*Title Page and Abstract

1 # Hepatocyte growth factor is elevated in amniotic fluid from obese
2 # women and regulates placental glucose and fatty acid metabolism

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14 **Running title:** Hepatocyte Growth Factor and placental metabolism

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27

Abstract

Introduction: To evaluate the impact of the pro-inflammatory cytokine hepatocyte growth factor (HGF) on the regulation of glucose and lipid placental metabolism.

Methods: HGF levels were quantified in amniotic fluid and placenta from control and obese women. 2-deoxy-glucose (2-DOG) uptake, glycolysis, fatty acid oxidation (FAO), fatty acid esterification, *de novo* fatty acid synthesis, triglyceride levels and carnitine palmitoyltransferase activities (CPT) were measured in placental explants upon addition of pathophysiological HGF levels.

Results: In obese women, total- and -activated-HGF levels in amniotic fluid were elevated ~24%, and placental HGF levels were ~3-fold higher than in control women. At a similar dose to that present in amniotic fluid of obese women, HGF (30 ng/mL) increased Glut-1 levels and 2-DOG uptake by ~25-30% in placental explants. HGF-mediated effect on 2-DOG uptake was dependent on the activation of phosphatidylinositol 3-kinase signaling pathway. In addition, HGF decreased ~20% FAO, whereas esterification and *de novo* fatty acid synthesis increased ~15% and ~25% respectively, leading to 2-fold triglyceride accumulation in placental explants. In parallel, HGF reduced CPT-I activity ~70%.

Discussion: HGF is a cytokine elevated in amniotic fluid and placental tissue of obese women, which through its ability to stimulate 2-DOG uptake and metabolism impairs FAO and enhances esterification and *de novo* fatty acid synthesis, leading to accumulation of placental triglycerides.

Keywords: Placental inflammation, fatty acid metabolism, glucose metabolism, Hepatocyte Growth Factor.

*Highlights (for review)

Highlights

Maternal obesity is linked with elevated placental HGF.

The cytokine HGF is elevated in amniotic fluid and placenta of obese pregnant women.

HGF regulates placental fatty acid and glucose metabolism.

The mechanism relies on enhanced glucose uptake and inhibition of CPT-I activity.

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28 ## Abstract

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48 **Keywords:** Placental inflammation, fatty acid metabolism, glucose metabolism, Hepatocyte
49 Growth Factor.

50 # Introduction

51 Obesity is a growing health concern in women of reproductive age because is associated with a
52 broad range of maternal and fetal complications, such as macrosomia, a condition characterized by
53 excessive fetal fat accretion that predispose the newborn to suffer metabolic diseases later in life [1-
54 4].

55 The mechanistic link between maternal obesity and fetal macrosomia is poorly understood.
56 Maternal obesity is usually associated with hyperglycemia and hypertriglyceridemia, which may
57 result in augmented transplacental nutrient transfer to the fetus. In the obesogenic-diabetogenic
58 hypothesis proposed by Catalano et al. [2], changes in maternal availability of lipid surplus would
59 facilitate non-esterified free fatty acids delivery to the adipocytes of the fetus, whereas maternal
60 hyperglycemia and hyperinsulinemia would enhance lipogenesis leading to fetal adiposity. Hence,
61 maternal hyperinsulinemia, and excessive circulating levels of glucose and lipids would play a direct
62 role in the accumulation of fat in fetal adipose tissue. However, in pregnancies complicated with
63 obesity, the expression of placental pro-inflammatory cytokines and certain immune cell populations
64 are elevated leading to a chronic inflammatory milieu in which the fetus develops [5-7]. These
65 observations have propelled the question whether excessive fetal adiposity can be explained solely as
66 a result of higher circulating nutrients in maternal blood. Thus, it has been proposed that placental
67 inflammation observed in obese women may modify the availability of nutrient supply at the
68 maternal-fetal interface leading to augmented transplacental nutrient transfer to the fetus [2].
69 Unfortunately, this question remains to be addressed, and the contribution of intrauterine pro-
70 inflammatory milieu on placental metabolism regulation has not been extensively investigated.

71 HGF is a pro-inflammatory cytokine elevated in serum of obese patients, which decline with
72 weight loss and reduced body fat mass that occurs after gastroplasty [8-10]. The adipose tissue of
73 these patients abnormally produces and secretes HGF, contributing to augmented serum HGF levels
74 [9, 11]. HGF is activated by serine proteases, such as the HGF activator (HGFA), to exert its
75 biological functions [12]. Although HGF was initially identified as a circulating factor that stimulates
76 hepatocyte proliferation after liver injury [13], HGF exhibits pleiotropic biological functions in a
77 broad range of cell types. Among them, HGF is a potent regulator of glucose and lipid metabolism in
78 pancreatic β-cells, intestine epithelial cells, adipocytes and skeletal muscle cells [14].

79 HGF is also expressed in placental mesenchymal cells, syncytiotrophoblast cells of the chorionic
80 villi and in amniotic epithelium cells [15]. The biological effects of HGF are mediated by its receptor
81 (c-met) a transmembrane protein encoded by the MET proto-oncogene [16]. The receptor for HGF is
82 primary localized in placental cytotrophoblast cells and to a lesser degree in syncytiotrophoblast cells
83 [17]. In the literature, it can be found few and contradictory reports about plasma HGF levels during
84 normal pregnancy and in pregnancies complicated with obesity. In normal pregnancy, HGF levels
85 increased with gestational age until term [18, 19] or there was no change during pregnancy [20]. In
86 pregnancies complicated with obesity, HGF levels were similar to normal women and remained
87 unchanged with increasing BMI during the second trimester of pregnancy [21]. These results are in
88 disagreement with the notion that in obese patients serum HGF associated with obesity [9, 11] and
89 had a linear relationship with BMI [9]. Finally, total-HGF and activated-HGF blood levels in
90 neonates are regulated in a time-dependent manner along fetal development [22].

91 In this study, we aimed to further understand the mechanistic link between maternal obesity,
92 through its associated inflammatory uteroplacental environment, and the regulation of placental

93 metabolism. To this end, we tested the hypothesis that the pro-inflammatory cytokine HGF alters
94 placental glucose and lipid metabolism leading to accumulation of placental triglycerides.

95 Methods

96 **Study subjects**

97 The study was performed on placentas and amniotic fluid from pregnant women recruited at the
98 Department of Obstetrics and Gynecology, University Hospital "Puerta del Mar" (HUPM). Patient
99 samples were obtained after written informed consent in accordance with the HUPM Ethics
100 Committee requirements and the Declaration of Helsinki. Specific exclusion criteria included women
101 under the age of 18, smokers, a history of long-chain 3-hydroxyacyl-CoA deficiency, hemolysis
102 elevated liver function syndrome or acute fatty liver of pregnancy, preeclampsia, chronic
103 hypertension, pregestational diabetes, GDM, other co-morbid disease, abnormal karyotype, fetal
104 malformations and multiple pregnancy.

105 In the studies conducted using placental explants, pregnant women who planned to deliver by an
106 elective Caesarean section due to clinical reasons such as breech presentation or prior Caesarean
107 section were recruited. All Caesarean sections were performed at term. Placental samples and fasting
108 maternal blood samples were obtained at the time of the elective Caesarean section. Neonatal
109 anthropometric measurements were performed immediately at delivery as usual. In total, 26 women
110 with no pregnancy complications (BMI 20-24.9) participated in the study for placental explants
111 experiments. In addition, placentas from 10 obese women (pre-pregnancy BMI >30) were used in
112 experiments showed in Figure 1. Randomly chosen subsets of placentas were used for the

113 experiments as indicated in the figure legends. In table 1 are listed demographics and baseline data,

114 as well as perinatal variables.

115 In the studies conducted using amniotic fluid, patients were eligible among pregnant women

116 attending our antenatal clinic undergoing elective amniocentesis at 15-20 weeks of gestation for

117 karyotype analysis, most of them due to advanced maternal age or combined screening showing high

118 risk for trisomy 21. All fetuses were chromosomally and anatomically normal at delivery. Women

119 were asked to give an extra amount of 3 mL of amniotic fluid for the study. Women were divided

120 into two groups according to their pre-pregnancy BMI: the control group composed of 29 normal

121 weight women (BMI 20-24.9); and the obese group, composed of 12 obese pregnant women (BMI

122 >30). Demographics and baseline data, as well as perinatal variables, are shown in Table 2. Specific

123 exclusion criteria included women under the age of 18, smokers, a history of long-chain 3-

124 hydroxyacyl-CoA deficiency, hemolysis elevated liver function syndrome or acute fatty liver of

125 pregnancy, preeclampsia, chronic hypertension, pregestational diabetes, GDM, other co-morbid

126 disease, abnormal karyotype, fetal malformations and multiple pregnancy.

