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4 # Expression of genes related to prostaglandin synthesis
5 or signaling in human subcutaneous and omental
6 adipose tissue: depot differences and modulation by
7 adipogenesis

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38 **ABSTRACT**

39 **Objectives:** 1) To examine depot-specific PGE_2 and $PGF_{2\alpha}$ release and mRNA
40 expression of enzymes or receptors involved in PG synthesis or signaling in human
41 adipose tissues; 2) To identify changes in expression of these transcripts through
42 preadipocyte differentiation; and 3) To examine associations between adipose tissue
43 mRNA expression of these transcripts and adiposity measurements. **Methods:** Fat
44 samples were obtained surgically in women. PGE_2 and $PGF_{2\alpha}$ release by preadipocytes
45 and adipose tissue explants was measured. Expression levels of mRNA coding for
46 enzymes or receptors involved in PG synthesis or signaling were measured by RT-PCR.
47 **Results:** Cultured preadipocytes and explants from omental fat released more PGE_2 and
48 $PGF_{2\alpha}$ than those from the subcutaneous depot and the corresponding transcripts showed
49 consistent depot differences. Following preadipocyte differentiation, expression of
50 PLA2G16 and PTGER3 mRNA was significantly increased whereas COX-1, COX-2,
51 PTGIS and PTGES mRNA abundance was decreased in both compartments ($p \leq 0.01$, for
52 all). Transcripts that were stimulated during adipogenesis were those that correlated best
53 with adiposity measurements. **Conclusion:** Cells from the omental fat compartment
54 release more PGE_2 and $PGF_{2\alpha}$ than those from the subcutaneous depot. Obesity
55 modulates expression of PG-synthesizing enzymes and PG receptors which likely occurs
56 through adipogenesis-induced changes in expression of these transcripts.

57

58 **Keywords:** prostaglandins, obesity, adipose tissue differentiation

59 **INTRODUCTION**

60 The metabolic and endocrine functions of adipose tissues are altered in obesity [1].
61 Excess non-esterified fatty acids, immune cell infiltration and the release of inflammatory
62 cytokines have been proposed as the link between obesity, insulin resistance and type 2
63 diabetes [2, 3]. In chronic overfeeding conditions, adipose tissue expansion occurs
64 through adipocyte hypertrophy and the differentiation of preadipocytes to mature, lipid-
65 storing adipocytes (hyperplasia) [4, 5]. The latter process involves important changes in
66 gene expression, such as proadipogenic transcription factors peroxisome proliferator-
67 activated receptor (PPAR) γ and CCAAT/enhancer-binding proteins (C/EBPs) [6, 7].

68

69 Prostaglandins (PGs) are lipid mediators secreted by various cell types in adipose tissue
70 [8, 9] and they appear to be involved in the regulation of inflammation and adipocyte
71 functions [10]. PGs are derived from arachidonic acid (AA), which is liberated by the
72 phospholipid membrane through the activity of phospholipase A2 (PLA2) [11]. Jaworski
73 et al. previously demonstrated that AdPLA (PLA2G16) is the main PLA2 enzyme in
74 adipose tissue of mice [12]. AA is consecutively converted into PGG_2 and PGH_2 by the
75 action of the two PGH synthases (PTGS), the constitutive cyclooxgenase (COX)-1 or the
76 inducible COX-2 [13]. PGH_2 is the common substrate for specific PG synthase enzymes
77 that synthesize PGs [11]. PGD synthase (PTGDS) catalyzes the isomerization of PGH_2 to
78 PGD_2, while prostacyclin synthase (PTGIS) catalyzes the isomerization of PGH_2 to PGI_2
79 [11]. The formation of PGE_2 from PGH_2 is catalyzed by PGE synthases (PTGES). Three
80 forms of PGE synthase have been identified, including cytosolic PGE synthase (cPGES),
81 which is constitutively and ubiquitously expressed [14], and the two membrane-bound

82 PGE synthases (mPGES-1 and mPGES-2) [14, 15]. Fujimori et al. demonstrated that
83 mPGES-1 acts as the main PGE_2 synthase in 3T3-L1 cells [16]. $PGF_{2\alpha}$ is synthesized by
84 enzymes of the aldo-keto reductase (AKRs) family through various pathways [17, 18].
85 The first PGF synthase identified in mammals was AKR1C3 [19]. However, we
86 demonstrated for the first time in models including human preadipocytes as well as
87 bovine and human endometrium that enzymes of the AKR1B family also show PGF
88 synthase activity [8, 20-22]. Specifically, we recently established in human adipose tissue
89 that preadipocytes from the omental fat compartment released more $PGF_{2\alpha}$ in response to
90 inflammatory stimuli compared to those from subcutaneous fat and that AKR1B1 may
91 have a predominant role in $PGF_{2\alpha}$ synthesis by human preadipocytes [8].

92

93 Taking into consideration that excess visceral adipose tissue accumulation is associated
94 with altered metabolic risk independent of total body fat mass [23], depot-specific
95 expression of key enzymes involved in cyclooxygenase-dependent pathways of PG
96 synthesis in adipose tissue may play a pathophysiological role in the development of
97 visceral obesity-associated alterations. PGs seem to alter adipose tissue function by acting
98 as modulators of PPARγ functions [10] and may be responsible for impairing fat storage
99 in abdominal obesity. *In vitro* studies indicated that PGE_2 and $PGF_{2\alpha}$ inhibit the early
100 phase of preadipocyte differentiation by binding to their specific receptors, the EP4
101 receptor [24] and the FP receptor [25-28] respectively, while others demonstrated that
102 PGD_2 enhances preadipocyte differentiation [29, 30]. Quinkler et al. previously
103 performed a detailed examination of depot-specific mRNA expression patterns of
104 enzymes involved in PGD_2 and PGJ_2 synthesis in human omental and subcutaneous

105 adipose tissues [31] and suggested that these enzymes may have an important role in
106 body fat distribution. However, whether expression of adipose tissue PG synthesizing-
107 enzymes and PG receptors are affected in human obesity and during preadipocyte
108 differentiation has never been clearly established.

109

110 Hence, the first aim of this study was to examine depot-specific PGE_2 and $PGF_{2\alpha}$ release
111 and mRNA expression patterns of phospholipase A2 (PLA2G16 and PLA2G4), COX-1,
112 COX-2, PTGDS, PTGIS, PTGFS (AKR1B1), PTGES, PTGES2, PTGES3, prostaglandin
113 FP receptor (PTGFR) and prostaglandin E receptors (PTGER1, PTGER2, PTGER3 and
114 PTGER4) in preadipocytes and whole adipose tissues. The second objective was to
115 identify changes in the expression of these enzymes through preadipocyte differentiation.
116 The third objective was to examine associations between whole adipose tissue mRNA
117 expression of these genes coding for key enzymes involved in PG synthesis or signaling
118 and measures of adiposity, body fat distribution or adipocyte size. We tested the
119 hypothesis that cells from the omental fat compartment release more anti-adipogenic
120 $PGF_{2\alpha}$ and PGE_2 than those from the subcutaneous depot and that corresponding
121 transcripts show consistent depot differences and modulation through adipogenesis. We
122 also hypothesized that abdominal obesity is related to altered expression of adipose tissue
123 PG-synthesizing enzymes and/or PG receptors.

124

125 **MATERIALS AND METHODS**

126 ***Participants***

127 The study sample included 46 healthy women (age 37.6-54.5 years) recruited through the
128 elective surgery schedule of the Gynecology Unit at CHU de Québec Medical Center.
129 Women of the study elected for total (n=45) or subtotal (n=1) abdominal hysterectomies,
130 some with salpingo-oophorectomy of one (n=6) or two (n=12) ovaries. A few weeks
131 before surgery and on the morning of surgery, detailed information was obtained on
132 medication use, reproductive, menstrual and medical history for each patient. Women
133 using medication affecting metabolic parameters (beta-blockers, ACE inhibitors, fibric
134 acid derivatives and statins) were not included in the present study. Women reporting use
135 of nonsteroidal anti-inflammatory medication a few weeks before the surgery were also
136 excluded from this analysis. To perform differentiation, explants and primary
137 preadipocyte experiments, a similar subsample of women undergoing gynecological
138 surgery was recruited (age 37.8-57.5 years, BMI 19.6-41.1 kg/m^2). Primary preadipocytes
139 were also obtained commercially (n=1 for SC, from a 43 year-old woman) (Zen Bio, NC,
140 USA). The study was approved by the Research Ethics Committees of CHU de Québec
141 Medical Center (protocol C09-08-086). We also included cultures from women
142 undergoing bariatric surgery (biliopancreatic diversion or sleeve gastrectomy) for the
143 treatment of severe obesity (n=8, age 26-54 years, BMI 40.0-52.7 kg/m^2) with approval
144 from the Research Ethics Committees of the Quebec Cardiology and Pulmonology
145 Institute (protocol CER-IUCPQ 21049). All subjects provided written informed consent
146 before their inclusion in the study.

