

1  
2  
3  
4 **Maternal serum IGF-1, IGFBP-1 and 3, and placental growth hormone at 20**  
5 **weeks' gestation in pregnancies complicated by preeclampsia**  
6

7 *Shutan Liao*<sup>1,2,6</sup>, *Mark H Vickers*<sup>1,2</sup>, *Rennae S Taylor*<sup>3</sup>, *Beatrix Jones*<sup>4</sup>, *Mhoyra Frasers*<sup>5</sup>, *Lesley*  
8 *ME McCowan*<sup>3</sup>, *Philip N Baker*<sup>1,2,7</sup>, *Jo K Perry*<sup>1,2</sup>  
9

10 <sup>1</sup>*Liggins Institute, University of Auckland, Auckland, New Zealand;*

11 <sup>2</sup>*Gravida: National Centre for Growth and Development, New Zealand;*

12 <sup>3</sup>*Department of Obstetrics and Gynaecology, University of Auckland, Auckland, New Zealand;*

13 <sup>4</sup>*Institute of Natural and Mathematical Sciences, Massey University, Auckland, New Zealand;*

14 <sup>5</sup>*Department of Physiology, University of Auckland, Auckland, New Zealand;*

15 <sup>6</sup>*The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China;*

16 <sup>7</sup>*College of Medicine, Biological Sciences and Psychology, University of Leicester, UK*  
17

18 **Abbreviated Title:** Maternal hormones in preeclampsia  
19

20 **Key words:** placental growth hormone, insulin-like growth factors, insulin-like growth factor  
21 binding proteins, pregnancy, preeclampsia  
22

23 **Word count: (including the abstract) 2622**  
24

25 **Number of figures and tables: 3**  
26

27 Correspondence and reprint requests to be addressed to:

28 Dr Jo. K. Perry, PhD

29 The Liggins Institute, University of Auckland

30 85 Park Rd, Private Bag 92019 Auckland 1142, New Zealand

31 Tel: +64(9) 9237873; Fax: +64(9) 3737497

32 Email: j.perry@auckland.ac.nz  
33  
34

35 This work was funded by The Maurice and Phyllis Paykel Trust and Gravida: National  
36 Centre for Growth and Development.  
37  
38

39 *Declaration of interest:*

40 The authors have nothing to declare.  
41

42 **Abstract**

43 **Objective:** To investigate whether maternal serum concentrations of placental growth  
44 hormone (GH-V), insulin-like growth factor (IGF) 1 and 2, and IGF binding proteins  
45 (IGFBP) 1 and 3 were altered in pregnancies complicated by later preeclampsia (PE).

46 **Study design:** In a nested case-control study, PE cases (n=71) and matched controls (n=71)  
47 were selected from the Screening for Pregnancy Endpoints (SCOPE) biobank in Auckland,  
48 New Zealand. Maternal serum hormone concentrations at 20 weeks of gestation were  
49 determined by ELISA.

50 **Results:** We found that maternal serum GH-V concentration at 20 weeks of gestation was  
51 unaltered in the PE group, compared to the control group (median, 1.78 ng/ml vs. 1.65 ng/ml,  
52  $p = 0.884$ ). Maternal IGF-1 and IGFBP-3 concentrations and the IGF-1/IGFBP-3 ratio in PE  
53 pregnancies were significantly higher than in controls (median, 253.1 ng/ml vs. 204.3 ng/ml,  
54  $p < 0.0001$ ; 8535 ng/ml vs. 7711 ng/ml,  $p = 0.0023$ ; 0.032 vs. 0.026,  $p < 0.0001$ ,  
55 respectively), whereas maternal IGFBP-1 concentration was significantly lower in PE  
56 pregnancies than in controls (median, 34.85 ng/ml vs. 48.92 ng/ml,  $p = 0.0006$ ).

57 **Conclusion:** Our findings suggest a potential role of IGFs and IGFBPs in the prediction of  
58 pregnancies complicated by PE. However, the maternal serum concentration of GH-V at 20  
59 weeks' gestation is unlikely to be useful in the early prediction of PE.

