and CD8+CD45RO+ T lymphocytes) and an increase in the TBF percentage (severity of obesity).

Conclusions. The correlations between lymphocyte subpopulations and metabolic, body composition, and clinical variables demonstrated the existence of a chronic, low-intensity inflammatory process in obesity. Therefore, measuring the immunometabolic profile by means of lymphocyte subpopulations in patients with severe obesity could be useful to determine the severity of the disease and the increased risk of presenting obesity-associated chronic degenerative diseases.

Subjects: Immunology and Metabolic Sciences

Key words: Obesity, immunometabolism, body fat, memory T cells, effector T cells.

Introduction

Obesity is a disease characterized by an excessive increase in total body fat (TBF), the result of a disequilibrium between energy ingested and energy spent; this disease is distinguished by being chronic and multifactorial (World Health Organization [WHO], 2020). There are different classes of obesity, and the most objective manner to diagnose and classify obesity is by determining the TBF percentage (Rosales, 2012; Suárez and Sánchez, 2018). It is determined through electrical bioimpedance (BIA) and can be related to the body mass index (BMI) (World Health Organization [WHO], 1995; Okorodudu et al., 2010; Fried et al., 2014), although TBF provides a more exact classification of obesity.

Obesity is an important public-health problem that is increasing worldwide, augmenting morbimortality and diminishing life expectancy as well as quality of life (Taylor, 2011). In addition, health costs regarding the treatment of obesity and its comorbidities were estimated at 200 million USD in 2019 alone, without counting the economic losses to the labor market, due to absenteeism, unemployment, and early retirement (Organization for Economic Cooperation and Development [OECD], 2019).

Adipose tissue is an endocrine organ that contains various types of cells, such as preadipocytes, adipocytes, fibroblasts, vascular endothelial cells, and immune cells (Lee et al., 2016; Liu and Nikolajczyk, 2019). Adipocyte hypertrophy and hyperplasia, the release of fatty acids, and the activation of innate and adaptive immune cells in the adipose tissue of individuals with obesity generate diverse inflammatory stimuli. These include the secretion of proinflammatory cytokines and the activation of signaling pathways such as c-Jun N-terminal kinase (JNK), IκB kinase beta (IKKβ), and nuclear factor kappa-light-chain-enhancer of the activated immunological cells (NF-κB). This activation increases the expression of target genes to produce more proinflammatory cytokines, such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and IL-1β. The consequent systemic inflammation acts on other organs such as skeletal muscle, the liver, and the vascular endothelium and generates insulin resistance (IR) (Zatterale et al., 2020; Hou et al., 2023). Therefore, obesity is considered a low-intensity, chronic inflammatory state, which is also known as metainflammation-related obesity (MIOR) (de Heredia, Gómez-Martínez & Marcos, 2012). It leads to the development of chronic degenerative diseases such as metabolic syndrome (MS) (World Health Organization [WHO], 2020), type 2 diabetes mellitus, systemic

94 arterial hypertension, cardiovascular disease, polycystic ovary syndrome, articular disease,

95 metabolic hepatic steatosis, obstructive sleep apnea, and gastroesophageal reflux disease, among

96 others. Similarly, due to this immunometabolic alteration, obesity also increases the rate of

97 infection and some types of cancer (Avgerinos et al., 2019; Fariñas Guerrero and López Gigosos,

98 2021).

99   Researchers have shown that in people with obesity, there are also changes in the

100 proportion and functionality of lymphocytes at the local level (in adipose tissue), as well as in

101 peripheral blood, and these variations are accentuated by an increase in BMI and adipose tissue

102 (de Heredia, Gómez-Martínez & Marcos, 2012). Indeed, MIOR in adipose tissue appears to be

103 the principal factor that influences the leukocyte count in peripheral blood. The increase in total

104 lymphocytes amplifies the inflammatory response, which plays a key role in the onset of obesity-

105 related comorbidities (Ryder et al., 2014; Rodríguez-López et al., 2018). We aimed to evidence

106 immunometabolic differences in individuals with different classes of obesity by determining

107 correlations between peripheral lymphocyte subpopulations and metabolic variables.

108

Materials & Methods

110 We conducted an observational, cross-sectional, and comparative study in 124 adults of both

111 sexes aged > 18 years, from whom we determined anthropometric and body composition, arterial

112 pressure, the blood glucose and lipid profiles, and lymphocyte subpopulations in peripheral

113 blood. All participants signed an informed consent letter, and the study was reviewed and

114 approved by the Ethics and Research Committee of the Hospital Gea González and the

115 Universidad Autónoma Metropolitana-Xochimilco (approval reference numbers: 46-119-2019

116 and Agreement 7/22.5.2).

117   The inclusion criteria were: patients with obesity in a protocol for bariatric surgery (class

118 3 and 4 obesity) of the Hospital Gea González Obesity Clinic, and students and/or personnel of

119 the Universidad Autónoma Metropolitana-Xochimilco, Mexico City (controls with normal TBF

120 and class 1 and 2 obesity), of both sexes. The exclusion criteria were: patients with infection,

121 pregnancy, autoimmune diseases, renal disease and/or cancer, or who were taking anti-

122 inflammatory or immunosuppressant drugs. The elimination criteria were: individuals who

123 desired to withdraw from the study, those with incomplete data, or those with any of the diseases

124 and/or treatments mentioned under the exclusion criteria.

125

126 **Anthropometric and body composition measurements**

127 We measured weight and height with a Seca 704s TM scale with a stadiometer (Seca, México).

128 We measured the waist circumference (WC) with a Executive 6FT W606P stainless-steel metric

129 tape measure (Lufkin, Englewood CO.USA). We followed the standardized protocol of the

130 International Society for the Advancement of Kinanthropometry (ISAK) when taking

131 measurements. We obtained BMI, TBF, Fat Free Mass (FFM), and visceral fat (VF) from an

132 InBody 720 body composition analyzer (BioSpace Co., Ltd.). We asked each patient not to

133 engage in intense physical exercise during the 24 hours prior to the study and to arrive at their

134 appointment having fasted for at least 4 hours. If not, the fluid distribution in the body change

135 and is underestimate or overestimate FFM. We used the TBF percentage and BMI to establish

136 the class of obesity based on the WHO (1995) criteria. The classes are defined as follows: class 1

137 and 2 obesity, a TBF percentage of 25%–34.9% for men and 30%–39.9% for women; class 3

138 obesity, a TBF percentage of 35%–39.9% for men and 40%–44.9% for women; and class 4

139 obesity, a TBF percentage of $\geq$ 40% for men and $\geq$ 45% for women (Table 1) (WHO, 1995;

140 Okorodudu et al., 2010; Fried et al., 2014).

141

142 Table 1

143

144 **Biochemical and arterial blood tests**

145 We collected peripheral blood samples in 5-mL Vacutainer™ tubes (BD, USA) from the

146 participants after they had fasted for at least 8 hours. We measured the following molecules in

147 peripheral blood: glucose, triglycerides (TG), total cholesterol, high-density lipoprotein

148 cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), glycated hemoglobin

149 (HbA1c), insulin, and C-reactive protein (CRP). We measured glucose, TG, total cholesterol,

150 HDL-c, and LDL-c with an automatized clinical biochemical analyzer iKem YY/T0654-2008

151 (KONTROLab, México). We determined the insulin concentration by using a simultaneous one-

152 step immunoenzymatic assay, HbA1c by using capillary electrophoresis, and CRP by using a

153 high-sensitivity immunoassay of near-infrared fluorescence probes. We determined arterial

154 pressure twice according to the guidelines of the Official Mexican Regulation for the Prevention,

155 Detection, Diagnosis, Treatment, and Control of Systemic Arterial Hypertension (PROY-NOM-

156 030-SSA2-2017).