147

148 ***Body fatness and body fat distribution measurements***

149 These measurements were performed on the morning of or a few days before surgery.
150 Measures of total body fat mass were determined by dual-energy x-ray absorptiometry
151 using a Hologic QDR-4500A densitometer and the whole-body software body fan
152 V8.269:3 (Hologic Inc., Bedford, MA, USA). Measurement of abdominal subcutaneous
153 and visceral adipose tissue cross-sectional areas at the L4-L5 vertebrae was performed by
154 computed tomography as previously described [32, 33].

155

156 ***Adipose tissue sampling***

157 Subcutaneous adipose tissue was collected at the site of surgical incision (lower
158 abdomen) and omental adipose tissue was collected at the distal portion of the greater
159 omentum. Adipose tissue samples were immediately carried to the laboratory. A portion
160 of the fresh sample was used to perform adipocyte and preadipocyte isolation and a
161 portion of fresh adipose tissue (30 mg) was cut into 5-10 mg pieces and placed in serum-
162 free Medium 199. Adipose tissue explants were kept in culture at 37°C under 5% CO_2
163 atmosphere. The remaining portion of the sample was immediately frozen for RNA
164 isolation and expression measurements.

165

166 ***Adipocyte isolation and adipocyte size measurement***

167 A portion of each fresh tissue sample was digested with type I collagenase in Krebs-
168 Ringer-Henseleit (KRH) buffer according to a modified version of the Rodbell method
169 [34]. Following digestion, cell suspensions were filtered through nylon mesh and mature
170 adipocytes were separated from the stromal-vascular fraction by floatation. Mature cells

171 were washed 3 times with KRH buffer. For cell size measurements, mature adipocyte
172 suspensions were visualized using a phase contrast microscope attached to a camera and
173 computer interface. Pictures of the suspensions were taken and the Scion Image software
174 (Scion Corporation, Frederick, MA, USA) was used to measure the diameter of 250
175 adipocytes for each tissue sample. Average adipocyte diameter was used in analyses.
176
177 ***Preadipocyte isolation and primary cultures***
178 Preadipocytes were isolated from the stromal-vascular fraction using a modification of
179 the Van Harmelen method [35]. The residual KRH buffer of adipocyte isolation, which
180 contained the stromal-vascular fraction, was centrifuged and the pellet was washed in
181 DMEM-F12 culture medium supplemented with 10% calf serum, 2.5µg/ml amphotericin
182 B and 50µg/ml gentamicin. Stromal-vascular cells were then filtered through 140µm
183 nylon mesh to remove endothelial/mesothelial cells, placed in culture plates and cultured
184 at 37ºC under a 5% CO_2 atmosphere. Medium was changed every 2-3 days.
185
186 ***Induction of adipocyte differentiation***
187 Preadipocytes were seeded in 12-well plates to obtain full confluence within 48h.
188 Differentiation of fully confluent preadipocyte cultures was induced using standardized
189 differentiation medium and protocols for 0-8 days (Zen Bio, Durham, NC, USA).
190 Differentiation medium consisted of DMEM-F12 supplemented with a PPARγ agonist,
191 insulin, dexamethasone and 3-isobutyl-1-methylxanthine. To assess the extent of
192 differentiation, we measured mRNA expression of PPARγ. The accumulation of lipid
193 droplets in differentiated cells (after 8 days) was also assessed by Oil Red O-staining.

194 Cells were washed with PBS and fixed with formalin for 1 h. They were then incubated
195 for 2 h with a 4.9 mM Oil Red O solution and washed three times with ddH2O. Pictures
196 of differentiated cells were taken using a phase contrast microscopy at 20 X
197 magnification.

198

199 ***PGE_2 and $PGF_{2\alpha}$ measurements***

200 PGE_2 and $PGF_{2\alpha}$ release by subcutaneous and omental primary preadipocytes were
201 measured in cells incubated for 24 hours at 37°C in DMEM-F12 medium. PGE_2 and
202 $PGF_{2\alpha}$ release by subcutaneous and omental primary organ cultures were also measured
203 in serum-free Medium 199 incubated for 24 hours at 37°C with the tissue samples. The
204 incubation time was established according to a time-course experiment. PGE_2 and $PGF_{2\alpha}$
205 release during various stages of omental or subcutaneous preadipocyte differentiation (0,
206 6h, 1day and 8 days) was also measured in differentiation medium from ZenBio. On the
207 basis of these measurements, omental and subcutaneous PGE_2 and $PGF_{2\alpha}$ production rate
208 was calculated. PGE_2 and $PGF_{2\alpha}$ content in the media was measured by enzyme
209 immunoassay and acetylcholinesterase-linked PGE_2 and $PGF_{2\alpha}$ tracer (Cayman
210 Chemicals, Ann Arbor, MI, USA) as previously described [36]. Considering the nature
211 and cultivability of primary preadipocytes and adipose tissue explants, PGE_2 and $PGF_{2\alpha}$
212 release by cultured primary preadipocytes was expressed as pg/ml*µg protein*24h and
213 PGE_2 and $PGF_{2\alpha}$ release by adipose tissue explants was expressed as pg/ml*mg
214 tissue*24h. Omental and subcutaneous PGE_2 and $PGF_{2\alpha}$ production rate during
215 differentiation was expressed as pg/ml*µg protein*hour.

216

217 ***Messenger RNA expression by quantitative realtime RT-PCR***

218 Total RNA was isolated from whole subcutaneous and omental adipose tissue or from
219 primary differentiated and non-differentiated cultures using the RNeasy lipid tissue
220 extraction kit and on-column DNase treatment (Qiagen, Hilden, DE) following the
221 manufacturer's recommendations. RNA quality and concentration was assessed using the
222 Agilent Technologies 2100 bioanalyzer (Agilent, Santa Clara, CA, USA).
223 Complementary DNA was generated from total RNA using random hexamers, oligo dT_{18}
224 and Superscript III Rnase H-RT (Invitrogen Life Technologies, Burlington, ON,
225 CANADA) and purified with QIAquick PCR Purification Kit (Qiagen, Hilden, DE).
226 Realtime cDNA amplification was performed in duplicate using the LightCycler 480
227 (Roche Diagnostics, Indianapolis, IN, USA) and the SYBRGreen I Master (Roche
228 Diagnostics, Indianapolis, IN, USA). The conditions for PCR reactions were: 45 cycles,
229 denaturation at 95°C for 10 sec, annealing at 60°C for 10 sec, elongation at 72°C for 14
230 sec and then 74°C for 5 sec (reading). A melting curve was performed to assess non-
231 specific signal. Calculation of copy number for each transcript was performed according
232 to Luu-The et al. [37] using the second derivative method and a standard curve of Cp
233 versus logarithm of the quantity. The standard curve was established using known
234 amounts of purified PCR products and a LightCycler 480 v1.5 program provided by the
235 manufacturer (Roche Diagnostics, Mannheim, DE). PCR amplification efficiency was
236 verified. Target gene amplifications were normalized using housekeeping gene
237 expression levels of ATP synthase O subunit (*ATP5O*) for whole tissue extracts or
238 Glucose-6-phosphate dehydrogenase (*G6PD*) for preadipocyte differentiation. Expression
239 levels of *ATP5O* were not different in omental versus subcutaneous adipose tissue in our

