Abdominal Adipocyte Populations in Women with Visceral Obesity

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29 **ABSTRACT**

30 Visceral obesity is independently related to numerous cardiometabolic alterations, with adipose
31 tissue dysfunction as a central feature. **Objective**: To examine whether omental (OM) and
32 subcutaneous (SC) adipocyte size populations in women relate to visceral obesity,
33 cardiometabolic risk factors and adipocyte lipolysis independent of total adiposity. **Design and**
34 **Methods:** OM and SC fat samples were obtained during gynecological surgery in 60 women
35 [mean age: 46.1±5.9 years; mean BMI: 27.1±4.5 kg/m^2 (range: 20.3-41.1 kg/m^2)]. Fresh samples
36 were treated with osmium tetroxide and were analyzed with a Multisizer Coulter. Cell size
37 distributions were computed for each sample with exponential and Gaussian function fits.
38 **Results:** Computed tomography-measured visceral fat accumulation was the best predictor of
39 larger cell populations as well as the percentage of small cells in both OM and SC fat ($p<0.0000$
40 for all). Accordingly, women with visceral obesity had larger cells in the main population and
41 higher proportion of small adipocytes independent of total adiposity ($p\leq0.05$). Using linear
42 regression analysis, we found that women characterized by larger-than-predicted adipocytes in
43 either OM or SC adipose tissue presented higher visceral adipose tissue area, increased
44 percentage of small cells and $HOMA_{ir}$ index as well as higher OM adipocyte isoproterenol-,
45 forskolin- and dibutyryl cAMP- stimulated lipolysis compared to women with smaller-than-
46 predicted adipocytes, independent of total adiposity ($p\leq0.05$). **Conclusion:** Excess visceral
47 adipose tissue accumulation is a strong marker of both adipocyte hypertrophy and increased
48 number of small cells in either fat compartment, which relates to higher insulin resistance index
49 and lipolytic response, independent of total adiposity.

50

51

52 # INTRODUCTION

53 Excess fat accumulation on the greater omentum and mesentery has been recognized as an
54 important predictor of cardiometabolic abnormalities such as dyslipidemia, insulin resistance and
55 type 2 diabetes mellitus, hypertension, inflammation and a pro-thrombotic profile [1-3]. Numerous
56 studies have now proposed visceral obesity as a marker of adipose tissue storage dysfunction and
57 fat deposition in ectopic locations [3, 4]. Despite the fact that overall and abdominal obesity are
58 both related to metabolic risk, increases in adipocyte size within abdominal adipose tissue
59 compartments may also represent a significant marker of these metabolic alterations [5-8].

60

61 Adipose tissue expansion in response to a positive energy imbalance results from adipocyte
62 hypertrophy (enlargement of existing mature adipocytes) and adipocyte hyperplasia (recruitment
63 of new cells and differentiation of preadipocytes) [5, 9, 10]. Thus, the total amount of body fat mass
64 may be examined through both adipocyte size and adipocyte number. Fat cell size is influenced
65 by various factors including sex, regional localization and obesity degree [3, 8, 11, 12]. Size of cells
66 from all anatomical localizations generally increases with obesity level [3, 5, 6, 12, 13]. Nevertheless,
67 there is a wide inter-individual variability in adipocyte size at any given obesity level and values
68 tend to reach a plateau in massively obese subjects, indicating that an increase in adipocyte
69 number is also required for the expansion of body fat mass [3, 5, 6, 13].

70

71 Many previous studies [5, 6, 14-19] and one recent review [8] reported that increased adipocyte size, in
72 either the subcutaneous (SC) or visceral fat compartment, was associated with metabolic
73 alterations and markers of adipose tissue dysfunction, independent of overall adiposity.
74 However, in most of these studies, detailed variations in fat cell size populations were not

75 considered [8]. Osmium tetroxide fixation of adipose tissue and Multisizer Coulter analysis
76 generate a bimodal distribution of adipocyte sizes, with a population of large adipocytes and a
77 population of small adipocytes [20, 21]. To our knowledge, only a few studies in humans have used
78 this technique to examine the link between bimodal distribution of adipocyte sizes and type 2
79 diabetes [22, 23], insulin resistance [24-28], inflammation [29] and patient response to pioglitazone [30].
80 According to available studies, larger adipocytes and/or high proportion of small cells seem to be
81 associated with metabolic alterations such as insulin resistance [22, 24-26, 28]. However, most of the
82 studies were performed exclusively in SC adipose tissue [22-25, 28-30] and did not take into
83 consideration visceral adipocyte size populations. Thus, how population dynamics of abdominal
84 adipocyte size affect the pathophysiology of the metabolic alterations associated with obesity
85 remains unclear.
86
87 Our objective was to examine whether variations in omental (OM) and SC adipocyte size
88 distribution in women are related to visceral obesity, cardiometabolic risk factors and adipocyte
89 lipolysis, independent of total adiposity. Considering its well-established link with adipose tissue
90 dysfunction, we tested the hypothesis that visceral fat accumulation is a strong predictor of larger
91 cell populations and increased percentage of small cells in both fat compartments. Furthermore,
92 since limited expandability of adipose tissue has been proposed as a major determinant linking
93 abdominal obesity and metabolic alterations [3], we tested the hypothesis that the presence of
94 larger adipocytes is associated with altered cardiometabolic risk and high adipocyte lipolytic
95 responsiveness independent of total adiposity.

96 # SUBJECTS AND METHODS

97 ***Participant recruitment***

98 We enrolled 60 women electing for total (n=57) or subtotal (n=2) abdominal hysterectomies or
99 myomectomy (n=1) at the CHU de Québec. A few weeks before surgery and on the morning of
100 surgery, detailed information was obtained on medication use as well as reproductive, menstrual,
101 and medical history for each woman. Exclusion criteria were the following: 1) women with
102 Cushing syndrome, hyperthyroidism, cancer, cardiovascular diseases, type 1 or 2 diabetes; and 2)
103 women who reported significant weight loss or gain in the past year. With the exception of their
104 gynecological condition, these women were generally healthy. Reasons for surgery included one
105 or more of the following: menorrhagia/menometrorrhagia (n=29), myoma (n=22), fibroids
106 (n=12), uterine myomatosis (n=1), incapacitating dysmenorrhea (n=6), pelvic pain (n=7),
107 endometriosis (n=8), pelvic adhesions (n=4), adenomyosis (n=1) or benign cystadenoma (n=6).
108 The study was approved by the Research Ethics Committees of this institution (C09-08-086). All
109 subjects provided written informed consent to participate in the study.

110 ***Anthropometrics, body composition and body fat distribution measurements***

111 On the morning of the surgery, body weight, height, waist circumference, resting systolic and
112 diastolic blood pressure were measured using standardized procedures. The number of criteria of
113 the metabolic syndrome (MetS) was assessed for each woman as follows: 1) waist circumference
114 $\geq$ 88 cm; 2) plasma triglycerides > 1.69 mmol/L; 3) HDL-cholesterol $\leq$ 1.29 mmol/L; 4) systolic
115 blood pressure (SBP) $\geq$130mmHg or diastolic blood pressure (DBP) $\geq$85mmHg; 5) fasting
116 glycemia $\geq$ 5.6 mmol/L. MetS+ women had to meet three or more criteria, as described by the
117 National Cholesterol Education Program (NCEP) Adult Treatment Panel (APT) III [31]. Body fat
118 distribution and body composition measurements were performed a few days before surgery

119 (14±13 days), according to standardized protocols. These measurements were performed after a
120 12h-overnight fast. Abdominal SC and visceral adipose tissue cross-sectional areas at the L4-L5
121 vertebrae level were determined by computed tomography using a GE Light Speed 1.1 CT
122 scanner (General Electric Medical Systems, Milwaukee, WI). Briefly, subjects were examined in
123 the supine position, with arms stretched above the head. Images from scans were used for the
124 quantification of total abdominal adipose tissue area using an attenuation range of -190 to -30
125 Hounsfield Units. Visceral adipose tissue area was measured by delineating the intra-abdominal
126 cavity at the internal-most aspect of the abdominal and oblique muscle walls surrounding the
127 cavity and the posterior aspect of the vertebral body. SC adipose tissue area was obtained by
128 subtracting visceral adipose tissue area from total abdominal area. Regions of interest were
129 delineated with the ImageJ 1.33u software (National Institutes of Health, USA). All analyses
130 were performed by the same investigator. Intra-observer coefficients of variation were 0%, 0.3%
131 and 1.3% for total, subcutaneous and visceral adipose tissue area respectively. Total body fat
132 mass, trunk fat mass and lean body mass were assessed by a dual energy X-ray absorptiometry
133 (DXA) [Hologic QDR-4500A densitometer with whole-body fan beam software v8.26a:3
134 (Hologic Inc., Bedford, MA)].

135 ***Plasma lipid profile and glucose homeostasis measurements***

136 Fasting blood samples were collected on the morning of surgery after a 12h-overnight fast. From
137 these samples, cholesterol and triglyceride levels in both plasma and lipoprotein fractions were
138 measured, as previously described [32]. Glucose was measured with a Modular P800 system
139 (Roche, Diagnostics, Laval, Canada). Insulin was measured by ELISA (Alpco, Salem, NH,
140 USA). HOMA insulin resistance index was calculated from fasting glucose and insulin levels [33].

141

142 ***Adipose tissue sampling***

143 SC and OM adipose tissue samples were collected during the surgical procedure at the site of
144 incision and at the distal portion of the greater omentum, respectively. The samples were
145 immediately carried to the laboratory.

146

147 ***Adipose cell size distribution measurements***

148 Fresh tissue samples (~ 50 mg) were fixed in collidine-HCL osmium tetroxide solution for at
149 least 96 hours at room temperature. Samples were then rinsed with phosphate buffer saline for 24
150 hours, incubated with 8M urea for 48 hours and rinsed with phosphate buffer saline 0.01% Triton
151 X-100. The resulting fixed cells were analyzed using a Beckman Coulter Multisizer IV
152 (Beckman Coulter, Villepinte, France), as previously described [34]. The range of cell sizes
153 analyzed was 25 to 240 µm. Cell size distributions were determined with at least 12 000 cells per
154 sample and were analyzed by fitting an exponential (small cells)-Gaussian (large adipocytes)
155 formula (non-linear least-squares function) as described by McLaughlin et al. [24]. Endpoints of
156 interest in the cell population-derived parameters were the mode (center of the Gaussian peak),
157 which is the median diameter of the large cells, the height and the width of the Gaussian
158 distribution as well as the nadir, which is the least probable cell size (in frequency) between the
159 two cell populations, and the percentage of small cells defined as the percentage of adipose cells
160 below the frequency nadir. We also calculated from these distributions the mean diameter of the
161 10% larger cells.

