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## Role of Cardiovascular Risk Factors on the Association Between Physical Activity and Brain Integrity Markers in Older Adults

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1 **Role of Cardiovascular Risk Factors on the Association Between Physical**  
2 **Activity and Brain Integrity Markers in Older Adults**

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20  
21 **Abstract**

22 **Objective:** Physical activity has been associated with a decreased risk for dementia,  
23 but the mechanisms underlying this association remain to be determined. Our  
24 objective was to assess whether cardiovascular risk factors mediate the association  
25 between physical activity and brain integrity markers in older adults.

26 **Methods:** Participants from the Age-Well study underwent, at baseline, a physical  
27 activity questionnaire, cardiovascular risk factors collection (systolic blood pressure,  
28 body mass index [BMI], current smoker status, HDL-cholesterol, total-cholesterol,  
29 insulin) and multimodal neuroimaging (structural-MRI, diffusion-MRI, FDG-PET,  
30 Florbetapir-PET). Multiple regressions were conducted to assess the association  
31 between physical activity, cardiovascular risk factors, and neuroimaging. Mediation

32 analyses were performed to test whether cardiovascular risk factors mediated the  
33 associations between physical activity and neuroimaging.

34 **Results:** 134 cognitively unimpaired older adults ( $\geq 65$  years) were included. Higher  
35 physical activity was associated with higher grey matter (GM) volume ( $\beta=0.174$ ,  
36  $p=0.030$ ) and cerebral glucose metabolism ( $\beta=0.247$ ,  $p=0.019$ ), but not with amyloid  
37 deposition or white matter integrity. Higher physical activity was associated with lower  
38 insulin and BMI, but not with the other cardiovascular risk factors. Lower insulin and  
39 BMI were related to higher GM volume, but not to cerebral glucose metabolism.  
40 When controlling for insulin and BMI, the association between physical activity and  
41 cerebral glucose metabolism remained unchanged, while the association with GM  
42 volume was lost. When insulin and BMI were entered in the same model, only BMI  
43 remained a significant predictor of GM volume. Mediation analyses confirmed that  
44 insulin and BMI mediated the association between physical activity and GM volume.  
45 Analyses were replicated within Alzheimer's disease-sensitive regions, and results  
46 remained overall similar.

47 **Conclusions:** The association between physical activity and GM volume is mediated  
48 by changes in insulin and BMI. In contrast, the association with cerebral glucose  
49 metabolism seems to be independent from cardiovascular risk factors. Older adults  
50 practicing physical activity have cardiovascular benefits, through the maintenance of  
51 a lower BMI and insulin, resulting in greater structural brain integrity. This study has  
52 strong implications as understanding how physical activity affects brain health may  
53 help developing strategies to prevent or delay age-related decline.

54 **Trial Registration Information:** EudraCT: 2016-002441-36; IDRCB: 2016-A01767-  
55 44; ClinicalTrials.gov Identifier: NCT02977819.

56

## 57 **Introduction**

58 Physical activity is important in preventing pathological aging and dementia, including  
59 Alzheimer's Disease (AD).<sup>1</sup> Growing evidence indicates that older adults engaging in  
60 a higher amount of physical activity have greater brain integrity,<sup>2,3</sup> including increased  
61 grey matter (GM) volume,<sup>4</sup> cerebral glucose metabolism,<sup>5,6,7</sup> WM microstructural  
62 integrity,<sup>8</sup> along with reduced amyloid burden<sup>7,6,9</sup> and white matter hyperintensities  
63 (WMH).<sup>8</sup> However, the relationship between physical activity and brain structure and  
64 function is still not fully understood as i) some inconsistencies remained<sup>10</sup> and ii) the  
65 mechanisms by which physical activity exerts its benefits on brain health remain to be  
66 determined. Animal studies suggest that physical activity promotes neurogenesis, cell  
67 survival, expression of neurotrophic factors, and synaptic plasticity,<sup>3</sup> while human  
68 studies also propose indirect effects of physical activity, via a reduction in  
69 cardiovascular risk factors.<sup>11,12</sup> In fact, physical activity has been proven to efficiently  
70 reduce cardiovascular risk factors,<sup>13,14</sup> which are known to increase dementia risk  
71 and negatively affect brain structure and function.<sup>15</sup> Nevertheless, the role of  
72 cardiovascular risk factors on the association between physical activity and brain  
73 integrity remains unknown. Most of the studies only controlled for cardiovascular risk  
74 factors and found no changes after such correction,<sup>16</sup> but contradictory findings have  
75 also been reported.<sup>17</sup>

76 The scarcity of studies combining several neuroimaging modalities in the same  
77 sample, and exploring the role of cardiovascular risk factors in the association  
78 between physical activity and neuroimaging biomarkers, make it difficult to  
79 understand how physical activity contributes to brain health. We investigated whether  
80 the association between physical activity and multimodal neuroimaging, including GM

81 volume, cerebral glucose metabolism, amyloid burden, WMH load, and WM  
82 microstructure, is mediated by cardiovascular risk factors.

83

## 84 **Methods**

### 85 **Participants**

86 Participants from the baseline visit of the Age-Well randomized clinical trial (Medit-  
87 Ageing European Project),<sup>18</sup> were included (Figure 1). Individuals were recruited from  
88 the general population from November 2016 until March 2018. They were all native  
89 French speakers, retired for at least 1 year, had at least 7 years of education, and  
90 performed within the normal range on standardized cognitive tests. The main  
91 exclusion criteria were antecedent of major neurological or psychiatric disorders  
92 (including alcohol or drug abuse), presence of a chronic disease or acute unstable  
93 illness (including cardiovascular and metabolic affections), and current or recent  
94 treatments that may interfere with cognitive functioning. All participants included  
95 underwent structural MRI, <sup>18</sup>F-florbetapir-PET and <sup>18</sup>F-fluorodeoxyglucose (FDG)-  
96 PET scans, along with a physical activity questionnaire, blood sampling and a clinical  
97 exam including information on cardiovascular risk factors, within a 3-months period.  
98 Baseline data were collected from November 2016 until April 2018.