60

61

62

63

64

65

66

## 67 **Introduction**

68 Preeclampsia (PE) is one of the leading causes of maternal, fetal, and neonatal mortality and  
69 morbidity. It affects 3–5% of pregnancies worldwide and is characterized by maternal  
70 hypertension, proteinuria, and if left untreated it can progress to maternal multi-organ failure,  
71 coagulopathy and seizures [1]. Women whose pregnancies are complicated by PE also have  
72 an increased risk of diabetes mellitus, chronic hypertension, ischemic heart disease,  
73 cerebrovascular disease and kidney disease later in life [2, 3]. However, there are no  
74 therapeutic approaches available for treatment currently and there are limited options for  
75 prevention of PE in women identified to be at high risk [4]. Therefore, identification of  
76 biomarkers that predict the development of PE or improve detection of this life-threatening  
77 pregnancy disorder is warranted **in order to enable better monitoring of patients and reduce**  
78 **the occurrence of adverse complications for them and their babies.**

79 The human growth hormone (GH) locus contains two evolutionarily related growth hormone  
80 genes: the pituitary GH gene (GH-N; GH1) and a gene for the placental growth hormone  
81 variant (GH-V; GH2) [5, 6]. GH-V binds the GH receptor with similar affinity to GH-N, but  
82 has lower affinity for the prolactin receptor [5, 6]. During pregnancy, GH-V is secreted from  
83 the syncytiotrophoblast cells of the placenta, and gradually replaces GH-N as the main form  
84 of GH in the maternal circulation [5, 6]. Despite this intriguing switch in expression from  
85 pituitary to placental expression of GH, very little is known about the role GH-V plays in  
86 human pregnancy. However, it is thought that GH-V plays a role in maternal adaptation to  
87 pregnancy. Studies investigating the association between maternal serum GH-V and PE are  
88 limited, and results have been conflicting. Two studies observed increased GH-V levels in  
89 maternal serum at mid to late gestation in pregnancies complicated by PE [7, 8]. Sifakis *et al.*  
90 found no changes in GH-V concentration in maternal serum taken at 11-13 weeks in PE cases

91 when compared to controls [9]. Mannik *et al.* demonstrated reduced GH-V expression in  
92 placentas from pregnancies complicated by PE [10].

93 In the current study, we aimed to determine whether there was an association between  
94 maternal serum concentration of GH-V at 20 weeks of gestation and the subsequent  
95 development of PE. We hypothesised that maternal serum GH-V concentrations were altered  
96 in pregnancies with later PE. Serum concentrations of related GH/ insulin-like growth factor  
97 (IGF) axis proteins: IGF-1, IGF-2, and their binding proteins (IGFBP) 1 and 3 were also  
98 measured.

99

## 100 **Materials and Methods**

101 Ethical approval was obtained from New Zealand Health and Disability Ethics Committees  
102 (AKX/02/00/364/AM03), and all women provided written informed consent. Between  
103 November 2004 and October 2007, 2,032 nulliparous women with singleton pregnancies  
104 were recruited to the Screening for Pregnancy Endpoints (SCOPE) study in Auckland, New  
105 Zealand. The inclusion criteria has been described previously [11].

106 Participants were interviewed and examined by a SCOPE research midwife at 15 and 20  
107 weeks of gestation. At the first visit, detailed clinical and demographic data were collected  
108 and entered into an internet accessed, central database with a complete audit trail  
109 (MedSciNet, Stockholm, Sweden). A family history of gestational hypertensive disorders was  
110 defined as a mother and/or sister(s) who had a history of either gestational hypertension or  
111 PE. Mean arterial pressure (MAP) was measured by mercury or aneroid sphygmomanometer  
112 at the 15 and 20 weeks SCOPE research visits. Umbilical artery resistance index (RI, defined  
113 as maximum – minimum velocity/maximum velocity) and mean uterine artery RI were  
114 measured using Doppler ultrasound at 20 weeks. Maternal serum samples were collected at  
115 20 weeks and stored at -80 °C. The specimens did not undergo any freeze/thaw cycles prior

116 to these analyses. Birth weight was recorded to the nearest **gram** using electronic scales at the  
117 time of birth.