127 **Materials**

128 Cell culture reagents (RPMI-1640 medium without glucose) and fetal bovine serum were from

129 Invitrogen/Gibco, California, USA. The [9,10-^{3}H]-palmitic acid, [^{3}H]-H_2O, 2-[1,2-^{3}H]-deoxy-D-

130 glucose, [1-^{14}C]-mannitol, [5-^{3}H]-glucose, [1,2-^{14}C]-acetic acid, L-[N-methyl-^{14}C]-carnitine-HCl and

131 liquid scintillation counting cocktail were from PerkinElmer, Massachusetts, USA. Methotrexate,

132 Wortmannin, 2-deoxy-glucose (2-DOG) and cytochalasin B were purchased from Sigma-Aldrich

133 (Madrid, Spain). Recombinant human HGF was purchased from Millipore Iberica (Madrid, Spain).

134 **Biochemical parameters and HGF determination**

135 All biochemical parameters were analyzed at the clinical laboratory, HUPM, using reagents and
136 modular systems from Roche Diagnostics as described previously [23]. Low-density lipoprotein
137 cholesterol (LDL-c) was calculated using the Friedewald-Fredrickson formula.

138 Human total-HGF (t-HGF) and activated-HGF (a-HGF) were measured by ELISA using a
139 commercially available kit supply by IBL International GmbH (Hamburg, Germany). Each assay
140 required 100 μL of amniotic fluid to determine the levels of t-HGF and a-HGF.

141 **Placental explants culture**

142 Term placenta obtained from elective Caesarean section was placed on ice and arrived to the
143 laboratory within 10-15 minutes of delivery. Afterwards, placental villous explants (~100 mg wet
144 weight) were dissected and cultured in 6-well plate containing 2 mL of culture medium (RPMI-1640
145 supplemented with 5 mmol/l glucose, 10% FBS (vol/vol), 100 units/ml penicillin G, and 100 μg/ml
146 streptomycin) as described previously [23].

147 **Fatty acid oxidation assay**

148 Stock of fatty acid solution was prepared by conjugating palmitate with essentially fatty acid-free
149 bovine serum albumin (BSA) to generate a stock solution of 25% (wt/vol) BSA, 4 mmol/L palmitate
150 in culture medium were prepared as described previously [23]. Mitochondrial fatty acid oxidation
151 (FAO) assays were performed in placental explants as described previously [23-25]. Briefly, freshly
152 isolated villous explants were incubated in culture media in the presence or absence of HGF, plus
153 1.25% BSA, 0.1 mmol/l cold palmitate, and 18500 Bq/ml [^{3}H]-palmitate at 37ºC for 18h. At the end
154 of the incubation period, the medium was collected, and tritiated water determined by the vapor-

155 phase equilibration method of Hughes et al [26]. FAO was defined as nmol of palmitate per mg of
156 tissue per hour.

157 **Esterification into total lipids assay**

158 Incorporation of [^{3}H]-palmitate into total lipids was determined as previously described [23, 24].
159 Briefly, after similar incubation conditions to those used for measurements of β-oxidation, in the
160 presence or absence of HGF, plus 0.1 mmol/L cold palmitate and 18500 Bq/mL for 18h, explants
161 were washed 3 times with 2 mL of ice-cold PBS and homogenized in 500 µL of PBS. An aliquot of
162 100 µL was used to extract the lipid content from samples according to Bligh and Dyer [27].
163 Afterwards, the radioactive content was determined by liquid scintillation counting. Esterification
164 was plotted as % of control.

165 ***De novo* lipid synthesis assay**

166 *De novo* lipid synthesis was determined using [^{14}C]-acetate in placental explants according to the
167 procedure described previously [23, 24]. Villous placental explants were incubated in RPMI-1640
168 culture media in the presence or absence of HGF plus 1850 Bq/mL [^{14}C]-acetate at 37ºC for 18h. At
169 the end of the incubation period, culture media were discarded followed by tissue homogenization in
170 500 µL of PBS. After a total lipid extraction (as described for measurements of placental
171 esterification rate), the radioactive content was determined.

172 **Glycolysis assay**

173 Glucose utilization (equivalent to glycolysis) was determined as the production of tritiated water
174 (as for FAO experiments) after incubation of placental explants for 18h in the presence or absence of
175 HGF, plus 240500 Bq/mL [5-^{3}H]-glucose [24, 28].

176 **Glucose transport assay**

177 Uptake of $[^{3}H]$-2-DOG was performed ex vivo in placental explants, as described previously
178 [14], with the following modifications. Freshly isolated placental explants were preincubated for 18h
179 in culture medium in the presence or absence of HGF, plus cytochalasin B, a potent inhibitor of
180 glucose transport mediated via facilitative glucose transporters. Afterwards, explants were washed in
181 transport solution buffer (20 mmol/L Hepes-Na pH 7.4, 140 mmol/L NaCl, 5 mmol/L KCl, 2.5
182 mmol/L $MgSO_4$, 1 mol/L $CaCl_2$) at room temperature, and immediately incubated in transport
183 solution buffer plus 10 μmol/L 2-DOG (18500 Bq/mL $[^{3}H]$-2-DOG) and 39 mmol/L mannitol
184 (11840 Bq/mL $[^{14}C]$-mannitol) with or without HGF for 1 min. Then, explants were removed
185 rapidly, rinsed with cold 0,9% NaCl to stop reactions, blotted, digested in 1 mol/L NaOH and
186 analyzed for ^{14}C and ^{3}H content. Glucose uptake was defined as nmol of 2-DOG per mg of protein
187 per minute.

188 **Quantification of triglyceride content**

189 Placental triglyceride content was determined, as previously described by Perdomo et al. [29], in
190 explants preincubated in the presence or absence of HGF for 18h. Placental tissues (~100 mg) were
191 homogenized in 400 µl HPLC-grade acetone, and aliquots of 5 µl of acetone-extracted lipid
192 suspension were used to determined triglyceride concentrations using a triglyceride reagent kit
193 (Biosystems, Barcelona, Spain). Proteins were quantified using the bicinchoninic acid method
194 (Thermo Scientific, Madrid, Spain). Placental lipid content was defined as mg of triglyceride per mg
195 of total placental proteins.

196 **Western blot analysis**

197 Placental explants from control and obese women were dissected and washed with ice-cold PBS,
198 followed by homogenization in lysis buffer (20 mmol/l Tris-HCl pH 7.5, 150 mmol/l NaCl, 1 mmol/l
199 EDTA, 1 mmol/l EGTA, 1% (v/v) Triton X-100, 2.5 mmol/l sodium pyrophosphate, 1 mmol/l β-
200 glycerophosphate, 1 mmol/l Na_3VO_4, 1 μg/ml leupeptin, 1 mmol/l phenylmethylsulfonyl fluoride))
201 plus protease inhibitors (Protease Inhibitor Cocktail, Sigma, St. Louis, MO). After 10 min. on ice,
202 extracts were sonicated and centrifugated at 18,000 X *g* for 10 minutes at 4ºC. Pellets were discarded
203 and solubilized proteins (40-60 μg/sample) were resolved by 10% SDS-PAGE for HGF and
204 electrotransferred onto polyvinylidene difluoride (PVDF) filters for immunoblotting by conventional
205 means. After probing with specific antibodies [glucose transporter 1 (Glut-1; 1:2000, Abcam,
206 Cambridge, UK) HGF antibody (1:1000, Santa Cruz Biotechnology, Inc., Heidelberg, Germany) and
207 protein kinase B (PKB; 1:1000, Cell Signaling, Barcelona, Spain)], the membranes were stripped and
208 reprobed against phospho-PKB at Ser473 (p-PKB; 1:1000, Cell Signaling, Barcelona, Spain), or
209 actin (1:3000, Sigma, Madrid, Spain). Signals were detected by chemiluminiescence (Immun-Start
210 western chemiluminiescence kit, Bio-Rad, Madrid, Spain), and band densitometry was quantified
211 with the ImageJ software (NIH, USA).

212 **CPT assay**

213 Activities of carnitine palmitoyltransferase I (CPT-I) and carnitine palmitoyltransferase II (CPT-
214 II) were determined in the direction of acyl-carnitine formation, using [^{14}C]-carnitine as substrate in
215 placental explants as described previously [23, 25, 30]. Briefly, explants were preincubated in RPMI-
216 1640 culture media in the presence or absence of HGF (30 ng/mL) at 37ºC for 18h. At the end of the
217 incubation period, culture media were discarded, and explants were washed with ice-cold PBS prior

218 to homogenization in lysis buffer (5 mmol/l Tris-HCl, pH 7.2, 150 mmol/l KCl) with a glass
219 homogenizer. For CPT-I assay, 100 µl of cell homogenate, in which the mitochondria remain largely
220 intact, was incubated in the presence of 50 µmol/l palmitoyl-CoA, 500 µmol/l carnitine, and
221 9250Bq/ml [^{14}C]-carnitine in a 30ºC shaking water bath for 10 min. For CPT-II assay, a portion of
222 the homogenate was adjusted to 1 % (wt/v) of the detergent octylglucoside, which solubilizes the
223 mitochondrial membranes, inactivating CPT-I and releasing CPT-II from the mitochondrial matrix in
224 active form. Afterward, reactions were stopped by adding 500 µl of 1-butanol. Radioactive content
225 was determined by liquid scintillation counting.