99

### 100 **Standard Protocol Approvals, Registrations, and Patient Consents**

101 The Age-Well randomized clinical trial was approved by local ethics committee  
102 (Comité de Protection des Personnes Nord-Ouest III, Caen, France; trial registration  
103 number: EudraCT: 2016-002441-36; IDRCB: 2016-A01767-44; ClinicalTrials.gov  
104 Identifier: NCT02977819) and all participants gave their written informed consent  
105 prior to the examinations.

106

## 107 **Image Acquisition**

108 All participants were scanned on the same magnetic resonance imaging (MRI; Philips  
109 Achievia 3.0T scanner) and positron emission tomography (PET; Discovery RX VCT  
110 64 PET-CT scanner, General Electric Healthcare) cameras at the Cyceron Center  
111 (Caen, France).

112 A high-resolution T1-weighted structural image using a 3D fast-field echo sequence  
113 (sagittal; repetition time (TR): 7.1 ms, echo time (TE): 3.3ms, field of view (FOV):  
114 256×256 mm<sup>2</sup>, 180 slices, voxel size: 1×1×1 mm<sup>3</sup>) and a 3D fluid-attenuated  
115 inversion recovery (FLAIR; sagittal; TR: 4800 ms, TE: 272 ms, inversion time: 1650  
116 ms; FOV: 250×250 mm<sup>2</sup>, 180 slices, voxel size: 0.98×0.98×1 mm<sup>3</sup>) were acquired for  
117 133 participants. An echo-planar imaging/spin echo diffusion weighted sequence  
118 (DKI) was then performed at multiple shells for 133 participants: 3b-values (0, 1000,  
119 2000 s/mm<sup>2</sup>) (axial; 30 directions, TR: 6100 ms, TE: 101 ms, flip angle, 90°, FOV:  
120 216×216 mm<sup>2</sup>, 48 slices, voxel size: 2.7×2.7×2.7 mm<sup>3</sup>) and additional blips images  
121 with b=0 s/mm<sup>2</sup> (number of signal averages, 9) were acquired in reverse phase  
122 encoding direction for susceptibility distortion.

123 Florbetapir- and FDG-PET scans were acquired in two separate sessions, with a  
124 resolution of 3.76×3.76×4.9 mm<sup>3</sup> (FOV: 157 mm). Forty-seven planes with a voxel  
125 size of 1.95×1.95×3.27 mm<sup>3</sup> were obtained. Before the PET acquisition, a  
126 transmission scan was performed for attenuation correction. For Florbetapir-PET,  
127 each participant (n=133) underwent a 10-min PET scan beginning 50 minutes after  
128 the intravenous injection of ~4MBq/Kg of Florbetapir. For the FDG-PET scan,  
129 participants (n=92) were fasted for at least 6 hours before scanning. After a 30-min  
130 resting period in a quiet and dark environment, ~180 MBq of FDG were intravenously

131 injected as a bolus and a 10-min PET acquisition scan was acquired 50 minutes after  
132 injection.

133

### 134 **Image processing**

135 T1-weighted MRIs were segmented using FLAIR images and normalized to the  
136 Montreal Neurological Institute (MNI) space. GM normalized segments were  
137 modulated to correct for nonlinear warping effects using the Statistical Parametric  
138 Mapping (SPM12) software's multiple channels segmentation procedure  
139 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm12>).

140 Raw FLAIR images were coregistered onto their corresponding native space T1-  
141 weighted MRI, and WMH were segmented by the lesion prediction algorithm LPA<sup>19</sup>  
142 implemented in the Lesion Segmentation Toolbox version 2.0.15 ([www.statistical-](http://www.statistical-modelling.de/ist.html)  
143 [modelling.de/ist.html](http://www.statistical-modelling.de/ist.html)) for SPM12. A minimum extend threshold of 0.01 cm<sup>3</sup> was set.  
144 Lesion probability maps were binarized by applying a threshold of 0.5 and lesions  
145 masks were thus generated. Lesion masks were then visually inspected and  
146 corrected for false positives in corticospinal tracts if necessary, using a specific  
147 corticospinal tract mask for each participant.<sup>20</sup>

148 DKI images were corrected for susceptibility artifacts, eddy current distortions and  
149 subject motion using Functional Magnetic Resonance Imaging of the Brain diffusion  
150 toolbox (FSL 5.0.9, <http://www.fmrib.ox.ac.uk/fsl>). DKI data were then processed  
151 using Matlab R2012b (MathWorks, Natick, MA) and the Diffusional Kurtosis Estimator  
152 software (DKE: Version 2.6; <http://nitrc.org/projects/dke>) to estimate the diffusional  
153 kurtosis tensor.<sup>21</sup> Images were smoothed with a 3.375×3.375×3.375 mm full-width at  
154 half-maximum (FWHM) Gaussian filter. Mean kurtosis (MK) and fractional anisotropy  
155 (FA) maps, reflecting WM microstructural integrity,<sup>22</sup> were then extracted from the

156 diffusional kurtosis estimator. These maps were coregistered to the T1-weighted MRI  
157 and normalized to the MNI template by applying the deformation parameters from the  
158 corresponding T1-weighted MRI.

159 PET data were coregistered onto their corresponding MRI, corrected for partial  
160 volume effects (PVE) using the Muller-Gartner method, and normalized using the  
161 deformation parameters defined from the T1-weighted procedure. Resulting images  
162 were quantitatively normalized using the cerebellar GM as the reference region.

163 Averaged global neuroimaging values were obtained by applying a binary mask of  
164 GM for preprocessed T1-weighted and PET images, and a binary mask of WM, for  
165 preprocessed DKI images. The cerebellum was excluded from both masks, and only  
166 voxels with a probability >60% of being GM and WM were included, to exclude non-  
167 GM or WM voxel and reduce the probability of overlap with other tissue classes.