240 study sample and were used to normalize whole tissue expression levels. *G6PD* mRNA
241 expression was not significantly modulated during preadipocyte differentiation and was
242 used as control in these experiments. Primer sequences were designed using Gene Tools
243 2.0 software (Biotools Inc, Edmonton, AB, CA) and their specificity was verified by blast
244 in the GenBank database. The synthesis was performed by IDT (Integrated DNA
245 Technology, Coralville, IA, USA). The transcripts examined were phospholipase A2
246 (PLA2G16 and PLA2G4), cyclooxygenase 1 and 2 (PTGS1 and PTGS2), PGF synthase
247 aldo-keto reductase 1B1 (AKR1B1), PGI synthase (PTGIS), prostaglandin D synthase
248 (PTGDS), prostaglandin E synthases (PTGES, PTGES2 and PTGES3), PGE receptors 1,
249 2, 3 and 4 (PTGER1, PTGER2, PTGER3 and PTGER4) as well as prostaglandin FP
250 receptor (PTGFR). Primer sequences are listed in **Supplemental Table 1**. Quantitative
251 Realtime PCR measurements were performed by the CHU de Québec Research Center
252 Gene Expression Platform (Quebec City, Quebec, Canada).
253

254 ***Statistical analyses***

255 Student's t-tests were performed to examine depot differences in mean basal PGE_2 and
256 $PGF_{2\alpha}$ release by primary preadipocytes or adipose tissue explants, mRNA expression
257 levels of the transcripts examined in cultured preadipocytes and mean PGE_2 and $PGF_{2\alpha}$
258 production rate during preadipocyte differentiation. To analyze significant changes
259 among scheduled endpoints (0, 6, 24 and 192 h after induction of differentiation) for two
260 depots, three experimental factors were defined: patient donor (random factor), depot and
261 measurements at the four time points (fixed factors). The latter was analyzed as a
262 repeated-measure factor with the use of an unstructured covariance matrix. The

263 interaction between depot and the scheduled endpoints was considered. Missing values
264 were not imputed. We used residual maximum likelihood as the method of estimation and
265 the Kenward–Roger method to estimate denominator degrees of freedom for the test of
266 fixed effect. The univariate normality assumption was verified with the Shapiro-Wilk
267 tests on the error distribution from the Cholesky factorization of the statistical model. The
268 Brown and Forsythe variation of Levene's test statistic was used to verify homogeneity of
269 variances. Student's paired t-tests were performed to assess depot differences in adipose
270 tissue mRNA expression levels of the transcripts examined in whole adipose tissue.
271 Pearson correlation coefficients were computed to quantify associations between adipose
272 tissue mRNA expression of the transcripts and adiposity measurements. Non-normally
273 distributed variables were log- or boxcox-transformed. All data are presented as mean ±
274 SEM. Statistical analyses were performed with the statistical packages R v3.0.2. (R
275 Foundation for Statistical Computing, Vienna, Austria), JMP software version 4.0 or SAS
276 v9.4 (SAS Institute Inc, Cary, NC, U.S.A.).
277

278 **RESULTS**

279 ***PGE_2 and $PGF_{2\alpha}$ release by adipose tissue explants and primary preadipocytes from***
280 ***the subcutaneous and omental compartments***

281 **Figure 1** shows PGE_2 and $PGF_{2\alpha}$ release by subcutaneous and omental explants or
282 primary preadipocyte cultures over 24 hours. Adipose tissue explants tended to release
283 more PGE_2 than $PGF_{2\alpha}$ in both adipose tissue compartments ($p \leq 0.10$) (**Figure 1A**). The
284 release of $PGF_{2\alpha}$ by omental explants was significantly higher compared to that of
285 subcutaneous adipose tissue explants ($p \leq 0.05$) (**Figure 1A**). Omental explants also
286 tended to have a higher PGE_2 release compared to subcutaneous explants ($p \leq 0.10$).
287 **Figure 1B** shows that preadipocyte cultures released more PGE_2 than $PGF_{2\alpha}$ in both
288 adipose tissue compartments ($p \leq 0.05$). Similar to adipose tissue explants, the release of
289 $PGF_{2\alpha}$ by omental preadipocytes was significantly higher compared to that of
290 subcutaneous preadipocytes ($p \leq 0.05$) (**Figure 1B**). Omental preadipocytes also tended to
291 release more PGE_2 compared to subcutaneous preadipocytes ($p \leq 0.10$).

292

293 ***Expression levels of genes related to PG synthesis or signaling in whole adipose tissue***
294 ***and primary preadipocytes from the subcutaneous and omental compartments***

295 Considering that omental PGE_2 and $PGF_{2\alpha}$ release by primary preadipocytes and adipose
296 tissue explants was higher compared to subcutaneous fat cells, we also examined depot-
297 differences in adipose tissue expression of several genes coding for enzymes involved in
298 PG synthesis or PG receptors. Messenger RNA abundance of these genes in whole
299 adipose tissues was assessed in 46 women. **Table 1** shows characteristics of this sample.

300 According to the mean BMI (28.0 kg/m^2) and body composition measurements, women
301 were overweight and covered a wide range of adiposity values.
302
303 The transcripts examined were detectable in whole tissues from both fat compartments
304 and most showed significant depot differences (**Figure 2**). Specifically, COX-1,
305 AKR1B1, PTGIS, PTGDS, PTGES3 and PTGER1 mRNA expression levels were
306 significantly higher in omental compared to subcutaneous adipose tissue ($p \leq 0.05$ for all).
307 Expression levels of PLA2G16, PTGFR, PTGES, PTGER3 and PTGER4 were
308 significantly higher in subcutaneous compared to omental adipose tissue ($p \leq 0.05$ for all).
309 No significant depot difference was observed in PLA2G4, COX-2, PTGES2 and
310 PTGER2 expression levels. PTGER1 mRNA abundance was weak in whole tissues (i.e.
311 below 2000 copies per ug of total RNA).
312
313 In a subsample of patients, expression of these transcripts was also examined in cultured
314 primary preadipocytes. The transcripts studied were detectable in primary preadipocytes
315 from both fat compartments and most showed significant depot differences (**Figure 3**).
316 Expression levels of COX-1, COX-2, AKR1B1 and PTGES were significantly higher in
317 omental compared to subcutaneous primary preadipocytes ($p \leq 0.05$ for all). A trend for
318 higher expression of PLA2G4A and PTGDS in omental preadipocytes was observed
319 ($p \leq 0.10$ for both). Messenger RNA expression of PTGER3 was significantly higher in
320 subcutaneous compared to omental preadipocytes ($p \leq 0.001$). A trend for higher
321 expression of PTGFR in subcutaneous preadipocytes was observed ($p \leq 0.10$). No
322 significant depot difference was observed in PLA2G16, PTGIS, PTGES2, PTGES3,

323 PTGER1 and PTGER2 mRNA expression levels. Messenger RNA expression levels of
324 PTGER1 and PTGER4 were very low in preadipocytes (i.e. below 2 500 copies per µg
325 total RNA for PTGER1 and below 300 copies per µg of total RNA for PTGER4).
326

327 ***Expression levels of PG enzyme- or receptor-coding genes during preadipocyte***
328 ***differentiation***

329 To examine whether expression of adipose tissue PG synthesizing-enzymes and PG
330 receptors are modulated during preadipocyte differentiation, we measured these
331 transcripts at 0h, 6h, 1 day and 8 days after induction of differentiation. As expected,
332 **Figure 4A** shows that mRNA expression of PPARγ was significantly induced in
333 differentiated cells from both fat compartments compared to undifferentiated cells, as
334 expected from treatments ($p<0.0001$). Consistent with these results, the accumulation of
335 lipid droplets in differentiated cells (after 8 days) was also observed by Oil Red O-
336 staining and phase contrast microscopy (**Figure 4B**). Subcutaneous PPARγ mRNA
337 abundance was significantly higher at all-time points tested compared to omental PPARγ
338 mRNA abundance, with a significant depot effect ($p=0.05$) (**Figure 4A**).
339

340 Eight days after the induction of preadipocyte differentiation, PLA2G16 and PTGER3
341 mRNA expression in both fat compartments were significantly increased (**Figure 5A and**
342 **L**) whereas COX-1, COX-2, PTGIS and PTGES mRNA abundance in both fat depots
343 were decreased ($p\leq0.01$ for all) (**Figure 5B, C, F and G)**. Interestingly, omental COX-2
344 mRNA was highly abundant before differentiation and significantly decreased 6 hours
345 after induction, with a significant time-by-depot interaction ($p=0.02$) (**Figure 5C**). Higher