226 **Statistical analysis**

227 Statistical analysis of data was performed using the SPSS software (SPSS 20.0, Inc., Chicago,
228 IL). Data were presented as mean ± SEM. Distributions were checked with a histogram and the
229 Kolmogorov-Smirnov test. Comparisons between two groups were done by unpaired Student´s t-test,
230 and comparisons between more than two groups were performed by ANOVA followed by Tukey's
231 Multiple Comparison Test. Differences were considered significant at $p<0.05$."

232 # Results

233 **HGF is elevated in amniotic fluid and placenta from obese pregnant women**

234 Obesity in pregnancy induces placental inflammation, which is associated with production of
235 pro-inflammatory mediators [5]. To determine the presence of the pro-inflammatory cytokine HGF
236 in the inflammatory milieu, in which the fetus develop, we measured t-HGF and a-HGF levels in
237 amniotic fluid from control and obese pregnant women during early- mid pregnancy. As shown in
238 Figure 1A-B, t-HGF and a-HGF are elevated up to ~30 ng/mL in amniotic fluid from obese women.

239 In addition, HGF levels in placentas from obese women were ~3-fold higher than in control women
240 (Fig. 1C).

241 **HGF increases glucose transport and metabolism in human placental explants**

242 HGF is a potent stimulator of glucose transport and metabolism in different cell types [14].
243 Because HGF is elevated in placental explants and amniotic fluid from obese women, we
244 hypothesized that HGF stimulates glucose uptake in human placenta. To test our hypothesis, we
245 preincubated placental explants, from pregnancies without complications, in the presence or absence
246 of HGF for 18h, and measured glucose transport and glycolysis. HGF significantly increased 2-DOG
247 uptake in human placental explants and the effect was maximal (~30%) at 30 ng/mL (Fig. 2A).
248 Interestingly, in the presence of 10μM cytochalasin B (CB; an inhibitor of facilitative glucose
249 transport), 2-DOG uptake was suppressed by ~60%, whereas in the presence of 50μM CB B by
250 ~90% (Fig. 2B). Likewise, CB completely abolished the effect of HGF on 2-DOG uptake, suggesting
251 that HGF-mediated 2-DOG uptake in human placental explants was likely mediated by a facilitative
252 glucose transport (Fig. 2B). Finally, HGF-mediated effect on 2-DOG uptake was accompanied by a
253 significant augment (~20%) on glycolysis in placental explants (Fig. 2C). Collectively, these results
254 demonstrate a novel role of HGF in the regulation of placental glucose metabolism.

255 **HGF-mediated glucose uptake in human placental explants is Glut-1 and phosphatidylinositol**
256 **3-kinase (PI3K) dependent**

257 Glucose transport is mainly regulated by Glut-1 in human placenta [31]. Consequently, we
258 hypothesized that pathophysiological levels of HGF stimulates glucose uptake in human placental
259 explants through up-regulation of Glut-1. To test this hypothesis, we analyzed Glut-1 levels in

260 protein extracts prepared from explants after chronic exposure to HGF for 18h. As shown in Figure
261 3A, treatment with HGF increased by ~25% Glut-1 levels.

262 To gain further insight into the molecular mechanism by which HGF regulates glucose transport
263 and Glut-1 levels, we analyzed the possible signaling pathways downstream of c-met in placental
264 explants. To this end, explants were preincubated in the presence or absence of HGF (30 ng/mL) in a
265 time dependent manner (from 0 to 60 min), and PI3K signaling pathway was studied by immunoblot
266 analysis. HGF potently and rapidly stimulated PKB phosphorylation in a time-dependent manner
267 (Fig. 3B). To reveal whether the inhibition of this signaling pathway could reverse the effect of HGF
268 on glucose transport, we preincubated placental explants with 100 nM wortmannin (WM; a PI3K
269 inhibitor) for 30 min before and during chronic (18h) incubation with HGF (30ng/mL). At the end of
270 the incubation period, the 2-DOG uptake was measured in these cells. As shown in Figure 3C,
271 wortmannin completely abolish the effect of HGF on glucose transport. Taken together, these results
272 indicate that PI3K signaling pathway is involved in HGF-mediated 2-DOG uptake in human
273 placental explants.

274 **HGF alters the metabolic partitioning of fatty acids in human placental explants**

275 We showed above that HGF enhanced glucose transport and utilization in placental explants.
276 Therefore, it is reasonable to hypothesize that HGF-mediated accelerated glucose metabolism would
277 inhibit fatty acid oxidation leading to accumulation of placental triglycerides. To test this hypothesis
278 we measured the effect of chronic exposure (18h) to pathophysiological levels of HGF (30 ng/mL)
279 on FAO in placental explants. As shown in Figure 4A, HGF significantly reduced the FAO rate by
280 ~20% in placental explants. In parallel, HGF enhanced fatty acid esterification by ~15% (Fig. 4B).
281 Surprisingly, HGF also augmented *de novo* fatty acid synthesis by ~25% (Fig. 4C). Finally,

282 consistently with the expectation that fatty acids are preferentially directed towards esterification,
283 and that HGF stimulated *de novo* fatty acid synthesis, placental triglyceride content significantly
284 increased 2-fold in placental explants (Fig. 4D). Taken together, these data indicate that HGF alters
285 the metabolic partitioning of fatty acids towards triglyceride accumulation through inhibition of FAO
286 and stimulation of esterification and *de novo* fatty acid synthesis.

287 **HGF decreases carnitine palmitoyltransferase I activity in human placental explants**

288 Because HGF increased glucose transport and utilization, in parallel with diminished FAO, we
289 hypothesized that the mechanistic link between accelerated glucose metabolism and lower FAO was
290 inhibition of CPT-I activity by its physiological inhibitor malonyl-CoA, which is synthesized from
291 glucose-derived acetyl-CoA by acetyl-CoA carboxylase (ACC) activity. Therefore, to gain further
292 insight into the molecular mechanism by which HGF inhibits FAO in placental explants, we
293 measured the activity of CPT-I and CPT-II in placental explants preincubated without (control) or
294 with HGF (30 ng/mL) for 18h. As shown in Figure 7, HGF reduced by ~70% the activity of CPT-I
295 (Fig. 5A), whereas CPT-II activity remained unchanged as expected (Fig. 5B).

296 Discussion

297 In obese women, the risk of macrosomia is 2-fold higher than in control women [2], leading to
298 heavier neonates because of an increase in fat but not lean body mass [32]. Excessive fat
299 accumulation in adipose tissue of newborns predispose them to suffer from metabolic diseases later
300 in life [4], which perpetuates a vicious cycle of obesity and diabetes. At first glance,
301 hypertriglyceridemia in plasma from obese women, secondary to maternal insulin resistance, would
302 facilitate non-esterified free fatty acids delivery to the adipocytes of the fetus through potential

303 mechanisms such as alteration of concentration gradients across the placental barrier, and changes in
304 the levels and/or activity of lipoprotein lipase, fatty acid-binding proteins and fatty acid transporters
305 in syncytiotrophoblast cells [33]. Thus, it has been shown that maternal obesity modulates placental
306 fatty acid transporters and placental fatty acid transport and metabolism [34, 35].

307 Maternal obesity during pregnancy is also associated with placental accumulation of immune
308 cells, such as macrophages and neutrophils, lipid accumulation in the placental villi stroma and
309 syncytium, and elevated pro-inflammatory cytokines leading to lipotoxicity and inflammation [5-7,
310 33, 36]. In this work, we showed that HGF is elevated in placental tissue and amniotic fluid of obese
311 women during early- mid pregnancy, and identified mechanisms by which HGF regulated placental
312 lipid accumulation. However, the molecular and biochemical mechanisms by which obesity is related
313 to enhance pro-inflammatory cytokines expression in placenta have not been entirely clarified. A
314 conceivable mechanism by which placental HGF production is augmented could be related to the
315 lipotoxic and pro-inflammatory placental environment. Excessive circulating maternal free fatty
316 acids and/or elevated glucose levels, which reduce fatty acid oxidation and enhance esterification
317 through inhibition of carnitine palmitoyltransferase I [23], would lead to the accumulation of
318 triglycerides in placenta. This accumulation of lipids may trigger activation of toll-like receptors
319 (TLR) in trophoblast and/or macrophages, which would signal through phosphorylation of the c-Jun
320 N-terminal kinase (JNK) and the inhibitor of κB (IκB), leading to activation of the nuclear factor-kB
321 (NF-κB) and activator protein 1 (AP-1), the primary mediators of inflammatory responses. This
322 signaling pathway could be further stimulated by interleukin 1-beta (IL-1β), tumor necrosis alpha
323 (TNF-α) and interleukin 6 (IL-6), constituting a vicious cycle. Nonetheless, signaling pathways
324 involved in the production/ secretion of HGF in placentas from obese women remains unknown.