168 For FLAIR data, the total volume of WMH was extracted, as fully described  
169 elsewhere.<sup>20</sup>

170 To investigate how the results could apply to AD, neuroimaging values were also  
171 extracted in AD-sensitive regions. More specifically, we extracted GM volume in the  
172 hippocampus (ROI), glucose metabolism SUVR in the precuneus (from the AAL  
173 atlas) and in a posterior cingulate and temporoparietal regions previously  
174 determined,<sup>23</sup> as well as FA in the cingulum (from the JHU WM atlas).

175

## 176 **Physical activity**

177 Physical activity over the last 12 months was assessed using a French version of the  
178 Modifiable Activity Questionnaire (MAQ),<sup>24</sup> adapted to be self-administered.<sup>25</sup> This  
179 version of the questionnaire allows assessing the amount of physical activity,  
180 including both leisure activities and work activities. Since participants were retired,

181 only the leisure-related physical activity was considered in the present study.  
182 Participants had to report, for each activity they did at least 10 times over the last 12  
183 months (e.g., walking, hiking, biking, gardening, jogging), how much time they spent  
184 doing that activity (i.e., how many months per year, time per month and minutes each  
185 time). The total physical activity score corresponds to the average hours per week of  
186 leisure-time physical activity over the last 12 months. The averaged hours per week  
187 was calculated for each activity as follows [(number of months per year) x (number of  
188 times per month) x (minutes per time) / 60 (*min*)] / 52 (*week per year*), and which  
189 were summed to obtain a total score of physical activity.

190

### 191 **Cardiovascular risk factors**

192 Cardiovascular risk factors measures included insulin, cholesterol (total and HDL),  
193 systolic blood pressure, BMI and smoking habits.

194 Fasting blood sample were obtained for all participants. The plasma concentration of  
195 Insulin (pmol/L) was performed by chemiluminescence assay on automated analyzer  
196 COBAS 6000 (Roche diagnostics, Meylan, France), using ready-made commercial  
197 reagent kits (Insulin ROCHE). The quantitative determination of total cholesterol  
198 (mmol/L) and high-density lipoprotein (HDL; mmol/L) cholesterol concentrations in  
199 serum were carried out by an enzymatic staining test in Beckman Coulter Clinical  
200 Chemistry AU analyzers (Beckman Coulter, Villepinte, France). Systolic blood  
201 pressure (SBP; mm Hg) was averaged over three consecutive assessments at two  
202 different times in a seated position at rest. The BMI was objectively obtained during  
203 the medical interview and was calculated as weight in kilograms divided by height in  
204 meters squared ( $\text{Kg}/\text{m}^2$ ). Participants were questioned on their smoking habits and  
205 were classified as currently smokers (yes) or not (no).

206

## 207 **Statistical analysis**

208 We carried out multiple linear regressions, in order to assess whether there was a  
209 specific association between (1) physical activity and each neuroimaging value  
210 separately, (2) physical activity and each cardiovascular risk factor separately and 3)  
211 cardiovascular risk factors and neuroimaging values that were significantly  
212 associated with physical activity. Next, to better understand the specific implication of  
213 each cardiovascular risk factor to these effects, we replicated the same multiple  
214 regression by including cardiovascular risk factors of interest (i.e. found to be  
215 associated in the previous analyses) in the same model. All analyses were controlled  
216 for age, sex, and education. Analyses with insulin, total and HDL-cholesterol, and  
217 SBP were further controlled for hypo-glycaemic, hypo-cholesterol and high-blood  
218 pressure treatments, respectively.

219 Models with polynomial terms of different orders for physical activity were considered,  
220 to assess which function best described the association between physical activity and  
221 neuroimaging global values (Supplemental materials).

222 Analyses were conducted using the R software (R Core Team, 2019) and considered  
223 significant at a  $p < .05$ .

224 Finally, we used causal mediation analyses to examine whether the cardiovascular  
225 risk factors mediated these associations.

226 We tested the mediation effect using “mediation analysis”, and we reported the  
227 average direct effects and average causal mediation effect estimated using  
228 nonparametric bootstrapping based on 5000 bootstrap samples,  $p < .05$ .

229

## 230 **Data availability**

231 Data is available on request following a formal data sharing agreement and approval  
232 by the consortium and executive committee. The data sharing request form can be  
233 downloaded at <https://silversantestudy.eu/2020/09/25/data-sharing/>.

234

## 235 **Results**

236 Data of 134 cognitively unimpaired older adults ( $\geq 65$  years) were analyzed.

237 Participants' characteristics are detailed in Table 1. Because physical activity, insulin  
238 and BMI were not normally distributed, values were log-transformed. One participant  
239 had a physical activity score of zero, which cannot be log-transformed. As a result, to  
240 be able to include this subject in the analyses, a constant of 1 was added to all  
241 physical activity scores before transformation.

242

### 243 *Association between physical activity and global neuroimaging measures*

244 Higher physical activity was correlated to higher global GM volume (Figure 2A) and  
245 cerebral glucose metabolism (Figure 2B), but not with amyloid burden, WMH volume  
246 or WM microstructural integrity (MK; Table 2).

247 The association between physical activity and GM volume was best described by a  
248 linear function. The association between glucose metabolism and physical activity  
249 seemed also better described by a linear association, even though some indices  
250 suggest that the quadratic model cannot be completely rejected (Supplemental  
251 materials, eTable 1).

252

### 253 *Association between physical activity and cardiovascular risk factors*

254 Higher physical activity was associated with lower insulin (Figure 2C) and BMI  
255 (Figure 2D), but not with total and HDL-cholesterol, SBP or current smoker status

256 (Table 2). Results remained similar when further controlling for treatments  
257 (Supplemental materials, eTable 2).  
258 When insulin and BMI were entered in the same model, they were no longer  
259 associated with physical activity, suggesting that the link of physical activity with  
260 insulin and BMI is not independent one from another. Consistently, insulin and BMI  
261 were highly correlated.