118 In this nested case-control study, 71 PE cases were selected from the New Zealand SCOPE  
119 cohort and matched by ethnicity, age ( $\pm 3$  years) and body mass index (BMI) ( $\pm 3$  kg/m<sup>2</sup>) to  
120 71 controls with uncomplicated pregnancies. PE was defined as gestational hypertension  
121 (systolic blood pressure  $\geq 140$  mmHg or diastolic blood pressure  $\geq 90$  mmHg on at least 2  
122 occasions 4 hours apart after the 20 week visit in previous normotensive women) with either  
123 proteinuria ( $\geq 2+$  dipstick or urine protein creatinine ratio  $\geq 30$  mg/mmol or 24 hours urinary  
124 protein excretion  $\geq 0.3$  g) or multisystem disease (thrombocytopenia, renal insufficiency,  
125 impaired liver function, pulmonary, cerebral or visual symptoms) [12].

## 126 **Materials**

127 Recombinant human GH-V (22 kDa) was purchased from Protein Laboratories Rehovot  
128 (Rehovot, Israel) and was reconstituted in 0.4% NaHCO<sub>3</sub> pH 9 [13]. Human GH-V  
129 monoclonal antibodies 78.8E8 (E8; MCA5827G) and 78.7C12 (7C12; MCA5828G) were  
130 obtained from Bio-Rad AbD Serotec (NC, US). E8 does not cross react with GH-N or  
131 prolactin; 7C12 shows some cross reactivity with GH-N (5%) as per manufacturer's  
132 documentation. Antibody 7C12 was biotinylated using a LYNX Rapid Biotin Antibody  
133 Conjugation Kit (Bio-Rad AbD Serotec) according to the manufacturer's instructions.

## 134 **GH-V ELISA procedure**

135 We have previously described the development and validation of an in-house enzyme-linked  
136 immunosorbent assay (ELISA) for the measurement of GH-V in serum [14]. In brief,  
137 microtiter plates were coated with antibody E8 diluted in phosphate buffer (0.1M Sodium  
138 Carbonate, pH 9.5) at a concentration of 2  $\mu$ g/ml by overnight incubation at 4°C. Coated  
139 plates were washed three times with wash buffer (PBS-T; 10 mM phosphate buffer pH 7.4,  
140 150 mM NaCl, 0.05% Tween 20). Blocking was achieved by 1 hour incubation at room

141 temperature with Ultrablock (Bio-Rad AbD Serotec). Standards were prepared from GH-V  
142 solution with a range from 5 to 0.078 ng/ml. Standards and 1:2 diluted serum samples were  
143 incubated for 2 hours at room temperature, then washed three times. 8 µg/ml biotinylated  
144 antibody C12 was added and incubated for 1 hour. After being washed three times, 200 ng/ml  
145 horseradish peroxidase conjugated streptavidin (Bio-Rad AbD Serotec) was added and  
146 incubated for 30 min. The microtiter plates were washed four times. End-point detection was  
147 processed by using 3, 3', 5, 5'-Tetramethylbenzidine (TMB) Substrate Reagent Set (BD  
148 Biosciences) and stop solution (2N H<sub>2</sub>SO<sub>4</sub>). Absorbance was read at 450nm and 590 nm  
149 within 30 min of stopping reaction. Serum samples were spiked with GH-V and the average  
150 recovery rate was 106%. Coefficients of variation (CV) of intra-assay and inter-assay were  
151 4.8% and 6.8%, respectively.

#### 152 **Serum analysis**

153 Serum total IGF-1, total IGF-2, IGFBP-1 and IGFBP-3 were assayed with human-specific  
154 ELISA as per the manufacturer's instructions (Mediagnost, Germany).

#### 155 **Statistical analysis**

156 GH-V, IGF-1, IGFBP-1 and IGFBP-3 data were log-transformed to improve the  
157 approximation of normal distribution and linearize relationships. Data are expressed as means  
158 ± **standard deviation (SD)** and median unless stated otherwise. Group means were compared  
159 using a Student's *t* test. Categorical variables were compared using chi-square or Fisher's  
160 exact test. Pearson's coefficient was used to determine correlations between variables,  
161 presented as *r* values. All analyses were conducted using IBM SPSS Statistics 21. A *p*-value  
162 of <0.05 was accepted as statistically significant.

