

Gut permeability, inflammation, and bone density across the menopause transition

Albert Shieh, ... , Arun S Karlamangla, Gail A. Greendale

JCI Insight. 2019. <https://doi.org/10.1172/jci.insight.134092>.

Clinical Medicine

In-Press Preview

Bone biology

Endocrinology

Background: Inflammation is implicated in many aging-related disorders. In animal models, menopause leads to increased gut permeability and inflammation. Our primary objective was to determine if gut permeability increases during the menopause transition (MT) in women. Our exploratory objectives were to examine whether greater gut permeability is associated with more inflammation and lower bone mineral density (BMD). **Methods:** We included 65 women from the Study of Women's Health Across the Nation. Key measures were markers of gut permeability (gut barrier dysfunction [fatty acid binding protein 2 [FABP2]) and immune activation secondary to gut microbial translocation (lipopolysaccharide binding protein [LBP], soluble CD14 [sCD14]); inflammation (high-sensitivity CRP); and lumbar spine (LS) or total hip (TH) BMD. **Results:** In our primary analysis, FABP2, LBP, and sCD14 increased by 22.8% ($P = 0.001$), 3.7% ($P = 0.05$), and 8.9% ($P = 0.0002$), respectively from pre- to postmenopause. In exploratory, repeated measures, mixed-effects linear regression (adjusted for age at the premenopausal visit, body mass index, race/ethnicity, and study site), greater gut permeability was associated with greater inflammation, and lower LS and TH BMD. **Conclusions:** Gut permeability increases during the MT. Greater gut permeability is associated with [...]

Find the latest version:

<https://jci.me/134092/pdf>



1 **TITLE**

2 Gut permeability, inflammation, and bone density across the menopause transition

3

4 **AUTHORS AND AFFILIATIONS**

5 Albert Shieh MD¹, Marta Epeldegui PhD², Arun S. Karlamangla MD PhD¹, and Gail A.

6 Greendale MD¹

7 1. Division of Geriatrics, Department of Medicine, David Geffen School of Medicine at

8 University of California, Los Angeles

9 2. Department of Obstetrics and Gynecology, David Geffen School of Medicine at

10 University of California, Los Angeles

11

12 **CORRESPONDING AUTHOR/CONTACT FOR REPRINT REQUESTS**

13 Albert Shieh:

14 • ashieh@mednet.ucla.edu

15 • 781-254-5034

16 • UCLA Division of Geriatrics

17 10945 Le Conte Avenue, Suites 2339-2345

18 Los Angeles, CA 90095-1687

19

20 **CONFLICT OF INTEREST STATEMENT**

21 The authors have declared that no conflict of interest exists.

22

23 **ABSTRACT**

24 **Background:** Inflammation is implicated in many aging-related disorders. In animal models,
25 menopause leads to increased gut permeability and inflammation. Our primary objective was to
26 determine if gut permeability increases during the menopause transition (MT) in women. Our
27 exploratory objectives were to examine whether greater gut permeability is associated with more
28 inflammation and lower bone mineral density (BMD).

29
30 **Methods:** We included 65 women from the Study of Women’s Health Across the Nation. Key
31 measures were markers of gut permeability (gut barrier dysfunction [fatty acid binding protein 2
32 [FABP2]) and immune activation secondary to gut microbial translocation (lipopolysaccharide
33 binding protein [LBP], soluble CD14 [sCD14]); inflammation (high-sensitivity CRP); and
34 lumbar spine (LS) or total hip (TH) BMD.

35
36 **Results:** In our primary analysis, FABP2, LBP, and sCD14 increased by 22.8% (p=0.001), 3.7%
37 (p=0.05), and 8.9% (p=0.0002), respectively from pre- to postmenopause. In exploratory,
38 repeated measures, mixed-effects linear regression (adjusted for age at the premenopausal visit,
39 body mass index, race/ethnicity, and study site), greater gut permeability was associated with
40 greater inflammation, and lower LS and TH BMD.

41
42 **Conclusions:** Gut permeability increases during the MT. Greater gut permeability is associated
43 with more inflammation and lower BMD. Future studies should examine the longitudinal
44 associations of gut permeability, inflammation, and BMD.

45

46 **Funding:** NIH, Department of Health and Human Services, through the National Institute on
47 Aging, National Institute of Nursing Research, and NIH Office of Research on Women's Health
48 (U01NR004061, U01AG012505, U01AG012535, U01AG012531, U01AG012539,
49 U01AG012546, U01AG012553, U01AG012554, U01AG012495).

50 **INTRODUCTION**

51 Inflammation contributes to the pathogenesis of numerous medical disorders that commonly
52 affect older adults, including osteoporosis, diabetes mellitus, cardiovascular disease, and
53 dementia (1-10); this is often referred to as “inflammaging” (3, 11). Beyond chronological aging,
54 the postmenopausal state may independently contribute to greater inflammation in older women.
55 In animal models, chemical or surgical menopause leads to down-regulation of epithelial
56 junction proteins, gut barrier dysfunction and increased gut permeability (12). This, in turn,
57 permits the translocation of microbes from the intestinal lumen into the subepithelial space,
58 triggering immune cells to produce pro-inflammatory cytokines (12). Whether an increase in gut
59 permeability accompanies the menopause transition (MT) in humans is uncertain. If gut
60 permeability does increase, whether it is associated with inflammation and end organ
61 manifestations is also unknown.

62
63 The overarching goals of this pilot study were to determine if gut permeability increases during
64 the MT, and if such an increase were confirmed, to explore whether gut permeability is
65 associated with inflammation and bone mineral density (BMD). In this report, subsequent use of
66 the term “gut permeability” refers to its indirect assessment using these blood markers. We used
67 bone as a model end-organ system because increased gut permeability mediates inflammation
68 and hypogonadal BMD loss in rodents, and probiotics that reduce gut permeability (12) or
69 inflammation (13-15) can decrease bone resorption (12-15) and prevent bone loss (12, 15). Our
70 primary hypothesis was that gut permeability increases from pre- to postmenopause. Our
71 exploratory hypothesis was that greater gut permeability is associated with greater inflammation

72 (assessed by high-sensitivity CRP [hs-CRP]) and lower BMD (assessed by dual-energy X-ray
73 absorptiometry [DXA]).

74

75 The parent study for this pilot was the Study of Women's Health Across the Nation (SWAN), a
76 multi-racial/ethnic, longitudinal cohort study of the MT. We obtained two stored plasma samples
77 for each study participant from the SWAN Repository: one from premenopause (operationalized
78 as 3-5 years before the final menstrual period [FMP]), and one from postmenopause
79 (operationalized as 3-5 years after the FMP). To assess gut permeability, we assayed indirect
80 markers of gut barrier dysfunction (fatty acid binding protein 2 [FABP2]) and immune activation
81 secondary to gut microbial translocation (lipopolysaccharide binding protein [LBP], soluble
82 CD14 [sCD14]). FABP2 is considered a marker of gut epithelial cellular dysfunction because it
83 is expressed by enterocytes, and released into the circulation when gut epithelial cells are
84 damaged (16, 17). LBP and sCD14 are deemed markers of immune activation secondary to gut
85 microbial translocation because they are produced by hepatocytes and monocytes/macrophages,
86 respectively, in response to lipopolysaccharide (a product of gram negative bacterial, of which
87 the gut is the predominant source) (18, 19).

88 **RESULTS**

89 ***Participant Characteristics in Pre- to Postmenopause***

90 We included a total of 65 women. At the premenopausal visit, subjects were, on average, 49.9
91 years of age, and 3.8 years before their FMP. At the postmenopausal visit, mean age was 57.7
92 years and mean time after the FMP was 4.0 years (Table 1).