262

### 263 *Association between cardiovascular risk factors and global neuroimaging measures*

264 We then examined whether the cardiovascular risk factors (insulin, BMI) and the  
265 global neuroimaging values (GM volume and cerebral glucose metabolism) found to  
266 be associated with physical activity were correlated to each other. Lower insulin  
267 (Figure 2E) and BMI (Figure 2F) were both correlated with higher GM volume, but  
268 neither of them was associated with cerebral glucose metabolism (Table 3). When  
269 BMI and insulin were included in the same model, the correlation of GM volume with  
270 insulin was lost, while the association with BMI remained unchanged, suggesting that  
271 the association between insulin and GM volume was driven by BMI. Results  
272 remained similar when further controlling for treatments (Supplemental materials,  
273 eTable 1).

274

### 275 *Influence of cardiovascular risk factors on the association between physical activity* 276 *and neuroimaging*

277 When the association between physical activity and GM volume was controlled for  
278 insulin and BMI separately, or simultaneously, the association was lost. On the other  
279 hand, the association with cerebral glucose metabolism remained significant when  
280 controlling for insulin, BMI or both.

281

### 282 *Mediation analyses*

283 To directly assess whether the association between physical activity and global GM  
284 volume was mediated by insulin and BMI, we performed mediation analyses. The  
285 relationship between physical activity and GM volume was fully mediated by insulin  
286 (Figure 3, Model 1) and BMI (Figure 3, Model 2). The indirect effects of insulin and  
287 BMI separately were significant (Table 4).

288

### 289 ***AD-sensitive region of interest***

290 To further investigate whether the effect of physical activity, cardiovascular risk  
291 factors and the mediations were present in regions specifically involved in AD,  
292 analyses were replicated on GM volume, glucose metabolism and WM  
293 microstructural integrity in AD-sensitive ROIs.

294 As depicted in Table 1, higher physical activity was correlated to higher hippocampal  
295 volume and glucose metabolism in the precuneus (Supplemental materials, eFigure  
296 1A,B), but not to global glucose metabolism, suggesting that the link found in the  
297 main analyses involves only in part AD-sensitive regions.

298

299 We then examined whether the cardiovascular risk factors (insulin, BMI) and the  
300 neuroimaging values (hippocampal volume and glucose metabolism of the  
301 precuneus) found to be associated with physical activity were correlated to each  
302 other. Lower insulin and BMI were correlated with higher hippocampal volume (Table  
303 2; Supplemental materials, eFigure 1C,D). Of note, when adding insulin and BMI into  
304 the model, BMI was the best predictor of hippocampal volume. On the other hand,  
305 neither insulin, nor BMI were associated with glucose metabolism of the precuneus.

306

307 When insulin and BMI were added separately or simultaneously (Table 2) in the  
308 regression model between physical activity and hippocampal volume, the association  
309 was not significant anymore (although close to the threshold of 0.05).

310

311 Mediation analyses confirmed that insulin (Figure 2, Model1) and BMI (Figure2,  
312 Model 2) fully mediated the association between physical activity and hippocampal  
313 volume. The indirect effects of insulin and BMI separately were significant (Table 3).

314

315 Complementary analyses:

316 Analyses were replicated on FDG-PET data not corrected for PVE, and show similar  
317 results (Supplemental materials, eTable 3, eTable 4, eFigure 2).

318 In addition, to further investigate whether other lifestyle factors drive the association  
319 between physical activity and brain integrity, analyses were replicated adjusting for  
320 Mediterranean diet adherence and lifetime cognitive activity. Results remained similar  
321 (Supplemental materials, eTable 5, eTable 6), suggesting that the associations  
322 previously highlighted are not driven by other lifestyle factors.

323

## 324 **Discussion**

325 Our multimodal neuroimaging study provides an integrated view of the differential  
326 association between physical activity and complementary measures of brain integrity.

327 In cognitively unimpaired older adults, we found that physical activity was directly  
328 associated with cerebral glucose metabolism, while the association with global GM  
329 volume was mediated by cardiovascular risk factors, and more specifically insulin and  
330 BMI. Similar results were found when focusing on AD-sensitive brain regions.

331

332 The finding that physical activity is associated with higher GM volume is in line with  
333 previous literature in cognitively unimpaired older adults using self-reported physical  
334 activity and related measures such as exercise and fitness.<sup>26,27,28,6</sup> Importantly, this  
335 association was mediated by the effect of insulin and BMI. These results tie well with  
336 prior studies showing that increased physical activity in older adults was  
337 accompanied with lower insulin<sup>9</sup> and BMI<sup>29</sup> and other evidence showing that lower  
338 insulin and BMI<sup>30</sup> were linked to greater GM volume in older adults. One conceivable  
339 explanation is that physical activity leads to better cardiovascular health, which could  
340 in turn lead to a greater preservation of brain structure.<sup>31</sup>

341 Obesity and insulin dysfunction may develop with aging,<sup>32</sup> and are risk factors for  
342 brain atrophy, cognitive impairment and dementia, including AD.<sup>33,30</sup> Obesity is the  
343 most common cause of insulin resistance<sup>34,32</sup> and they are both source of  
344 inflammation and oxidative stress,<sup>35</sup> that negatively impact brain health and increase  
345 AD risk.<sup>36</sup> Insulin action in the brain includes neuroprotective, neurotrophic and  
346 neuromodulatory functions, and its perturbation may accelerate brain atrophy in AD.<sup>33</sup>

347 Insulin resistance is the main characteristic of Type 2 Diabetes, a disease associated  
348 not only with aging, obesity and physical inactivity,<sup>37</sup> but also with brain atrophy and  
349 increased dementia risk.<sup>38</sup> Recently, active lifestyle has been found to protect against  
350 the deleterious effects of diabetes on dementia over 12 years, likely through its  
351 positive impact on reduced brain volume loss.<sup>39</sup> Interestingly, insulin and BMI were  
352 strongly associated in our study, and when insulin and BMI were included in the  
353 same model to predict GM volume, BMI remained the only predictor. Therefore, we  
354 argue that maintenance of a lower BMI through physical activity could help

355 preventing disturbed insulin metabolism observed in aging, thus promoting brain  
356 health.