325 Using placental explants from women with no pregnancy complications, we demonstrate that at
326 similar concentration to that present in amniotic fluid of obese women during early- mid pregnancy,
327 HGF inhibited FAO and increased triglyceride accumulation. A limitation of this study is that we
328 performed a cross-sectional study regarding HGF levels in amniotic fluid. Thus, in the absence of a
329 longitudinal study, we cannot assume that HGF levels during the third trimester are similar to those
330 measured during the second trimester. Nonetheless, these HGF levels augmented Glut-1 protein
331 levels, glucose transport and metabolism. We evaluated whether the mechanistic link between HGF
332 and lower FAO was inhibition of CPT-I activity by its physiological inhibitor malonyl-CoA, which
333 is synthetized from glucose-derived acetyl-CoA by ACC. This mechanism results in a shift of fatty
334 acid partitioning away from the β-oxidation pathway toward esterification, allowing the
335 accumulation of triglycerides in placenta. This notion is in agreement with our findings in placentas
336 from pregnancies complicated by GDM, where we showed that accelerated glucose metabolism in
337 placental explants lowered FAO [23]. The impact of other pro-inflammatory cytokines on lipid
338 metabolism has not been extensively studied. In human trophoblast cell cultures IL-6, but not TNF-α,
339 stimulated fatty acid accumulation [37]. This effect was not explained by an increased expression of
340 lipoprotein lipase or fatty acid binding proteins. Although the mechanism by which IL-6 stimulates
341 trophoblast fatty acid accumulation remains to be established, it is plausible to hypothesize that
342 would be mediated by inhibition of FAO and/or stimulation of de novo fatty acid synthesis. Finally,
343 this knowledge about HGF-mediated accelerated glucose metabolism and its impact on placental
344 lipid metabolism, may offer new therapeutic targets for clinical management of pregnancies
345 complicated by obesity.

346 Earlier studies suggested that *de novo* fatty acid synthesis pathway plays a minor role on
347 accumulation of triglycerides in placentas from pregnancies complicated with diabetes [23, 38].
348 However, we demonstrated that HGF stimulated *de novo* fatty acid synthesis in placental explants
349 from women with no pregnancy complication. If this HGF-mediated regulation on fatty acid
350 synthesis would translate directly into the *in vivo* situation of placentas from obese women, the
351 glucose-derived malonyl-CoA, synthesized by the activity of ACC, would not merely serve as a
352 regulator of FAO pathway; but in addition, it would serve as a regulator of the *de novo* fatty acid
353 synthesis. Consequently, *de novo* fatty acid synthesis pathway in placenta from pregnancies
354 complicated with obesity would significantly contribute to placental triglycerides accumulation and
355 augmented transplacental fatty acids delivery to fetal adipocytes.
356 Although it is known that HGF participates in the regulation of glucose transport in different
357 cells types [14], no study has addressed weather HGF might regulate glucose metabolism in human
358 placenta. The current study demonstrates for the first time that HGF increases glucose uptake and
359 utilization in human placental explants *ex vivo*. Our studies establish that, in addition to behaving as
360 a growth factor, HGF has additional biological features as a modulator of glucose metabolic flux in
361 placental cells engaged in glucose homeostasis control. Interestingly, HGF significantly increased
362 total Glut-1 expression in human placental explants, which is associated with augmented glucose
363 uptake. Furthermore, inactivation of PI3K signaling pathway completely abolished HGF-mediated
364 effect on glucose uptake. We have previously showed that HGF also activates PI3K and ERK1/2
365 signaling pathways in parallel with up-regulation of Glut-1 levels in skeletal muscle cells [14].
366 Likewise, pharmacological inhibition of these pathways also abolished glucose uptake in these cells
367 [14]. Also, Di Simone et al. showed that resistin, a cytokine elevated in serum of third trimester

368 normal pregnancies, stimulated glucose uptake and Glut-1 levels in trophoblast cells through
369 activation of ERK1/2 pathway [39]. Collectively, these results highlight an underappreciated role for
370 cytokines in the control of glucose transport and metabolism in human placenta.

371 In conclusion, HGF is a mechanistic link between the intrauterine pro-inflammatory milieu of
372 pregnancies complicated with obesity and its associated placental lipotoxicity. Because most of
373 maternal cytokines do not cross the human placental barrier, we propose that synthesized HGF
374 within the placenta itself exacerbates lipotoxicity through biochemical mechanisms involving
375 augmented glucose transport, fatty acid esterification and *de novo* fatty acid synthesis, which lead to
376 placental triglyceride accumulation. Finally, our results suggest that placental inflammation may
377 play an indirect role on fetal macrosomia modifying the availability of nutrient supply to the fetus.

378

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386 # References

387 [1] Catalano PM. Obesity, insulin resistance, and pregnancy outcome. Reproduction.
388 2010;140(3):365-71.

389 [2] Catalano PM and Hauguel-De Mouzon S. Is it time to revisit the Pedersen hypothesis in the face
390 of the obesity epidemic? Am J Obstet Gynecol. 2011;204(6):479-87.

391 [3] King JC. Maternal obesity, metabolism, and pregnancy outcomes. Annu Rev Nutr. 2006;26:271-
392 91.

393 [4] Yogev Y and Visser GH. Obesity, gestational diabetes and pregnancy outcome. Semin Fetal
394 Neonatal Med. 2009;14(2):77-84.

395 [5] Challier JC, Basu S, Bintein T, Minium J, Hotmire K, Catalano PM and Hauguel-de Mouzon S.
396 Obesity in pregnancy stimulates macrophage accumulation and inflammation in the placenta.
397 Placenta. 2008;29(3):274-81.

398 [6] Hauguel-de Mouzon S and Guerre-Millo M. The placenta cytokine network and inflammatory
399 signals. Placenta. 2006;27(8):794-8.

400 [7] Roberts KA, Riley SC, Reynolds RM, Barr S, Evans M, Statham A, Hor K, Jabbour HN, Norman
401 JE and Denison FC. Placental structure and inflammation in pregnancies associated with obesity.
402 Placenta. 2011;32(3):247-54.

403 [8] Jung C, Fritzenwanger M, Fischer N and Figulla HR. Hepatocyte growth factor is elevated in
404 obese adolescents. J Pediatr Endocrinol Metab. 2009;22(7):645-51.

405 [9] Rehman J, Considine RV, Bovenkerk JE, Li J, Slavens CA, Jones RM and March KL. Obesity is
406 associated with increased levels of circulating hepatocyte growth factor. J Am Coll Cardiol.
407 2003;41(8):1408-13.

408 [10] Swierczynski J, Korczynska J, Goyke E, Adrych K, Raczynska S and Sledzinski Z. Serum
409 hepatocyte growth factor concentration in obese women decreases after vertical banded gastroplasty.
410 Obes Surg. 2005;15(6):803-8.

411 [11] Bell LN, Ward JL, Degawa-Yamauchi M, Bovenkerk JE, Jones R, Cacucci BM, Gupta CE,
412 Sheridan C, Sheridan K, Shankar SS, Steinberg HO, March KL and Considine RV. Adipose tissue
413 production of hepatocyte growth factor contributes to elevated serum HGF in obesity. Am J Physiol
414 Endocrinol Metab. 2006;291(4):E843-8.

415 [12] Kataoka H, Miyata S, Uchinokura S and Itoh H. Roles of hepatocyte growth factor (HGF)
416 activator and HGF activator inhibitor in the pericellular activation of HGF/scatter factor. Cancer
417 Metastasis Rev. 2003;22(2-3):223-36.

418 [13] Matsumoto K and Nakamura T. Hepatocyte growth factor (HGF) as a tissue organizer for
419 organogenesis and regeneration. Biochem Biophys Res Commun. 1997;239(3):639-44.

420 [14] Perdomo G, Martinez-Brocca MA, Bhatt BA, Brown NF, O'Doherty RM and Garcia-Ocana A.
421 Hepatocyte growth factor is a novel stimulator of glucose uptake and metabolism in skeletal muscle
422 cells. J Biol Chem. 2008;283(20):13700-6.

423 [15] Wolf HK, Zarnegar R, Oliver L and Michalopoulos GK. Hepatocyte growth factor in human
424 placenta and trophoblastic disease. Am J Pathol. 1991;138(4):1035-43.

425 [16] Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF and Aaronson
426 SA. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product.
427 Science. 1991;251(4995):802-4.

428 [17] Kauma S, Hayes N and Weatherford S. The differential expression of hepatocyte growth factor
429 and met in human placenta. J Clin Endocrinol Metab. 1997;82(3):949-54.

430 [18] Clark DE, Salvig JD, Smith SK and Charnock-Jones DS. Hepatocyte growth factor levels during
431 normal and intra-uterine growth-restricted pregnancies. Placenta. 1998;19(8):671-3.