163

#### 164 **Results**

165 The demographic and clinical details are shown in Table 1. There were no significant  
166 differences between the two groups except for MAP at 15 and 20 weeks', birth weight and  
167 gestational age at birth.

### 168 **Serum concentrations of maternal GH-related hormones**

169 Maternal serum GH-V concentrations varied considerably between individuals (range: 0.69-  
170 8.37 ng/ml). There was no significant difference in maternal serum GH-V concentrations at  
171 20 weeks of gestation in the PE group when compared to the control group (median, 1.78  
172 ng/ml vs. 1.65 ng/ml,  $p = 0.884$ ) (Fig. 1A). Maternal IGF-1 and IGFBP-3 concentrations and  
173 the IGF-1/IGFBP-3 ratios in PE pregnancies were significantly higher than in control  
174 (median, 253.1 ng/ml vs. 204.3 ng/ml,  $p < 0.0001$ ; 8535 ng/ml vs. 7711 ng/ml,  $p = 0.0023$ ;  
175 0.032 vs. 0.026,  $p < 0.0001$ , respectively) (Fig. 1B, E and F). Maternal IGFBP-1  
176 concentrations were significantly lower in PE pregnancies compared to the controls (median,  
177 34.85 ng/ml vs. 48.92 ng/ml,  $p = 0.0006$ ) (Fig. 1D). There was no significant difference in  
178 IGF-2 concentrations between groups (Fig. 1C).

179 The PE group was separated into early-onset PE patients (defined as PE that developed  
180 before 34 weeks of gestation) and late-onset PE (defined as PE which developed at or after 34  
181 weeks of gestation). Early-onset PE patients had increased IGF-1 and IGFBP-1  
182 concentrations compared to late-onset PE patients (IGF-1: median, 313.5 ng/ml vs. 251.3  
183 ng/ml,  $p = 0.0268$ ; IGFBP-1: median, 9372 ng/ml vs. 8461 ng/ml,  $p = 0.0469$ ), although the  
184 number of patients in the early-onset PE group was much lower ( $n = 8$  vs.  $n = 63$ ).

### 185 **Correlation analysis**

186 In both the control and PE groups, maternal IGF-1 concentrations were positively related to  
187 the changes in IGFBP-3 but negatively related to IGFBP-1 concentrations. There was also an  
188 association between the concentrations of GH-V and IGF-1 in the control group, but not in  
189 the PE group (Table 2).

190 Interestingly, in the PE group, maternal IGF-1 had a weak positive association with MAP at  
191 15 and 20 weeks' ( $r = 0.276$ ,  $p = 0.021$ ;  $r = 0.24$ ,  $p = 0.046$ ); while maternal GH-V was  
192 negatively associated with mean uterine artery RI ( $r = -0.367$ ,  $p = 0.002$ ) but not with  
193 umbilical artery RI ( $r = 0.043$ ,  $p = 0.72$ ) at 20 weeks. The associations of IGF-1 with MAP  
194 and of GH-V with uterine artery RI were still significant after adjusting for maternal age,  
195 ethnicity, BMI, family history of gestational hypertensive disorders, smoking and drinking  
196 habits.

197

## 198 **Discussion**

199 The precise aetiology of PE remains elusive. A long standing hypothesis is that PE develops  
200 as a consequence of an immunologically-initiated impaired trophoblast invasion, shallow  
201 implantation and inadequate remodelling of the uterine spiral arteries, leading to a high-  
202 resistance uteroplacental circulation [15, 16]. Subsequent oxidative stress and inflammation  
203 in the placenta alters expression of pro-inflammatory, anti-angiogenic and angiogenic factors,  
204 contributing to endothelial cell dysfunction and an inflammatory response [17]. Trophoblast  
205 migration and invasive capacity has been shown to be modulated by a number of factors,  
206 including oxygen concentration [18], interleukin and transforming growth factor [19], IGF-2  
207 and IGFBP-1 [20], epidermal and hepatocyte growth factors [21, 22]. A potential role of GH-  
208 V in the regulation of trophoblast invasion is also suggested by the presence of the GH  
209 receptor in the placenta [23] and the stimulation of trophoblast invasion by GH-V *in vitro*  
210 [24]. Further, GH-V is a target gene of peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ )  
211 and has been proposed to be involved in the PPAR $\gamma$ -mediated inhibition of trophoblast  
212 invasion in an autocrine manner [25]. It is possible that circulating GH-V concentrations may  
213 not reflect the effect of GH-V on trophoblast invasion.