162

163

164 ***Adipocyte isolation and adipocyte lipolysis measurement***

165 Adipocytes were isolated with collagenase digestion in Krebs-Ringer-Henseleit (KRH) buffer for
166 45 minutes at 37°C according to a modified version of the Rodbell method [12]. For lipolysis
167 experiments, cell suspensions were incubated for 2 h at 37°C in KRH buffer, with or without β-
168 adrenergic receptor agonist isoproterenol (10^{-10} to 10^{-5} mol/L), post-receptor-acting agents
169 dibutyryl cyclic AMP (10^{-3} mol/L) or forskolin (10^{-5} mol/L). Glycerol release in the medium
170 was measured by bioluminescence with the nicotinamide adenine dinucleotide-linked bacterial
171 luciferase assay as described [12]. Lipid weight of the cell suspension was measured using Dole's
172 extraction. Data were expressed in μmol per 10^{6} cells per 2h [12, 14].

173 ***Statistical analyses***

174 Quantitative analysis of cell population-derived parameters was performed by mathematical
175 modeling using SAS software V9.4 (SAS Institute, Carry, NC). Student paired *t*-tests were
176 computed to examine regional differences in adipocyte size distribution parameters (n=54).
177 Pearson correlation coefficients were computed to assess associations between adipocyte size
178 distribution parameters, age, body fatness, body fat distribution and cardiometabolic risk. Partial
179 Pearson correlation coefficients were computed to assess associations between adipocyte size
180 distribution parameters and cardiometabolic risk after statistical adjustments for BMI. Multiple
181 linear regressions were performed to identify the best predictor of the mode, the nadir, the height,
182 the width, the 10% larger cells and the percentage of small cells in the OM or SC adipose tissues.
183 These regression models included age, BMI, total body fat mass, menopausal status, VAT area
184 and SAT area. To identify participants with visceral obesity, a separate linear regression analysis
185 was computed to predict visceral fat accumulation using BMI. The subjects were then stratified
186 in two subgroups according to the residuals of this regression. Women with a positive residual

187 were considered as having higher-than-predicted visceral fat accumulation for a given BMI, and
188 women with a negative residual were considered as having lower-than-predicted visceral fat
189 accumulation for a given BMI. Such stratification generated two subgroups of women matched
190 for BMI but with either low or high VAT area. There was no significant difference between the
191 two subgroups for total body fat mass, age and SAT area, even if these variables were not
192 included in the regression model. Student's *t*-tests were used to compare women with versus
193 without visceral obesity. Furthermore, an independent linear regression analysis was performed
194 to predict the mode in each fat compartment separately using BMI to control for total adiposity [5,
195 6]. In each fat compartment, women with a positive residual were considered as having larger-
196 than-predicted mode value for a given BMI, whereas women with negative residual were
197 considered as having smaller-than-predicted mode value for a given BMI. Using this
198 stratification, we obtained two subgroups of women matched for overall adiposity but with either
199 a large or small mode in each adipose tissue compartment. There was no significant difference
200 between the two subgroups for total body fat mass, age, menopausal status and SAT area, even if
201 these variables were not included in the models. In each fat compartment, *t*-tests were used to
202 compare adipocyte size distribution parameters, $HOMA_{ir}$ index as well as isolated adipocyte
203 lipolysis between the two subgroups of women. T-tests were also performed to compare
204 adipocyte size distribution parameters, adiposity variables and metabolic alterations between
205 MetS+ or MetS- women. Non-normally distributed variables were log10- or Box-Cox-
206 transformed. Statistical analyses were performed using JMP software (SAS Institute, Carry, NC).
207

208 **RESULTS**

209 ***Clinical characteristics of participants***

210 The average age of the women was 46.1 years and they were slightly overweight according to a
211 mean BMI of 27.1 kg/m^2 (**Table 1**). Women were covering a wide range of adiposity values (the
212 spectrum from lean to obese), as shown by BMI values spanning from 20.3 to 41.1 kg/m^2.
213 According to BMI categories of the World Health Organization, 24 women were lean, 22 women
214 were overweight and 14 women were obese. These women were also covering a large range of
215 body fat distribution as shown by ranges of trunk-to-total body fat mass ratio and adipose tissue
216 areas.

217

218 ***Regional differences in adipocyte populations of abdominal adipose tissues***

219 Bimodal distributions were found for all the samples examined, with a population of large
220 adipocytes corresponding to the Gaussian distribution and a population of small adipocytes
221 corresponding to cells below the frequency nadir and the exponential curve fit. **Figure 1A** shows
222 OM and SC mean adipocyte size bimodal distributions, which is the mean relative frequency for
223 each cell size diameter. Adipocyte size distributions from the OM compartment were different
224 from those of the SC compartment, showing a shift to the right for SC adipocyte populations.
225 According to parameters obtained from the distributions, the mode ($p \leq 0.001$) (**Figure 1B**), the
226 10% larger cells ($p \leq 0.0001$, data not shown), the width ($p \leq 0.0001$, data not shown) and the nadir
227 of the distributions ($p \leq 0.001$) (**Figure 1C**) as well as the percentage of small cells ($p \leq 0.05$)
228 (**Figure 1D**) were all significantly higher in SC compared to OM fat samples. The height of the
229 distribution was significantly lower in SC compared to OM fat samples ($p \leq 0.0001$, data not
230 shown). Strong positive correlations were observed between OM and SC adipose tissue for the

231 mode (r=0.57, p<0.0001), the nadir (r=0.70, p<0.0001) as well as the proportion of small cells
232 (r=0.75, p<0.0001) (**Figure 1E, F, G**). When mean cell size distribution curves were expressed
233 as percentage of the volume occupied for each cell diameter (**Figure 1H**), we observed that the
234 volume occupied by the population of small cells was negligible (very low) despite the large
235 number of these cells.

236

237 **Figure 2** illustrates associations between the mode, the nadir or the percentage of small cells in
238 both OM and SC depots. Highly significant positive correlations were observed between the
239 mode and the nadir of the distributions in both adipose tissue compartments (p<0.0001 for both).
240 In addition, the mode and the nadir were positively and significantly associated with the
241 percentage of small cells in both fat depots (p<0.0001 for all). Size of the 10% larger cells was
242 also positively and significantly related to the nadir, the mode and the percentage of small cells
243 in both fat depots (p<0.0001 for all, data not shown). The height of the Gaussian distribution was
244 negatively correlated with the nadir, the mode, the 10% larger cells and the percentage of small
245 cells in both fat depots (p<0.0001 for all, data not shown).

246

247 ***Adipocyte populations in relation with body fatness or visceral obesity***

248 **Table 2** shows Pearson correlation coefficients between OM and SC adipocyte size distribution
249 parameters and age or body fatness (BMI, total body fat mass) and body fat distribution
250 measurements (computed tomography-derived adipose tissue areas). No significant association
251 was found between age and adipocyte size distribution parameters. Increased weight, waist
252 circumference, BMI, total body fat mass and computed tomography-derived adipose tissue areas
253 were strongly associated with larger cells of the main population (mode and 10% larger cells),

254 larger nadir size and greater percentage of small cells in both fat compartments ($p \leq 0.05$).
255 Inversely, increased weight, BMI, waist circumference, total body fat mass as well as computed
256 tomography-measured adipose tissue areas were related to lower height of the main cell
257 population in both adipose tissue compartments ($p \leq 0.05$). The width of the Gaussian in both
258 depots was positively and significantly correlated with waist circumference and measurements of
259 body fat distribution ($p \leq 0.05$). Overall, increased adiposity variables were associated with
260 enlargement of larger cells, lower height of the main cell population and greater percentage of
261 small cells. Similar patterns of correlations were observed in each BMI category (lean,
262 overweight and obese).

263

264 To identify the strongest predictors of adipocyte size distribution parameters in both depots,
265 multiple linear regression models were computed. Regression models included VAT area, SAT
266 area, total body fat mass, BMI, age and menopausal status (**Table 3**). VAT area was the best
267 correlate of the mode, the nadir, the percentage of small cells, the 10% larger cells, the height
268 and the width of the Gaussian curve in OM adipose tissue, explaining respectively 62.1%
269 ($p<0.0001$), 63.6% ($p<0.0001$), 43.9% ($p<0.0001$), 45.8% ($p<0.0001$), 34.2% ($p<0.0001$) and
270 17.1% ($p=0.002$) of the variance (data not shown for the 10% larger cells, the height and the
271 width). Interestingly, VAT area was also the best predictor of the mode, the nadir, the percentage
272 of small cells, the 10% larger cells, peak height and width in the SC depot, explaining
273 respectively, 43.7% ($p<0.0001$), 41.5% ($p<0.0001$), 23.6% ($p<0.0001$), 39.2% ($p<0.0001$),
274 24.8% ($p<0.0001$) and 12.3% ($p=0.008$) of the variance (data not shown for the 10% larger cells,
275 peak height and width).