93

94 ***Results for Primary Objective: Change in Gut Permeability during the Menopause Transition***

95 From pre- to postmenopause, median E2 decreased from 51.7 to 15.5 pg/ml ($p < 0.0001$,
96 Wilcoxon signed-rank test), and FSH increased from 14.8 to 84.3 mIU/ml ($p < 0.0001$, Wilcoxon
97 signed-rank test) (Table 1). E2 and FSH had skewed distributions, so were log-transformed for
98 analyses. During the same period, FABP2, LBP, and sCD14 increased from 1,298 to 1,595
99 pg/ml, 5892 to 6,112 ng/ml, and 948 to 1,032 ng/ml, respectively.

100

101 In our primary analysis, we used the paired t-test at a two-sided alpha of 0.05 to determine if the
102 rise in each gut permeability marker was statistically significant. Using this approach, the
103 increases in FABP2 ($p = 0.001$), LBP ($p = 0.05$), and sCD14 ($p = 0.0002$) were considered
104 statistically significant. We further examined the associations of these markers with E2 and FSH
105 (log transformed), using repeated measures, mixed effects linear regression (Table 2). Adjusted
106 for chronological age at the premenopausal visit, race/ethnicity, BMI, and study site, lower E2
107 and greater FSH (tested separately) were associated with greater FABP2 and sCD14. Each 50%
108 decrement in E2 was associated with 77 pg/ml greater FABP2 ($p = 0.02$), and 28 ng/ml greater
109 sCD14 ($p = 0.001$). Analogously, each two-fold increment in FSH was associated with 113 pg/ml

110 greater FABP2 ($p=0.001$), and 34 ng/ml greater sCD14 ($p<0.0001$). Neither E2 nor FSH was
111 significantly associated with LBP.

112

113 ***Results for Exploratory Objective: Associations of Gut Permeability with Inflammation and***
114 ***Bone Mineral Density***

115 Median hs-CRP increased from 1.4 mg in premenopause to 1.6 mg/L in postmenopause, but this
116 increase did not reach statistical significance ($p=0.06$, Wilcoxon signed-rank test). The
117 distribution of hs-CRP was skewed. During the same period, mean LS and TH BMD decreased
118 from 1.104 to 0.986 and from 0.964 to 0.901 g/cm², respectively ($p<0.00001$ for both sites).
119 Scatter plots of FABP2, LBP, and sCD14 vs. hs-CRP are presented in Figure 1. Figure 2 contains
120 scatter plots of FABP2, LBP, sCD14 and hs-CRP vs. BMD; we present plots for LS only, as
121 those for FN were similar (data not shown).

122

123 To test whether greater FABP2, LBP, or sCD14 is associated with greater hs-CRP, we used
124 repeated measures, mixed effects linear regression (Table 3). hs-CRP was log transformed for
125 these analyses because of skewed distributions. Adjusted for age at the premenopausal visit,
126 race/ethnicity, BMI, and study site, each SD increment in LBP and sCD14 was associated with
127 2.32- ($p<0.001$) and 1.44- ($p=0.001$) fold greater hs-CRP, respectively. FABP2 was not
128 associated with hs-CRP.

129

130 To examine the associations of FABP2, LBP, sCD14 and hs-CRP with BMD, we again used
131 repeated measures, mixed effects linear regression. Adjusted for the same covariates listed
132 above, each SD increment in FABP2 was associated with 0.021 and 0.017 g/cm² lower LS

133 (p=0.05) and TH BMD (p=0.02), respectively. Similarly, each SD increment in sCD14 was
134 associated with 0.051 lower LS BMD (p=0.0001) and 0.025 g/cm² lower TH BMD (p=0.004).
135 LBP was not associated with BMD. In contrast, each two-fold increment in hs-CRP was
136 associated with 0.024 g/cm² lower LS (p<0.0001) BMD; hs-CRP was not associated with TH
137 BMD.

138 **DISCUSSION**

139 The primary objective of this longitudinal, pilot study was to determine if gut permeability
140 (assessed non-invasively using markers of gut barrier dysfunction [FABP2] and immune
141 activation secondary to microbial translocation [LBP, sCD14]) increases during the MT. Upon
142 observing this increase, our exploratory objective was to examine whether greater gut
143 permeability was associated with greater inflammation and lower BMD. Our primary analysis
144 supports the hypothesis that, in humans, gut permeability increases from pre- to postmenopause.
145 Exploratory analyses suggest that greater gut permeability (LBP and sCD14) is associated with
146 greater inflammation (hs-CRP), and both greater gut permeability and inflammation (FABP2,
147 sCD14, hs-CRP) are associated with lower BMD.

148
149 Several prior studies have reported the effects of aging, but not the MT, on gut permeability in
150 humans. One cross-sectional analysis found that gut permeability was higher in older vs. younger
151 men and women (20). Subsequently, the longitudinal Cardiovascular Health Study determined
152 that sCD14 increased by 0.4% per year in men and women over 65 years ($p < 0.0001$) (21). This is
153 the first human study, to our knowledge, to demonstrate that gut permeability (assessed
154 indirectly) increases during the MT. This finding is additionally supported by data that lower E2
155 and greater FSH are associated with greater FABP2 and sCD14.

156
157 In rodent models, increased gut permeability underlies an MT-related increase in inflammation.
158 Chemical or surgical menopause leads to decreased expression of epithelial junction proteins
159 (e.g., claudins 1, 2, 3, and Jam3), and increased gut permeability (12). This, in turn, permits the
160 translocation of microbes from the intestinal lumen into the subepithelial space, triggering

161 immune cells to produce pro-inflammatory cytokines (12). Our exploratory analyses suggest that
162 immune activation from gut microbial translocation (LBP and sCD14) is associated with greater
163 inflammation (hs-CRP). The implications are that a mechanism that contributes to inflammation
164 in rodents may similarly be present in humans, and that MT-related changes in gut physiology
165 warrants further research as a potential therapeutic target for preventing inflammation.

166

167 The physiologic importance of increased gut permeability and inflammation is highlighted in
168 rodent studies in which probiotics that reduce gut permeability (12) or inflammation (13-15) can
169 decrease bone resorption (12-15) and prevent bone loss (12, 15). In humans, a prior SWAN study
170 reported that greater inflammation (assessed by hs-CRP) is associated with lower BMD (cross-
171 sectional), lower hip strength (cross-sectional), and more future fractures (4). We also have
172 ongoing analyses using the full SWAN Bone Cohort to examine the associations of change in hs-
173 CRP and IL-6 with change in BMD. Here, we report that greater FABP2 and sCD14 are
174 associated with lower LS and TH BMD, and greater hs-CRP is associated with lower LS BMD.
175 While these findings suggest a link between gut permeability, inflammation and BMD, this study
176 was not sufficiently powered to explicitly relate changes in FABP2, LBP, sCD14, and hs-CRP to
177 concurrent change in BMD. We also could not discern whether inflammation vs. circulating
178 bacterial products contribute more to lower BMD.