214 Doppler ultrasonography can assess uteroplacental and fetoplacental blood flows and has  
215 been suggested as a screening method for PE as increased uterine artery blood flow resistance  
216 had been observed prior to the onset of PE [26-28]. One study by Schiessl *et al.* demonstrated  
217 a strong correlation between decreasing uterine and peripheral arterial resistance and  
218 increasing maternal serum GH-V concentration [29]. In our study, we found that maternal  
219 GH-V concentration was negatively associated with mean uterine artery RI in preeclamptic  
220 pregnancies, suggesting a potential role of GH-V in the alteration of maternal arterial  
221 resistance.

222 IGF-1 and IGF-2 mediate a range of actions in many tissues including stimulation of cell  
223 growth, cell survival and differentiation. During human pregnancy, maternal IGF-1 is  
224 believed to originate mainly from the maternal liver, and GH-V has been suggested to be a  
225 main regulator of its synthesis as serum concentrations during pregnancy are highly  
226 correlated [30-32]. The majority of circulating IGF-1 is bound to IGFBP-3 and the acid labile  
227 subunit to form a 150 kDa ternary complex and is the major storage form of IGF-1 in the  
228 circulation [33]. This complex prolongs the half-life of circulating IGF-1 and facilitates its  
229 endocrine actions. IGF-1/IGFBP-3 ratio correlates with the amount of free, biologically  
230 active IGF-1[34]. IGFBP-1 binds to only a small proportion of circulating IGF-I but is  
231 considered to be important for short-term regulation of IGF bioactivity [35, 36]. The placenta  
232 expresses considerable amounts of IGFs and IGFBPs; however, it is unclear if the placenta-  
233 derived IGFs and IGFBPs serve local function by paracrine or autocrine regulation, or if they  
234 are secreted into the maternal or fetal circulation. Nevertheless, IGFs and IGFBPs are crucial  
235 for fetal growth and placental development since they regulate trophoblast migration at the  
236 maternal-fetal interface [20, 37]. In addition, previous studies also provide evidence in  
237 support of a potential role of IGFs and IGFBPs in pregnancies complicated by PE. Increased  
238 IGF-2 and decreased IGFBP-1 mRNA expression were observed in the placentae of women

239 with PE [38, 39]. Transgenic mice overexpressing human IGFBP-1 exhibit a PE phenotype  
240 [40]. However, maternal serum concentrations of IGFs and IGFBPs in PE pregnancies vary  
241 across different studies. Maternal serum IGF-1 and IGFBP-1 were decreased and IGFBP-3  
242 was increased at 11-13 weeks in pregnancies that subsequently developed PE in several  
243 studies [41-43]. However, two studies observed **decreased** IGF-1 and IGFBP-3, as well as  
244 increased IGFBP-1 levels in the third trimester in PE patients [44, 45]. Consistent with our  
245 study, increased IGF-I and decreased IGFBP-1 from the first to second trimester were  
246 associated with a higher risk of PE [46, 47]. Further, a progressive increase in maternal  
247 circulating IGFBP-1 concentrations from 16 to 36 weeks' was also observed in those  
248 pregnancies **complicated by** PE [48]. Contributing factors to this observed variation may  
249 include the sample size, the time of sampling and the onset and severity of PE where  
250 placental integrity is compromised, and consequently the secretion of placental hormones is  
251 also compromised.

252 There is clear evidence that maternal MAP in the second trimester is associated with the later  
253 development of PE, although the predictive ability of blood pressure alone is low [49-51].  
254 The vasoactive effects of IGF-1 indicate that IGF-1 can influence blood pressure [52, 53].  
255 Increased IGF-1 levels have also been reported in patients with hypertension [54, 55]. In our  
256 study, we found that maternal IGF-1 concentration was positively associated with MAP in the  
257 PE group only. However, the role of circulating IGF-1 in the pathogenesis of PE still needs  
258 further investigation.