346 expression of AKR1B1 mRNA in both fat compartments was observed one day after
347 induction of differentiation ($p<0.0001$) (**Figure 5D)**, but this transcript was significantly
348 lower in differentiated cells (after 8 days) compared to undifferentiated cells (p=0.01). A
349 similar pattern of expression was observed for PTGFR. This transcript was slightly
350 decreased after 6 hours, especially in the subcutaneous depot, but significantly increased
351 after one day in both depots (p=0.0001) (**Figure 5J**). PTGDS mRNA expression tended
352 to increase in omental differentiated cells only (time-by-depot interaction, p=0.06)
353 (**Figure 5E**). PTGES2 mRNA expression in both fat compartments was significantly
354 increased after one day compared to undifferentiated cells (p=0.05) (**Figure 5H**). No
355 significant difference was observed in PGTES3 mRNA expression during differentiation
356 (**Figure 5I**). PTGER2 mRNA expression in both fat compartments was significantly
357 increased 6 hours after the induction of differentiation, but this transcript was decreased
358 in differentiated cells, especially in the subcutaneous depot (time-by-depot interaction,
359 p=0.06) (**Figure 5K**). PTGER1 and PTGER4 were only weakly expressed in
360 undifferentiated and differentiated cells (i.e. below 2 000 copies per μg of total RNA for
361 PTGER1 and below 1000 copies per μg of total RNA for PTGER4). The level of
362 PTGER1 was gradually decreased after 8 days of differentiation in both fat compartments
363 (p=0.006, data not shown). Significantly lower expression of PTGER4 was observed 6
364 hours after the induction of differentiation compared to control in both fat compartments
365 (p=0.006). However, this transcript tended to be higher in differentiated cells compared to
366 control (p=0.09, data not shown).

367

368 Depot-differences in these transcripts were also observed during preadipocyte
369 differentiation. More specifically, subcutaneous PLA2G16 mRNA expression was
370 significantly higher compared to omental PLA2G16 mRNA expression (p=0.001)
371 (**Figure 5A**). Omental COX-1, COX-2, AKR1B1 and PTGES mRNA expression were
372 also significantly higher compared to that of subcutaneous cells ($p \leq 0.05$, for all) (**Figure**
373 **5B, C, D and G**). Subcutaneous PTGER3 mRNA abundance was significantly higher
374 compared to omental PTGER3 mRNA expression after induction of differentiation
375 (p=0.003) (**Figure 5L**). No significant depot difference was observed for mRNA
376 abundance of PTGDS, PTGIS, PTGES2, PTGES3, PTGFR and PTGER2 during the
377 induction of preadipocyte differentiation (**Figure 5E, F, H, I, J and K**).
378

379 PGE_2 and $PGF_{2\alpha}$ release by these cells was also measured in the medium at 0, 6h, 1 day
380 and 8 days after the induction of differentiation. To examine depot differences, omental
381 and subcutaneous PGE_2 and $PGF_{2\alpha}$ production rate was calculated on the basis of these
382 measurements. Omental PGE_2 secretion rate was significantly higher compared to
383 subcutaneous PGE_2 secretion rate (p=0.04, **Figure 6A**). Furthermore, omental $PGF_{2\alpha}$
384 secretion rate tended to be higher compared to subcutaneous $PGF_{2\alpha}$ secretion rate
385 (p=0.14, **Figure 6B**).
386

387 ***Whole adipose tissue expression levels of PG enzyme- or receptor-coding genes in***
388 ***relation with body fatness and body fat distribution***

389 Associations between expression levels of PG enzyme- or receptor-coding genes in whole
390 adipose tissue and body composition or fat distribution measurements was performed to

391 test the hypothesis that abdominal obesity is related to altered expression of adipose
392 tissue PG-synthesizing enzymes and/or PG receptors and that the transcripts that are
393 stimulated through adipogenesis are those that correlate best with the degree of obesity.
394 **Table 2** shows Pearson correlation coefficients between mRNA expression levels of
395 several transcripts examined and body composition or fat distribution measurements.
396 PLA2G16 mRNA expression in omental adipose tissue was positively and significantly
397 correlated with total adipose tissue area and adipocyte diameters ($p \leq 0.05$, for all).
398 AKR1B1 mRNA expression in both fat compartments was positively and significantly
399 associated with lean body mass ($p \leq 0.05$). Expression of this transcript in subcutaneous
400 adipose tissue was also positively and significantly correlated with total body fat mass,
401 adipose tissue areas and adipocyte sizes ($p \leq 0.05$, for all). PTGDS mRNA expression in
402 omental adipose tissue was positively associated with visceral adipose tissue area
403 ($p \leq 0.05$). Trends were observed between omental PTGDS mRNA expression and total
404 body fat mass as well as omental adipocyte diameter ($p \leq 0.10$, for both). Expression of
405 this transcript in subcutaneous adipose tissue was not significantly related to body
406 composition and fat distribution measurements. PTGER3 mRNA expression in omental
407 adipose tissue was positively correlated with total and visceral adipose tissue areas as
408 well as adipocyte sizes ($p \leq 0.05$, for all). PTGER4 mRNA abundance in both fat depots
409 was associated with body fat distribution indices and omental adipocyte size ($p \leq 0.05$, for
410 all). PTGER4 mRNA expression in subcutaneous adipose tissue was positively correlated
411 with subcutaneous adipocyte size, body composition and lean body mass ($p \leq 0.05$, for
412 all). PTGES2 mRNA expression in omental adipose tissue was positively associated only
413 with visceral adipose tissue area ($p \leq 0.05$, data not shown). No significant associations

414 were found between COX-1, COX-2 PTGIS, PTGES, PGTES3, PTGFR, PTGER1 or
415 PTGER2 mRNA expression in either fat compartment and body composition or fat
416 distribution measurements (data not shown).
417

418 **DISCUSSION**

419 To our knowledge, this is the first study to clearly investigate fat depot-specific PGE_2 and
420 $PGF_{2\alpha}$ release combined with expression patterns of several enzymes involved in PG
421 synthesis or signaling in human preadipocytes or whole adipose tissues. In addition, we
422 determined which transcripts were modulated in response to preadipocyte differentiation
423 and we examined the association between body composition and fat distribution
424 measurements and the expression levels of these transcripts in human subcutaneous and
425 omental adipose tissues. We found that cultured preadipocytes and explants from the
426 omental fat compartment release more PGE_2 and $PGF_{2\alpha}$ than those from the subcutaneous
427 depot and the corresponding transcripts show consistent depot differences. During
428 preadipocyte differentiation, PGE_2 and $PGF_{2\alpha}$ secretion rates were higher in omental
429 compared to subcutaneous cells and consistent depot differences in transcripts involved in
430 PGE_2 and $PGF_{2\alpha}$ synthesis were observed. Transcripts that were stimulated during
431 adipogenesis were those that correlated best with adiposity measurements, suggesting that
432 obesity-related changes in cell number and size affect PG-synthesizing genes and PG
433 receptor expression.