179

180 Gut permeability can be assessed by various methods. Histologic and electron microscopic
181 evaluation of biopsy samples provides direct information on gut barrier integrity and mucosal
182 tight junctions (22). Alternatively, enteral administration of non-digestible markers affords a
183 functional assessment of gut permeability (22-24). However, neither methodology is well-suited

184 to community-based cohort designs. For this pilot study, we indirectly assessed gut permeability
185 using markers of gut barrier dysfunction (FABP2) and immune activation secondary to gut
186 microbial translocation (LBP, sCD14). These markers are associated with adverse outcomes in
187 pathologic conditions characterized by increased gut permeability (e.g., liver cirrhosis,
188 inflammatory bowel disease, HIV, and sepsis) (18, 25-28). That FABP2, LBP, and sCD14
189 increase from pre- to postmenopause suggests that they are sensitive and precise enough to
190 examine within-individual changes in gut physiology during the MT. Ideally, prior to large-scale
191 implementation of these blood markers in observational research, their correlation with
192 functional measurements of gut permeability using non-digestible markers should be determined.

193

194 While FABP2 is specific to the intestine (17), LBP and sCD14 are not; they are produced by
195 hepatocytes and immune cells, respectively (18, 19, 22). We presume that increases in LBP and
196 sCD14 reflect immune activation from gut microbial translocation because the gut is the primary
197 source of gram negative microbes (29). However, the vaginal flora becomes more gram negative
198 during postmenopause (30). Thus, gram negative microbial translocation could also occur across
199 the vaginal epithelium. Future studies should consider whether postmenopausal loss of epithelial
200 integrity contributes to a greater inflammatory burden.

201

202 The primary strength of this pilot is that we examined longitudinal changes in markers of gut
203 permeability in a well-characterized sample of women transitioning through the MT. In addition,
204 in exploratory analyses, we related these gut permeability markers to a marker of inflammation
205 and BMD. This study is a critical first step in elucidating whether a mechanism that contributes
206 to inflammation and disease in animal models is similarly present in humans.

207

208 The principal limitation of this study is the modest sample size of 65 women, which was,
209 nonetheless, powered to our primary objective: to determine if gut permeability increases during
210 the MT. Our primary analysis upheld that all tested markers of gut permeability increase from
211 pre- to postmenopause. While our exploratory analyses suggest a link between gut permeability,
212 inflammation and BMD, not all predictor-outcome associations were significant. In addition, this
213 study was not sufficiently powered to explicitly examine the longitudinal associations between
214 within-woman changes in exposures and outcomes. Nonetheless, our pilot data provide a strong
215 rationale to conduct future studies of these questions using larger study samples. Specifically,
216 future investigations should explore the trajectories of change in gut permeability across the MT,
217 and relate the longitudinal changes in gut permeability and inflammation to bone loss and other
218 end-organ manifestations. Lastly, we measured a limited number of gut permeability markers.
219 Future work should also assay markers such as zonulin and claudin 3, which reflect gut epithelial
220 paracellular integrity (31, 32), since decreased estrogen down-regulates gut epithelial junction
221 proteins (12).

222

223 In summary, our findings support that gut permeability increases from pre- to postmenopause,
224 and exploratory results suggest a relation between gut permeability, inflammation, and BMD,
225 that should be further investigated in larger study samples. To our knowledge, this is the first
226 demonstration of a MT-related “gut leak” in humans. If, indeed, increased gut permeability
227 during the MT leads to a state of immune activation and inflammation with negative health
228 consequences, the clinical implications could be substantial: interventions that target gut

229 physiology during and after the MT could lessen inflammation and multiple disorders that plague
230 older adults.
231

232 **METHODS**

233 SWAN, the parent study for this pilot, is a multi-center, longitudinal cohort study of the MT in
234 3,302 ambulatory, multi-racial/ethnic (Black, Chinese, Japanese, White) women. The SWAN
235 cohort has been described in detail (33). In brief, at SWAN baseline, participants were in pre-
236 (no change in menstrual bleeding in the past year) or early perimenopause (less predictable
237 menstrual bleeding at least once every 3 months), and between 42 to 52 years of age. After 2
238 decades of observational follow-up, consisting of 17 serial visits, all SWAN women have
239 transitioned to postmenopause. To access BMD data, we selected our pilot sample from the
240 SWAN Bone Cohort, a subset of 2,365 participants, in whom BMD was measured at each study
241 visit. The SWAN Repository provided previously collected and frozen plasma samples to
242 measure the various markers of gut permeability (33, 34).

243

244 ***Study Sample***

245 Eligibility: To be included in the pilot, participants were required to: 1) have undergone natural
246 menopause; 2) have a known final menstrual period (FMP) date; 3) have at least one visit in
247 premenopause (operationalized as having occurred 3-5 years prior to the FMP) and one visit in
248 postmenopause (operationalized as having occurred between 3-5 years after the FMP) from
249 which previously collected plasma could be accessed through the SWAN Repository to measure
250 FABP2, LBP, and sCD14; 4) have pre-existing measures of E2, FSH, hs-CRP and BMD
251 corresponding to the plasma sample times; and 5) have not used sex steroid medications or bone
252 modifying agents in between their pre- and postmenopausal pilot study visits. We selected the
253 timing of the pre- and postmenopausal samples (relative to the FMP date) based on the

254 trajectories of change in estradiol across the MT: estradiol does not decrease significantly until
255 3 years pre-FMP and nadirs at about 3 years after the FMP (35).

256

257 Sample Derivation: Figure 3 depicts the derivation of the analysis sample. In total, 621 women
258 met our eligibility criteria. From this potential pool of subjects, the SWAN Repository randomly
259 selected 65 women for this pilot study (see Analysis for sample size considerations).

260

261 ***Phlebotomy***

262 Every effort was made to perform phlebotomy fasting, and before 10:00 AM during the early
263 follicular phase (EFP, between days 2 and 5) of a spontaneous menstrual cycle (prior to
264 postmenopause). If an EFP sample could not be obtained after 2 attempts, a random fasting
265 sample was taken within a 90-day window of the anniversary of the baseline visit. Collected
266 specimens were initially stored between -20 to -80 degrees Celsius at individual study sites for
267 up to 30 days, and then shipped to a central lab (either Medical Research Lab [Highland Heights,
268 KY] or CLASS Laboratory at the University of Michigan [Ann Arbor, MI]). Once at the central
269 lab, samples were stored at -80 degrees Celsius.

270

271 ***Marker of Gut Permeability***

272 To assess gut permeability, we measured markers of gut barrier dysfunction (FABP2) and
273 immune activation secondary to microbial translocation (LBP, sCD14) using citrated plasma
274 samples that were accessed from the SWAN Repository. FABP2 was measured using the
275 Quantikine Human FABP2/I-FABP ELISA (R&D Systems). The lower limit of detection (LLD)
276 for this assay is 6.21 pg/mL. The intra-assay coefficient of variation (CV) was <4.1%. LBP and

277 sCD14 were assayed in multiplex, using the Luminex platform with custom-made panels (R&D
278 Systems). This platform uses microparticles that are pre-coated with analyte-specific antibodies
279 and incubated with diluted plasma samples, followed by a biotin antibody, and lastly by a
280 streptavidin-phycoerythrin conjugate. The fluorescence intensity of each analyte's microparticles
281 are then quantified using a Bioplex 200 (Luminex) System Analyzer, and the data analyzed using
282 Bioplex Manager software. The LLD for the LBP is 0.0839 ng/ml, and the intra-assay CV was
283 <2.9%. The LLD for sCD14 is 0.0366 ng/ml, and the intra-assay CV was <2.9%.