432 [19] Horibe N, Okamoto T, Itakura A, Nakanishi T, Suzuki T, Kazeto S and Tomoda Y. Levels of
433 hepatocyte growth factor in maternal serum and amniotic fluid. Am J Obstet Gynecol. 1995;173(3 Pt
434 1):937-42.

435 [20] Watanabe T, Sakata Y, Matsubara S, Yamagishi T, Nagaike K, Kuwata T and Suzuki M.
436 Changes in plasma levels of hepatocyte growth factor and its associated factors during pregnancy. J
437 Obstet Gynaecol Res. 2006;32(1):10-4.

438 [21] Madan JC, Davis JM, Craig WY, Collins M, Allan W, Quinn R and Dammann O. Maternal
439 obesity and markers of inflammation in pregnancy. Cytokine. 2009;47(1):61-4.

440 [22] Jimenez-Gomez G, Benavente-Fernandez I, Lubian-Lopez SP, Matias-Vega M, Lechuga-
441 Campoy JL, Saez-Benito A, Lechuga-Sancho AM and Perdomo G. Hepatocyte growth factor as an
442 indicator of neonatal maturity. J Pediatr Endocrinol Metab26(7-8):709-14.

443 [23] Visiedo F, Bugatto F, Sanchez V, Cozar-Castellano I, Bartha JL and Perdomo G. High glucose
444 levels reduce fatty acid oxidation and increase triglyceride accumulation in human placenta. Am J
445 Physiol Endocrinol Metab. 2013;305(2):E205-12.

446 [24] Brown NF, Stefanovic-Racic M, Sipula IJ and Perdomo G. The mammalian target of rapamycin
447 regulates lipid metabolism in primary cultures of rat hepatocytes. Metabolism. 2007;56(11):1500-7.

448 [25] Perdomo G, Commerford SR, Richard AM, Adams SH, Corkey BE, O'Doherty RM and Brown
449 NF. Increased beta-oxidation in muscle cells enhances insulin-stimulated glucose metabolism and
450 protects against fatty acid-induced insulin resistance despite intramyocellular lipid accumulation. J
451 Biol Chem. 2004;279(26):27177-86.

452 [26] Hughes SD, Quaade C, Johnson JH, Ferber S and Newgard CB. Transfection of AtT-20ins cells
453 with GLUT-2 but not GLUT-1 confers glucose-stimulated insulin secretion. Relationship to glucose
454 metabolism. J Biol Chem. 1993;268(20):15205-12.

455 [27] Bligh EG and Dyer WJ. A rapid method of total lipid extraction and purification. Can J
456 Biochem Physiol. 1959;37(8):911-7.

457 [28] Sipula IJ, Brown NF and Perdomo G. Rapamycin-mediated inhibition of mammalian target of
458 rapamycin in skeletal muscle cells reduces glucose utilization and increases fatty acid oxidation.
459 Metabolism. 2006;55(12):1637-44.

460 [29] Perdomo G, Kim DH, Zhang T, Qu S, Thomas EA, Toledo FG, Slusher S, Fan Y, Kelley DE
461 and Dong HH. A role of apolipoprotein D in triglyceride metabolism. J Lipid Res. 2010;51(6):1298-
462 311.

463 [30] Brown NF. Expression, purification, and reconstitution of rat liver carnitine palmitoyltransferase
464 I. Methods Mol Biol. 2003;228:281-301.

465 [31] Illsley NP. Glucose transporters in the human placenta. Placenta. 2000;21(1):14-22.

466 [32] Sewell MF, Huston-Presley L, Super DM and Catalano P. Increased neonatal fat mass, not lean
467 body mass, is associated with maternal obesity. Am J Obstet Gynecol. 2006;195(4):1100-3.

468 [33] Saben J, Lindsey F, Zhong Y, Thakali K, Badger TM, Andres A, Gomez-Acevedo H and
469 Shankar K. Maternal obesity is associated with a lipotoxic placental environment. Placenta.
470 2014;35(3):171-7.

471 [34] Dube E, Gravel A, Martin C, Desparois G, Moussa I, Ethier-Chiasson M, Forest JC, Giguere Y,
472 Masse A and Lafond J. Modulation of fatty acid transport and metabolism by maternal obesity in the
473 human full-term placenta. Biol Reprod. 2012;87(1):14, 1-1.

474 [35] Zhu MJ, Ma Y, Long NM, Du M and Ford SP. Maternal obesity markedly increases placental
475 fatty acid transporter expression and fetal blood triglycerides at midgestation in the ewe. Am J
476 Physiol Regul Integr Comp Physiol. 2010;299(5):R1224-31.

477 [36] Bugatto F, Fernandez-Deudero A, Bailen A, Fernandez-Macias R, Hervias-Vivancos B and
478 Bartha JL. Second-trimester amniotic fluid proinflammatory cytokine levels in normal and
479 overweight women. Obstet Gynecol. 2010;115(1):127-33.

480 [37] Lager S, Jansson N, Olsson AL, Wennergren M, Jansson T and Powell TL. Effect of IL-6 and
481 TNF-alpha on fatty acid uptake in cultured human primary trophoblast cells. Placenta.
482 2011;32(2):121-7.

483 [38] Coltart TM and Bateman C. Carbohydrate-induced lipogenesis in the human placenta of normal
484 and diabetic pregnancies. Br J Obstet Gynaecol. 1975;82(6):471-5.

485 [39] Di Simone N, Di Nicuolo F, Marzioni D, Castellucci M, Sanguinetti M, D'Lppolito S and
486 Caruso A. Resistin modulates glucose uptake and glucose transporter-1 (GLUT-1) expression in
487 trophoblast cells. J Cell Mol Med. 2009;13(2):388-97.

488

489 # Figure legends

490 **Figure 1. HGF levels in amniotic fluid and placentas from obese pregnant women.** HGF
491 levels in amniotic fluid. t-HGF (A) and a-HGF (B) were measured in amniotic fluid from control
492 (n=29) and obese (n=12) women by ELISA (see details in Table 2). Values are means ± SEM.
493 *p<0.05 relative to control group by unpaired t-test. (C) Western blot analysis of HGF in placental

494 tissue from control and obese women. Frozen placental tissues (~100 mg) from control (n=8) and
495 obese (n=10) groups (see details in Table 1) were used to quantify placental HGF content. β-actin
496 expression was determined to ensure similar protein loading. Top: the y-axis represents the ratio of
497 HGF vs. β-actin in arbitrary units (A.U). Bottom: a representative picture of the western blot is
498 shown. Data are means ± SEM.*p<0.05 relative to control group by unpaired t-test.

499 **Figure 2. HGF stimulates glucose uptake and metabolism in human placental explants.** (A)
500 Stimulation of glucose uptake in placental explants. Placental explants from control women (n=3;
501 Table 1) were incubated with different concentrations of HGF for 18h, and 2-DOG uptake was
502 measured. Values are means ± SEM for 3 independent experiments in triplicate. *p<0.05 relative to
503 control without HGF by ANOVA. (B) Effect of cytochalasin B (CB) on 2-DOG uptake mediated by
504 HGF. Placental explants from control women (n= 4; Table 1) were treated with HGF (30 ng/ml) for
505 18h in the presence or the absence of CB at 10 μM or 50 μM. Afterward, 2-DOG uptake was
506 determined. Values are means± SEM for 4 independent experiments in triplicate. *p<0.05 relative to
507 control without CB and without HGF; †p<0.05 relative to HGF without CB by ANOVA. (C) Effect
508 of HGF on glycolysis in placental explants. Placental explants from control women (n=7; Table 1)
509 were treated with HGF (30ng/ml) for 18h. Afterward, glycolysis was determined. Values are
510 represented as percentage of control for 7 independent experiments in triplicate. *p<0.05 relative to
511 control by unpaired t-test.

512 **Figure 3. Augmented Glut-1 protein levels and activation of PI3K signaling pathway are**
513 **required for HGF-mediated stimulation of glucose transport in human placental explants.** (A)
514 Western blot analysis of Glut-1 in placental explants. Explants from control women (n=4; Table 1)
515 were preincubated with or without HGF (30 ng/mL) for 18h, and protein extracts were subjected to

516 immunoblot analysis using anti-Glut-1 antibody. Top: the y-axis represents the ratio of Glut-1 vs. β-
517 actin in arbitrary units (A.U). Bottom: a representative picture of the western blot is shown. Data are
518 means ± SEM for 4 independent experiments. *p<0.05 relative to control by unpaired t-test. (B)
519 Western blot analysis of PI3K-signaling pathway activation mediated by HGF in placental explants.
520 Explants from control women (n=4; Table 1) were preincubated without (time 0) or with HGF
521 (30ng/mL) for the indicated times. Afterward, protein extracts were subjected to immunoblot
522 analysis using anti-PKB or anti-p-PKB antibody. Top: the y-axis represents the ratio of p-PKB vs.
523 PKB in arbitrary units (A.U). Bottom: a representative picture of the western blot is shown. Data are
524 means ± SEM for 4 independent experiments. *p<0.05 relative to time 0 by ANOVA. (C) Effect of
525 wortmannin-mediated inhibition of PI3K signaling pathway on 2-DOG uptake. Placental explants
526 from control women (n=4; Table 1) were preincubated with 100 nmol/L wortmannin (WM) for 30
527 min before and during chronic (18h) incubation with HGF (30 ng/mL). At the end of the incubation
528 period, the 2-DOG uptake was measured. Values are means ± SEM for 4 independent experiments in
529 triplicate. *p<0.05 relative to control without HGF; †p<0.05 relative to HGF without WM by
530 ANOVA.