157

158 **Analysis of lymphocyte populations by flow cytometry**

159 We collected samples of peripheral blood in 5 mL Vacutainer™ tubes (BD). To identify the

160 different cellular subpopulations, we employed a mixture of commercial monoclonal antibodies

161 conjugated with fluorochromes (BD). We utilized the following combinations of conjugated

162 antibodies: control isotype with forward scatter (FSC) determines cell size and side scatter (SSC)

163 determines complexity cell (to identifies by morphology total lymphocytes, monocytes, and

164 granulocytes); FITC-anti-CD3/PE-anti-(CD16+CD56)/PerCP-anti-CD19 (identifies T

165 lymphocytes, natural killer [NK] cells, and B lymphocytes); FITC-anti-CD4/PE-anti-

166 CD62L/APC-anti-CD3 (identifies activated T lymphocytes with helper functions); FITC-anti-

167 CD8/PE-anti-CD28/APC-anti-CD3 (identifies activated T lymphocytes with cytotoxic

168 functions); FITC-anti-CD45RA/PE-anti-CD45RO/PerCP-anti-CD4/APC-anti-CD3 (identifies

169 naïve helper T lymphocytes and memory T lymphocytes); and FITC-anti-CD45RA/PE-anti-

170 CD45RO/PerCP-anti-CD8/APC-anti-CD3 (identifies cytotoxic naïve and memory T

171 lymphocytes) (Fig. 1S).

172   We incubated the cells with the combination of antibodies. Subsequently, we applied the

173 lysis solution, washed the cells in phosphate-buffered saline (PBS), and fixed the cells with 1%

174 paraformaldehyde containing 0.1% sodium azide (NaN_3). We analyzed the samples in a flow

175 cytometer (FACScanto TM II; BD) within 24 h of staining. The analysis included a total of

176 10,000 cells for each event. We used Forward-Scatter and FL-3-Scatter to obtain the percentages

177 of the desired cellular populations and then constructed bi-fluorescent dot plot graphs to delimit

178 regions´ lymphocytes subpopulations (Nájera-Medina et al., 2017; Rodríguez-López et al., 2018)

179 utilizing FACSDiva version 6.1.3 statistical software (Fig. 1S).

180   We calculated the absolute number of each lymphocyte subpopulation as: (the percentage

181 of the required lymphocyte subpopulation × the total number of lymphocytes) / 100. The

182 absolute values are expressed in cells/μL

183

184 **Statistical analysis**

185 We analyzed the results after divided the participants into classes of obesity based on TBF

186 percentages. We used the adjusted Kolmogorov–Smirnov method to determine the normality of

187 the data. We transformed the variables that did not pass the normality test to achieve a normal

188 distribution. The normally distributed data are presented as the mean and standard deviationof

189 the mean, and the non-normally distributed variables are presented as the median and

190 interquartile interval. We applied one-way analysis of variance (ANOVA) to each variable to

191 estimate the difference among the groups. We applied two-way ANOVA to each variable to

192 estimate the difference among the groups by sex. We used the Bonferroni post hoc test to

193 determine intergroup mean differences. To determine relationships between lymphocyte

194 subpopulations and metabolic variables, we utilized a Pearson correlation matrix and identified

195 correlations greater than ± 0.6. For all tests, we considered $p < 0.05$ to indicate a significant

196 difference. We used the Phyton Environment v3.6.7 statistical package (CreateSpace 2009,

197 USA) to transform variables and products graphs, and IBM SPSS Statistics Version 25.0 (USA)

198 for the remainder of the analyses.

199

200 **Results**

201 We evaluated 124 individuals with a mean age of 34.3 ± 8.8 years, most of whom were women

202 (64.5%). Overall, 21.0% (n = 26) of the participants formed the control group (normal TBF

203 percentage), 20.2% (n = 25) had class 1 and 2 obesity, 25.0% (n = 31) had class 3 obesity, and

204 33.8% (n = 42) had class 4 obesity. All of the patients with class 3 and 4 obesity had MS and

205 received treatment for obesity. There were no significant differences in age and sex between the

206 groups (data not shown).

207

208 **Metabolic and body composition variables**

209 There were significant differences in most metabolic, body composition, and clinical variables,

210 except for total cholesterol, between the obesity classes. People with class 4 obesity presented

211 higher mean weight, BMI (Fig. 1A), WC, VF, glucose (Fig. 1B), LDL-c (only a significant

212 difference by sex) TG, CRP (Fig. 1C), and systolic blood pressure (SBP). On the other hand,

213 among the obesity classes, people with class 3 obesity presented the highest FFM, HbA1c,

214 insulin, and diastolic blood pressure (DBP), and the lowest HDL-c (Table 2). The normal body

215 fat group presented the highest FFM of all participants (Fig. 1D).

216

217 Table 2

218 Fig 1

219

220 **Immune cell counts**

221 We analyzed the percentages and absolute numbers of cellular populations in peripheral blood

222 for each group (classified based on the TBF percentage). We found significant differences in

223 leukocytes, total lymphocytes, CD19+ lymphocytes (B cells), CD4+ lymphocytes (helper T

224 lymphocytes), CD4+CD62- lymphocytes (effector helper T lymphocytes), CD8+ lymphocytes

225 (cytotoxic T lymphocytes), and CD8+CD45RA+ lymphocytes (naïve cytotoxic T lymphocytes)

226 (Table 3). The percentage of CD19+ lymphocytes and CD8+ lymphocytes decreased as the

227 degree of obesity increased; however, the same did not occur with the absolute numbers of these

228 cells (Table 3). The percentages of CD4+ (Fig. 2A), CD4+CD62- (Fig. 2B), and

229 CD8+CD45RO+ (Fig. 2C) T lymphocytes increased as the degree of obesity increased. The

230 percentage of CD8+CD45RA+ T lymphocytes was lower in individuals with class 3 obesity

231 compared with the rest of the groups (Fig. 2D).

232

233 Table 3

234

235 Fig 2

236

237 **Correlations between cellular populations and immunological, body composition, and**

238 **metabolic variables**

239 In participants with class 1 and 2 obesity, we found positive correlations between the percentage

240 of CD3+ T lymphocytes and age (Fig. 3A), between the percentage of CD8+CD45RO+ T

241 lymphocytes and WC (Fig. 3B), and between the percentage of CD4+CD45RO+ T lymphocytes

242 and FFM (Fig. 3C), and a negative correlation between CD4+CD45RA+ T lymphocytes and

243 FFM (Fig. 3D).

244

245 Fig 3

246

247   In the class 3 obesity group, we found positive correlations between leukocytes and VF

248 and CRP (Fig. 4A & 4B) and between granulocytes and CRP (Fig. 4C), and a negative

249 correlation between CD8+CD45RA+CD45RO+ T lymphocytes and VF (Fig. 4D). In the class 4

250 obesity group, we found positive correlations between the absolute number of CD4+CD45RO+

251 T lymphocytes and TBF (Fig. 4E), and a negative association between the absolute number of

252 CD8+CD28- lymphocytes and age (Fig. 4F). Considering all participants, we found a positive

253 correlation between the absolute number of CD8+CD45RO T lymphocytes and BMI and WC

254 (Fig. 4G and 4H).