276

277 To better examine cell populations in visceral obesity, women were stratified in two subgroups
278 according to the residuals of the regression between VAT area and BMI. Such stratification
279 generated two subgroups of women matched for overall adiposity but with either low (n=28) or
280 high (n=31) VAT area (**Figure 3**). No significant difference was found between women with
281 versus those without visceral obesity in BMI (26.9±4.4 vs. 27.4±4.5 kg/m^2 respectively, p=0.66),
282 total body fat mass (24.3±7.7 vs. 26.1±6.3 kg respectively, p=0.32), age (45.6±5.2 vs. 47.9±6.5
283 years respectively, p=0.17) and SAT area (291±101 vs. 328±103 cm^2 respectively, p=0.16). As
284 expected from the stratification model, VAT area was significantly higher in women with than
285 those without visceral obesity (120±45 vs. 71±33 cm^2 respectively, p≤0.0001). Women with
286 visceral obesity had significantly larger cells of the main population (mode and 10% larger cells)
287 (**Figure 3B and C**), larger nadir size (**Figure 3D**) as well as greater percentage of small cells
288 (**Figure 3E**) in both adipose tissue depots (p≤0.05), independent of overall adiposity. Differences
289 between the two subgroups were more significant in the OM fat compartment (p≤0.001 for all).
290 Height of the Gaussian curve in both fat depots was significantly lower in women with visceral
291 obesity (p≤0.01, data not shown). Regional differences in adipocyte size distribution curves were
292 observed between the two subgroups. Indeed, no significant depot difference was observed for
293 the percentage of small cells (p=0.37) in visceral obese women, although this percentage was
294 significantly higher in SC compared to OM fat in women without visceral obesity (p=0.002).
295 The mode, size of the 10% larger cells and nadir were higher in the SC versus OM depot in both
296 subgroups (p≤0.01 for all), whereas height of the distribution was significantly lower in SC
297 compared to OM fat samples (p≤0.0001). As expected, women with visceral obesity had higher
298 fasting glucose (5.13±0.36 vs. 5.48±0.45mmol/L, p=0.002), fasting insulin (6.47±4.40 vs.

299 7.89±3.62μIU/mL, $p=0.06$) and $HOMA_{ir}$ index (1.49±1.05 vs. 1.91±0.87, $p=0.03$) compared to
300 women with normal visceral fat accumulation (data not shown).
301
302 ***Adipocyte populations in relation with cardiometabolic risk factors***
303 Strong correlations were observed between metabolic alterations and adipocyte size distribution
304 parameters in each depot (**Table 4)**. Larger cells of the main population (mode and nadir) and
305 greater percentage of small cells in both depots were associated with higher values of plasma
306 triglycerides, cholesterol-to-HDL-cholesterol ratio and $HOMA_{ir}$ index ($p \leq 0.05$ for all, except a
307 trend between values of plasma triglycerides and the mode in the SC depot, $p<0.10$). These cell
308 size distribution parameters were also negatively related to concentrations of HDL-cholesterol
309 ($p \leq 0.05$). The distribution width and size of the 10% larger cells in both depots were also
310 negatively associated with values of HDL-cholesterol ($p \leq 0.05$). In OM adipose tissue only, the
311 width of the distribution and size of the 10% larger cells were positively associated with
312 concentrations of plasma triglycerides and $HOMA_{ir}$ index ($p \leq 0.05$). In both depots, size of the
313 10% larger cells was also positively related to the total-to-HDL-cholesterol ratio ($p \leq 0.05$).
314 Inversely, higher height of the main distribution in both fat compartments was correlated with
315 lower concentrations of plasma triglycerides, total-to-HDL-cholesterol ratio and $HOMA_{ir}$ index
316 as well as higher values of HDL-cholesterol ($p \leq 0.05$ for all, except a trend between values of
317 plasma triglycerides and height in the SC depot, $p<0.10$). Statistical adjustment for BMI did not
318 substantially change these associations (**Table 4**). When associations were examined within each
319 BMI category, we found similar patterns of correlations than those presented in **Table 4**. Overall,
320 OM adipocyte size distribution parameters were most strongly associated with $HOMA_{ir}$ index,

321 whereas SC adipocyte size distribution parameters were most strongly associated with values of
322 HDL-cholesterol.
323
324 To further examine the link between adipocyte populations and cardiovascular risk factors,
325 women were stratified in two subgroups according to the presence (MetS+) or the absence
326 (MetS-) of metabolic syndrome. As expected, MetS+ women were characterized by altered
327 cardiometabolic risk factors and increased adiposity. In both fat compartments, MetS+ women
328 (n=17) were characterized by larger mode (OM: 111.4±15.7 vs. 96.0±18.5 µm, p=0.008; SC:
329 133.1±15.8 vs. 113.7 ±15.5 µm, p<0.0001), larger nadir (OM: 70.4±14.8 vs. 52.2±14.1 µm,
330 p=0.003; SC: 80.6±11.2 vs. 63.8±12.6 µm, p<0.0001), larger 10% largest cells (OM: 151.1±19.8
331 vs. 132.0±24.6 µm, p=0.009; SC: 175.7±18.0 vs. 153.7±17.3 µm, p<0.0001), lower Gaussian
332 peak height (OM: 1.4±0.5 vs. 1.9±0.7, p=0.01; SC: 1.0±0.2 vs. 1.4±0.3, p<0.0001) and a greater
333 proportion of small cells (OM: 34.5±16.5 vs. 20.4±14.9%, p=0.005; SC: 39.3±13.6 vs.
334 22.1±11.6%, p<0.0001) compared to MetS- women (n=43). Statistical adjustment for BMI did
335 not markedly change the differences between the two subgroups.
336
337 ***Adipocyte populations in relation with adipocyte lipolysis independent of total adiposity***
338 To examine whether OM and SC adipocyte hypertrophy are related to adipocyte lipolysis
339 measurements independent of total adiposity, women were stratified in two subgroups according
340 to residuals of the regression between the mode of the large cell population and BMI. For each
341 fat depot, women with a positive residual were considered as having larger-than-predicted mode
342 value for a given BMI, whereas women with a negative residual were considered as having
343 smaller-than-predicted mode value for a given BMI. Such stratification generated two subgroups

344 of women with either large or small mode values of the main cell population, but matched for
345 total adiposity (**Figures 4 and 5**).
346
347 **Figure 4A** shows mean OM adipocyte size distribution curves of these subgroups. The two
348 curves were markedly different, showing a shift to the right for OM cell size distributions in
349 women characterized by larger-than-predicted OM adipocytes. As expected from the
350 stratification model, women with larger-than-predicted OM adipocytes had significantly larger
351 mode in OM adipose tissue ($p \leq 0.0001$) (**Figure 4B**). These women also had a higher nadir
352 ($p \leq 0.0001$) (**Figure 4B**), higher mean size of the 10% larger cells ($p \leq 0.0001$) (**Figure 4B**), lower
353 height of the main distribution ($p \leq 0.0001$, data not shown) and greater percentage of small cells
354 in OM adipose tissue ($p \leq 0.0001$) (**Figure 4C**), as well as higher $HOMA_{ir}$ index ($p=0.002$)
355 (**Figure 4D**) and higher OM adipocyte isoproterenol-(10^{-10} to 10^{-5} mol/L), forskolin- and
356 dibutyryl cAMP-stimulated lipolysis ($p \leq 0.05$) (**Figure 4E and F**) compared to women
357 characterized by smaller-than-predicted OM adipocytes, independent of overall adiposity.
358
359 Very similar results were observed when women were stratified according to SC adipocyte sizes
360 (**Figure 5**). The two mean adipocyte size distribution curves were different, showing a shift to
361 the right in women with larger-than-predicted SC adipocytes (**Figure 5A**). As expected, women
362 characterized by larger-than-predicted SC adipocytes had significantly larger mode, nadir and
363 size of 10% larger cells in SC adipose tissue ($p \leq 0.0001$ for all) (**Figure 5B**). These women also
364 had greater percentage of small cells in SC adipose tissue ($p=0.001$) (**Figure 5C**), lower height
365 of the main distribution ($p \leq 0.0001$), higher $HOMA_{ir}$ index ($p=0.06$) (**Figure 5D**) as well as
366 higher SC adipocyte basal as well as isoproterenol- (10^{-10} to 10^{-5} mol/L), forskolin- and dibutyryl

367 cAMP-stimulated lipolysis ($p \leq 0.05$) (**Figure 5E and F**) compared to women characterized by
368 smaller-than-predicted SC adipocytes, independent of overall adiposity.
369

370 **DISCUSSION**

371 The main objective of the present study was to examine whether variations in OM and SC
372 adipocyte size distributions in women are related to visceral obesity, cardiometabolic risk factors
373 and adipocyte lipolysis independent of total adiposity. Using osmium tetroxide fixation of
374 adipose tissue and Multisizer Coulter analysis, we demonstrated that adipocyte size distributions
375 from the OM compartment were significantly different from those of the SC compartment.
376 Furthermore, strong correlations were observed between parameters of adipocyte size
377 distributions in both depots and adiposity measurements. As shown with our regression analyses,
378 visceral fat accumulation was the best predictor of all parameters of the larger cell distributions
379 as well as the percentage of small cells in both OM and SC fat. Accordingly, we also
380 demonstrated that women with visceral obesity had significantly larger cells and lower height of
381 the main population as well as higher proportion of small cells in both adipose tissue
382 compartments, independent of total adiposity. Using linear regression analysis, we found that
383 women with larger-than-predicted adipocytes in either OM or SC adipose tissue presented higher
384 VAT area and were characterized by increased percentages of small cells and $HOMA_{ir}$ index as
385 well as a high adipocyte lipolytic responsiveness compared to women with smaller-than-
386 predicted adipocytes, independent of total adiposity.

387

388 The relationship between adipocyte size populations assessed with osmium tetroxide and glucose
389 homeostasis has been investigated in a few studies [22, 24-26, 28, 30]. More specifically, McLaughlin et
390 al. [24] originally demonstrated that mean fat cell size of the larger SC cells was not significantly
391 different between insulin resistant and insulin sensitive patients. In the latter study, they also
392 showed that insulin resistance in obese subjects was associated with higher percentage of small

393 cells in SC adipose tissue [24]. In a more recent study, the same group demonstrated that insulin
394 resistance was related to both enlargement of large adipocytes and increased proportion of small
395 adipose cells in SC adipose tissue, independent of body fat mass and sex [25]. Authors suggested
396 that the small number of patients in the first study may explain the discrepancy observed
397 between these reports [25]. In the current analysis, we also found that both hypertrophic adipocytes
398 and higher percentage of small cells in OM and SC fat depots were strongly associated with
399 blood lipid and glucose homeostasis alterations. Statistical adjustment for BMI did not markedly
400 change the strongest associations. To support this finding, women with the metabolic syndrome
401 were characterized by increased adiposity variables, altered lipid profile and glucose homeostasis
402 as well as enlargement of larger adipocytes, lower height of the Gaussian distribution and greater
403 percentage of small cells. Consistent with the results of McLaughlin et al. in 2014 [25], we
404 demonstrated using a linear regression analysis that women with large adipocytes in either OM
405 or SC depot are characterized by increased small cell numbers and insulin resistance, as well as
406 high adipocyte lipolytic responsiveness compared to women with smaller-than-predicted
407 adipocytes, independent of total adiposity. Consistently, Pasarica et al. [22] previously
408 demonstrated that patients with type 2 diabetes mellitus had larger very large adipocytes and
409 lower total adipocyte number, but a greater proportion of small cells compared to BMI-matched
410 obese subjects. Taken together, these data suggest an important effect of adipocyte cellularity
411 independent of obesity level. McLaughlin et al [24] were the first group to propose that these small
412 adipocytes below the frequency nadir represent immature adipocytes that are unable to correctly
413 store excess energy [24, 25]. We also observed that even if obese women had an increased
414 proportion of small cells and larger adipocytes of the main population, the volume occupied by
415 the small cells was very low, suggesting that these cells may not significantly contribute to the