434

435 One important finding in this study is that preadipocytes and adipose tissue explant from
436 the omental fat compartment both released more PGE_2 and $PGF_{2\alpha}$ than those from the
437 subcutaneous depot. The findings of higher expression of COX-1, AKR1B1, PTGES3,
438 PTGIS and PTGDS in omental vs. subcutaneous adipose tissue as well as higher
439 expression of COX-1, COX-2, AKR1B1, PTGES and PTGDS in omental vs.
440 subcutaneous cultured preadipocytes are consistent with these results. Several studies

441 previously established that the two COXs are the rate-limiting enzymes in the synthesis
442 of PG [11, 16]. In our study, higher expression of COX isoforms in visceral fat may
443 explain depot differences in PG synthesis. Farb et al.[38] recently demonstrated in
444 severely obese subjects that expression of COXs, PTGES, PTGDS and PTGIS were up-
445 regulated in visceral adipose tissue, which is highly consistent with our results. They also
446 observed that the release of PGE_2 and 6-keto $PGF_{1\alpha}$ were significantly higher in visceral
447 compared to subcutaneous fat after 48-hours of culture [38]. Quinkler et al. also reported
448 that COX-1 and PTGDS mRNA expression levels were higher in omental compared to
449 subcutaneous adipose tissue [31]. Furthermore, we previously found that visceral
450 preadipocytes were more responsive to inflammatory signals in terms of $PGF_{2\alpha}$ release
451 [8]. In the present study, our results suggest a predisposition of omental fat cells to
452 release COX-derived prostaglandins, which represents convincing evidence supporting
453 the notion that omental fat is distinct from subcutaneous adipose tissue in terms of
454 endocrine function and that visceral adipose tissue contributes to the inflammatory
455 phenotype [39]. Depot-specific expression patterns of key enzymes involved in PG
456 synthesis or signaling may play a pathophysiological role in visceral obesity. In
457 agreement with this hypothesis, Farb et al. established that PGs of the cyclooxygenase
458 pathway are involved in vascular endothelial dysfunction of visceral adipose tissue [38].
459
460 In the present study, we observed that subcutaneous mRNA expression of PPARγ was
461 significantly higher compared to that of the omental fat compartment. In agreement with
462 previous studies regarding depot-differences in adipogenesis [40-44], preadipocytes from
463 the subcutaneous fat compartment appeared to have a higher ability to differentiate
464 compared to preadipocytes from visceral fat. Interestingly, during preadipocyte

465 differentiation, we found that the production rate of PGE_2 and $PGF_{2\alpha}$ was lower in the
466 subcutaneous fat compartment and consistent depot differences were also observed in
467 transcripts involved in PGE_2 and $PGF_{2\alpha}$ synthesis (COXs, AKR1B1 and PTGES). These
468 depot differences in PG synthesis during the induction of preadipocyte differentiation are
469 consistent with the notion that PGE_2 and $PGF_{2\alpha}$ may have anti-adipogenic functions.
470 Indeed, as mentioned, *in vitro* studies demonstrated that PGE_2 and $PGF_{2\alpha}$ inhibit the
471 early-phase of preadipocyte differentiation by acting through their specific receptor [24-
472 28, 45]. Considering the design of our study, we cannot conclude on cause-and-effect
473 relationships. However, our findings do not exclude a potential impact of PGs on
474 adipocyte differentiation. As impaired adipogenesis is a critical factor linking obesity and
475 metabolic complications [40], further studies are necessary to examine how these two
476 PGs might modulate the expandability of adipose tissue in humans.
477
478 To our knowledge, this is the first study to clearly report associations between obesity or
479 visceral fat accumulation and the expression of several PG-synthesizing enzymes or PG
480 receptors in human adipose tissues. Many significant correlations were found between
481 expression levels of several transcripts and adiposity measurements. AKR1B1 expression
482 levels in both fat compartments were positively related to measures of total and visceral
483 adiposity. Only omental PTGDS and PLA2G16 expression were correlated with adiposity
484 indices. Expression levels of the receptors EP3 and EP4 were also positively correlated
485 with body composition and fat distribution indices, especially in the omental
486 compartment for EP3 receptor and in both fat compartments for EP4 receptor. A striking
487 aspect of these results is that all the significant correlations observed were positive. The

488 multiple significant positive correlations with adipocyte size indirectly suggested that
489 expression patterns of PG-synthesizing enzymes or PG receptors in adipose tissue might
490 reflect the proportion of large, mature adipocytes in each adipose tissue sample. In obese
491 individuals, expansion of adipose tissue leads to increased adipocyte size (hypertrophy)
492 and increased adipocyte number (hyperplasia) [4, 5]. We propose that the correlation
493 pattern we describe reflects a modulating effect of obesity on adipose tissue cell
494 composition, which in turn, affects PG-synthesizing enzymes and PG receptors.
495

496 Consistent with a modulating effect of obesity on gene expression, transcripts that were
497 significantly correlated with adiposity were also highly expressed in differentiated cells
498 compared to undifferentiated cells. In contrast, most of the enzymes that were not
499 associated to adiposity were weakly expressed in differentiated cells compared to
500 undifferentiated cells. In a similar manner, transcripts that were not modulated during
501 differentiation such as PTGES3 and subcutaneous PTGDS were not significant correlates
502 of body composition or fat distribution. AKR1B1 seems to be an exception to this pattern
503 as its expression was significantly reduced 8 days after differentiation. However, we also
504 found a transient increase in its expression 1 day after differentiation. This could explain
505 the positive associations between AKR1B1 expression in whole tissue and body
506 composition indices in our correlation analysis. In agreement with these results, Fujimori
507 et al. showed that Akr1b3, the murine ortholog of human AKR1B1, was weakly
508 expressed in 3T3-L1 preadipocytes, but increased after the initiation of differentiation
509 and then quickly decreased [46]. In the latter study, COX-2 expression was modulated in
510 a similar manner [46]. Our findings are consistent as expression levels of COX-1 and 2

511 decreased during differentiation. Xie et al. also previously observed that both COXs were
512 down-regulated after differentiation of 3T3-L1 cells [47]. On the basis of the transient
513 expression of Akr1b3 in differentiating 3T3-L1 cells, Fujimori et al. showed that Akr1b3
514 is involved in the suppression of the early phase of adipogenesis through $PGF_{2\alpha}$ synthesis
515 and signaling through FP receptors [46]. As mentioned, we also reported that AKR1B1 is
516 a functional PG synthase in humans [8, 20, 21]. These findings on AKR1B1 in adipose
517 tissue raise a potential role of this enzyme in the expandability of adipose tissue and the
518 metabolic complications of abdominal obesity.
519
520 The expression of PTGES has been previously investigated in adipocytes [45, 48], but the
521 specific isoform involved in the production of PGE_2 has never been clearly identified in
522 human adipose tissue. Fujimori et al. recently demonstrated that PTGES is responsible
523 for PGE_2 synthesis in 3T3-L1 cells [16]. Hétu et al. demonstrated that expression of
524 PTGES was greatly decreased in murine obese tissues, whereas PTGES2 and PTGES3
525 expression showed minor or no changes [48]. Fain et al. also showed that adipocytes
526 isolated from human subcutaneous and visceral adipose tissue of morbidly obese
527 individuals tended to release less PGE_2 than those from leaner individuals [49]. Our
528 results are not entirely concordant with these previous analyses as we found no
529 correlations between PTGES, PTGES2 or PTGES3 mRNA expression and total adiposity
530 measurements. The fact that we studied women who were in the lean to moderately obese
531 range as opposed to morbidly obese individuals may possibly explain these differences.
532 Xie et al. revealed that PTGES expression is enhanced while level of the cytosolic form
533 (PTGES3) is unchanged during murine 3T3-L1 differentiation [47]. Conversely, Fujimori

534 et al. indicated that the protein and mRNA levels of all three isoforms of PTGES (1, 2
535 and 3) were constitutively expressed during adipogenesis [16], which is not consistent
536 with our results. Regarding PGE_2 receptors, we demonstrated that EP1 receptor was
537 weakly expressed both in whole adipose tissue and preadipocytes. We also found that
538 EP4 receptor was rapidly decreased 6h hours after the induction of differentiation, which
539 is consistent with a study demonstrating that PGE_2-EP4 signaling suppresses adipocyte
540 differentiation [16]. Furthermore, we found that the expression of EP3 receptor was
541 significantly higher in differentiated cells, indirectly supporting the notion that EP3
542 receptor is specific to mature adipocytes [12].
543
544 Limitations of the study should be acknowledged. We only have expression data and
545 posttranscriptional or translational modifications may affect protein levels differently. In
546 the context of our study, we examined a rather large sample of patients for which we also
547 had detailed data on obesity levels and adipose tissue distribution as well as
548 visceral/subcutaneous adipose tissue samples. However, we cannot extrapolate our
549 findings to men. Furthermore, we could not determine whether the main PG-secreting
550 cells are in the stromal-vascular fraction of adipose tissue as done by others [50-52]. This
551 is due to the fact that PG release by preadipocytes and adipose tissue explants were not
552 normalized for comparable values. Indeed, PGs release by adipose tissue explants were
553 normalized by milligrams of tissue while PG release by adherent preadipocytes in
554 primary cultures were expressed as a function of total protein amount. Additional studies
555 are needed to understand the mechanisms underlying changes in the expression of these
556 enzymes in abdominal obesity and through adipocyte differentiation.