284

285 ***Markers of Ovarian Function***

286 To examine whether reduced ovarian function is associated with greater gut permeability across
287 the MT, we used previously measured E2 and FSH. Serum E2 was measured in duplicate with a
288 modified, off-line ACS:180 (E2-6) immunoassay using an ACS:180 automated analyzer (Bayer
289 Diagnostics Corp., Tarrytown, New York). The average between duplicates was recorded in the
290 dataset and used in analyses. The LLD was 1.0 pg/ml, and the intra-assay CV was 6.4%. Serum
291 FSH was measured in singlicate with a 2-site chemiluminometric assay (Bayer Diagnostics
292 Corp., Tarrytown, New York). The LLD was 1.05 mIU/ml, and intra-assay CV was 6.0%. For
293 this pilot, we only included women whose premenopausal E2 and FSH measurements were
294 obtained in the EFP of a spontaneous menstrual cycle.

295

296 ***Marker of Inflammation: High-sensitivity C-reactive Protein (hs-CRP)***

297 To assess whether the associations of gut permeability with inflammation, and of inflammation
298 with BMD, we used hs-CRP. CRP was measured at SWAN baseline, and at follow-up visits 1, 3-
299 7, 9, 10, 12, and 15. Owing to the long duration of follow up, the laboratories that measured CRP

300 and the assays employed changed with time. SWAN used 3 CRP assays and a fourth, hs-CRP
301 assay as the calibration standard to harmonize all assays. The fourth assay was also used to
302 obtain results for analytes that had been below the LLD on each of the 3 original assays. From
303 baseline to follow-up visit 7, CRP was measured by Medical Research Laboratories. CRP assays
304 for samples collected at follow-up visit 9 and later were conducted by the CLASS Laboratory at
305 the University of Michigan (Ann Arbor, MI) using the Alfa Wassermann ACE analyzer. Assays
306 and their calibration are described below.

307
308 CRP assay 1: From baseline to follow-up visit 7, CRP was measured using an ultrasensitive rate
309 immunonephelo-metric method, with a LLD of 0.3 mg/L (BN100; Dade-Behring, Marburg,
310 Germany). The intra-assay CV at CRP concentrations of 0.5 and 22.0 mg/L were 10–12% and 5–
311 7%, respectively. CRP assay 2: For follow-up visit 12, CRP was assayed using the ACE
312 *UltraWide Range* assay, a latex-enhanced turbidimetric *in vitro* immunoassay (Alfa
313 Wassermann, West Caldwell, NJ). The LLD was 0.1 mg/L, and the intra-assay CV at CRP
314 concentrations of 0.5 mg/L and 9.8 mg/L were 5.7-7.0% and 1.2%, respectively. CRP Assay 3:
315 Samples from follow-up visit 9, 10 and 15 were assayed using a high sensitivity immunoassay
316 (Alfa Wassermann, West Caldwell, NJ). The LLD was 0.1 mg/L, and the intra-assay CV at CRP
317 concentrations of 0.5 and 9.8 mg/L were 5.7-7.0% and 1.2%, respectively. CRP assay 4: For
318 approximately 25% of all samples run between baseline and follow-up 15, CRP was below the
319 LLD of the original 3 assays outlined above. In these instances, an additional sample was
320 retrieved from the Repository and CRP was measured using the Human High Sensitivity CRP
321 ELISA (R&D Systems, DCRP00), a quantitative sandwich enzyme immunoassay. The lower
322 LLD for this assay was 0.10 pg/ml. The remaining 75% of results (those above the LLD for the 3

323 original assays) were calibrated to the high sensitivity ELISA by simultaneously assaying 600
324 paired samples (representing the full range of results from each of the original 3 assays) and the
325 high sensitivity assay; thus, there were 200 paired samples for each original vs. high sensitivity
326 ELISA. After calibration, correlations between the first 3 CRP assays and the fourth, high
327 sensitivity ELISA were ≥ 0.94 .

328

329 ***Bone Mineral Density***

330 BMD at the lumbar spine (LS) and total hip (TH) BMD was measured by DXA. At study
331 inception, the Pittsburgh and Oakland sites used the Hologic QDR 2000 machine, and the
332 Boston, Los Angeles, and Michigan sites used the Hologic QDR 4500A machine. At follow-up
333 visit 8, Pittsburgh and Oakland upgraded to the 4500A models. Boston and Los Angeles
334 upgraded to the Hologic Discovery model at follow-up visit 13. To develop cross-calibration
335 regression equations, each site obtained duplicate scans using the old and new hardware within
336 90 days in at least 40 volunteers (N=40 for Oakland, Pittsburgh; N=41 for Boston; and N=50 for
337 Los Angeles). To determine the short-term *in vivo* precision error, each study site measured LS
338 and TH BMD twice in 5 women with complete subject repositioning between duplicate scans.
339 Using the root mean square SD approach, the precision error in SWAN was 1.4% at the LS and
340 2.2% at the TH. An anthropomorphic spine phantom was circulated between sites for cross-site
341 calibration. Standard quality control phantom scans were conducted before each BMD
342 measurement session. If necessary, these were used to adjust for longitudinal machine drift.

343

344 ***Additional Measures and Variables***

345 The following were included in analyses as covariates: age (years), race/ethnicity (Black,
346 Chinese, Japanese, White), and body mass index (BMI [weight in kilograms/(height in
347 meters)²]), based on height and weight measured using standardized protocols and with
348 participants wearing light clothing and no shoes.

349

350 *Statistical Analyses*

351 Sample Size: The sample size of 65 subjects was predicated on funding constraints and the
352 power to detect change in our primary outcomes. Because there are no published cross-sectional
353 or longitudinal studies examining whether gut permeability (assessed directly or indirectly)
354 increase across the MT, we relied on change in sCD14 with aging published in the
355 Cardiovascular Health Study (CHS) (21). In CHS, sCD14 increased by 0.4% per year (21),
356 which would result in a gain of 2.4 to 4.0% over a 6 to 10-year period (the approximate time
357 interval between our pre- to postmenopausal time points). A sample size of 65 yielded 90%
358 power at a 2-sided alpha of 0.05 to detect 4.5%, 4.4%, and 2.4% changes in FABP2, LBP, and
359 sCD14, respectively, using the inter-assay CV for each analyte.

360

361 Data Analyses: We generated descriptive statistics for all variables and assessed the distributions
362 of continuous variables for normality. E2, FSH, and hs-CRP had skewed distributions, and were
363 thus log-transformed for relevant analyses. For all analyses, a p-value of ≤ 0.05 was considered to
364 be statistically significant.

365

366 To assess our primary hypothesis, we used the paired t-test at a 2-sided alpha of 0.05 to test the
367 mean within-person change in FABP2, LBP, and sCD14 from pre- to postmenopause against the

368 null hypothesis of zero change. We considered a p-value of 0.05 and lower to be statistically
369 significant. To additionally examine whether ovarian function is associated with gut
370 permeability, we used repeated measures, mixed effects linear regressions with E2 or FSH as
371 continuous primary predictors, and FABP2, LBP, or sCD14 as continuous outcome measures
372 (with each primary predictor-outcome pair modeled separately). Models included a random
373 intercept at the individual level to account for clustering. Covariates were chronologic age at the
374 premenopausal visit, BMI, race/ethnicity, and SWAN study site.

375

376 To test our secondary hypothesis, we used repeated measures, mixed effects linear regression
377 with FABP2, LBP, sCD14, or hs-CRP as continuous primary predictors, and hs-CRP or LS or
378 TH BMD as continuous outcomes variables. As above, each primary predictor-outcome pair was
379 modeled separately, and all models included a random intercept at the individual level.

380 Covariates were chronologic age at the premenopausal visit, BMI, race/ethnicity, and study site.

381

382 For all relational analyses stipulated above, we used mixed effects, repeated measures linear
383 regression because this approach is best suited for detecting predictor-outcome associations in
384 small samples. This is because this type of model accounts for the associations of both between-
385 women and within-woman differences in the predictor with corresponding differences in the
386 outcome.