416 storage of triglycerides. Thus, higher proportion of small adipocytes and hypertrophic mature
417 adipocytes may both be markers of impaired adipogenesis, which is known to be involved in
418 insulin resistance. Further studies are clearly needed to characterize the function of these small
419 adipocytes and their impact on metabolic disease risk.
420
421 The notion that adipocyte size increases as a function of obesity levels is now well-supported in
422 the literature [3, 8]. Our findings that the mode of adipocyte size distributions increases with overall
423 and regional adiposity measurements are expected and consistent with this concept. We also
424 found that with increasing adiposity level, enlargement of larger adipocytes (mode and size of
425 10% larger cells) was associated with higher nadir, lower height of the Gaussian distribution as
426 well as greater percentage of small cells, suggesting that the shape of the distribution is
427 influenced by obesity level. The physiological meaning of these results is complex to interpret.
428 Only a portion of the large adipocytes seem to be able to expand their size to reach extreme
429 values of the distribution [35]. Various factors may explain the inability of some adipocytes to
430 further expand through hypertrophy such as angiogenesis, extra-cellular matrix remodeling,
431 hypoxia or inflammation [8, 32, 36].
432
433 Our analysis also demonstrated that VAT area was the strongest predictor of both hypertrophic
434 adipocytes of the large cell population and percentage of small cells in both fat compartments.
435 Using linear regression analysis, we consistently reported that women with high VAT area were
436 characterized by larger cells of the main population as well as higher percentage of small cells in
437 both adipose tissue depots compared to women with low VAT area, independent of total
438 adiposity. The difference in VAT area between women characterized by larger- and smaller-

439 than-predicted adipocyte (especially in OM adipose tissue) also supports these findings. In
440 agreement with our results, Kursawe et al [27] demonstrated that adolescents with high VAT
441 accumulation, according to the ratio of VAT to VAT+SAT, were characterized by higher
442 proportion of small adipose cells, hypertrophy of the largest cells and lower expression of
443 adipogenesis and lipogenesis genes in SC adipose tissue compared to adolescents with low VAT.
444 Taken together, these findings suggest that excess visceral fat accumulation may be an important
445 predictor of hypertrophy of the cells and increased number of small cells either in the SC and
446 OM fat compartment. These results are consistent with the notion that excess visceral adipose
447 tissue accumulation is a strong marker of dysfunctional adipose tissue and limited capacity of
448 adipose tissues to store excess of energy, which can lead to ectopic fat deposition and metabolic
449 alterations [1, 8, 12, 32, 37].
450
451 A few studies also reported that fat cell size seems to be a marker of altered adipocyte lipid
452 metabolism [3, 8, 14, 15, 19]. Previous studies reported that large adipocytes were characterized by
453 higher rates of glycerol release compared to smaller adipocytes from the same fat compartment
454 [15, 38]. Our study was not designed to compare adipocyte function between small adipocytes and
455 large adipocytes. Nevertheless, our study demonstrated that lipolytic response to β-adrenergic
456 receptor agonist stimulation was increased in women with larger-than predicted adipocytes
457 independent of total adiposity. Consistent with our results, Laurencikiene et al. [19] reported that
458 large adipocytes had increased hormone-induced lipolysis rates and protein expression of
459 hormone-sensitive lipase (HSL), perilipin (PLIN) and adipose triglyceride lipase (ATGL)
460 compared to small adipocytes in SC adipose tissue. Furthermore, in a previous sample of
461 women, we also demonstrated that women with adipocyte hypertrophy in either OM or SC

462 adipose tissues, had higher adipocyte lipolytic responses and had altered adipose tissue
463 expression of genes involved in lipid storage, inflammation and angiogenesis [14]. Our results
464 highlighted once again the concept that hypertrophied adipocytes are more lipolytic compared to
465 smaller and more abundant adipocytes, which may reflect the link between adipocyte
466 hypertrophy, altered lipid handling and the development of insulin resistance.
467
468 Previous studies demonstrated that OM mean adipocyte size was lower (around 20%) compared
469 to SC adipocyte size in women, while this depot difference was not observed in men [3, 8]. These
470 studies support our results in women showing that the mode, size of the 10% larger cells, the
471 nadir and the percentage of small cells were all lower in OM compared to SC fat samples. To our
472 knowledge, only one study also reported OM and SC differences in cell size parameters using
473 detailed analysis of adipose cell size distributions [26]. Consistent with our findings, they
474 demonstrated in 11 obese insulin resistant women that the peak diameter of large cells and the
475 nadir between the two cell populations were significantly lower in OM compared to SC adipose
476 tissue. However, they found that the percentage of small cells was similar between the two tissue
477 depots [26]. In our sample, women with visceral obesity showed no significant regional difference
478 for the percentage of small cells which is consistent with these previous findings.
479
480 Limitations of the study should be acknowledged. We cannot conclude on cause-and-effect
481 relationships as our results are cross-sectional. However, we investigated a relatively large
482 number of women for whom we obtained visceral/subcutaneous adipose tissue samples. These
483 women were also very well-characterized for body fatness and body fat distribution. Our study
484 only included women because of the difficulty to perform similar studies in lean-to-moderately

485 obese men. Strengths of the study included the quantitative method used to measure adipocyte
486 size which allowed the characterization of large and very small fat cells. Other groups have
487 clearly demonstrated the existence of small cells in human adipose tissue [18, 24]. However, to
488 discriminate mature adipocytes and artefacts, fat cells under 25 µm were not considered in our
489 analysis [8]. Even if our results are consistent with the notion that impaired adipogenesis may
490 contribute to the development of metabolic complications, additional studies are needed to better
491 understand the mechanisms involved in adipose tissue lipid storage.

492

493 In conclusion, our results support the hypothesis that excess visceral fat accumulation is the best
494 predictor of both larger adipocytes and increased percentage of small adipocytes in OM and SC
495 adipose tissues. Women with larger-than-predicted adipocytes in either OM or SC adipose tissue
496 have larger adipocytes, greater percentage of small adipocytes, higher insulin resistance index
497 and higher adipocyte lipolytic responsiveness, independent of total adiposity. On the basis of
498 these results and other studies of our group [3, 32, 37], excess visceral adipose tissue accumulation,
499 adipocyte hypertrophy and increased proportion of small adipocytes, in both OM and SC adipose
500 tissues, seem to represent strong markers of dysfunctional adipose tissue and limited capacity of
501 adipose tissues to store excess dietary energy.

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511 **AUTHOR CONTRIBUTIONS**

512 A. Michaud: Performed the experiment; analysis and interpretation of data; manuscript writing;
513 critical revision of the manuscript; final approval. S. Laforest: Analysis and interpretation of
514 data; critical revision of the manuscript; final approval. M. Pelletier: Performed the experiment;
515 critical revision of the manuscript; final approval. M. Nadeau: Performed the experiment; critical
516 revision of the manuscript; final approval. S. Simard: Statistical analysis of data; critical revision
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519 Medical coverage; supervision of clinical aspects of the study; sample acquisition; critical
520 revision of the manuscript; final approval. H. Vidal: Analysis and interpretation of data; critical
521 revision of the manuscript; final approval. A. Géloën: Performed the experiment; data collection
522 and analysis; interpretation of data; manuscript writing; critical revision of the manuscript; final
523 approval. A. Tchernof: Study funding, design and conduction of the study; data collection and
524 analysis; interpretation of data; manuscript writing; critical revision of the manuscript; final
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635

636 # FIGURE LEGENDS

637 **Figure 1 :** Comparison of adipocyte size bimodal distribution parameters between SC and OM
638 adipose tissue (n=54). (**A**) Mean cell size distribution curves obtained from raw data [which is
639 the mean relative frequency for each cell size diameter (25-240μm) ± SEM], (**B**) mode of the
640 fitted-distribution, (**C**) nadir of the fitted-distribution and (**D**) percentage of small cells in OM
641 and SC adipose tissue (n=54). Means ± SEM are shown $*p \leq 0.05$, $***p \leq 0.001$ (**E**) Correlations
642 between the mode of large cells in OM adipose tissue and the mode of large cells in SC adipose
643 tissue; (**F**) Correlations between the nadir of the distribution in OM adipose tissue and the nadir
644 of the distribution in SC adipose tissue; and (**G**) Correlations between the percentage of small
645 cells in OM adipose tissue and the percentage of small cells in SC adipose tissue. Pearson
646 correlation coefficients and P values are shown. (**H**) Mean cell size distribution curves expressed
647 as percentage of the volume occupied at cell size diameter (25-240μm) ± SEM. (**B-G**) Cell
648 population-derived parameters were obtained by mathematical modeling.

649 **Figure 2**: Correlations between the mode of large adipocyte populations, the nadir of the
650 distribution or the percentage of small cells in OM (n=55) (**A**) or SC (n=59) (**B**) adipose tissues.
651 Pearson correlation coefficients and P values are shown.

652 **Figure 3:** Comparison of OM and SC adipocyte size bimodal distribution parameters between
653 women characterized by normal (n=28) or visceral adiposity (n=31), but with similar overall
654 adiposity. Means ± SEM are shown $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, [a] significant difference
655 between women characterized by normal vs. visceral adiposity ($p \leq 0.01$). (**B-E**) Cell population-
656 derived parameters were obtained by mathematical modeling.