557

558 In conclusion, cells from the omental fat compartment release more $PGF_{2\alpha}$ and PGE_2 than
559 those from the subcutaneous depot and the corresponding transcripts show consistent
560 depot differences. Obesity modulates adipose tissue expression of PG-synthesizing
561 enzymes and PG receptors. This likely occurs through adipogenesis-induced changes in
562 expression of these transcripts.

563

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568

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573

574 **DISCLOSURES**

575 The authors have no conflict of interests regarding the current manuscript.

576

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735
736

737 **FIGURE HEADINGS**

738 **Figure 1:** Basal release of PGE_2 and $PGF_{2\alpha}$ by subcutaneous and omental (**A**) adipose
739 tissue explants (n=8) or (**B**) primary preadipocyte cultures (OM: n=11 and SC: n=9).
740 PGE_2 and $PGF_{2\alpha}$ release by adipose tissue explants was expressed as pg/ml*mg
741 tissue*24h and PGE_2 and $PGF_{2\alpha}$ release by cultured primary preadipocytes was expressed
742 as pg/ml*µg protein*24h. Results are expressed as mean ± SEM $^{\dagger}p<0.10$; $^{*}p\leq0.05$

743

744 **Figure 2:** Omental and subcutaneous adipose tissue mRNA expression of several
745 transcripts coding for enzymes involved PG synthesis or PG receptors in women (n=46).
746 Expression levels relative to ATP5O mRNA abundance are shown. PLA2G4
747 (phospholipase A2), PLA2G16 (phospholipase A2), PTGS1 (cyclooxygenase 1), PTGS2
748 (cyclooxygenase 2), AKR1B1 (PGF synthase), PTGFR (Prostaglandin FP receptors),
749 PTGIS (PGI synthase), PTGDS (PGD synthase), PTGES, PTGES2 and PTGES3 (PGE
750 synthase), PTGER1, PTGER2, PTGER3 and PTGER4 (Prostaglandin E receptors). Mean
751 ± SEM are shown. * $p\leq0.05$

752

753 **Figure 3:** Omental (n=4) and subcutaneous (n=5) preadipocyte mRNA expression of
754 several transcripts coding for enzymes involved PG synthesis or PG receptors.
755 Expression levels relative to G6PD mRNA abundance are shown. PLA2G4
756 (phospholipase A2), PLA2G16 (phospholipase A2), PTGS1 (cyclooxygenase 1), PTGS2
757 (cyclooxygenase 2), AKR1B1 (PGF synthase), PTGFR (Prostaglandin FP receptors),
758 PTGIS (PGI synthase), PTGDS (PGD synthase), PTGES, PTGES2 and PTGES3 (PGE
759 synthase), PTGER1, PTGER2, PTGER3 and PTGER4 (Prostaglandin E receptors). Mean
760 ± SEM are shown. † $p<0.10$; * $p\leq0.05$, ** $p\leq0.01$

761

762 **Figure 4: A)** PPARγ, mRNA expression during omental (n=4) or subcutaneous (n=5)
763 preadipocyte differentiation (0h, 6h, 1d, 8d); and **B)** At day 8 of differentiation,
764 preadipocyte differentiation efficiency was assessed using phase contrast microscopy at
765 20 X magnification imaging of differentiated, Oil Red O-stained cells. These pictures
766 were obtained in a representative subcutaneous culture. Expression levels relative to
767 G6PD mRNA abundance. Mean ± SEM are shown. All variables were log-transformed to
768 stabilize variances in statistical analyses. Reported p-values are based on transformed
769 variables.

770

771 **Figure 5: A)** PLA2G16, **B)** COX-1 (PTGS1), **C)** COX-2 (PTGS2), **D)** AKR1B1, **E)**
772 PTGDS, **F)** PTGIS, **G)** PTGES, **H)** PTGES2, **I)** PTGES3 **J)** PTGFR, **K**) PTGER2 and **L**)
773 PTGER3 mRNA expression during omental (n=4) or subcutaneous (n=5) preadipocyte
774 differentiation (0h, 6h, 1d, 8d). Expression levels relative to G6PD mRNA abundance.
775 Mean ± SEM are shown. All variables were log-transformed to stabilize variances.
776 Reported p-values are based on transformed values.

777

778 **Figure 6:** PGE_2 **(A)** and $PGF_{2\alpha}$ **(B)** production rate during omental (n=4) or subcutaneous
779 (n=5) preadipocyte differentiation (0h to 8 days). PGs production rate was expressed as
780 pg/ml*µg protein*hour. Mean ± SEM are shown. *$p \leq 0.05$

781

782

783

784

785 **TABLES**

786 **Table 1: Characteristics of the sample (n=46)**

Variables	Mean ± SD	Range (min-max)
Age and anthropometrics		
Age (yrs)	46.8 ± 4.0	37.6-54.5
Weight (kg)	73.6 ± 17.0	48.0-133.0
Waist circumference (cm)	93.9 ± 14.7	71.5-147.0
BMI (kg/m²)	28.0 ± 6.4	19.5-50.1
Body composition		
Total body fat mass (kg)[a]	27.4 ± 8.8	10.6-50.4
Lean body mass (kg)[a]	43.6 ± 6.8	29.3-55.3
Adipose tissue areas (cm²)		
Total[c]	407 ± 146	93-773
Subcutaneous[c]	319 ± 120	59-555
Visceral[a]	98 ± 57	35-266
Adipocyte diameter (µm)		
Subcutaneous[b]	101 ± 14	61-131
Omental[b]	87 ± 16	50-121

787 [a] n=45, [b] n=44, [c] n=43

788 **Table 2: Pearson correlation coefficients between PLA2G16, AKR1B1, PTGDS, PTGER3 or**
789 **PTGER4 mRNA expression level in subcutaneous (SC) or omental (OM) adipose tissue and body**
790 **composition or fat distribution (n=46)**

Variables	PLA2G16		AKR1B1		PTGDS		PTGER3		PTGER4	
	OM	SC	OM	SC	OM	SC	OM	SC	OM	SC
Body composition										
Total body fat mass [a]	-	-	-	0.39*	0.26†	-	-	0.26†	0.27†	0.47**
Lean body mass [a]	-	-	0.31*	0.38*	-	-	-	-	-	0.36*
Adipose tissue areas										
Total [c]	0.31*	-	-	0.35*	-	-	0.35*	-	0.41*	0.51**
Visceral [a]	-	-	0.26†	0.41*	0.43*	-	0.43*	-	0.38*	0.48**
Subcutaneous [c]	0.24†	-	-	0.31*	-	-	-	-	0.31*	0.52**
Adipocyte diameter										
Omental [b]	0.31*	-	-	0.47**	0.25†	-	0.51**	-	0.33*	0.54**
Subcutaneous [a]	0.30*	-	-	0.37*	-	-	0.35*	-	-	0.35*

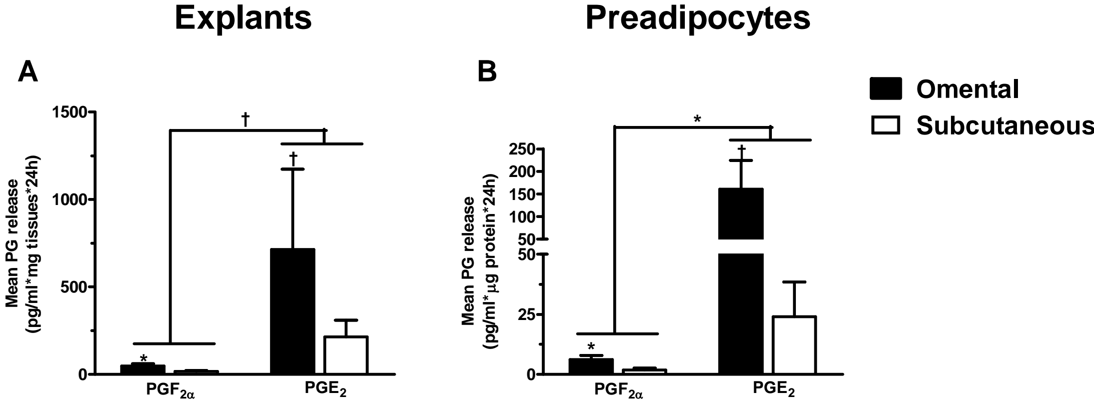
791 PLA2G16 (phospholipase A2), AKR1B1 (PGF synthase), PTGDS (PGD synthase), PTGER3 (PGE
792 receptor) and PTGER4 (PGE receptor). Expression levels relative to ATP5O mRNA expression
793 [a] n=45, [b] n=44, [c] n=43, ** p≤0.001, *p≤0.05, †p≤0.10, (-) No significant association.