531 **Figure 4. HGF inhibits fatty acid oxidation and increases triglycerides content in human**
532 **placental explants.** (A) Effect of HGF on fatty acid oxidation. Placental explants from control
533 women (n=7; Table 1) were incubated in the absence (control) or presence of HGF (30 ng/mL) for
534 18h. Afterward, [^{3}H]-water was determined. Values are means ± SEM for 7 independent experiments
535 in triplicate. Significance is indicated *p<0.05 relative to control by unpaired t-test. (B) Effect of
536 HGF on fatty acid esterification. The same subset of placental explants described in panel A was
537 used to assess esterification into total lipids. Values are represented as percentage of control for 7

538 independent experiments in triplicate. Significance is indicated *p<0.05 relative to control by
539 unpaired t-test. (C) Effect of HGF on de novo lipid synthesis. Placental explants from control women
540 (n=7; Table 1) were used to assess de novo fatty acid synthesis in the absence (control) or presence
541 of HGF (30 ng/mL) plus [^{14}C]-acetate for 18h. Afterward, [^{14}C]-acetate incorporation into total lipids
542 was determined. Values are represented as percentage of control for 7 independent experiments in
543 triplicate. Significance is indicated *p<0.05 relative to control by unpaired t-test. (D) Placental
544 explants from control women (n=4; Table 1) were used to assess the effect of HGF on placental TG
545 accumulation. Values are means ± SEM for 4 independent experiments in triplicate. Significance is
546 indicated *p<0.05 relative to control by unpaired t-test.

547 **Figure 5. HGF inhibits carnitine palmitoyltransferase I activity.** (A) Effect of HGF on CPT-I
548 activity. Placental explants from control women (n=4; Table 1) were incubated in the absence
549 (control) or presence of HGF (30ng/mL) for 18h. Afterward, CPT-I activity was determined. Values
550 are means ± SEM for 4 independent experiments in duplicated. Significance is indicated *p<0.05
551 relative to control by unpaired t-test. (B) Effect of HGF on CPT-II activity. The same placental
552 explants used in panel A were used to assess CPT-II activity. Values are means ± SEM for 4
553 independent experiments in duplicated. Significance is indicated *p<0.05 relative to control by
554 unpaired t-test.

555

556 **Contributors**

557 Francisco Visiedo, I declare that I participated in sample collection, experimental work, data
558 analysis and figures/tables and manuscript elaboration, and that I have seen and approved the final
559 version. I have no conflicts of interest to declare.

560 Fernando Bugatto, I declare that I participated in sample collection, experimental work, data
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566 Ana Sáez-Benito, I declare that I participated in sample collection, experimental work, data
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575 José L. Bartha, I declare that I participated in sample collection, data analysis and figures/tables
576 and manuscript elaboration, and that I have seen and approved the final version. I have no conflicts
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578 Germán Perdomo, I declare that I participated in work conceptualization, experimental design,
579 experimental work, data analysis and figures/tables, and I wrote the manuscript, and that I have seen
580 and approved the final version. I have no conflicts of interest to declare.
581

Table-1
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Table 1. Placental explants samples. Maternal and fetal characteristics from the studied population used for placental explants experiments.

	Control group (n=26)	Obese group (n=10)
Delivery mode	Caesarean section No labour	Caesarean section No labour
Maternal age (yr)	30.0 ± 5.7	31.8 ± 4.0
Gestational age at delivery (wk)	38.3 ± 1.81	38.6 ± 0.9
Maternal pregravid BMI (kg/m^2)	21.4 ± 1.5	34.2 ± 4.1*
Maternal plasma glucose (mg/dL)	72.8 ± 6.6	80.6 ± 15.3
Maternal plasma insulin (pmol/L)	6.5 ± 3.7	16.1 ± 9.4*
Maternal plasma triglycerides (mg/dL)	182.0 ± 35.8	226.5 ± 48.6
Maternal plasma total cholesterol (mg/dL)	249.8 ± 32.0	288.4 ± 35.1*
Maternal plasma HDL-cholesterol (mg/dL)	73.3 ± 21.2	68.0 ± 18.0
Maternal plasma LDL-cholesterol (mg/dL)	144.9 ± 24.3	158.7 ± 40.5
Placental weight (g)	528 ± 106	617 ± 42*
Birthweight (g)	3225 ± 309	3235 ± 279

Data are given as mean ± SEM. *BMI*, Body mass index; *HDL*, High-density lipoprotein; *LDL*, Low-density lipoprotein. *p<0.05 *vs.* control group by unpaired t-test.

Table-2
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Table 2. Amniotic fluid samples. Maternal and fetal characteristics from the studied population used for amniotic fluid collection.

	Control group (n=29)	Obese group (n=12)
Maternal age (yr)	35.61 ± 5.02	35 ± 1.1
Gestational age at amniocentesis (wk)	16 (15-17)	16 (16-17)
Gestational age at delivery (wk)	40.0 ± 1	39 ± 1.7
Body mass index (kg/m^2)	22.69 ± 1.3	34.6 ± 2.56*
Newborn weight (g)	3258.52 ± 475	3357 ± 579

Data are given as mean ± SE. Gestational age at amniocentesis is listed as median (interquartile range). *$p<0.05$ *vs.* control group by unpaired t-test.

Figure-1

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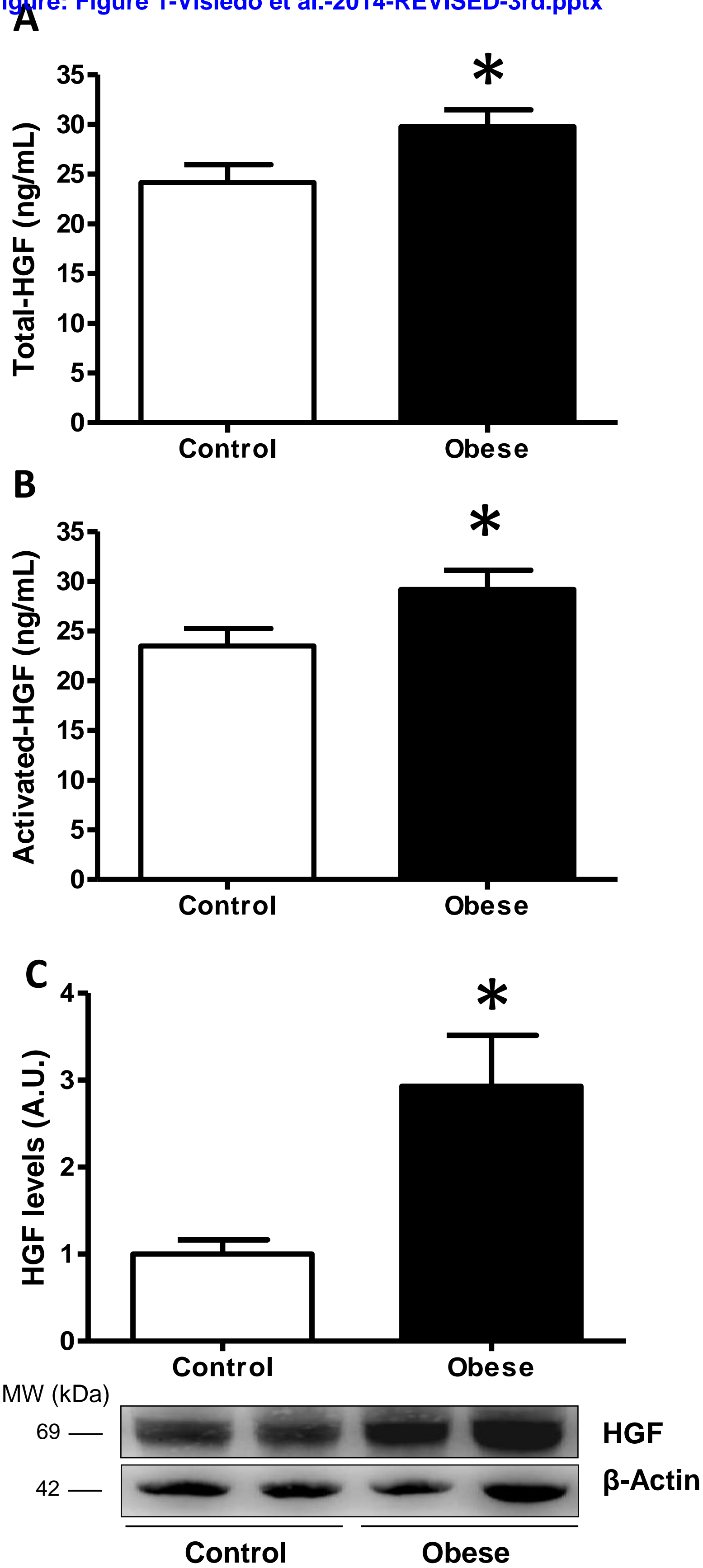