259 **Our study provides further evidence for differences in concentrations of IGF-1 and IGFBPs at**  
260 **20 weeks of gestation in pregnancies with later PE.** However, maternal GH-V concentration  
261 was not altered and is unlikely to be useful in the early prediction of PE.

262

263 **Acknowledgments**

264 The New Zealand SCOPE study was funded by the New Enterprise Research Fund,  
265 Foundation for Research Science and Technology; Health Research Council (04/198); Evelyn  
266 Bond Fund, Auckland District Health Board Charitable Trust. This work was funded by The  
267 Maurice and Phyllis Paykel Trust and Gravida: National Centre for Growth and  
268 Development.

269

270 **Conflict of Interest**

271 The authors declare that they have no conflict of interest.

272

273 **References**

- 274 [1] S. Friedman, R. Taylor, J. Roberts, Pathophysiology of preeclampsia, *Clinics in*  
275 *perinatology* 18(4) (1991) 661-682.
- 276 [2] L.M. Amaral, M.W. Cunningham, Jr., D.C. Cornelius, B. LaMarca, Preeclampsia: long-  
277 term consequences for vascular health, *Vasc Health Risk Manag* 11 (2015) 403-15.
- 278 [3] E.F. Funai, Y. Friedlander, O. Paltiel, E. Tiram, X. Xue, L. Deutsch, S. Harlap, Long-term  
279 mortality after preeclampsia, *Epidemiology* 16(2) (2005) 206-15.
- 280 [4] J.T. Henderson, E.P. Whitlock, E. O'Connor, C.A. Senger, J.H. Thompson, M.G.  
281 Rowland, Low-dose aspirin for prevention of morbidity and mortality from preeclampsia: a  
282 systematic evidence review for the U.S. Preventive Services Task Force, *Ann Intern Med*  
283 160(10) (2014) 695-703.
- 284 [5] D. Haig, Placental growth hormone-related proteins and prolactin-related proteins,  
285 *Placenta* 29 Suppl A(Suppl A) (2008) S36-41.
- 286 [6] M.C. Lacroix, J. Guibourdenche, J.L. Frendo, F. Muller, D. Evain-Brion, Human  
287 placental growth hormone--a review, *Placenta* 23 Suppl A(Suppl A) (2002) S87-94.
- 288 [7] P. Mittal, J. Espinoza, S. Hassan, J.P. Kusanovic, S.S. Edwin, J.K.A. Nien, F. Gotsch,  
289 N.G. Than, O. Erez, S. Mazaki-Tovi, R. Romero, Placental growth hormone is increased in  
290 the maternal and fetal serum of patients with preeclampsia, *J Matern-Fetal Neo M* 20(9)  
291 (2007) 651-659.
- 292 [8] E. Papadopoulou, S. Sifakis, E. Giahnakis, Y. Fragouli, N. Karkavitsas, E. Koumantakis,  
293 M. Kalmanti, Increased human placental growth hormone at midtrimester pregnancies may  
294 be an index of intrauterine growth retardation related to preeclampsia, *Growth Horm IGF Res*  
295 16(5-6) (2006) 290-6.
- 296 [9] S. Sifakis, R. Akolekar, N. Mantas, D. Kappou, K.H. Nicolaides, Maternal Serum Human  
297 Placental Growth Hormone (hPGH) at 11 to 13 Weeks of Gestation in Preeclampsia,  
298 *Hypertens Pregnancy* 30(1) (2011) 74-82.
- 299 [10] J. Mannik, P. Vaas, K. Rull, P. Teesalu, M. Laan, Differential placental expression  
300 profile of human Growth Hormone/Chorionic Somatomammotropin genes in pregnancies  
301 with pre-eclampsia and gestational diabetes mellitus, *Mol Cell Endocrinol* 355(1) (2012) 180-  
302 7.
- 303 [11] L. McCowan, R. North, R. Taylor, Screening for pregnancy endpoints: preeclampsia,  
304 growth restricted baby and spontaneous preterm birth. Actrn12607000551493. Australia New  
305 Zealand Clinical Trial Registry, 2007.
- 306 [12] M.A. Brown, M.D. Lindheimer, M. de Swiet, A. Van Assche, J.M. Moutquin, The  
307 classification and diagnosis of the hypertensive disorders of pregnancy: statement from the  
308 International Society for the Study of Hypertension in Pregnancy (ISSHP), *Hypertens*  
309 *Pregnancy* 20(1) (2001) IX-XIV.
- 310 [13] G. Solomon, S. Reicher, E.E. Gussakovsky, J.B. Jomain, A. Gertler, Large-scale  
311 preparation and in vitro characterization of biologically active human placental (20 and 22K)  
312 and pituitary (20K) growth hormones: placental growth hormones have no lactogenic activity  
313 in humans, *Growth Horm IGF Res* 16(5-6) (2006) 297-307.
- 314 [14] S. Liao, M.H. Vickers, R.S. Taylor, B. Jones, M. Fraser, L.M. McCowan, P.N. Baker,  
315 J.K. Perry, Human placental growth hormone is increased in maternal serum at 20 weeks of  
316 gestation in pregnancies with large-for-gestational-age babies, *Growth Factors* 2016  
317 Dec;34(5-6):203-209. doi(2017 Jan 25) 10.1080/08977194.2016.1273223.
- 318 [15] P. Kaufmann, S. Black, B. Huppertz, Endovascular trophoblast invasion: implications  
319 for the pathogenesis of intrauterine growth retardation and preeclampsia, *Biol Reprod* 69(1)  
320 (2003) 1-7.