357 To date, only one study specifically looked at and found insulin sensitivity to mediate  
358 the relation between physical activity and anterior cingulate cortex volume.<sup>40</sup> Thus,  
359 our study extends these results to a global measure of GM volume. Only a small  
360 number of studies accounted for the effect of BMI on the association between  
361 physical activity and GM volume,<sup>6,27,16</sup> but they failed to find a dependent effect of  
362 BMI on this association. This contradictory finding might be due, in part, to smaller  
363 physical activity assessment periods (e.g., last 7 days) considered in other  
364 research,<sup>16</sup> which may have hampered an effect on BMI and, by extension, any  
365 possible mediation on the link between physical activity and GM volume. Others  
366 included a smaller<sup>27</sup> or younger sample than the one included here.<sup>6</sup> Our findings go  
367 beyond previous reports, providing evidence of indirect, cardiovascular risk factors-  
368 dependent, association between physical activity and GM volume.

369

370 As a second key finding, physical activity was associated with greater cerebral  
371 glucose metabolism in a subsample of 92 participants, in agreement with FDG-PET  
372 neuroimaging modality;<sup>5,6,7</sup> but see.<sup>10</sup> Most importantly, the relation between physical  
373 activity and cerebral glucose metabolism did not depend on insulin or BMI. The  
374 benefits of physical activity on cerebral glucose metabolism may rather act through  
375 more direct neuronal effects, such as increased neurogenesis, cell survival,  
376 expression of neurotrophic factors, and synaptic plasticity.<sup>3</sup> The increased nutrient  
377 and energy demands of these neural processes are met with increased expression of  
378 enzymes implicated in glucose use and metabolism.<sup>13</sup> Alternatively, benefits of  
379 physical activity on cerebral glucose metabolism could be mediated by other factors

380 not measured here. With regards to insulin, the literature is controversial. Some  
381 animal studies highlighted the role of insulin in the regulation of brain glucose uptake  
382 and metabolism.<sup>41</sup> However, evidence in Humans is contradictory, with studies  
383 reporting an association of brain glucose uptake with insulin<sup>42</sup> and insulin  
384 resistance,<sup>43</sup> while others found no correlation with neither of them.<sup>44</sup> Our results  
385 align with prior observations showing that neuron glucose uptake, transport and use  
386 can be influenced by, but would not depend on, insulin.<sup>33</sup> Similarly, evidence on the  
387 link between BMI and cerebral glucose metabolism in aging individuals is weak and  
388 heterogeneous. Higher BMI has been found to be associated with both increased<sup>45</sup>  
389 and reduced<sup>5</sup> cerebral glucose metabolism, preventing from drawing clear  
390 conclusions. Only a few studies previously examined the association between  
391 physical activity and cerebral glucose metabolism in older participants.<sup>5,6,7</sup> Among  
392 them, none controlled for insulin, making it difficult to assess whether it might have  
393 influenced that relationship. On the other hand, in the ones controlling for BMI, the  
394 association between physical activity and cerebral glucose metabolism remained  
395 significant.<sup>16</sup> Overall, it remains unclear how much insulin and BMI are involved in the  
396 regulation of cerebral glucose metabolism. In the present study however, we did not  
397 find these cardiovascular risk factors to be related to cerebral glucose metabolism  
398 and neither to mediate the link between physical activity and cerebral glucose  
399 metabolism.

400

401 Taken together, these two first findings are important to help our understanding of the  
402 mechanisms by which physical activity benefits brain health. As a plausible  
403 interpretation, some authors proposed that direct and indirect mechanisms are likely  
404 interconnected via the modulation of growth factor signaling.<sup>13</sup> Accordingly, physical

405 activity directly induces the release of neurotrophic factors, which are supposed to  
406 activate a cascade of brain responses (i.e. neurogenesis, synaptic plasticity) that  
407 independently protect the brain. Physical activity also helps reducing pro-  
408 inflammatory conditions (induced by risk factors such as obesity and related insulin  
409 disturbances), which in turn indirectly improves growth factor signaling<sup>13</sup> and  
410 mitigates their deleterious effects on brain health.<sup>36</sup> Nevertheless, while results  
411 discussed were all statistically significant, it should be mentioned that they were of  
412 relatively modest magnitude (small effect sizes).

413

414 Of note, our multimodal study suggests that self-reported physical activity is  
415 associated with GM volume and cerebral glucose metabolism, but does not correlate  
416 with amyloid or markers of WM integrity (WMH and WM microstructure). Even if the  
417 effect size ( $\beta$  coefficient) of the link between physical activity and WMH was close to  
418 that of the link with GM volume, they did not reach the statistical threshold selected in  
419 this study. Previous literature has produced mixed results.<sup>10,8</sup> Methodological  
420 differences with our study,<sup>7,9,6,8</sup> including instruments (fitness test, accelerometry,  
421 exercise interventions), time intervals (physical activity on the last 7 days), and mean  
422 age of participants (younger than our sample) could possibly explain the divergent  
423 findings. Moreover, while higher physical activity was associated with reduced insulin  
424 and BMI, we found no associations with SBP, total and HDL-cholesterol or smoking  
425 status. Despite prior studies suggesting benefits of physical activity on SBP, total and  
426 HDL-cholesterol,<sup>14</sup> and smoking habits,<sup>46</sup> evidence in older individuals is scarce<sup>47</sup> and  
427 conflicting data have been also reported.<sup>14</sup> Furthermore, only a minority of studies  
428 examined these cardiovascular risk factors as primary outcomes. Further research is

429 warranted to disentangle the influence of physical activity on amyloid burden, WM  
430 integrity and cardiovascular risk factors.