657

658 **Figure 4:** Comparison of OM adipocyte size bimodal distribution parameters (**A-C**), $HOMA_{ir}$
659 index (**D**) and isolated OM adipocyte lipolysis (**E-F**) in women characterized by larger- (n=26)
660 or smaller- (n=29) than-predicted OM adipocytes, but similar overall adiposity. No significant
661 difference was found between subgroups for BMI (27.0±4.8 vs. 27.3±4.4 kg/m^2 respectively,
662 p=0.76), total body fat mass (25.6±6.0 vs. 24.8±7.6 kg respectively, p=0.65), age (47.6±6.5 vs.
663 47.3±4.6 years old respectively, p=0.82) and SAT area (318±92 vs. 305±108 cm^2 respectively,
664 p=0.66). VAT area was significantly higher in women with larger-than-predicted compared to
665 those with smaller-than-predicted OM adipocytes (118±48 vs. 83±35 cm^2 respectively, p=0.002).
666 (**B-C**) Cell population-derived parameters were obtained by mathematical modeling. DBC=post-
667 receptor-acting agent dibutyryl cyclic AMP (10^{-3} mol/L), FSK= post-receptor-acting agent
668 forskolin (10^{-5} mol/L). Means ± SEM are shown *p≤0.05, **p≤0.01, ***p≤0.001

669 **Figure 5:** Comparison of SC adipocyte size bimodal distribution parameters (**A-C**), $HOMA_{ir}$
670 index (**D**) and isolated SC adipocyte lipolysis (**E-F**) in women characterized by larger- (n=33) or
671 smaller- (n=26) than-predicted SC adipocytes, but similar overall adiposity. No significant
672 difference was found between subgroups for BMI (26.7±4.2 vs. 27.7±4.8 kg/m^2 respectively,
673 p=0.41), total body fat mass (25.1±6.0 vs. 25.3±8.3 kg respectively, p=0.65), age (46.8±6.3 vs.
674 46.5±5.3 years old respectively, p=0.85) and SAT area (311±101 vs. 309±110 cm^2 respectively,
675 p=0.94) (data not shown). A trend was observed for higher VAT area in women characterized by
676 larger-than-predicted SC adipocytes (103±47 vs. 87±42 cm^2 respectively, p=0.10). (**B-C**) Cell
677 population-derived parameters were obtained by mathematical modeling. DBC=post-receptor-
678 acting agent dibutyryl cyclic AMP (10^{-3} mol/L), FSK= post-receptor-acting agent forskolin (10^{-5}
679 mol/L). Means ± SEM are shown *p≤0.05, **p≤0.01, ***p≤0.001

680

681 # TABLES

682 **Table 1: Characteristics of the 60 women of the study**

Variables	Mean ± SD	Range (min-max)
Age (yrs)	46.1 ± 5.9	35.2-59.3
Waist circumference (cm)	92.3 ± 11.4	68-124
BMI (kg/m²)	27.1 ± 4.5	20.3-41.1
Total body fat mass (kg)	25.2 ± 6.9	9.6-47.3
Trunk fat mass (kg)	11.6 ± 4.0	3.3-24.1
Trunk fat mass/total body fat mass	0.5 ± 0.1	0.3-1.1
Adipose tissue area (cm²)[a]		
Total	407 ± 140	92-725
Subcutaneous	311 ± 103	71-568
Visceral	97 ± 45	21-278
Plasma lipid profile		
Cholesterol (mmol/L)		
Total	4.92 ± 0.82	3.21-6.99
VLDL	0.40 ± 0.27	0.05-1.23
LDL	3.06 ± 0.76	1.65-4.94
HDL	1.46 ± 0.39	0.83-2.57
Triglycerides (mmol/L)	1.12 ± 0.55	0.40-3.32
Cholesterol/HDL-cholesterol	3.53 ± 0.89	2.01-6.15
Glucose homeostasis		
Fasting glucose (mmol/L)	5.3 ± 0.4	4.5-6.6
Fasting insulin (µIU/mL)[b]	7.3 ± 4.0	1.5-21.4
HOMA index[b]	1.7 ± 1.0	0.3-4.9

683 BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-

684 density lipoprotein; HOMA, homeostasis model assessment index; [a] n=59, [b] n=58

685

Table 2: Pearson correlation coefficients between OM and SC adipocyte size bimodal distribution parameters and age or adiposity measurements.

	Omental adipose tissue					
Variables	**Mode**	**10% larger cells**	**Width**	**Height**	**Nadir**	**% small cells**
Age	-0.15	-0.13	-0.18	0.03	-0.05	0.03
Weight	0.56***	0.48**	0.24*	-0.31*	0.58***	0.39**
Waist circumference	0.65***	0.57***	0.34*	-0.47***	0.66***	0.51***
BMI	0.55***	0.44**	0.19	-0.25*	0.54***	0.32*
Total body fat mass	0.63***	0.52***	0.23#	-0.30*	0.64***	0.42**
Total adipose tissue area	0.71***	0.59***	0.36**	-0.46***	0.69***	0.52***
Visceral adipose tissue area	0.77***	0.66***	0.40**	-0.58***	0.77***	0.65***
SC adipose tissue area	0.63***	0.55***	0.35*	-0.42**	0.61***	0.45***

	Subcutaneous adipose tissue					
Variables	**Mode**	**10% larger cells**	**Width**	**Height**	**Nadir**	**% small cells**
Age	-0.01	0.10	0.22#	-0.12	-0.08	-0.01
Weight	0.56***	0.46**	0.22#	-0.34**	0.56***	0.35**
Waist circumference	0.65***	0.56***	0.32*	-0.49***	0.64***	0.47***
BMI	0.59***	0.46**	0.26*	-0.36**	0.55***	0.33*
Total body fat mass	0.63***	0.52***	0.30*	-0.40**	0.60***	0.39**
Total adipose tissue area	0.68***	0.58***	0.34**	-0.47***	0.65***	0.44***
Visceral adipose tissue area	0.65***	0.62***	0.34**	-0.48***	0.63***	0.47***
SC adipose tissue area	0.65***	0.52***	0.31*	-0.45***	0.62***	0.42**

# p<0.10, * p<0.05, **p<0.01, ***p<0.001

Table 3: Multiple linear regression analysis for Mode, Nadir and % of small cells in SC and OM adipose tissues

Dependent variables	Independent variables	Partial (r^2x100)	Total (r^2x100)	*p* values
Model 1				
Mode OM	Visceral adipose tissue area	62.11	65.38	<0.0001
	Age	1.98		NS
	Menopausal status	1.29		NS
Model 2				
Nadir OM	Visceral adipose tissue area	63.58	63.58	<0.0001
Model 3				
% small cell OM	Visceral adipose tissue area	43.85	49.42	<0.0001
	BMI	3.45		0.08
	Subcutaneous adipose tissue area	2.12		NS
Model 4				
Mode SC	Visceral adipose tissue area	43.65	50.44	<0.0001
	Subcutaneous adipose tissue area	6.79		0.01
Model 5				
Nadir SC	Visceral adipose tissue area	41.54	49.30	<0.0001
	Subcutaneous adipose tissue area	5.39		0.02
	Age	2.37		NS
Model 6				
% small cell SC	Visceral adipose tissue area	23.59	23.59	<0.0001

690 Regression models included age, BMI, total body fat mass, visceral adipose tissue area,
691 subcutaneous adipose tissue area, and menopausal status.

692

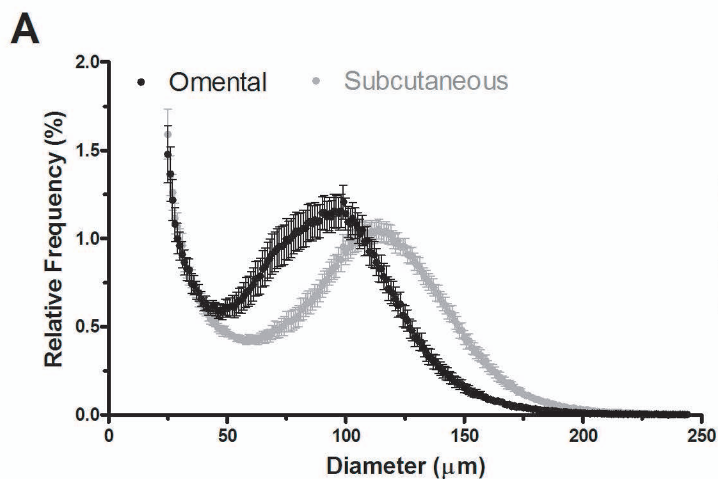
Table 4: Pearson correlation coefficients between OM or SC adipocyte sizes bimodal distribution parameters and metabolic alterations.

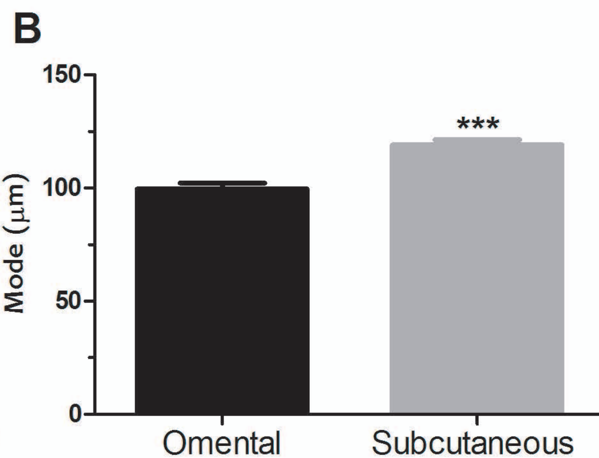
Variables	Omental adipose tissue					
	Mode	**10% larger cells**	**Width**	**Height**	**Nadir**	**% small cells**
HDL-cholesterol	- 0.37**	- 0.36**	- 0.31*	0.37** [a]	- 0.32*	- 0.30*
Plasma triglycerides	0.31*	0.28*	0.32* [a]	- 0.38** [a]	0.32*	0.35** [a]
Total-to-HDL chol. ratio	0.38**	0.36**	0.25[#]	- 0.39** [a]	0.42** [a]	0.40** [a]
$HOMA_{ir}$ index	0.49*** [a]	0.44** [a]	0.44** [a]	- 0.47*** [a]	0.46*** [a]	0.47*** [a]