255

256 Fig 4

257

258 **Discussion**

259 **Metabolic profile and body composition worsens with the severity of obesity**

260 We conducted this study to identify the immunological, metabolic, and body composition

261 changes in individuals with different classes of obesity, particularly those with severe obesity. In

262 the present work, the anthropometric parameters (weight, WC, and BMI) and body composition

263 (VF) significantly increased according to the TBF percentage increase (Table 2). Only FFM did

264 not demonstrate this pattern: The FFM percentage decreased significantly as the severity of

265 obesity increased (Fig. 1D). These data are consistent with other studies: An increase in obesity

266 is associated with an increase in weight, BMI, WC (Bauce & Moya, 2019), and VF (Valentino et

267 al., 2015) as well as a decrease in FFM, which indicates sarcopenic obesity (Barazzoni et al.,

268 2018; Poggiogalle et al., 2020), and an increased risk of presenting comorbidities associated with

269 obesity (Valentino et al., 2015; Servant et al., 2021).

270   Regarding the metabolic biochemical and clinical parameters, we observed that the

271 individuals with class 3 obesity had higher mean HbA1c, insulin, and DBP, and lower mean

272 HDL-c (Table 2). The worse profile metabolic profile in patients with class 3 compared with

273 class 4 obesity could be explained by the fact that individuals with class 4 obesity are asked to

274 lose 5% of their body weight before bariatric surgery (Hutcheon et al., 2018). Hence, they adhere

275 better to the dietary recommendations and therefore their metabolic profile improves. In contrast,

276 individuals with class 3 obesity are selected as candidates for bariatric surgery precisely because

277 they have comorbidities associated with obesity and can undergo bariatric surgery sooner,

278   without requiring weight loss. There is an adaptation or metabolic flexibility in individuals with

279   long-term obesity; this could be another reason why individuals with class 4 obesity show a

280   better metabolic profile than individuals with class 3 obesity (Goodpaster & Sparks, 2017).

281   Therefore, timely interventions for the diagnosis of comorbidities and for the treatment of

282   obesity (before it becomes severe) are critical to prevent and improve immunometabolic

283   alterations (Hutcheon et al., 2018).

284

285   **The severity of obesity leads to an increase in the percentages and absolute numbers of T**

286   **lymphocytes in peripheral blood**

287   T lymphocytes are part of the adaptive immune system. They are produced in the bone marrow

288   and mature in the thymus (lymphoid organ)—hence their name—are identified by the presence

289   of CD3+ on their surface. They are classified into T helper (CD4+), cytotoxic T (CD8+),

290   memory T (CD45RO+), naïve T (CD45RA+), regulatory T (Treg), and other lymphocytes. They

291   are responsible for cellular immunity by destroying infected cells or activating macrophages,

292   CD19+ lymphocytes, or other T lymphocytes by cytokines (messenger proteins responsible for

293   the communication between different cell types) and other costimulatory proteins that are found

294   on their cell membrane (Dvorkin et al., 2011). In individuals with obesity, those previously

295   mentioned factors contribute to generate and perpetuate chronic inflammation (Touch et al.,

296   2017; Schäfer and Zernecke, 2021).

297   Effector T lymphocytes, denotes as CD4+CD62-, are a subclass of helper lymphocytes

298   and are involved in the activation of other immune cells, which are particularly important in the

299   adaptive immune response. They are essential to stimulate B lymphocytes to produce antibodies,

300   to activate cytotoxic T lymphocytes, and for increased macrophage activity. In obesity, IR, and

301   MS, effector T lymphocytes promote the production of proinflammatory T helper cytokines and

302   directly influence obesity-associated inflammation, especially systemic inflammation that causes

303   numerous comorbidities (Touch et al., 2017; Liu et al., 2022). We observed that as the degree of

304   obesity increased, CD4+ lymphocytes and CD4+CD62- T lymphocytes (effector helper

305   lymphocytes) also increased (Table 3 and Fig. 2B). This finding is consistent with other studies

306   in which the authors observed an increase in helper T lymphocytes in peripheral blood of

307   individuals with obesity and increased VF (Rodríguez-López et al., 2018) was positive

308   associated with the presence of systemic inflammatory markers such as CRP in plasma and with

309 IR (Mclaughlin et al., 2014). It is important to point out that in these studies, the authors

310 determined obesity based on BMI, unlike our study, in which we classified it based on the TBF

311 percentage. However, the correlation between BMI and TBF in our study is high and significant

312 (r > 0.8, p < 0.001), so it is possible to compare our results with the aforementioned studies.

313   Regarding cytotoxic T lymphocytes, we observed that as the degree of obesity increased

314 (TBF), memory T lymphocytes (CD8+CD45RO+) also increased (Table 3 & Fig. 2C) but naïve

315 T lymphocytes (CD8+CD45RA+) decreased (Table 3 & Fig. 2D). We also found the higher the

316 WC and BMI, the higher the peripheral CD8+CD45RO+ T lymphocyte count (Figs. 3B, 4G, &

317 4H). This finding is in line with other studies: In individuals with obesity and MS, there are

318 many cytotoxic T lymphocytes and memory T lymphocytes in visceral adipose tissue (VAT) and

319 peripheral blood (Cancello et al., 2005; O'Rourke et al., 2011; Anderson et al., 2013; Ryder et

320 al., 2014; Patel et al., 2023) there are associated positively with MIOR. Stimulation of T

321 lymphocytes with IL-15 (a proinflammatory cytokine abundant in individuals with obesity and

322 fatty liver, and who consume a high-fat diet; Cepero-Donates et al., 2016) increases the

323 expression of the *CPT1A* gene, which, in turn, promotes the oxidation of fatty acids, which are

324 vital for providing energy for cytotoxic T lymphocyte proliferation and survival (Van Der Windt

325 et al., 2013). Activation of cytotoxic T lymphocytes constitutes one of the first events in the

326 inflammatory response associated with obesity, because it promotes the recruitment and

327 differentiation of macrophages in adipose tissue (Nishimura et al., 2009). Finally, cytotoxic

328 memory T lymphocytes promote chronic systemic inflammation (Schäfer and Zernecke, 2021).

329   We found that younger patients with class 4 obesity had higher blood levels of

330 CD8+CD28- T lymphocytes (cytotoxic effector T lymphocytes) (Fig. 4F). These lymphocytes

331 are responsible for the effects and functions of cellular immunity; they destroy targeted cells by

332 releasing substances stored in previously performed granules. In addition, they are capable of

333 secreting proinflammatory cytokines such as IFN-γ. VAT can activate cytotoxic to effector T

334 cells, its infiltration is an early event in the development of the inflammatory response associated

335 with obesity since it promotes the recruitment and differentiation of macrophages, therefore it

336 has essential functions in the initiation and maintenance of the inflammation of the BP and at a

337 systemic level in the development of comorbidities such as IR (Nishimura et al., 2009).

338   In addition, an increase in cytotoxic effector and memory T lymphocytes in peripheral

339 blood is the cause of acute endothelial injury that leads to circulatory diseases, such as

340 atherosclerosis. In individuals with severe obesity, this endothelial damage produced by CD8+ T

341 lymphocytes and is compounded by high concentrations of LDL-c and low concentrations of

342 HDL-c (Schäfer & Zernecke, 2021). Finally, it is interesting to mention that the increase,

343 activation, and proliferation of memory T lymphocytes (CD8+CD45RO+, CD4+CD45RO+, and

344 CD8+CD28-) in patients with obesity during chronic inflammation results in the loss of

345 telomerase (memory T lymphocytes with short telomeres), and this in turn leads to decreased

346 naïve T lymphocytes (with long telomeres). These changes condition people with obesity to

347 experience an increased risk of diseases associated with aging and chronic inflammation. This

348 premature aging favors the generation of dysfunctional mitochondria, which leads to the

349 production of reactive oxygen species (ROS) and activation of NF-κB that contribute to

350 perpetuate MIOR. For this reason, people with obesity are physiologically and metabolically

351 more likely to age faster than people with normal TBF (Santos & Sanchari, 2021).