431 Results were also present when focusing on AD-sensitive brain regions. More  
432 specifically, physical activity was associated with higher hippocampal volume. This is  
433 in agreement with previous literature in cognitively unimpaired older adults, using  
434 both self-reported and objective measures of physical activity.<sup>7,16</sup> Physical activity  
435 was also associated with cerebral glucose metabolism in the precuneus, which is in  
436 line with previous studies.<sup>48</sup> Our study further indicates that the association between  
437 physical activity and hippocampal volume is mediated by a reduction in insulin and  
438 BMI, while it was not the case for glucose metabolism in the precuneus. This  
439 suggests that results previously discussed for global neuroimaging measures could  
440 be extended to AD-sensitive regions. However, physical activity was neither  
441 associated with temporo-parietal cerebral glucose metabolism, nor with WM integrity  
442 of the cingulum (FA), suggesting that the results could not be generalized to all AD-  
443 related regions.

444 Our study has some limitations. First, we used a subjective (self-reported  
445 questionnaire) instead of an objective measure of physical activity. However, self-  
446 reported physical activity has been extensively used in the literature and  
447 questionnaires have an acknowledged clinical validity. The one that we used in this  
448 study evaluates physical activity over the past year, whereas objective measures  
449 often assess physical activity over a short time interval, which could be not enough to  
450 detect any changes in the variables examined. Even though imprecision in the  
451 measure is likely to occur, validity and reliability of the questionnaire have been  
452 previously established.<sup>24</sup> In addition, as participants are cognitively unimpaired, it  
453 seems unlikely that major recollection issue affected the questionnaire. Second, we

454 used BMI as indicator of obesity in line with most of the studies. However,  
455 controversy exists in the literature as BMI might not be a sensitive index at older ages  
456 as it would be instead protective.<sup>49</sup> BMI is nonetheless considered a valuable  
457 measure that is widely used, allowing for a better comparison with previous studies.  
458 Third, the cross-sectional nature of the design prevents us from inferring causal  
459 relationship regarding the association of physical activity with GM volume and  
460 cerebral glucose metabolism. Thus, we cannot exclude that older adults with a better  
461 brain health stay more physically active. Of note, while mediation analyses provide  
462 statistical support to theoretically driven hypothesis, they do not explain the biological  
463 mechanism underlying these associations. Further studies, including longitudinal and  
464 interventional designs should allow answering these questions, and further  
465 understanding the factors explaining the effects of physical activity on brain health.  
466 Accumulating evidences highlight the impact of midlife cardiovascular risk factors on  
467 brain health. However, we did not address this issue since only current  
468 cardiovascular risk factors were available here.<sup>50</sup> The population of our study is  
469 relatively healthy, with no severe cardiovascular risk factors. This could prevent  
470 generalizability of the results, and explain discrepancy with previous studies including  
471 individuals with more severe cardiovascular diseases. Finally, no correction was  
472 made for multiple comparisons.

473

474 Overall, our results suggest that the associations between physical activity and global  
475 GM, including hippocampal volume, are mediated by changes in insulin and BMI.  
476 Thus, older adults practicing physical activity have cardiovascular benefits, through  
477 the maintenance of lower insulin and BMI, which could in turn promote greater  
478 structural brain integrity. In contrast, the association with global and precuneus

479 cerebral glucose metabolism seems to be independent from cardiovascular risk  
480 factors. This study has strong implications as understanding how physical activity  
481 affects brain health may help developing strategies to prevent, or delay, age-related  
482 brain decline.

483

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501

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697 **Appendix 2 : Coinvestigators**

698 The Medit-Ageing Research Group: Institut National de la Santé et de la Recherche

699 Médicale, Caen, France:

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<b>Name</b>	<b>Location</b>	<b>Role</b>	<b>Contribution</b>
Eider M. Arenaza-Urquijo, PhD	Institut National de la Santé et de la Recherche Médicale, Caen, France	Postdoctoral researcher	Study design; acquisition, analysis, or interpretation of data
Florence Allais, BA	EUCLID/F-CRIN Clinical Trials Platform, Bordeaux, France	Data manager	Data management
Claire André, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	PhD student	Acquisition, analysis, or interpretation of data
Julien Asselineau, PhD	EUCLID/F-CRIN Clinical Trials Platform, Bordeaux, France	Statistician	Analysis of data
Sebastian Baez Lugo, MSc	University of Geneva, Geneva, Switzerland	PhD student	Acquisition, analysis, or interpretation of data
Martine Batchelor	Bordeaux, France	Independent meditation teacher	Meditation teacher
Axel Beaugonin	Caen, France	Independent meditation teacher	Meditation teacher
Alexandre Bejanin, PhD	Institut National de la Santé et de la Recherche Médicale, Caen, France	Postdoctoral researcher	Acquisition, analysis, or interpretation of data
Pierre Champetier, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	PhD student	Acquisition, analysis, or interpretation of data
Fabienne Collette, PhD	University of Liege, Liege, Belgium	Group leader	Obtained funding, study design
Sophie Dautricourt, MD, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	PhD student	Acquisition, analysis, or interpretation of data
Robin De Flores PhD	Institut National de la Santé et de la Recherche Médicale, Caen, France	Postdoctoral Researcher	Acquisition, analysis, or interpretation of data; administrative, technical, or material support
Pascal Delamillieure, MD, PhD	Centre Hospitalier Universitaire de Caen, Caen, France	Psychiatrist	Meditation teacher
Marion Delarue, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	Neuropsychologist	Acquisition, analysis, or interpretation of data
Yacila I. Deza-Araujo, PhD	University of Geneva, Geneva, Switzerland	Postdoctoral researcher	Acquisition, analysis, or interpretation of data
Hélène Esperou, MD	Institut National de la Santé et de la Recherche Médicale, Paris, France	Group leader	Sponsor