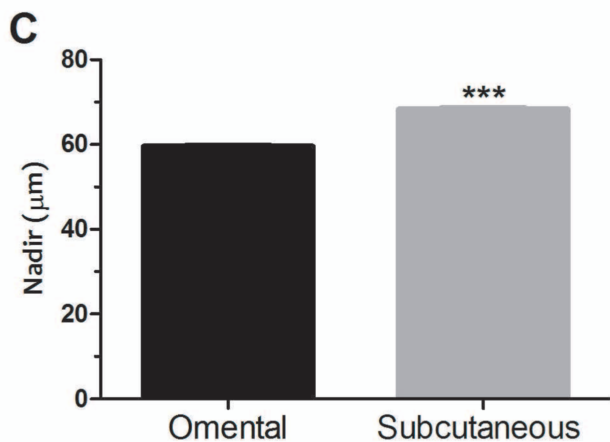
Variables	Subcutaneous adipose tissue					
	Mode	**10% larger cells**	**Width**	**Height**	**Nadir**	**% small cells**
HDL-cholesterol	- 0.43*** [a]	- 0.41* [a]	- 0.31* [a]	0.52*** [a]	- 0.47*** [a]	- 0.47*** [a]
Plasma triglycerides	0.19[#]	0.17	- 0.06	- 0.22[#]	0.30*	0.31*
Total-to-HDL chol. ratio	0.42***	0.45** [a]	0.27*	- 0.52*** [a]	0.46***	0.44*** [a]
$HOMA_{ir}$ index	0.28*	0.23[#]	0.01	- 0.29*	0.43*** [a]	0.47*** [a]

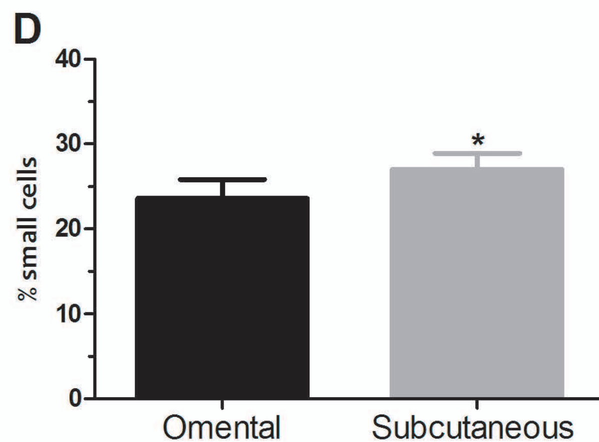
#: $p<0.10$, *: $p<0.05$, **: $p<0.01$, ***$p<0.001$

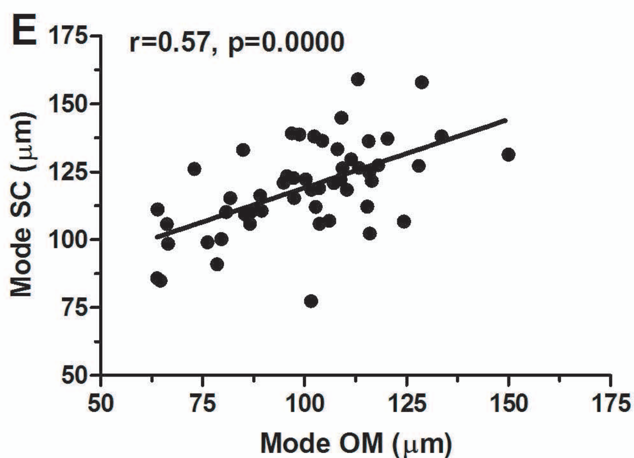
[a] remained significant after adjustment for BMI

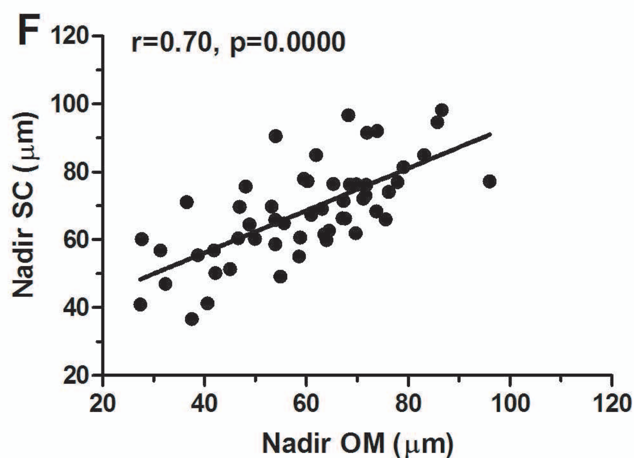

A
Omental
Subcutaneous
Relative Frequency (%)
Diameter (μm)

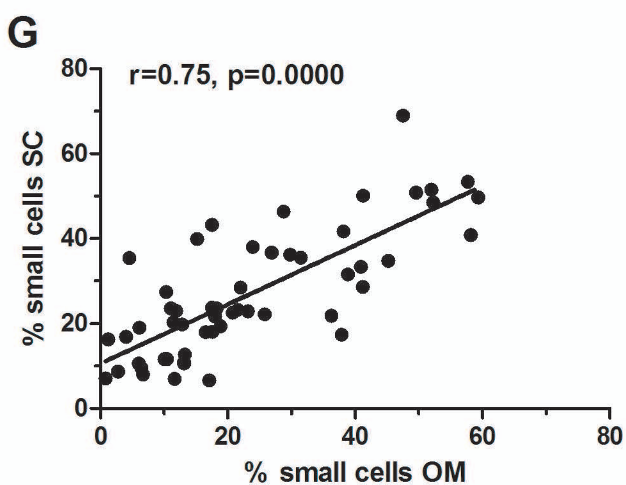

B
Mode (μm)
***
Omental
Subcutaneous

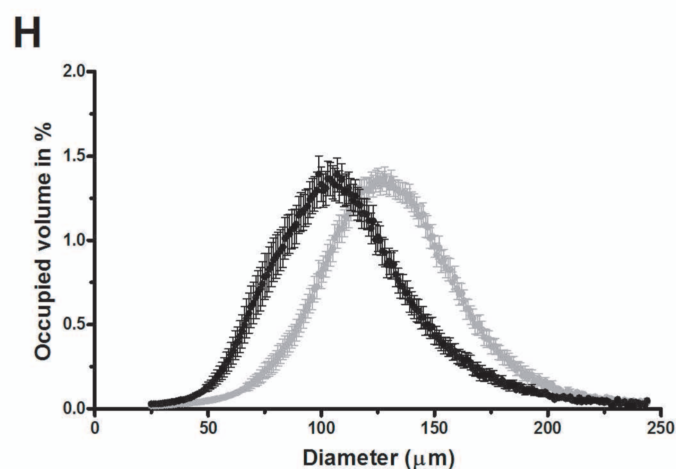

C
Nadir (μm)
***
Omental
Subcutaneous


D
% small cells
*
Omental
Subcutaneous


E
r=0.57, p=0.0000
Mode SC (μm)
Mode OM (μm)

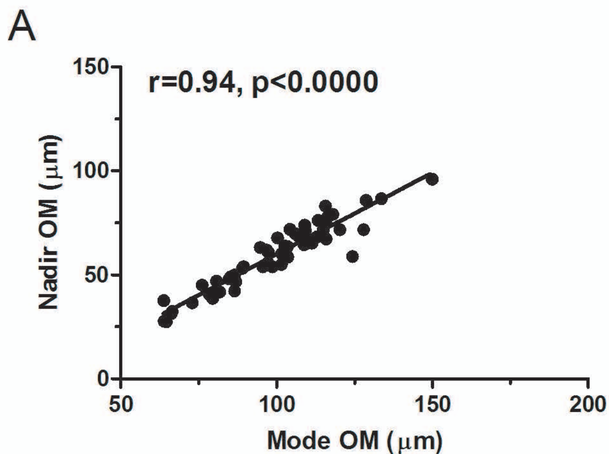

F
r=0.70, p=0.0000
Nadir SC (μm)
Nadir OM (μm)

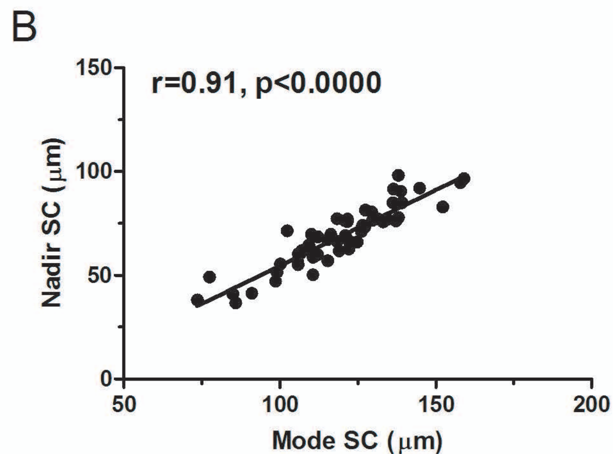

G
r=0.75, p=0.0000
% small cells SC
% small cells OM

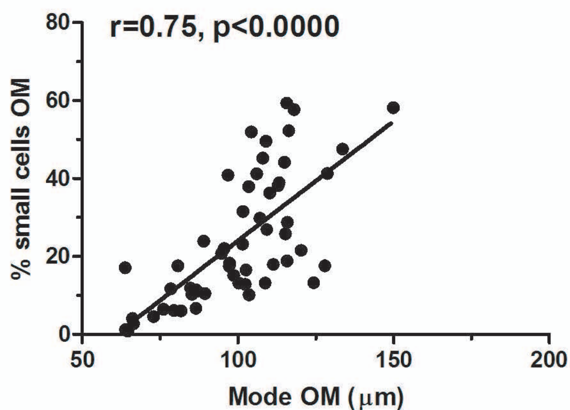

H
Occupied volume in %
Diameter (μm)