352

353 **The greater the severity of obesity, the lower the proportion of FFM, a phenomenon**

354 **associated with changes in memory and naïve T lymphocytes**

355 In individuals with severe obesity who present sarcopenic obesity, there are also endocrine

356 disorders, premature aging, and decreased physical activity that could generate changes in

357 immune cells (Kalinkovich & Livshits, 2017). In the class 1 and 2 obesity group, a decrease in

358 the FFM percentage was associated with a decrease in CD4+CD45RO+ T lymphocytes and an

359 increase in CD4+CD45RA+ T lymphocytes in peripheral blood (Fig. 3C & 3D). Obesity has also

360 been shown to promote increased infiltration of immune cells into muscle, a phenomenon that

361 might contribute to the chronic low-grade metainflammation associated with obesity (Patsouris

362 et al., 2014; Siervo et al., 2021). However, more studies are needed to explore this topic.

363

364 **Peripheral blood B lymphocytes change with the severity of obesity**

365 We observed that as the TBF percentage increased, the percentage of CD19+ lymphocytes

366 decreased and the absolute number increased in peripheral blood (Table 3). This finding is

367 consistent with the study by Frasca et al. (2016) carried out in an American population of 18

368 young adults between 20 and 40 years old and adults > 60 years old, where the percentage of B

369 lymphocytes was lower in individuals with obesity compared with lean individuals. However, in

370 this same study individuals with obesity showed a decrease in absolute number of B lymphocytes

371 compared with lean people (Frasca et al., 2016). Similarly, in a population of 169 young

372 Mexican adults, Rodríguez-López et al. (2018) reported a nonsignificant increase in the

373 percentage of total B lymphocytes in peripheral blood of individuals with high VAT. In a

374 population of 40 young American women with obesity (27–55 years old), Frasca et al. (2021a)

375 observed that the percentages of naïve B lymphocytes are higher and of memory B lymphocytes

376 are lower in peripheral blood compared with adipose tissue. Furthermore, they indicated that B

377 lymphocytes in the blood are metabolically less active regarding the production of

378 proinflammatory substances and in the expression of enzymes of glucose oxidation metabolism

379 than in adipose tissue (Frasca et al., 2021a). In other studies carried out in adult women between

380 40 and 55 years of age, Frasca et al. (2019, 2021b) found a higher percentage of senescent B

381 lymphocytes and double-negative B lymphocytes (a subset of B lymphocytes that secrete

382 autoimmune antibodies) in peripheral blood and adipose tissue of women with obesity compared

383 with thin women.

384   It has been demonstrated that as obesity increases, B lymphocytes increase because they

385 are among the first immune cells to infiltrate adipose tissue, mainly VAT (Harmon et al., 2016;

386 Srikakulapu and McNamara, 2020). Once inside adipose tissue and plasma of individuals with

387 obesity, B lymphocytes produce proinflammatory mediators that regulate inflammatory T

388 lymphocytes and macrophages and secrete adipocyte-specific autoimmune IgG antibodies

389 (Frasca et al., 2008; Frasca & Blomberg, 2020). In addition, in obesity and aging, the function of

390 B lymphocytes decreases, a phenomenon associated with deficient responses to infections and

391 vaccines (Muramatsu et al., 2000; Sayegh et al., 2003; Frasca et al., 2008, 2016; Zhai et al.,

392 2016).

393   Our study allows us to formulate two hypotheses as to why, in individuals with obesity,

394 the percentage of B lymphocytes in peripheral blood decreases but the absolute number

395 increases: 1) a large number of B lymphocytes and some of their phenotypic variants may be in

396 adipose tissue and 2) the number of T lymphocytes increases considerably as the severity of

397 obesity increases, and thus there is a decrease in B lymphocytes. However, it is important to add

398 that we included people with different degrees of obesity, including many with severe obesity.

399

400 **Conclusion**

401   We found significant differences in the immunometabolic profile of individuals with different

402   classes of obesity. Compared with the control group, an increase in the severity of obesity based

403   on the TBF percentage is associated with an increase in weight, BMI, WC, and VF; a reduction

404   in FFM (sarcopenic obesity); alterations in metabolic biochemical parameters; and changes in

405   lymphocyte subpopulation counts in peripheral blood. As the TBF percentage (severity of

406   obesity) increases, CD4+, CD4+CD62-, and CD8+CD45RO+ T lymphocytes increase in

407   peripheral blood, demonstrating the existence of an inflammatory process at the peripheral level

408   that is also associated with other variables such as WC, BMI, CRP, leukocytes, and age.

409   Therefore, evaluating the immunometabolic profile in patients with obesity can be clinically

410   useful to assess in a timely manner the risk of presenting inflammatory diseases associated with

411   obesity. However, to be more certain of the behavior of the immune cells of individuals with

412   severe obesity, it is important to continue with these studies to obtain the percentages and

413   absolute numbers of immune cells determine their phenotypic variants, their function, and their

414   correlation with metabolic markers in peripheral blood and adipose tissue.

415

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425

426   **Competing Interests**

427   The authors declare there are no competing interests.

428

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Figure 1

Differences in metabolic variables according to the total body fat (TBF) percentage

(A) BMI, (B) glucose, (C) C-reactive protein (CRP), and (D) fat free mass (FFM) percentage. The groups were compared with one-way analysis of variance. Abbreviations: C1-2 obesity, class 1 and 2 obesity; C3 obesity, class 3 obesity; C4 obesity, class 4 obesity; NBF, normal body fat. A one-way analysis (ANOVA) of variance was performed to estimate the difference in means. To estimate the difference in means between groups, the Bonferroni post hoc test was used (p < 0.05 indicate significant difference).

A

BMI (Kg/m²)

NBF   C1-2 obesity   C3 obesity   C4 obesity

B

Glucose (mg/dL)

NBF   C1-2 obesity   C3 obesity   C4 obesity

C

CRP (mg/dL)

NBF   C1-2 obesity   C3 obesity   C4 obesity

D

FFM (%)