Eric Frison, MD	EUCLID/F-CRIN Clinical Trials Platform, Bordeaux, France	Methodologist	Study design; interpretation of data
Francis Gheysen, MD	Caen, France	Independent meditation teacher	Meditation teacher
Marc Heidmann, MSc	Institut National de la Santé et de la Recherche Médicale, Lyon, France	PhD student	Acquisition, analysis, or interpretation of data
Thien (Titi) Huong Tran (Dolma)	Lyon, France	Meditation teacher	Meditation teacher
Frank Jessen, MD, PhD	Uniklinik Köln, Cologne, Germany	Cooperation Unit Leader	
Olga Klimecki, PhD	University of Geneva, Geneva, Switzerland	Neurologist	Obtained funding; study design
Pierre Krolak-Salmon, MD, PhD	Hospices Civils de Lyon, Lyon, France	Neurologist	Obtained funding; study design
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Gwendoline Le Du, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	Clinical research assistant	Acquisition, analysis, or interpretation of data; administrative, technical, or material support
Valérie LefranC, BA	Institut National de la Santé et de la Recherche Médicale, Caen, France	Clinical research assistant	Acquisition, analysis, or interpretation of data; Administrative, technical, or material support
Antoine Lutz, PhD	Institut National de la Santé et de la Recherche Médicale, Lyon, France	Group leader	Obtained funding; study design
Natalie MarchanT, PhD	University College London, United Kingdom	Group leader	Obtained funding; study design
Jose-Luis Molinuevo, MD, PhD	Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain	Group leader	Obtained funding; study design
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Valentin Ourry, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	PhD student	Acquisition, analysis, or interpretation of data
Léo Paly, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	Neuropsychologist	Acquisition, analysis, or interpretation of data
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Florence Requier, MSc	University of Liege, Liege,	PhD student	Acquisition, analysis, or

	Belgium		interpretation of data
Eric Salmon, MD, PhD	University of Liege, Liege, Belgium	Group leader	Obtained funding; study design
Raquel Sanchez, MD, PhD	Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain	Neurologist	Study design
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Siya Sherif, PhD	Institut National de la Santé et de la Recherche Médicale, Caen, France	Research engineer	Acquisition, analysis, or interpretation of data; administrative, technical, or material support
Edelweiss Touron, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	PhD student	Acquisition, analysis, or interpretation of data
Matthieu Vanhoutte, PhD	Institut National de la Santé et de la Recherche Médicale, Caen, France	Postdoctoral researcher	Acquisition, analysis, or interpretation of data; administrative, technical, or material support
Denis Vivien, PhD	Institut National de la Santé et de la Recherche Médicale, Caen, France	Group leader	Obtained funding; Administrative, technical, or material support
Patrik Vuilleumier, MD	University of Geneva, Geneva, Switzerland	Group leader	Obtained funding; study design
Caitlin Ware, MSc	Institut National de la Santé et de la Recherche Médicale, Caen, France	English teacher assistant	English teacher
Miranka Wirth, PhD	Deutsches Zentrum für Neurodegenerative Erkrankungen, Dresden, Germany	Group leader	Study design

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712 **Figure Caption**

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714 **Figure 1 Flow Diagram of the Inclusion Process.** Abbreviations: DKI = diffusion  
715 kurtosis imaging; FLAIR = Fluid Attenuated Inversion Recovery; FDG = <sup>18</sup>F-  
716 fluorodeoxyglucose; PET = positron emission tomography; WMH = white matter  
717 hyperintensities.

718

719 **Figure 2 Associations between physical activity, neuroimaging values, and**  
720 **cardiovascular risk factors.** Physical activity is associated with global neuroimaging  
721 values of gray matter volume (A) and glucose metabolism (B), as well as insulin (C)  
722 and body mass index (D). Global neuroimaging values of gray matter volume are  
723 associated with insulin (G) and body mass index (H).

724 Raw data (i.e., unadjusted) is plotted. Solid lines represent estimated regression lines  
725 and shaded areas represent 95% confidence intervals. Statistical values were  
726 obtained using multiple linear regressions controlling for age, sex, and education.  
727 Physical activity, insulin and body mass index values are log-transformed.  
728 Abbreviations: GM = gray matter.

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730 **Figure 3 Causal mediation analyses of the association between physical**  
731 **activity and global and hippocampal gray matter volume.** Direct effects in filled  
732 arrows (simple regressions between variables) are expressed as standardized  
733 regression coefficients, and indirect effects in dotted arrows (multiple regressions in  
734 which the predictor and the mediator are both added in the model) as partial  
735 correlation coefficients. All regressions are adjusted for age, sex and education.  
736 Physical activity, insulin and body mass index values are log-transformed. \*P > 0.05,  
737 \*\*P > 0.01, \*\*\*P < 0.001. Abbreviations: GM = gray matter; BMI = body mass index.

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751 **Tables**

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753 **Table 1 Demographics**

Age, y, mean $\pm$ SD (range)	68.90 $\pm$ 3.77 (64-83)
Female/Male (%)	82/52 (61/39)
Education, y, mean $\pm$ SD (range)	13.14 $\pm$ 3.09 (7-22)
Physical activity, mean $\pm$ SD (range)	6.31 $\pm$ 4.91 (0-23.08)
Insulin, pmol/L, mean $\pm$ SD (range)	65.08 $\pm$ 32.35 (14-201)
BMI, kg/m <sup>2</sup> , mean $\pm$ SD (range)	26.20 $\pm$ 4.31 (18.10-44.18)
Total Cholesterol, mmol/L, mean $\pm$ SD (range)	6.22 $\pm$ 1.14 (3.55-9.8)
HDL Cholesterol, mmol/L, mean $\pm$ SD (range)	1.66 $\pm$ 0.38 (0.94-3.08)
SBP, mm Hg, mean $\pm$ SD (range)	135 $\pm$ 20.08 (88-198)
Current smoker, Yes/No (%)	6/128 (4/96)

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755 Abbreviations: BMI = body mass index; HDL Cholesterol = high density lipoprotein cholesterol; SBP =  
756 systolic blood pressure; SD = standard deviation