- 321 [16] C.W. Redman, I.L. Sargent, Latest advances in understanding preeclampsia, *Science*  
322 308(5728) (2005) 1592-4.
- 323 [17] C. Lam, K.H. Lim, S.A. Karumanchi, Circulating angiogenic factors in the pathogenesis  
324 and prediction of preeclampsia, *Hypertension* 46(5) (2005) 1077-85.
- 325 [18] Y. Zhou, O. Genbacev, C.H. Damsky, S.J. Fisher, Oxygen regulates human  
326 cytotrophoblast differentiation and invasion: implications for endovascular invasion in  
327 normal pregnancy and in pre-eclampsia, *J Reprod Immunol* 39(1-2) (1998) 197-213.
- 328 [19] S. Karmakar, C. Das, Regulation of trophoblast invasion by IL-1beta and TGF-beta1,  
329 *Am J Reprod Immunol* 48(4) (2002) 210-9.
- 330 [20] G.S. Hamilton, J.J. Lysiak, V.K. Han, P.K. Lala, Autocrine-paracrine regulation of  
331 human trophoblast invasiveness by insulin-like growth factor (IGF)-II and IGF-binding  
332 protein (IGFBP)-1, *Exp Cell Res* 244(1) (1998) 147-56.
- 333 [21] K.E. Bass, D. Morrish, I. Roth, D. Bhardwaj, R. Taylor, Y. Zhou, S.J. Fisher, Human  
334 cytotrophoblast invasion is up-regulated by epidermal growth factor: evidence that paracrine  
335 factors modify this process, *Dev Biol* 164(2) (1994) 550-61.
- 336 [22] J.E. Cartwright, D.P. Holden, G.S. Whitley, Hepatocyte growth factor regulates human  
337 trophoblast motility and invasion: a role for nitric oxide, *Br J Pharmacol* 128(1) (1999) 181-9.
- 338 [23] F. Frankenne, E. Alsat, M.L. Scippo, A. Igout, G. Hennen, D. Evain-Brion, Evidence for  
339 the expression of growth hormone receptors in human placenta, *Biochem Biophys Res*  
340 *Commun* 182(2) (1992) 481-6.
- 341 [24] M.C. Lacroix, J. Guibourdenche, T. Fournier, I. Laurendeau, A. Igout, V. Goffin, J.  
342 Pantel, V. Tsatsaris, D. Evain-Brion, Stimulation of human trophoblast invasion by placental  
343 growth hormone, *Endocrinology* 146(5) (2005) 2434-44.
- 344 [25] T. Fournier, K. Handschuh, V. Tsatsaris, J. Guibourdenche, D. Evain-Brion, Role of  
345 nuclear receptors and their ligands in human trophoblast invasion, *J Reprod Immunol* 77(2)  
346 (2008) 161-70.
- 347 [26] W. Plasencia, N. Maiz, L. Poon, C. Yu, K.H. Nicolaides, Uterine artery Doppler at 11 +  
348 0 to 13 + 6 weeks and 21 + 0 to 24 + 6 weeks in the prediction of pre-eclampsia, *Ultrasound*  
349 *Obstet Gynecol* 32(2) (2008) 138-46.
- 350 [27] R. Napolitano, S. Santo, R. D'Souza, A. Bhide, B. Thilaganathan, Sensitivity of higher,  
351 lower and mean second-trimester uterine artery Doppler resistance indices in screening for  
352 pre-eclampsia, *Ultrasound Obstet Gynecol* 36(5) (2010) 573-6.
- 353 [28] K. Guzin, S. Tomruk, Y.A. Tuncay, M. Naki, S. Sezginsoy, E. Zemheri, N. Yucel, F.  
354 Kanadikirik, The relation of increased uterine artery blood flow resistance and impaired  
355 trophoblast invasion in pre-eclamptic pregnancies, *Arch Gynecol Obstet* 272(4) (2005) 283-8.
- 356 [29] B. Schiessl, C.J. Strasburger, M. Bidlingmaier, B. Gutt, S.E. Kirk, R. Oberhoffer, K.  
357 Friese, Role of placental growth hormone in the alteration of maternal arterial resistance in  
358 pregnancy, *J Reprod Med* 52(4) (2007) 313-6.
- 359 [30] A. Caufriez, F. Frankenne, G. Hennen, G. Copinschi, Regulation of maternal IGF-I by  
360 placental GH in normal and abnormal human pregnancies, *Am J Physiol* 265(4 Pt 1) (1993)  
361 E572-7.
- 362 [31] A. Caufriez, F. Frankenne, Y. Englert, J. Golstein, F. Cantraine, G. Hennen, G.  
363 Copinschi, Placental growth hormone as a potential regulator of maternal IGF-I during  
364 human pregnancy, *Am J Physiol* 258(6 Pt 1) (1990) E1014-9.
- 365 [32] A. Caufriez, F. Frankenne, G. Hennen, G. Copinschi, Regulation of maternal insulin-like  
366 growth factor I by placental growth hormone in pregnancy. Possible action of maternal IGF-I  
367 on fetal growth, *Horm Res* 42(1-2) (1994) 62-5.
- 368 [33] R.C. Baxter, Characterization of the acid-labile subunit of the growth hormone-  
369 dependent insulin-like growth factor binding protein complex, *J Clin Endocrinol Metab* 67(2)  
370 (1988) 265-72.