NBF   C1-2 obesity   C3 obesity   C4 obesity

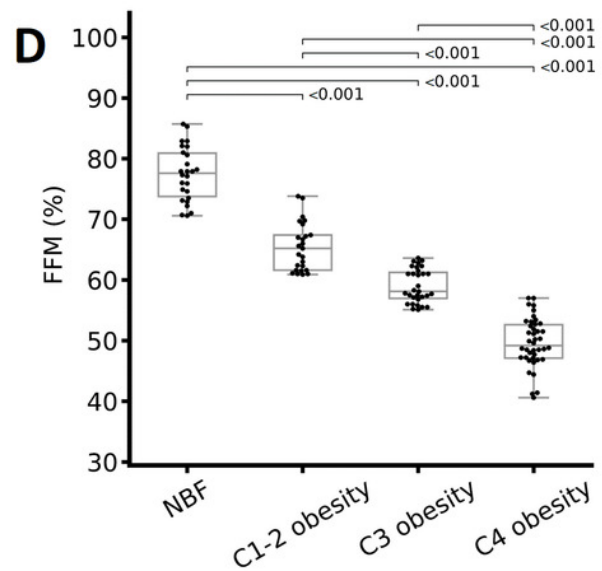
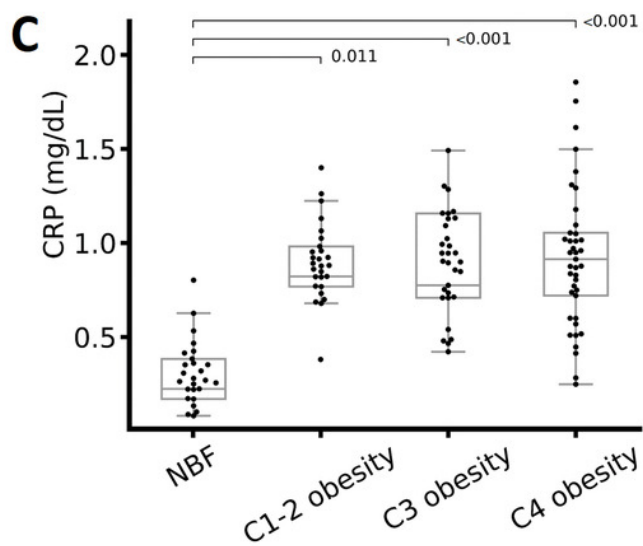
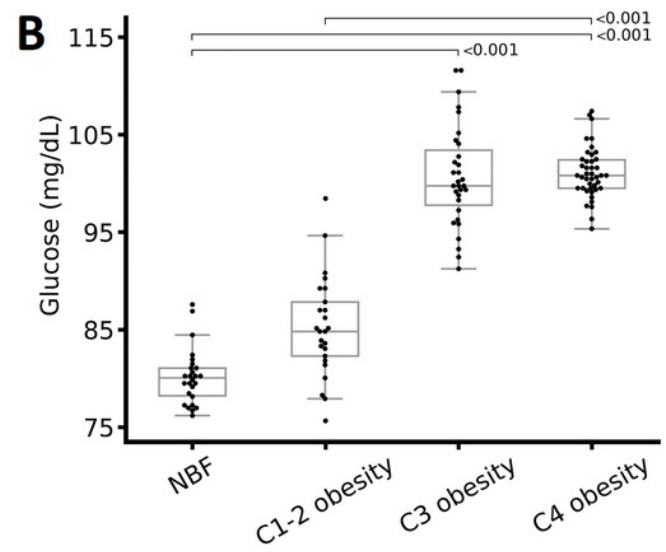
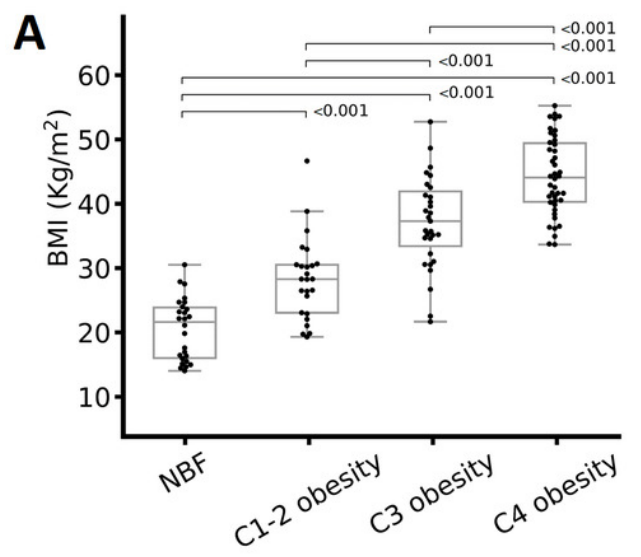



Figure 2

Differences in immune cells according to the total body fat (TBF) percentage

(A) CD4+ T lymphocytes, (B) CD4+CD62- T lymphocytes, (C) CD8+CD45RO+ T lymphocytes, and (D) CD8+CD45RA+ T lymphocytes. The groups were compared with one-way analysis of variance. Abbreviations: C1-2 obesity, class 1 and 2 obesity; C3 obesity, class 3 obesity; C4 obesity, class 4 obesity; NBF, normal body fat. A one-way analysis (ANOVA) of variance was performed to estimate the difference in means. To estimate the difference in means between groups, the Bonferroni post hoc test was used (p < 0.05 indicate significant difference)

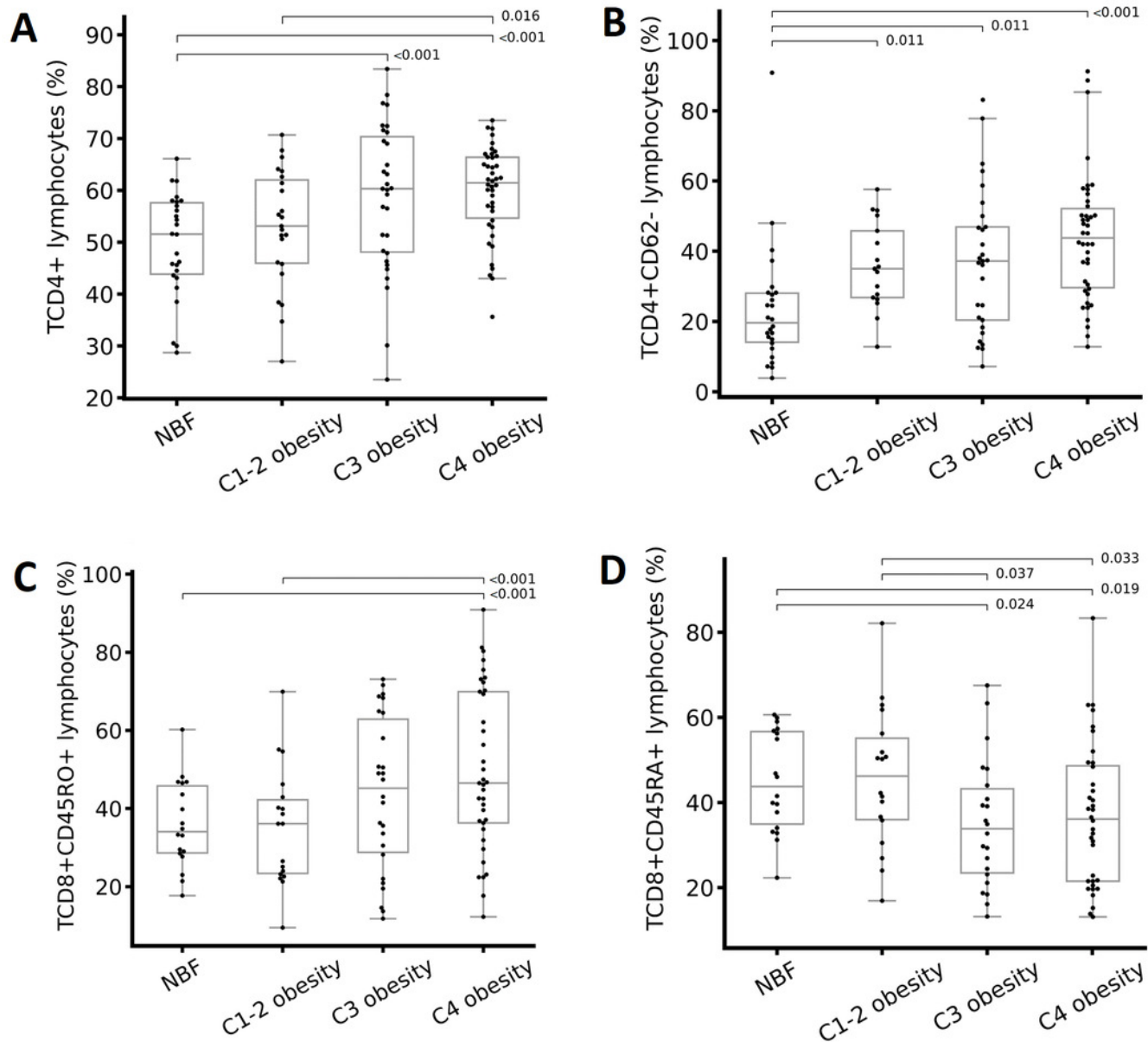



Figure 3

Correlation between immunological variables and metabolic and clinical variables of the class 1 and class 2 obesity group

(A) positive correlation between CD3+ T lymphocytes and age; (B) positive correlation between CD8+CD45RO+ T lymphocytes and waist circumference (WC); (C) positive correlation between CD4+CD45RO+ T lymphocytes and fat free mass (FFM); and (D) negative correlation between CD4+CD45RA+ T lymphocytes and FFM. The results show the correlation between two variables calculated with the Pearson correlation matrix, considering correlations (r) greater than ± 0.6.