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**Table 2 Multiple linear regressions between physical activity and neuroimaging values or cardiovascular risk factors**

		Physical activity				
		b	Std. Error	β	t Value	p Value
Neuroimaging values	Covariates					
GM volume	Age, sex, education	6900.768	3149.087	0.174	2.191	0.030*
	Age, sex, education, insulin	3992.5	3121.2	0.101	1.279	0.203
	Age, sex, education, BMI	2448.9	2882.4	0.062	0.850	0.397
	Age, sex, education, insulin, BMI	1944.8	2915.3	0.049	0.667	0.506
Glucose metabolism	Age, sex, education	0.028	0.012	0.247	2.383	0.019*
	Age, sex, education, insulin	0.029	0.012	0.253	2.387	0.019*
	Age, sex, education, BMI	0.030	0.012	0.262	2.479	0.015*
	Age, sex, education, insulin, BMI	0.030	0.012	0.261	2.439	0.017*
Amyloid deposition	Age, sex, education	-0.033	0.023	-0.122	-0.410	0.161
WMH	Age, sex, education	-2.087	1.094	-0.162	-1.907	0.059
MK	Age, sex, education	-0.002	0.006	-0.033	-0.380	0.705
<b>AD-sensitive regions</b>						
Hippocampal volume	Age, sex, education	127.828	47.025	0.219	2.718	0.007**
	Age, sex, education, insulin	86.231	46.802	0.148	1.842	0.068
	Age, sex, education, BMI	82.424	46.216	0.141	1.783	0.077
	Age, sex, education, insulin, BMI	69.057	46.348	0.118	1.490	0.139
Temporo-parietal glucose metabolism	Age, sex, education	0.023	0.018	0.142	1.327	0.188
Precuneus glucose metabolism	Age, sex, education	0.033	0.016	0.217	2.045	0.044*
	Age, sex, education, insulin	0.035	0.016	0.233	2.155	0.034*
	Age, sex, education, BMI	0.035	0.016	0.231	2.143	0.035*
Cingulum FA	Age, sex, education	-0.007	0.006	-0.103	-1.200	0.232
<b>Cardiovascular risk factors</b>						
Insulin	Age, sex, education	-0.202	0.066	-0.254	-3.059	0.003**
	Age, sex, education, BMI	-0.108	0.060	-0.135	-1.783	0.077
BMI	Age, sex, education	-0.061	0.020	-0.250	-2.995	0.003**
	Age, sex, education, insulin	-0.031	0.019	-0.128	-1.677	0.096
Total Cholesterol	Age, sex, education	0.061	0.141	0.034	0.431	0.668
HDL Cholesterol	Age, sex, education	0.040	0.045	0.068	0.888	0.376
SBP	Age, sex, education	-4.211	2.600	-0.135	-1.620	0.108
Smoking habits	Age, sex, education	-0.002	0.028	-0.006	-0.071	0.943

770 \*P > 0.05, \*\*P > 0.01, \*\*\*P < 0.001. Abbreviations: GM = gray matter; WMH = white matter hyperintensities; MK =  
771 mean kurtosis; FA = fractional anisotropy; AD = Alzheimer's disease; BMI = body mass index; HDL Cholesterol = high  
772 density lipoprotein cholesterol; SBP = systolic blood pressure.

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**Table 3 Multiple linear regressions between cardiovascular risk factors and neuroimaging values**

		Insulin					BMI				
		b	Std. Error	$\beta$	t Value	p Value	b	Std. Error	$\beta$	t Value	p Value
Neuroimaging values	Covariates										
GM volume	Age, sex, education	-15705.6	3882.6	-0.315	-4.045	<0.001***	-75345.5	11600.2	-0.465	-6.495	<0.001***
	Age, sex, education, insulin/BMI	-5128.0	4160.6	-0.103	-1.233	0.220	-66810.8	13489.9	-0.412	-4.953	<0.001***
Cerebral glucose metabolism	Age, sex, education	-0.002	0.015	-0.012	-0.113	0.910	0.018	0.053	0.038	0.349	0.728
<b>AD-sensitive regions</b>											
Hippocampal volume	Age, sex, education	-234.369	58.612	-0.320	-3.999	<0.001***	-829.482	187.768	-0.349	-4.418	<0.001***
	Age, sex, education, insulin/BMI	-139.909	66.606	-0.191	-2.101	0.038*	-596.625	215.956	-0.251	-2.763	0.007**
Precuneus glucose metabolism	Age, sex, education	0.009	0.020	0.049	0.450	0.654	0.027	0.070	0.043	0.390	0.697

778 \* $P > 0.05$ , \*\* $P > 0.01$ , \*\*\* $P < 0.001$ . Abbreviations: GM = gray matter; AD = Alzheimer's disease; BMI = body mass  
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792 **Table 4 Detailed statistics of causal mediation analyses**

Model	ADE			ACME		
	Estimate	CI 95%	P value	Estimate	CI 95%	P value
<b>Model 1</b>	3950	[-3100; 10188.12]	0.265	3050	[729; 6402.87]	0.005**
<b>Model 2</b>	2546.694	[-3252.001; 8271.47]	0.378	4454.972	[1444.678; 7962.51]	0.003**
<b>Model 3</b>	94.1836	[-11.4680; 182.31]	0.080	46.1605	[11.8789; 82.41]	0.004**
<b>Model 4</b>	81.751	[-16.646; 178.89]	0.098	48.594	[14.272; 86.05]	0.004**

793 In models 1 and 2, GM volume was entered as the dependent variable and physical activity as the independent  
 794 variable. In models 3 and 4, hippocampal volume was the dependent variable. Insulin and body mass index were  
 the mediators of model 1, 3 and model 2, 4, respectively. \* $P > 0.05$ , \*\* $P > 0.01$ , \*\*\* $P < 0.001$ . Abbreviations: ADE  
 = average direct effect; ACME = average causal mediation effect; CI = confidence interval.