387

388 ***Human Study Approval***

389 Each SWAN clinical site obtained Institutional Review Board approval, and all participants
390 provided written informed consent.

391 **AUTHOR CONTRIBUTIONS**

392 Participant recruitment for the parent SWAN study was contributed by GAG. Data management
393 and cleaning were contributed by AS, ASK, and GAG. Performance of gut permeability assays
394 was contributed by ME. Analytic design and statistical analysis were contributed by AS, ASK,
395 and GAG. Primary manuscript drafting by contributed by AS and GAG. Critical review and
396 revision of manuscript were contributed by AS, ME, ASK, and GAG.

397 **ACKNOWLEDGMENTS**

398 The Study of Women's Health Across the Nation (SWAN) has grant support from the National
399 Institutes of Health (NIH), DHHS, through the National Institute on Aging (NIA), the National
400 Institute of Nursing Research (NINR) and the NIH Office of Research on Women's Health
401 (ORWH) (Grants U01NR004061; U01AG012505, U01AG012535, U01AG012531,
402 U01AG012539, U01AG012546, U01AG012553, U01AG012554, U01AG012495). The content
403 of this article is solely the responsibility of the authors and does not necessarily represent the
404 official views of the NIA, NINR, ORWH or the NIH.

405 Clinical Centers: *University of Michigan, Ann Arbor – Siobán Harlow, PI 2011 – present,*
406 *MaryFran Sowers, PI 1994-2011; Massachusetts General Hospital, Boston, MA – Joel*
407 *Finkelstein, PI 1999 – present; Robert Neer, PI 1994 – 1999; Rush University, Rush University*
408 *Medical Center, Chicago, IL – Howard Kravitz, PI 2009 – present; Lynda Powell, PI 1994 –*
409 *2009; University of California, Davis/Kaiser – Ellen Gold, PI; University of California, Los*
410 *Angeles – Gail Greendale, PI; Albert Einstein College of Medicine, Bronx, NY – Carol Derby, PI*
411 *2011 – present, Rachel Wildman, PI 2010 – 2011; Nanette Santoro, PI 2004 – 2010; University*
412 *of Medicine and Dentistry – New Jersey Medical School, Newark – Gerson Weiss, PI 1994 –*
413 *2004; and the University of Pittsburgh, Pittsburgh, PA – Karen Matthews, PI.*

414 NIH Program Office: *National Institute on Aging, Bethesda, MD – Chhanda Dutta 2016-*
415 *present; Winifred Rossi 2012–2016; Sherry Sherman 1994 – 2012; Marcia Ory 1994 – 2001;*
416 *National Institute of Nursing Research, Bethesda, MD – Program Officers.*

417 Central Laboratory: *University of Michigan, Ann Arbor – Daniel McConnell (Central Ligand*
418 *Assay Satellite Services).*

419 Coordinating Center: University of Pittsburgh, Pittsburgh, PA – Maria Mori Brooks, PI 2012 -
420 present; Kim Sutton-Tyrrell, PI 2001 – 2012; New England Research Institutes, Watertown, MA
421 - Sonja McKinlay, PI 1995 – 2001.

422 Steering Committee: Susan Johnson, Current Chair

423 Chris Gallagher, Former Chair

424

425 **We thank the study staff at each site and all the women who participated in SWAN.**

426

427 This report is based on samples from the SWAN Core Repository. Scientists interested in
428 developing studies based on this resource can find a description of the SWAN Core Repository
429 and SWAN DNA Repository and information on obtaining access to the resources at

430 <https://www.nia.nih.gov/research/dgcg/study-womens-health-across-nation-swan-repository>

431

432 We thank Michelle Liao for figure preparation.

433 **REFERENCES**

- 434 1. Kennedy B, Berger S, Brunet A, Campisi J, Cuervo A, Epel E, et al. Geroscience: linking
435 aging to chronic disease. *Cell*. 2014;159(4):709-13.
- 436 2. Prasad S, Sung B, and Aggarwal B. Age-associated chronic diseases require age-old
437 medicine: role of chronic inflammation. *Prev Med*. 2012;54 Suppl:S29-S37.
- 438 3. Franceschi C, Garagnani P, Parini P, Giuliani C, and Santoro A. Inflammaging: a new
439 immune-metabolic viewpoint for age-related diseases. *Nature reviews Endocrinology*.
440 2018;14(10):576-90.
- 441 4. Ishii S, Cauley JA, Greendale GA, Crandall CJ, Danielson ME, Ouchi Y, et al. C-reactive
442 protein, bone strength, and nine-year fracture risk: data from the Study of Women's
443 Health Across the Nation (SWAN). *J Bone Miner Res*. 2013;28(7):1688-98.
- 444 5. Wang NC, Matthews KA, Barinas-Mitchell EJ, Chang CC, and El Khoudary SR.
445 Inflammatory/hemostatic biomarkers and coronary artery calcification in midlife women
446 of African-American and White race/ethnicity: the Study of Women's Health Across the
447 Nation (SWAN) heart study. *Menopause*. 2016;23(6):653-61.
- 448 6. Ding C, Parameswaran V, Udayan R, Burgess J, and Jones G. Circulating levels of
449 inflammatory markers predict change in bone mineral density and resorption in older
450 adults: a longitudinal study. *J Clin Endocrinol Metab*. 2008;93(5):1952-8.
- 451 7. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat
452 plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*.
453 2003;112(12):1821-30.

- 454 8. Ridker PM, Hennekens CH, Buring JE, and Rifai N. C-reactive protein and other markers
455 of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*.
456 2000;342(12):836-43.
- 457 9. Yaffe K, Kanaya A, Lindquist K, Simonsick EM, Harris T, Shorr RI, et al. The metabolic
458 syndrome, inflammation, and risk of cognitive decline. *Jama*. 2004;292(18):2237-42.
- 459 10. Woodard GA, Mehta VG, Mackey RH, Tepper P, Kelsey SF, Newman AB, et al. C-
460 reactive protein is associated with aortic stiffness in a cohort of African American and
461 white women transitioning through menopause. *Menopause*. 2011;18(12):1291-7.
- 462 11. Baylis D, Bartlett DB, Patel HP, and Roberts HC. Understanding how we age: insights
463 into inflammaging. *Longevity & healthspan*. 2013;2(1):8.
- 464 12. Li J, Chassaing B, Tyagi A, Vaccaro C, Luo T, Adams J, et al. Sex steroid deficiency-
465 associated bone loss is microbiota dependent and prevented by probiotics. *J Clin Invest*.
466 2016;126(6):2049-63.
- 467 13. Ohlsson C, Engdahl C, Fåk F, Andersson A, Windahl S, Farman H, et al. Probiotics
468 protect mice from ovariectomy-induced cortical bone loss. *PLoS One*. 2014;9(3):e92368.
- 469 14. Britton R, Irwin R, Quach D, Schaefer L, Zhang J, Lee T, et al. Probiotic *L. reuteri*
470 treatment prevents bone loss in a menopausal ovariectomized mouse model. *J Cell*
471 *Physiol*. 2014;229(11):1822-30.
- 472 15. Collins F, Irwin R, Bierhalter H, Schepper J, Britton R, Parameswaran N, et al.
473 *Lactobacillus reuteri* 6475 Increases Bone Density in Intact Females Only under an
474 Inflammatory Setting. *PLoS One*. 2016;11(4):e0153180.