A

r = 0.701
p = 0.014

TCD3+ lymphocytes (%)

Class 1 & 2 obesity

Age (years)

B

r = 0.666
p = <0.001

TCD8+CD45RO+ lymphocytes (%)

Class 1 & 2 obesity

WC (cm)

C

r = 0.769
p =<0.001

TCD4+CD45RO+ lymphocytes (%)

Class 1 & 2 obesity

FFM (%)

D

TCD4+CD45RA+ lymphocytes (%)

r = -0.704
p =<0.001

Class 1 & 2 obesity

FFM (%)

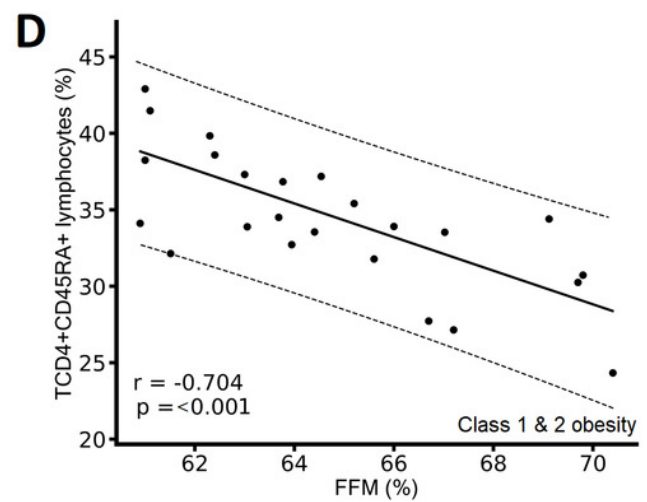
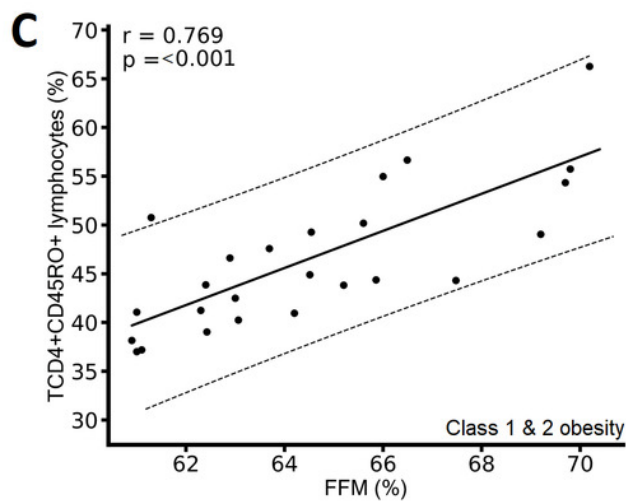
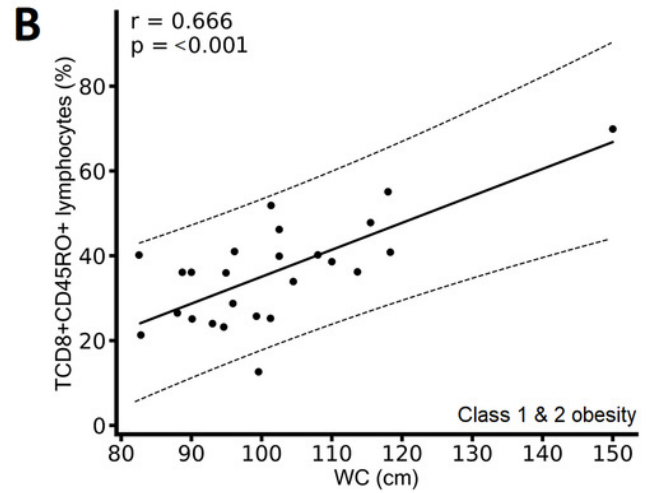
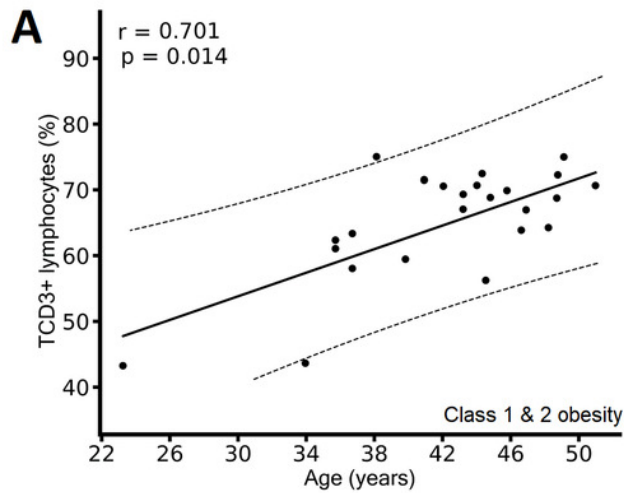
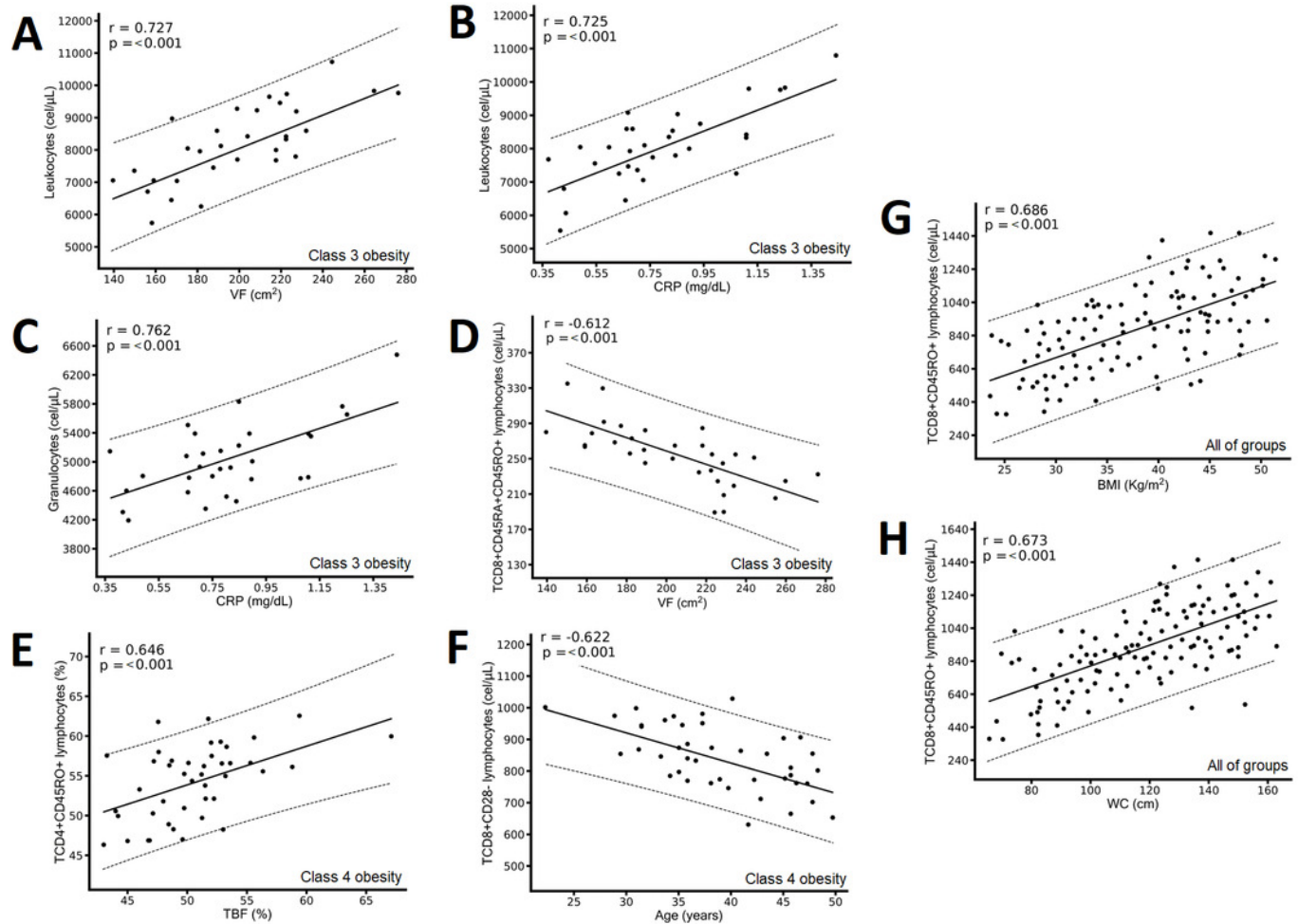