Figure 1

Figure-2

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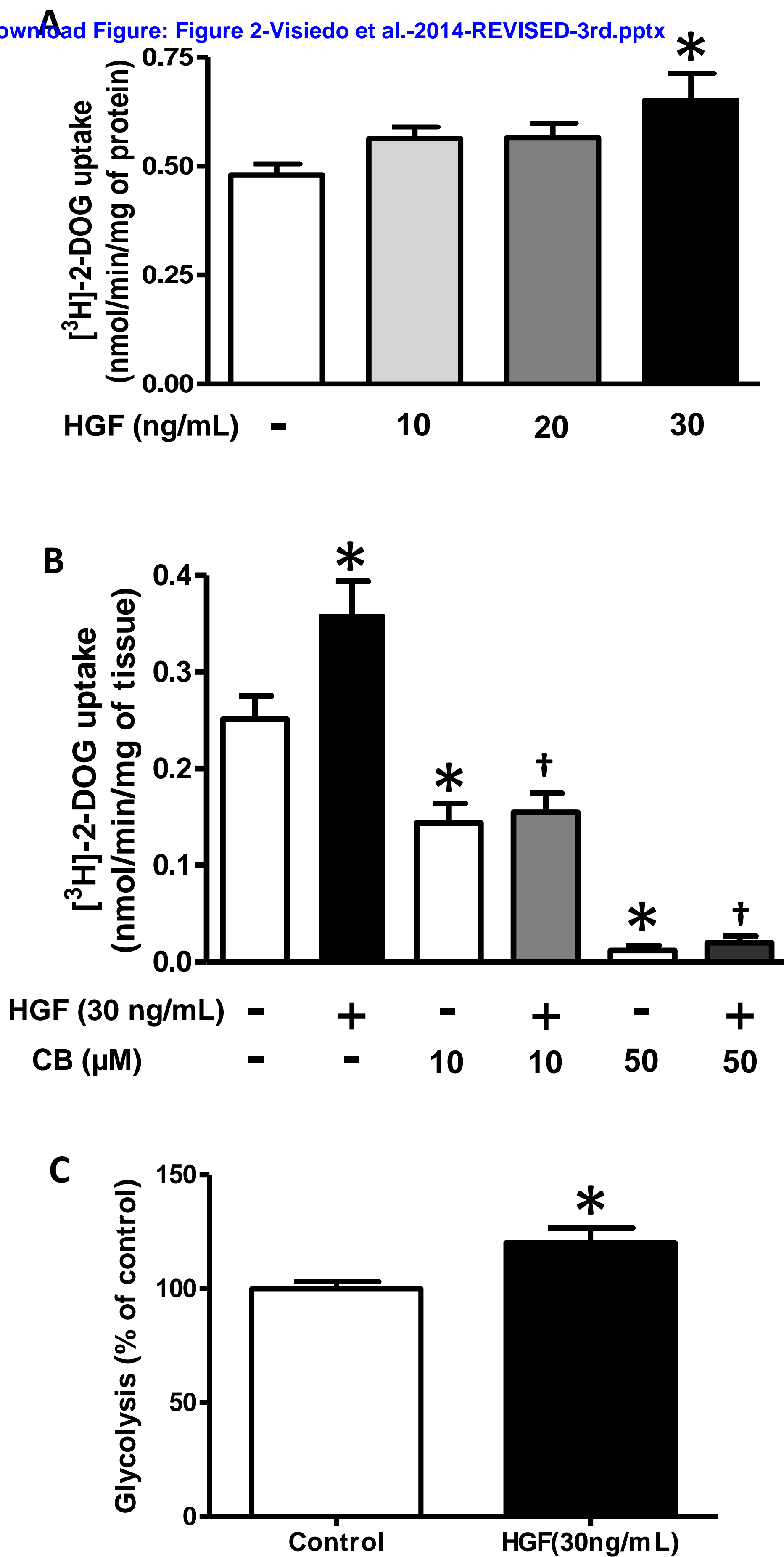

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Figure-3

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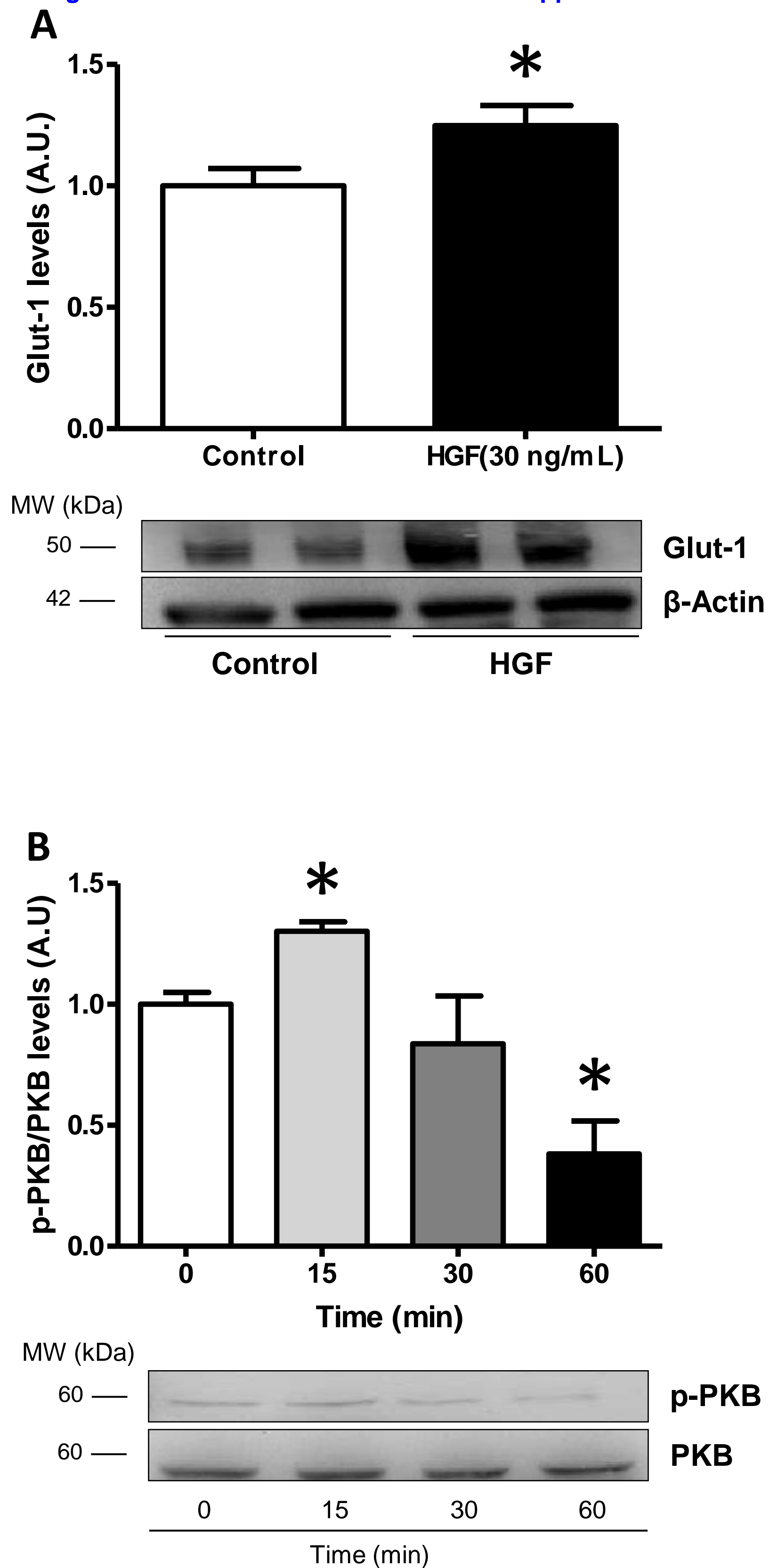