- 475 16. Pelsers MM, Namiot Z, Kisielewski W, Namiot A, Januszkiewicz M, Hermens WT, et al.
476 Intestinal-type and liver-type fatty acid-binding protein in the intestine. Tissue
477 distribution and clinical utility. *Clin Biochem.* 2003;36(7):529-35.
- 478 17. Stevens BR, Goel R, Seungbum K, Richards EM, Holbert RC, Pepine CJ, et al. Increased
479 human intestinal barrier permeability plasma biomarkers zonulin and FABP2 correlated
480 with plasma LPS and altered gut microbiome in anxiety or depression. *Gut.* 2017.
- 481 18. Epeldegui M, Magpantay L, Guo Y, Halec G, Cumberland WG, Yen PK, et al. A
482 prospective study of serum microbial translocation biomarkers and risk of AIDS-related
483 non-Hodgkin lymphoma. *AIDS.* 2018;32(7):945-54.
- 484 19. Pugin J, Schurer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, and Tobias PS.
485 Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by
486 lipopolysaccharide-binding protein and soluble CD14. *Proc Natl Acad Sci U S A.*
487 1993;90(7):2744-8.
- 488 20. Man A, Bertelli E, Rentini S, Regoli M, Briars G, Marini M, et al. Age-associated
489 modifications of intestinal permeability and innate immunity in human small intestine.
490 *Clin Sci (Lond).* 2015;129(7):515-27.
- 491 21. Reiner AP, Lange EM, Jenny NS, Chaves PH, Ellis J, Li J, et al. Soluble CD14:
492 genomewide association analysis and relationship to cardiovascular risk and mortality in
493 older adults. *Arterioscler Thromb Vasc Biol.* 2013;33(1):158-64.
- 494 22. Wang L, Llorente C, Hartmann P, Yang AM, Chen P, and Schnabl B. Methods to
495 determine intestinal permeability and bacterial translocation during liver disease. *Journal*
496 *of immunological methods.* 2015;421:44-53.

- 497 23. Peeters M, Hiele M, Ghooos Y, Huysmans V, Geboes K, Vantrappen G, et al. Test
498 conditions greatly influence permeation of water soluble molecules through the intestinal
499 mucosa: need for standardisation. *Gut*. 1994;35(10):1404-8.
- 500 24. Mattioli F, Fucile C, Marini V, Isola L, Montanaro F, Savarino V, et al. Assessment of
501 intestinal permeability using sugar probes: influence of urinary volume. *Clinical*
502 *laboratory*. 2011;57(11-12):909-18.
- 503 25. Sarikaya M, Ergul B, Dogan Z, Filik L, Can M, and Arslan L. Intestinal fatty acid
504 binding protein (I-FABP) as a promising test for Crohn's disease: a preliminary study.
505 *Clinical laboratory*. 2015;61(1-2):87-91.
- 506 26. Pastor Rojo O, Lopez San Roman A, Albeniz Arbizu E, de la Hera Martinez A, Ripoll
507 Sevillano E, and Albillos Martinez A. Serum lipopolysaccharide-binding protein in
508 endotoxemic patients with inflammatory bowel disease. *Inflammatory bowel diseases*.
509 2007;13(3):269-77.
- 510 27. Reiberger T, Ferlitsch A, Payer BA, Mandorfer M, Heinisch BB, Hayden H, et al. Non-
511 selective betablocker therapy decreases intestinal permeability and serum levels of LBP
512 and IL-6 in patients with cirrhosis. *Journal of hepatology*. 2013;58(5):911-21.
- 513 28. Opal SM, Scannon PJ, Vincent JL, White M, Carroll SF, Palardy JE, et al. Relationship
514 between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients
515 with severe sepsis and septic shock. *The Journal of infectious diseases*.
516 1999;180(5):1584-9.
- 517 29. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, and Gordon JI. The
518 human microbiome project. *Nature*. 2007;449(7164):804-10.

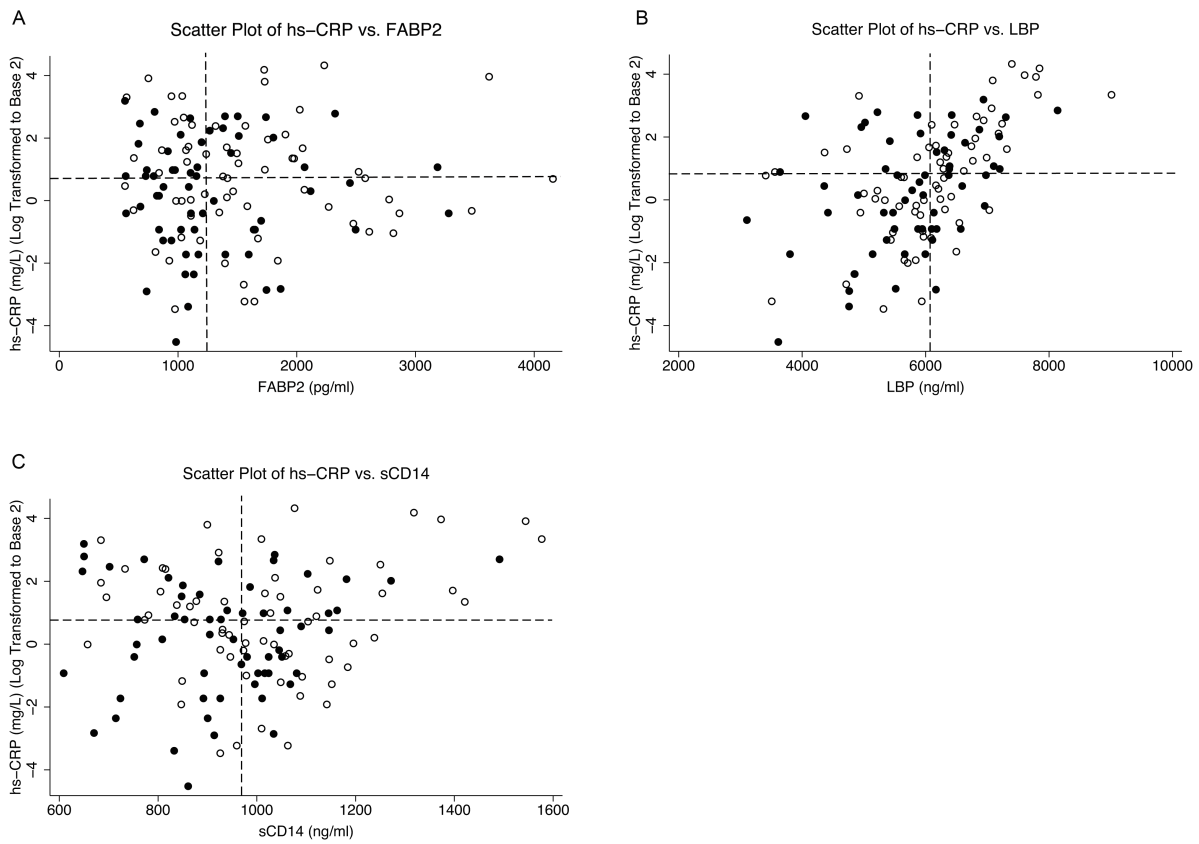
- 519 30. Pabich WL, Fihn SD, Stamm WE, Scholes D, Boyko EJ, and Gupta K. Prevalence and
520 determinants of vaginal flora alterations in postmenopausal women. *The Journal of*
521 *infectious diseases*. 2003;188(7):1054-8.
- 522 31. Fasano A. Intestinal permeability and its regulation by zonulin: diagnostic and
523 therapeutic implications. *Clin Gastroenterol Hepatol*. 2012;10(10):1096-100.
- 524 32. Patel RM, Myers LS, Kurundkar AR, Maheshwari A, Nusrat A, and Lin PW. Probiotic
525 bacteria induce maturation of intestinal claudin 3 expression and barrier function. *The*
526 *American journal of pathology*. 2012;180(2):626-35.
- 527 33. Sowers M, Crawford S, Sternfeld B, Morganstein D, Gold E, Greendale G, et al. In: Lobo
528 R, Kelsey J, and Marcus R eds. *Menopause: Biology and Pathobiology*. San Diego:
529 Academic Press; 2000:175-88.
- 530 34. Kardia SR, Chu J, and Sowers MR. Characterizing variation in sex steroid hormone
531 pathway genes in women of 4 races/ethnicities: the Study of Women's Health Across the
532 Nation (SWAN). *Am J Med*. 2006;119(9 Suppl 1):S3-15.
- 533 35. Randolph JJ, Zheng H, Sowers M, Crandall C, Crawford S, Gold E, et al. Change in
534 follicle-stimulating hormone and estradiol across the menopausal transition: effect of age
535 at the final menstrual period. *J Clin Endocrinol Metab*. 2011;96(3):746-54.