Figure 4

Correlation between immunological variables and the metabolic and clinical variables of the class 3 obesity group, the class 4 obesity group, and the entire study group

The class 3 obesity group has (A) a positive correlation between leukocytes and visceral fat (VF); (B) a positive correlation between leukocytes and C-reactive protein (CRP); (C) a positive association between granulocytes and CRP; and (D) a negative correlation between CD8+CD45RA+CD45RO+ T lymphocytes and VF. The class 4 obesity group has (E) a positive correlation between CD4+CD45RO+ T lymphocytes and total body fat (TBF) and (F) a negative correlation between CD8+CD28- T lymphocytes and age. The entire study group has (G) a positive correlation between CD8+CD45RO+ T lymphocytes and body mass index (BMI) and (H) a positive correlation between CD8+CD45RO+ T lymphocytes and waist circumference (WC). The results show the correlation between two variables calculated with the Pearson correlation matrix, considering correlations (r) greater than ± 0.6.

Diagnosis of the nutritional state based on body mass index (BMI) and total body fat percentage

Abbreviations: M, male; F, female

BMI (kg/m²)	Interpretation	Percentage of total body fat
< 18.5	Low body fat	M: < 14% F: < 15%
18.5–24.9	Normal or healthy body fat	M: 14%–17% F: 15%–24.9%
25–29.9	Normal or healthy body fat borderline high	M: 18%–24.9% F: 25%–29.9%
30–34.9	Class 1 obesity	M: 25%–34.9% F: 30%–39.9%
35–39.9	Class 2 obesity	
> 40–49.9	Class 3 obesity or morbid obesity	M: 35%–39.9% F: 40%–44.9%
> 50	Class 4 obesity or superobesity	M: ≥ 40% F: ≥ 45%

Metabolic, body composition, and clinical characteristics according to the total body fat percentage

The data are presented as mean ± standard deviation or median (interquartile range). Statistical analysis: p, one-way analysis of variance; p@, two-way analysis of variance (adjusted for sex). $p < 0.05$ is statistically significant.

[a] Significant difference versus individuals with normal body fat (Bonferroni test).

[b] Significant difference versus individuals with grade 1 and 2 obesity (Bonferroni test).

[c] Statistically significant difference versus individuals with grade 3 obesity (Bonferroni test).

Abbreviations: n, number of individuals; TBF, Total body fat; BMI, body mass index; WC, waist circumference; FFM, fat-free mass; VF, visceral fat; HbA1c, glycated hemoglobin; HDL-c, high-density cholesterol; LDL-c, low-density cholesterol;CRP, C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Variable (n = 124)	Normal body fat (n = 26)	Class 1 and 2 obesity (n = 25)	Class 3 obesity (n = 31)	Class 4 obesity (n = 42)	p	p[@]
TBF (%)	22.5 ± 4.4	34.5 ± 3.9[a]	40.9 ± 2.7[a,b]	50.7 ± 4.7[a,b,c]	**<0.001**	**<0.001**
Weight (kg)	62. ± 12.9	77.8 ± 22.2	104.4 ± 30.3[a,b]	117.1 ± 24.6[a,b]	**<0.001**	**<0.001**
BMI (kg/m^2)	22.7 (19.6–24.1)	28.1 (24.6–29.8)[a]	37.3 (32.9–42.6)[a,b]	43.6 (39.5–50.1)[a,b,c]	**<0.001**	**<0.001**
WC (cm)	78.5 ± 8.8	97.4 ± 14.6[a]	120.2 ± 20.1[a,b]	129.3 ± 16.0[a,b,]	**<0.001**	**<0.001**
FFM (kg)	48.4 (37.2–57.0)	47.0 (41.9–54.7)	55.7 (47.7–78.9) [a]	53.0 (48.5–60.6) [a]	**0.001**	**<0.001**
FFM (%)	77.4 ± 4.4	65.4 ± 3.9 [a]	59.0 ± 2.7 [a,b]	49.2 ± 4.7 [a,b,c]	**<0.001**	**<0.001**
VF (cm^2)	69.0 (43–95)	112.6 (98.5–137.7)[a,b]	173.0 (147.0–277.0)[a,b]	264 (199–290)[a,b,c]	**<0.001**	**<0.001**
Glucose (mg/dL)	80.2 (77.6–86.7)	85.3 (75.5–95.2)	99 (83.0–113.6)[a]	103 (95–111)[a,b]	**<0.001**	**<0.001**
HbA1c (%)	5.4 (5.3–5.5)	5.4 (5.3–10.5)[a]	5.8 (5.5–6.7)[b]	5.7 (5.5–6.0)[b,c]	**<0.001**	**0.040**
Insulin (μUI/mL)	6.3 (4.2–7.2)	14.9 (11.3–31.2) [a]	22.4 (12.7–27.6)[a]	21.1 (14.4–30.3)[a]	**<0.001**	**<0.001**
Total cholesterol (mg/dL)	164.5 ± 27.9	171.9 ± 35.8	173.3 ± 32.6	175.2 ± 31.3	0.595	0.080
HDL-c (mg/dL)	49.9 (44.6–60.6)	43.9 (34.5–53.4)	37 (33–42)[a]	41.4 (36.7–48.1)[a]	**<0.001**	**<0.001**

LDL-c (mg/dL)	86.1 ± 21.6	93.4 ± 24.1	97.9 ± 28.4	103.1 ± 27.8[a]	0.069	**0.027**
Triglycerides (mg/dL)	100 (81.8–141)	128.3 (107.5–184.4)[a]	138.8 (105.0–186.0)[a]	150.0 (106.7–195.2)[a,b]	**0.006**	**<0.001**
CRP (mg/dL)	0.077 (0.042–0.230)	0.720 (0.357–0.883)	0.600 (0.397–1.340)[a]	0.835 (0.439–1.155)[a]	**<0.001**	**<0.001**
SBP (mmHg)	106 (100–117)	110 (107–120)	120 (110–126)[a]	125 (110–132)[a,b]	**<0.001**	**<0.001**
DBP (mmHg)	70 (64–76)	76 (67–80)	80 (72–84) [a]	77 (70–82)	**0.017**	**0.002**

Percentages and absolute numbers of immune cells according to the total body fat percentage.