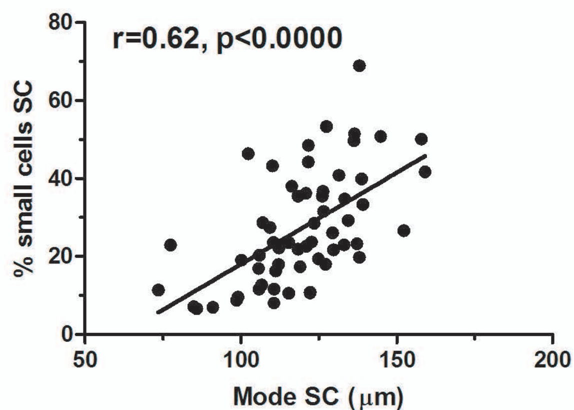
A

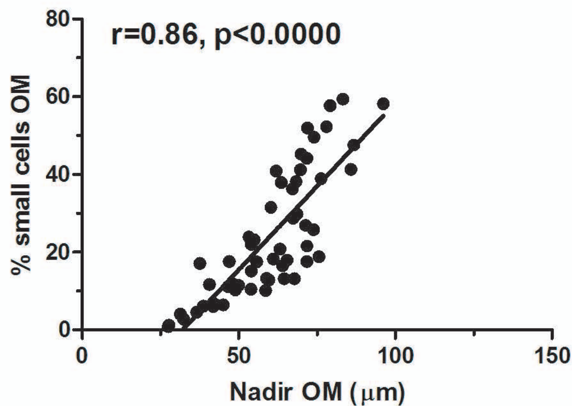
B

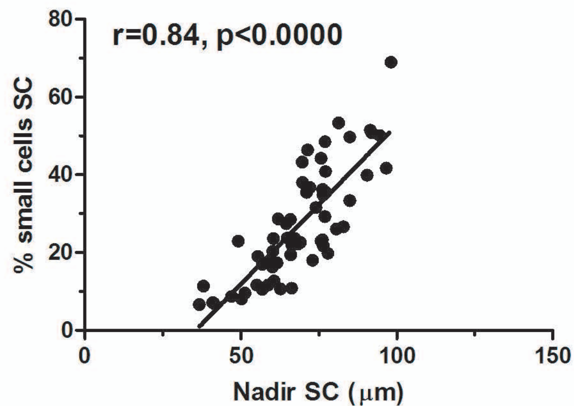


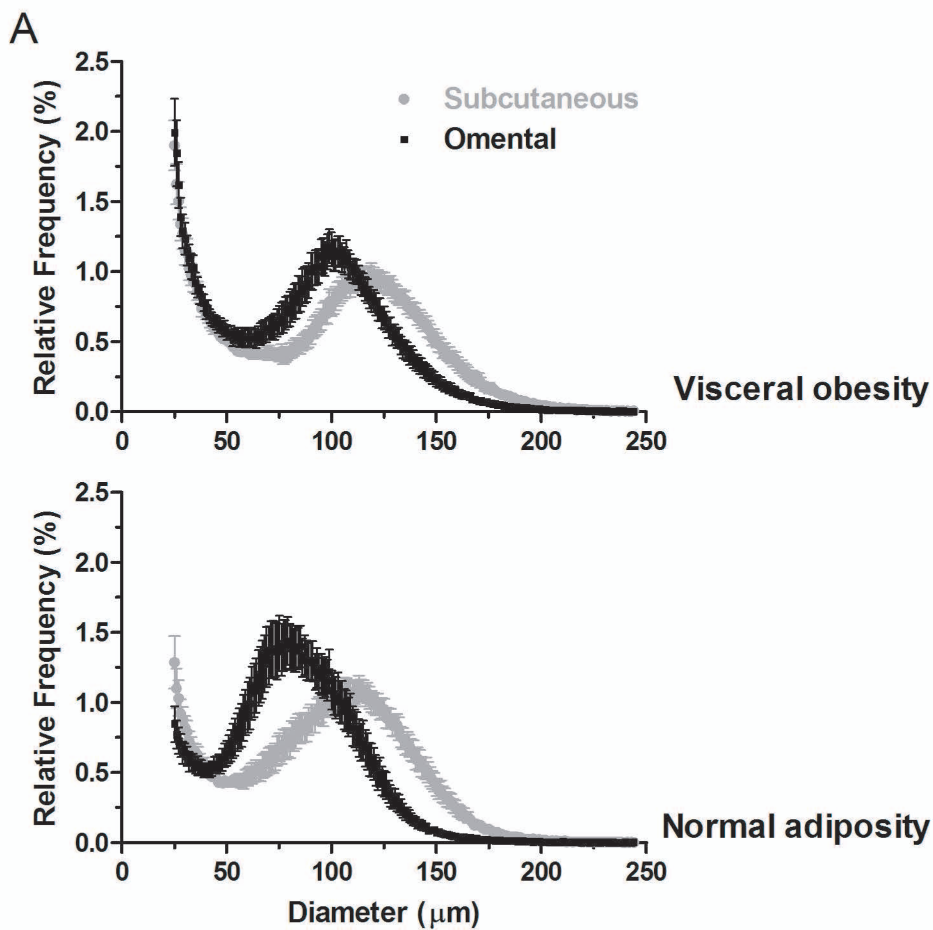


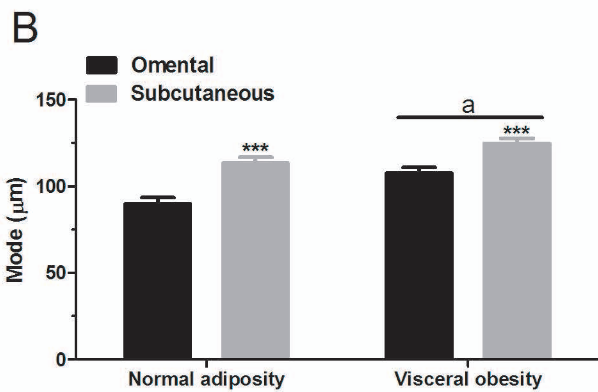


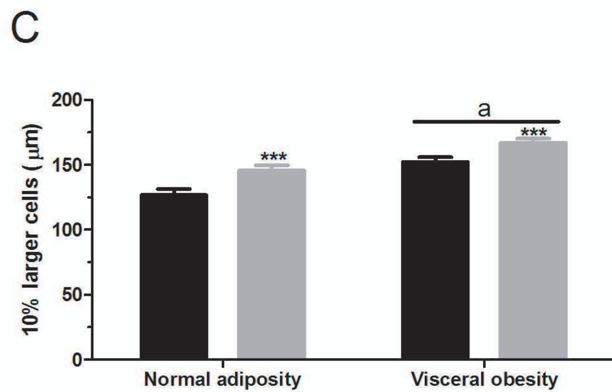


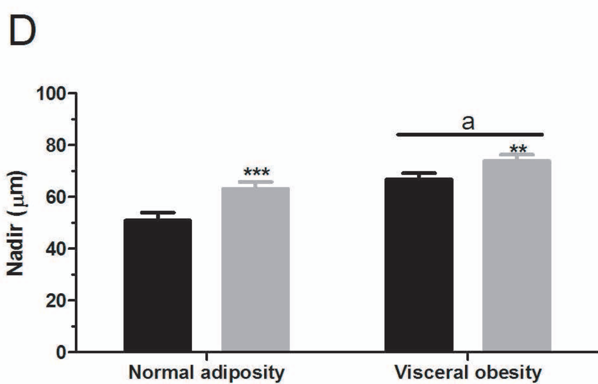


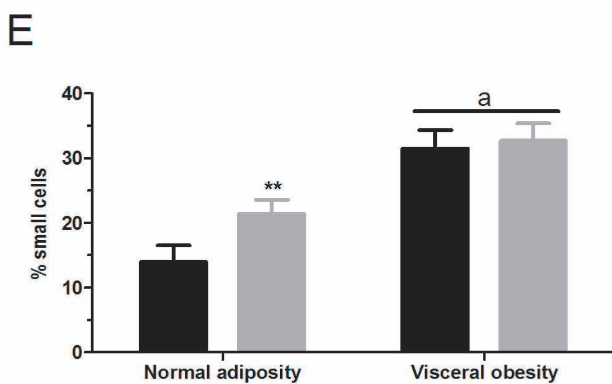


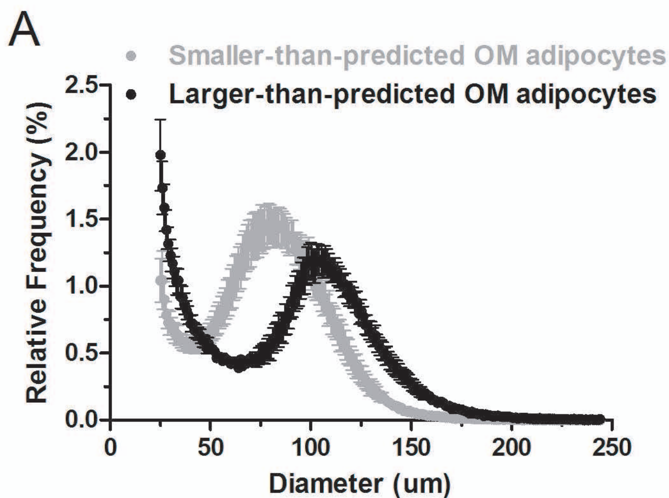

A
Subcutaneous
Omental
Relative Frequency (%)
Visceral obesity
Normal adiposity
Diameter (μm)