536

537 **FIGURE AND FIGURE LEGENDS**

538

539 **Figure 1**

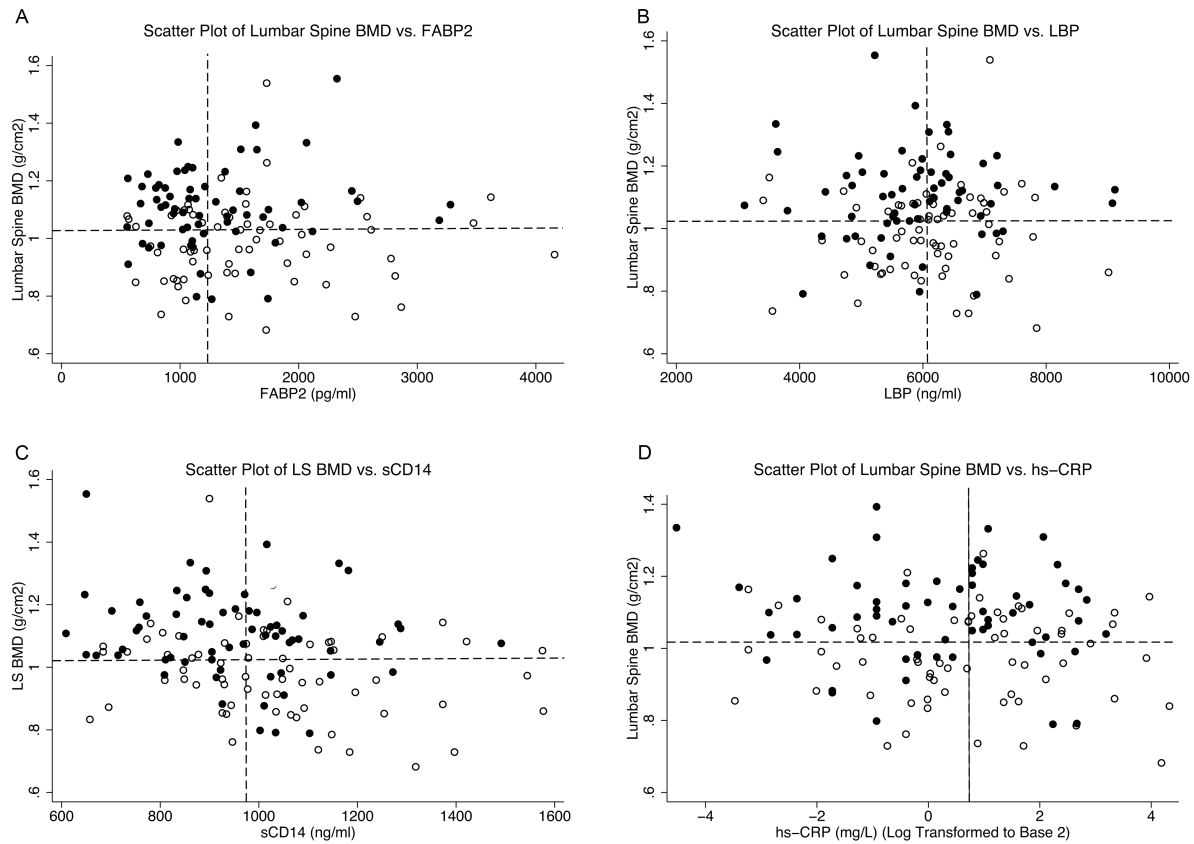


540

541 **Figure 1 Legend. Scatter plots of gut permeability markers vs. hs-CRP.**

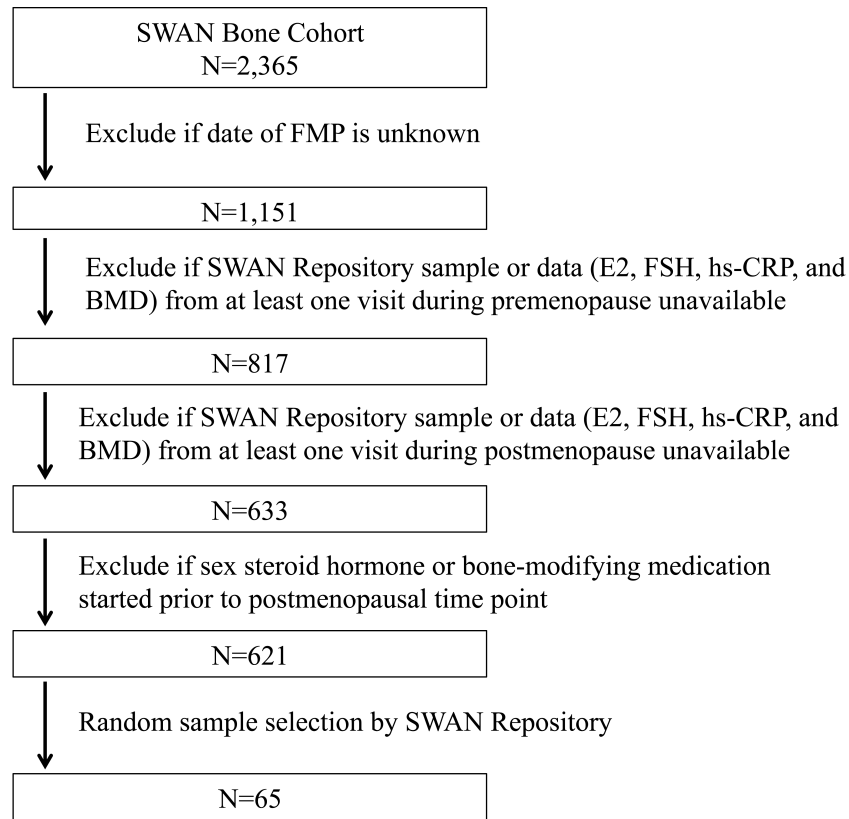
542 paired measures of FABP2 (panel A, gut barrier dysfunction marker), LBP (panel B, immune
543 activation/gut microbial translocation marker), or sCD14 (panel C, immune activation/gut
544 microbial translocation marker) vs. hs-CRP. Paired measures were obtained in premenopuase
545 (closed circles, 3-5 years before the final menstrual period) and postmenopause (open circles, 3-5
546 years after the final menstrual period). Vertical dashed lines indicate the median gut permeability
547 marker values, and horizontal dashed lines mark the median hs-CRP value. A total of 65 subjects
548 were included.