Figure 3

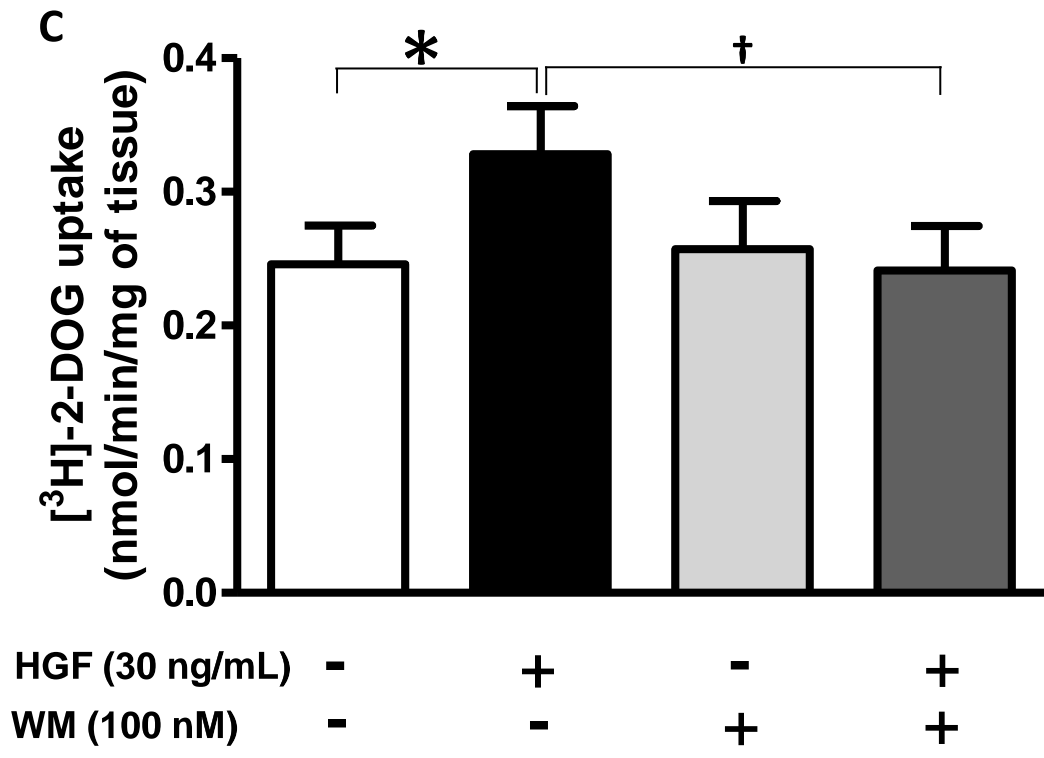

Figure 3

Figure-4

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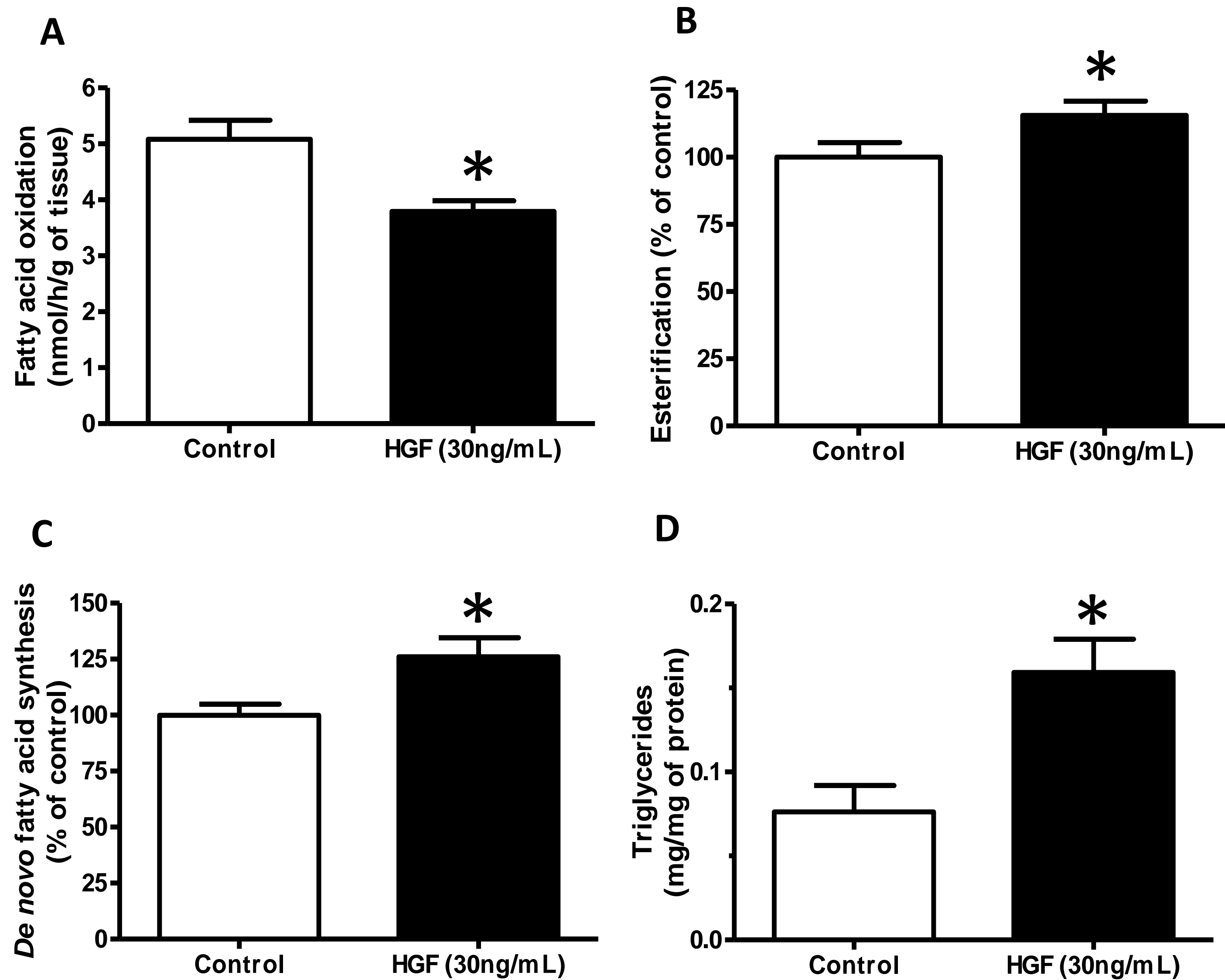

Figure 4

Figure-5

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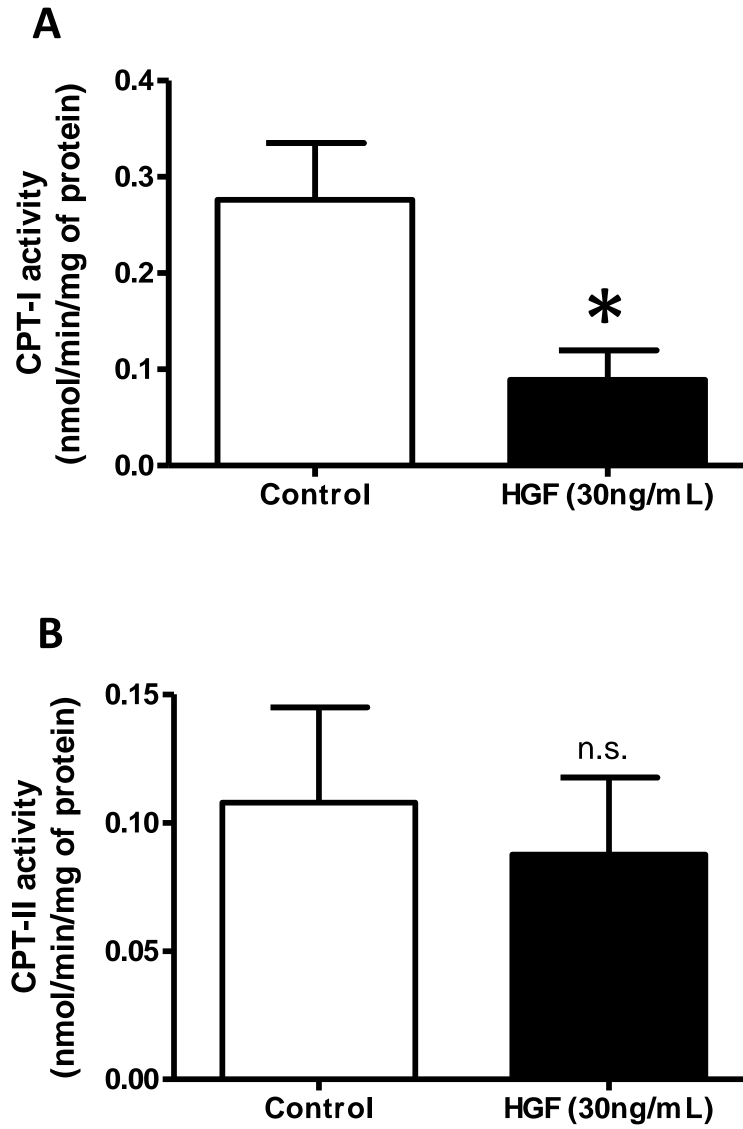

Figure 5