794 **FIGURES**

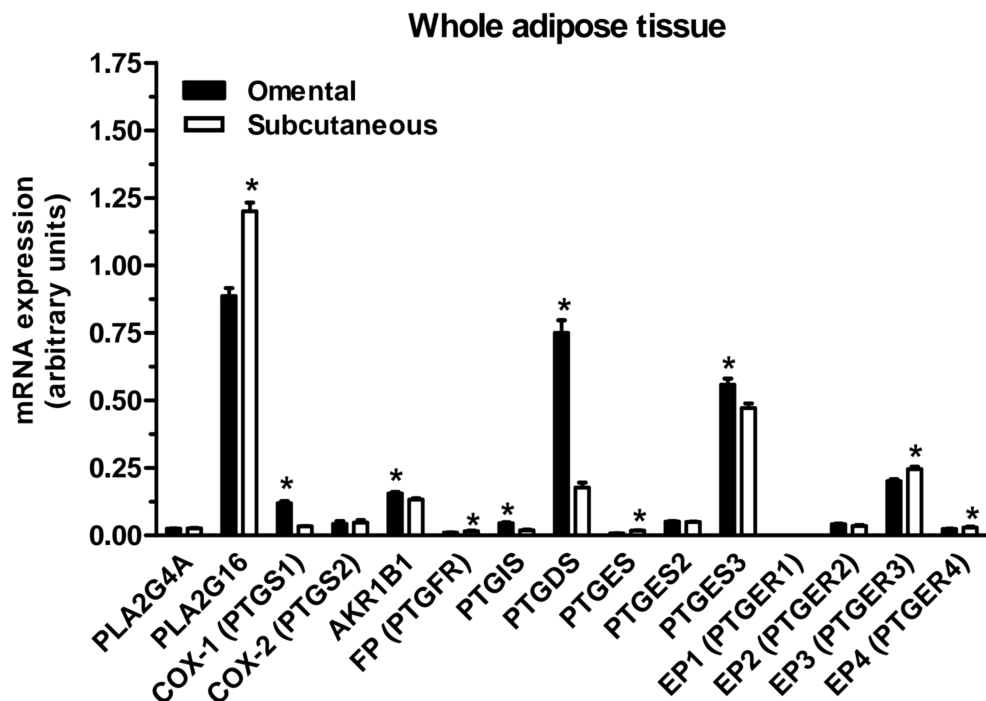
795 **Figure 1**

796

797

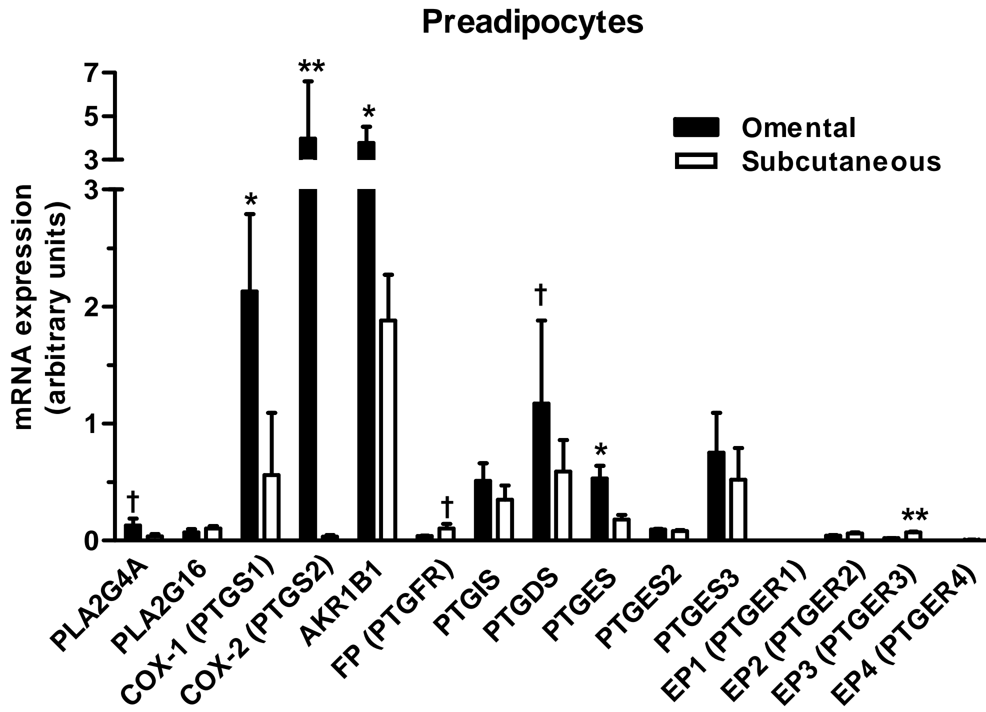


798 **Figure 2**



799

800 **Figure 3**



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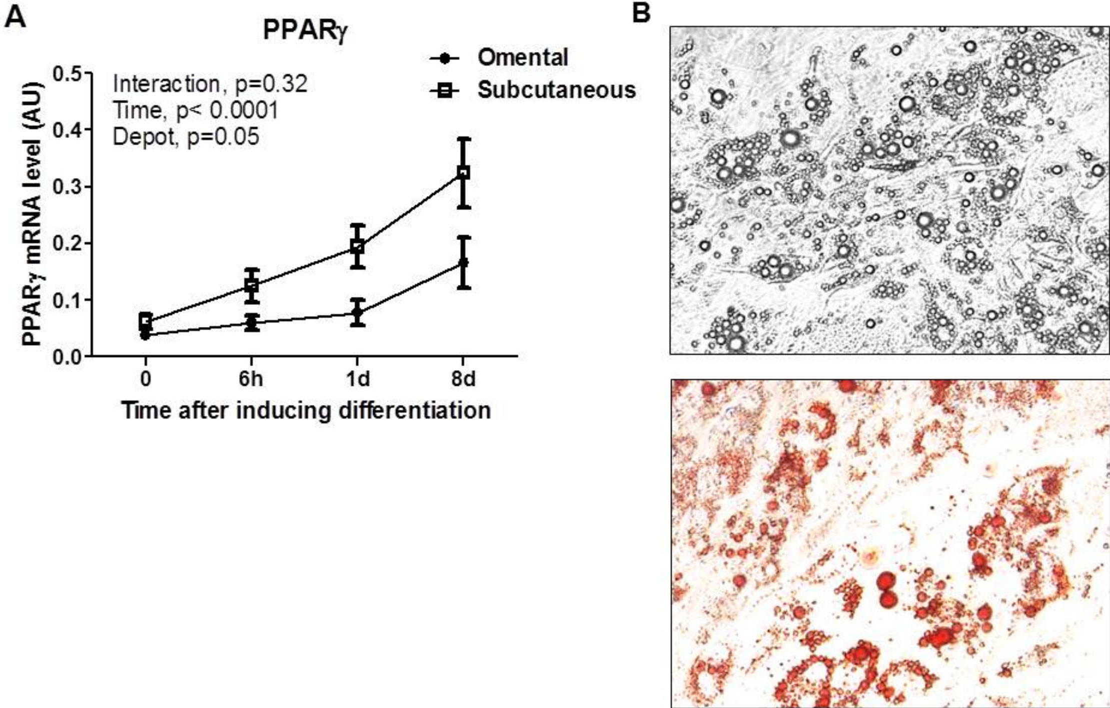
802