549 **Figure 2**



550
 551 **Figure 2 Legend. Scatter plots of gut permeability and inflammatory markers vs. lumbar**
 552 **spine bone mineral density.** Plots of paired measures of FABP2 (panel A, gut barrier
 553 dysfunction marker), LBP (panel B, immune activation/gut microbial translocation marker),
 554 sCD14 (panel C, immune activation/gut microbial translocation marker), or hs-CRP (panel D,
 555 inflammatory marker) vs. lumbar spine bone mineral density. Paired measures were obtained in
 556 premenopausal (closed circles, 3-5 years before the final menstrual period) and postmenopausal
 557 (open circles, 3-5 years after the final menstrual period). Vertical dashed lines indicate median
 558 gut permeability or inflammatory marker values, and horizontal dashed lines mark the median
 559 lumbar spine bone mineral density value. A total of 65 subjects were included.

560 **Figure 3**
561



562
563 **Figure 3 Legend. Analysis sample derivation.** This flow chart shows the derivation of the
564 analysis sample. In order to be included in the study, participants needed to meet the following
565 criteria: 1) have undergone natural menopause; 2) have a known final menstrual period (FMP)
566 date; 3) have at least one visit in premenopause and one visit in postmenopause from which
567 previously collected plasma could be accessed through the SWAN Repository to measure
568 FABP2, LBP, and sCD14; 4) have pre-existing measures of E2, FSH, hs-CRP and BMD
569 corresponding to the plasma sample times; and 5) have not used sex steroid medications or bone
570 modifying agents in between their pre- and postmenopausal pilot study visits. A total of 621
571 women met these criteria. From this potential pool of subjects, the SWAN Repository randomly
572 selected 65 women for this pilot study.

573 **TABLES**

574

575 **Table 1**576 **Descriptive statistics in pre- vs. postmenopause for analytic sample (N=65): Study of**577 **Women's Health Across the Nation (SWAN)**

	Premenopause ^a	Postmenopause ^b	p-value
Race/ethnicity			
Age (years) ^c	49.9 (1.9)	57.5 (1.8)	<0.0001
BMI (kg/m ²) ^c	27.6 (5.6)	28.9 (6.1)	
Time from final menstrual period (years) ^c	-3.8 (0.6)	+4.0 (0.6)	<0.0001
Ovarian function markers			
E2 (pg/ml) ^d	51.7 (32.3, 86.6)	15.5 (10.7, 20.1)	<0.0001
FSH (mIU/ml) ^d	14.8 (10.0, 21.8)	84.3 (64.1, 108.2)	<0.0001
Gut permeability markers			
FABP2 (pg/ml) ^c	1,298 (577)	1595 (754)	0.001
LBP (ng/ml) ^c	5,892 (1,139)	6,112 (1,037)	0.05
sCD14 (ng/ml) ^c	948 (176.2)	1,032 (212)	0.0002
Inflammation			
hs-CRP (mg/L) ^d	1.4 (0.5, 3.3)	1.6 (0.8, 3.3)	0.06
Bone mineral density			
Lumbar spine (g/cm ²) ^c	1.104 (0.139)	0.986 (0.142)	<0.0001
Total hip (g/cm ²) ^c	0.964 (0.123)	0.901 (0.124)	<0.0001

578

- 579 **a** Data obtained in premenopause, operationalized as a study visit from three to five years
580 before the final menstrual period (before ovarian function decreases)
- 581 **b** Data obtained in postmenopause, operationalized as a study visit from three to five years
582 After the final menstrual period (after ovarian function plateaus at its nadir)
- 583 **c** Variables with normal distribution, reported as mean (standard deviation). Within-
584 individual change from pre- to postmenopause assessed using the paired t-test.
- 585 **d** Variable with skewed distribution, reported as median (interquartile range). Within-
586 individual change from pre- to postmenopause assessed using the Wilcoxon rank-sum
587 test.

588 **Table 2**

589 **Associations of estradiol (E2) or follicle stimulating hormone (FSH) with gut permeability^a**

	Increment in FABP2 (pg/ml), LBP (ng/ml), or sCD14 (ng/ml) per 2-fold decrement in E2 or 2-fold increment in FSH (95% CI)^a	p-value
FABP2		
E2	77 (10, 143)	0.02
FSH	113 (47, 179)	0.001
LBP		
E2	33 (-41, 110)	0.3
FSH	21 (-55, 98)	0.5
sCD14		
E2	28 (12, 44)	0.001
FSH	34 (19, 50)	<0.0001

590

591 **a** Gut permeability assessed by FABP2 (barrier dysfunction), LBP and sCD14 (immune
592 activation secondary to gut microbial translocation).

593 **b** Associations assessed by repeated measured, mixed effect linear regression with FABP,
594 LBP, or sCD14 as continuous outcome variable, and E2 or FSH (log transformed) as
595 continuous primary predictors (each outcome/primary predictor pair modeled separately).

596 Analyses were adjusted for age at premenopausal time point, BMI, race/ethnicity, and

597 SWAN study site.

598 **Table 3**

599 **Associations of gut permeability^a with a marker of inflammation^b**

	“X”-fold increment in hs-CRP per SD increment in FABP2, LBP, or sCD14 (95% CI)^a	p-value
Gut barrier integrity or immune activation markers		
FABP2	-0.05 (-0.02, +0.02)	0.5
LBP	+2.32 (+1.87, +2.87)	0.0001
sCD14	+1.44 (+1.15, +1.80)	0.001

600

601 **a** Gut permeability assessed by FABP2 (barrier dysfunction), LBP and sCD14 (immune
602 activation secondary to gut microbial translocation).

603 **b** Inflammation assessed by hs-CRP.

604 **c** Associations assessed by repeated measured, mixed effect linear regression with hs-CRP
605 as continuous outcome variable, and FABP2, LBP, or sCD14 as continuous primary
606 predictors (each modeled separately). Analyses were adjusted for age at premenopausal
607 time point, BMI, race/ethnicity, and SWAN study site.

608

609 **Table 4**

610 **Associations of gut permeability^a with bone mineral density**

	Increment in lumbar spine or total hip bone mineral density (g/cm²) per SD increment in FABP2, LBP and sCD14, or per 2-fold increment in hs-CRP (95% CI)^a	p-value
FABP2		
Lumbar spine	-0.021 (-0.043, 0.000)	0.05
Total hip	-0.017 (-0.031 -0.003)	0.02
LBP		
Lumbar spine	-0.018 (-0.047, 0.010)	0.2
Total hip	-0.005 (-0.239, 0.015)	0.6
sCD14		
Lumbar spine	-0.051 (-0.076, -0.026)	0.0001
Total hip	-0.025 (-0.041, -0.008)	0.004
hs-CRP		
Lumbar spine	-0.024 (-0.038, -0.011)	<0.0001
Total hip	-0.008 (-0.018, 0.002)	0.1

611

612 **a** Gut permeability assessed by FABP2 (barrier dysfunction), LBP and sCD14 (immune
613 activation secondary to gut microbial translocation).

614 **b** Associations assessed by repeated measured, mixed effect linear regression with bone
615 mineral density at the lumbar spine or femoral as continuous outcome variables, and
616 FABP2, LBP, or sCD14 as continuous primary predictors (each modeled separately).
617 Analyses were adjusted for age at premenopausal time point, BMI, race/ethnicity, and
618 SWAN study site.