The data are presented as mean ± standard deviation or median (interquartile range). Statistical analysis: p, one-way analysis of variance; p@, two-way analysis of variance (adjusted for sex). $p < 0.05$ is statistically significant [a] Statistically significant difference versus individuals with normal body fat (Bonferroni test) [b] Statistically significant difference versus individuals with grade 1 and 2 obesity (Bonferroni test) [c] Statistically significant difference versus individuals with grade 3 obesity (Bonferroni test) Abbreviations: CD16+CD56+, Natural Killers lymphocytes; CD3+CD16+CD56+, Natural Killers T lymphocytes; CD19+, B lymphocytes; CD3+, T lymphocytes; CD4+, helper T lymphocytes; CD4+CD62+, non-effector helper T lymphocytes; CD4+CD62-,effector helper Tlymphocytes; CD4+CD45RA+,naive helper Tlymphocytes; CD4+CD45RO+,memory helper Tlymphocytes; CD4+CD45RA+CD45RO+, in transition from naïve to memory helper T lymphocytes; CD8+,cytotoxicTlymphocytes; CD8+CD28+, non-activated cytotoxic T lymphocytes; CD8+CD28-, activated cytotoxic T lymphocytes; CD8+CD45RA+, naive cytotoxic T lymphocytes; CD8+CD45RO+, memory cytotoxic T lymphocytes; CD8+CD45RA+CD45RO+, in transition from naive to memory cytotoxic T lymphocytes;NK, natural killer cells; NKT, natural killer T lymphocytes

Variable (%, cells/μL) (n = 124)	Normal body fat (n = 26)	Class 1 and 2 obesity (n = 25)	Class 3 obesity (n = 31)	Class 4 obesity (n = 42)	p	p[@]
Leukocytes	5800 (4900–6675)	6800 (6100–7642)	8200 (6400–10800)[a]	7965 (5825–9825)[a]	**0.013**	**0.001**
Monocytes	7.3 ± 1.8 419.5 (332–488)	7.7 ± 3.2 418.9 (339–644)	7.3 ± 2.7 569.0 (348–889)	7.6 ± 3.0 584.5 (383–797)[a]	0.916 0.138	0.233 **0.003**
Granulocytes	65.2 ± 11.4 4167.9 (3123–4585)	56.9 ± 14.3 4886.0 (4080–5214)	61.0 ± 11.4 4349.8 (3804–6511)	63.2 ± 11.4 4552.3 (3602–6548)	0.090 0.255	0.337 0.097
Total lymphocytes	27.0 ± 12.0 1188 (749–1725)	34.9 ± 13.5 1464 (844–2010)	31.6 ± 10.6 2365 (1613–3630)[a]	29.1 ± 11.4 2014 (1498–2704)[a]	0.095 **<0.001**	0.730 **<0.001**
CD16+CD56+	22.4 (11.3–30.2) 308 (188–420)	12.6 (9.3–18.7) 182 (145–388)	19.5 (12.2–25.1) 467 (397–726)[b]	18.4 (11.9–25.7) 332 (223–475)	0.105 **0.036**	0.618 0.086
CD3+CD16+CD56+	3.9 (1.4–5.4) 41 (20–101)	2.9 (2.3–6.7) 48 (20–93)	2.5 (1.1–9.8) 51 (27–174)	3.5 (1.6–5.3) 62 (26–103)	0.984 0.809	0.836 0.357
CD19+	14.6 (10.1–20.9) 154 (114–200)	9.8 (7.6–15.6) 172 (125–277)	9.6 (6.9–11.9) 237 (115–383)a	9.0 (6.4–12.6)[a] 205 (120–350)	**0.028** 0.310	**0.010** **0.043**
CD3+	59.2 (47.1–66.5)	77.5 (67.1–79.7)	73.2 (56.7–78.5)	68.3 (60.7–79.1)	0.130	0.063

	716 (390–973)	952 (543–1384)	1412 (863–2484)[a]	1438 (1058–2091)[a]	**<0.001**	**<0.001**
CD4+	49.4 ± 10.1	52.8 ± 11.3	58.7 ± 14.2[a]	59.7 ± 8.8[a]	**0.001**	**<0.001**
	507 (374–904)	804 (525–1076)	1122 (870–2213)[a]	1288 (931–1650)[a]	**<0.001**	**<0.001**
CD4+CD62-	23.6 ± 18.0	35.9 ± 12.4	37.2 ± 20.4[a]	44.0 ± 18.7[a]	**<0.001**	**<0.001**
	150 (92–401)	413 (330–723)	844 (598–1350)a	873 (667–1380)[a]	**<0.001**	**<0.001**
CD4+CD62+	75.8 ± 18.5	59.5 ± 13.2[a]	54.4 ± 21.9[a]	54.8 ± 18.6[a]	**<0.001**	**<0.001**
	877 (624–1478)	840 (393–1283)	1120 (730–2163)	1164 (694–1883)	0.258	0.009
CD4+CD45RA+	23 (13.5–32.5)	32.4 (21.3–48.9)	21.9 (14.9–32)	24.3 (14.3–42.3)	0.152	0.394
	307 (131–602)	392 (196–839)	459 (294–598)	539 (256–1118)[a]	**0.011**	**0.002**
CD4+CD45RO+	61.3 (55.8–73.9)	42.0 (34.7–62.4)	65.2 (41.4–74.7)	65.0 (43.1–72.5)	0.117	0.566
	773 (434–1083)	773 (414–1215)	1560 (1219–2497)[a]	1220 (707–1785)[a]	**<0.001**	**<0.001**
CD4+CD45RA+CD45RO+	9.6 (8.2–12.8)	13.2 (8.5–18.7)	11.9 (7.1–16.9)	12.8 (8.1–16.5)	0.523	0.324
	118 (69–141)	146.6 (102–485)	209.4 (131–504)[a,b]	247.4 (153–491)[a,b]	**<0.001**	**<0.001**
CD8+	56.8 (21.7–65.8)	36.1 (25.7–41.0)	31.6 (26.8–43.0)	29.0 (25.9–34.2)[a]	**0.001**	**<0.001**
	626 (493–859)	403 (222–866)	957 (439–1160)[b]	624 (416–834)	**0.045**	**0.015**
CD8+CD28-	47.6 ± 23.6	40.3 ± 16.7	45.6 ± 19.1	44.0 ± 19.7	0.571	0.275
	508 (251–875)	401 (247–1146)	955 (505–1660)[a]	798 (565–1075)[a]	**0.044**	**0.015**

CD8+CD28+	46.1 ± 21.4 338 (249–794)	57.3 ± 17.4 580 (515–1348)	49.6 ± 18.1 1075 (686–1843)[a]	53.5 ± 19.1 1016 (572–1656)[a]	0.191 **<0.001**	0.196 **<0.001**
CD8+CD45R A+	44.9 ± 11.9 495 (373–740)	45.8 ± 16.4 455 (218–826)	34.9 ± 14.9 702 (487–1107)	35.4 ± 17.3[b] 702 (436–1026)	**0.027** 0.196	**0.018** 0.066
CD8+CD45R O+	34 (28.3– 46.5) 392 (246–602)	36.1 (23– 43.7) 638 (438–799)	45.2 (26.6– 64.6) 931 (736–1587)[a]	46.5 (35.5– 70)[a,b] 1173 (713–1706)[a,b]	0.250 **<0.001**	**0.004** **<0.001**
CD8+CD45R A+CD45RO +	16.5 ± 9.4 178 (112–311)	15.1 ± 6.8 169 (76–516)	15.9 ± 9.0 220 (172–381)	15.5 ± 7.2 268 (178–580)[a]	0.840 **0.039**	0.794 **0.011**