803 **Figure 4**



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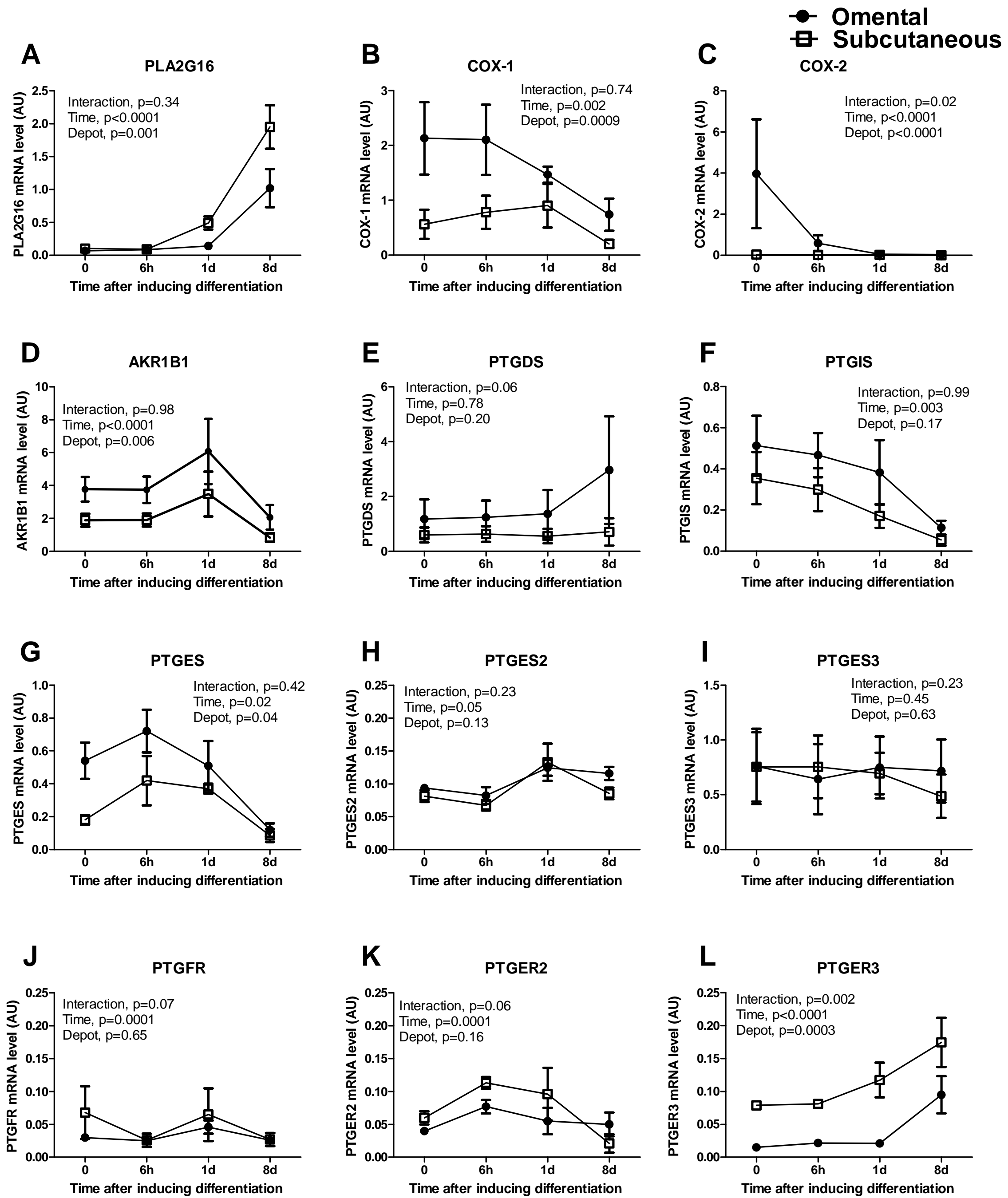
806 **Figure 5**



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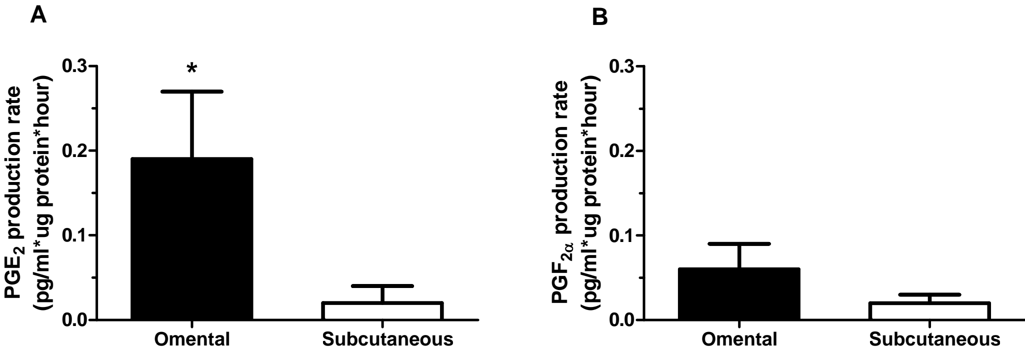
810 **Figure 6**



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813 **SUPPLEMENTAL DATA**

814 **Table 1. Oligonucleotides used in realtime RT-PCR quantification**

Gene Symbol	Description	GenBank	Oligonucleotide Sequence 5'→ 3' Sense/ Antisense
PLA2G4	Phospholipase A2, group IVA	NM_024420	AAGGACGTGCTGGGAAGGTACA/ GGGATACGGCAGGTTAAATGTG
PLA2G16	Homo sapiens phospholipase A2, group XVI (PLA2G16)	NM_007069	AGCCTAAGCCTGGAGACCTGAT/ GCCACATCATACAGCAATTCCTTCTT
PTGS1	Prostaglandin-endoperoxide synthase 1 (cyclooxygenase 1)	NM_000962	TTGGGGAGAGTATGATAGAGATTG/ CGGAAGGAAACGTAGGGACAG
PTGS2	Prostaglandin-endoperoxide synthase 2 (cyclooxygenase 2)	NM_000963	ATGGGTAATGTTATATGTTCTCCTGC/ TGGTGACTGTTTTAATGAGCTCTG
PTGES	Prostaglandin E synthase	NM_004878	GGCTATACCTGGGGACTTGATG/ CAGGAATCCAAGGGGCTAAGA
PTGES2	Prostaglandin E synthase 2	NM_025072	AGCCTTCCTCGACTTCCATGC/ GGTCTTGAGGGCGCTGATGAT
PTGES3	Prostaglandin E synthase 3	NM_006601	CAAGCATAAAAGAACGGACAGATCA/ AATCATCATCTGCTCCATCTACTTC
PTGIS	Prostaglandin I2 (prostacyclin) synthase	NM_000961	ATGCCTGCGAGAGACCCTACA/ GCAAGTCACCTCACCTCTCAGTT
PTGDS	Prostaglandin D2 synthase	NM_000954	TACAGCTACCGGAGTCCCCAC/ TATCCTCTGTGAAGCCCTGGG
AKR1B1	Aldo-keto reductase family 1B1	NM_001628	GATCGCAGCCAAGCACAATAA/ ACAGCTCAACAAGGCACAGAC
PTGFR	Prostaglandin F receptor (FP)	NM_000959	CCTTGCCATCGCCATTCTCAT/ CCAGAAAACACCATGCAGATACC
PTGER1	Prostaglandin E receptor 1 (EP1)	NM_000955	TCGCTTCGGCCTCCACCTTCT/ CAGCGCCACCAACACCAGCAT
PTGER2	Prostaglandin E receptor 2 (EP2)	NM_000956	TGCAACTTCAGTGTCATTCTCAACCT/ GCAGACGGCGAAGGTGATGGT
PTGER3	Prostaglandin E receptor 3 (EP3)	NM_198714	CTGGTCTCCGCTCCTGATAAT/ CAGCAGGTAAACCCAAGGATCC
PTGER4	Prostaglandin E receptor 4 (EP4)	NM_000958	CGCCGAGATCCAGATGGTCAT/ CGGCAGAAGAGGCATTTGATC
PPARG	Peroxisome proliferator-activated receptor gamma	NM_138712	GTCGGATCCACAAAAAAAGTAGAA/ AGCGGGAAGGACTTTATGTATGA
ATP5O	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit	NM_001697	ATTGAAGGTCGCTATGCCACAG/ AACGACTCCTTGGGTATTGCTTAA
G6PD	Glucose-6-phosphate dehydrogenase	NM_000402	GATGTCCCCTGTCCCACCAACTCTG/ GCAGGGCATTGAGGTTGGGAG

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