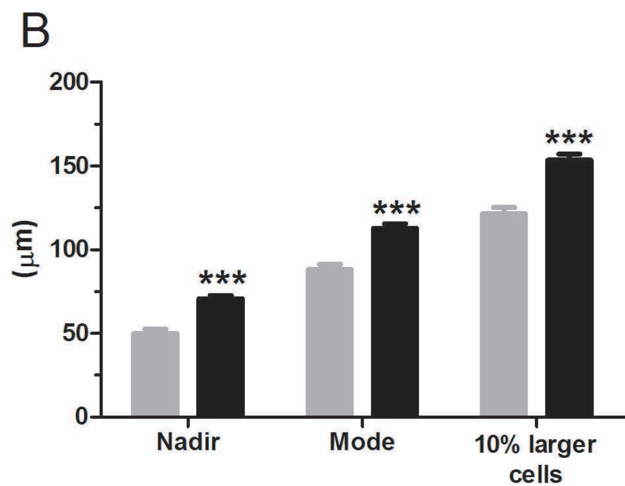

B
Omental
Subcutaneous
Mode (μm)
Normal adiposity
Visceral obesity

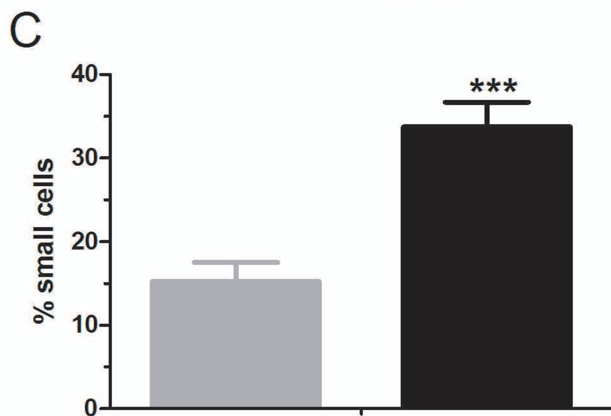

C
10% larger cells (μm)
Normal adiposity
Visceral obesity

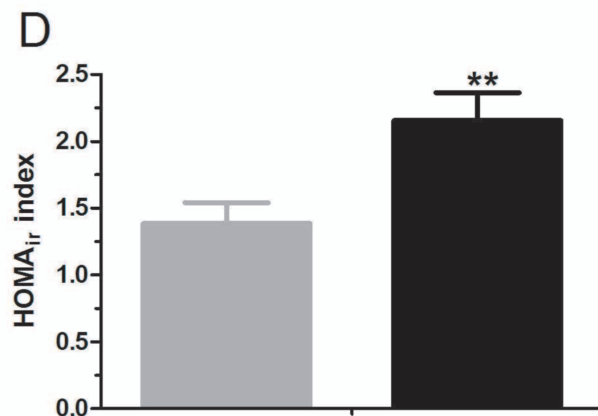

D
Nadir (μm)
Normal adiposity
Visceral obesity

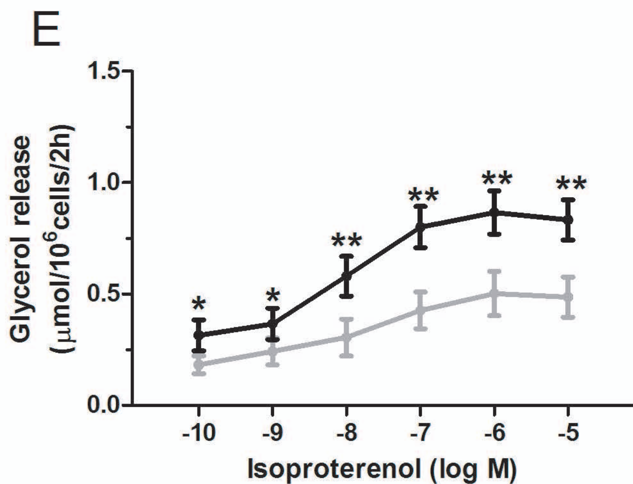

E
% small cells
Normal adiposity
Visceral obesity

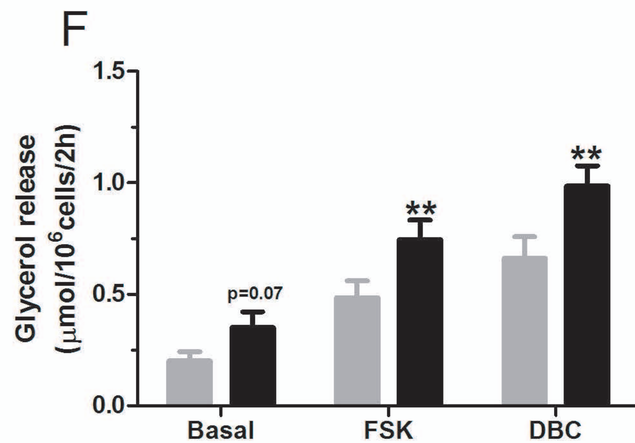

A
Smaller-than-predicted OM adipocytes
Larger-than-predicted OM adipocytes
Relative Frequency (%)
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
Diameter (um)

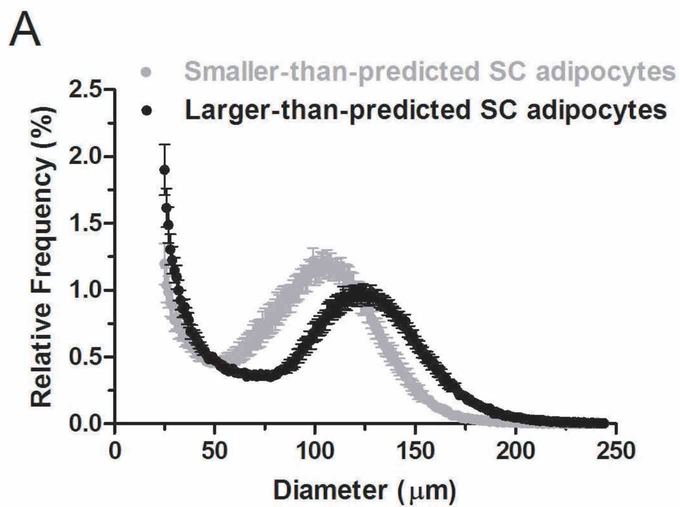

B
200
150
100
50
0
(μm)
***
***
***
Nadir
Mode
10% larger cells

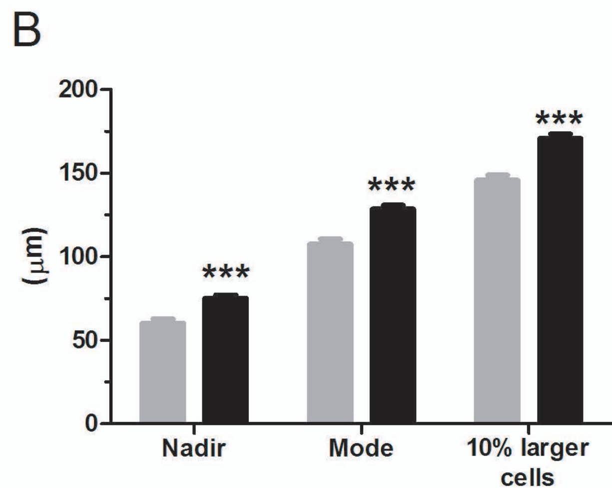

C
40
30
20
10
0
% small cells
***

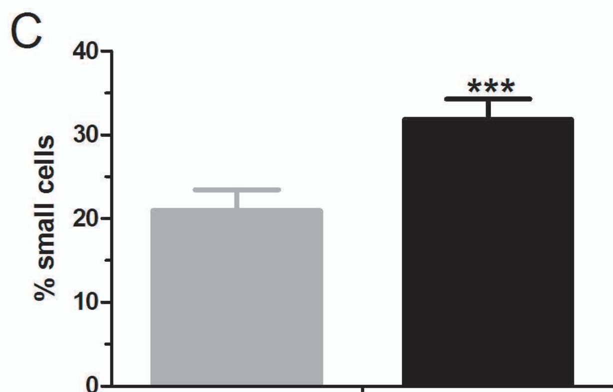

D
2.5
2.0
1.5
1.0
0.5
0.0
HOMA$_{ir}$ index
**

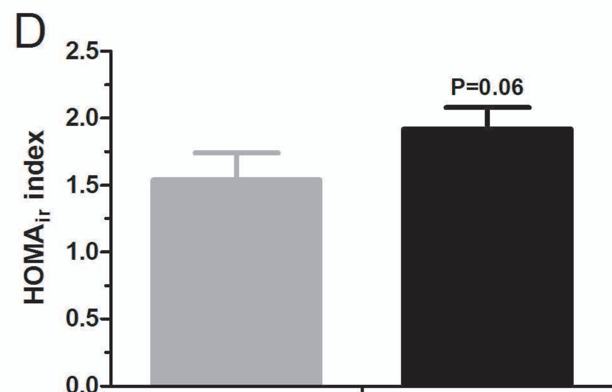

E
1.5
1.0
0.5
0.0
Glycerol release (μmol/10^{6}cells/2h)
*
*
**
**
**
**
-10
-9
-8
-7
-6
-5
Isoproterenol (log M)

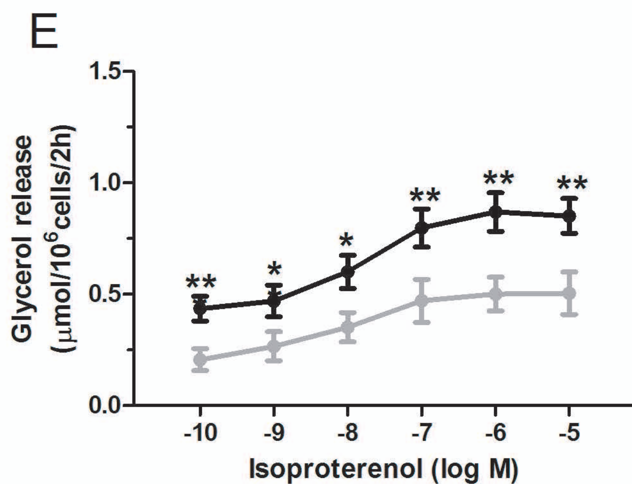

F
1.5
1.0
0.5
0.0
Glycerol release (μmol/10^{6}cells/2h)
p=0.07
**
**
Basal
FSK
DBC

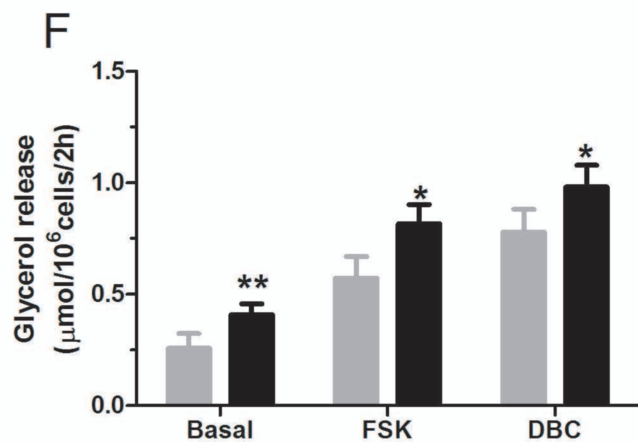

A
Smaller-than-predicted SC adipocytes
Larger-than-predicted SC adipocytes
Relative Frequency (%)
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
Diameter (μm)


B
200
150
100
50
0
(μm)
***
***
***
Nadir
Mode
10% larger cells


C
40
30
20
10
0
% small cells
***


D
2.5
2.0
1.5
1.0
0.5
0.0
HOMAir index
P=0.06


E
1.5
1.0
0.5
0.0
Glycerol release (μmol/10^6cells/2h)
**
*
*
**
**
**
-10
-9
-8
-7
-6
-5
Isoproterenol (log M)


F
1.5
1.0
0.5
0.0
Glycerol release (μmol/10^6cells/2h)
**
*
*
Basal
FSK
DBC