- 371 [34] A. Juul, K. Main, W.F. Blum, J. Lindholm, M.B. Ranke, N.E. Skakkebaek, The ratio  
372 between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins  
373 (IGFBP-1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic  
374 patients, *Clin Endocrinol (Oxf)* 41(1) (1994) 85-93.
- 375 [35] J. Frystyk, K. Hojlund, K.N. Rasmussen, S.P. Jorgensen, M. Wildner-Christensen, H.  
376 Orskov, Development and clinical evaluation of a novel immunoassay for the binary complex  
377 of IGF-I and IGF-binding protein-1 in human serum, *J Clin Endocrinol Metab* 87(1) (2002)  
378 260-6.
- 379 [36] R.C. Baxter, Insulin-like growth factor binding proteins as glucoregulators, *Metabolism*  
380 44(10 Suppl 4) (1995) 12-7.
- 381 [37] H. Lacey, T. Haigh, M. Westwood, J.D. Aplin, Mesenchymally-derived insulin-like  
382 growth factor 1 provides a paracrine stimulus for trophoblast migration, *BMC Dev Biol* 2  
383 (2002) 5.
- 384 [38] R.J. Gratton, H. Asano, V.K. Han, The regional expression of insulin-like growth factor  
385 II (IGF-II) and insulin-like growth factor binding protein-1 (IGFBP-1) in the placentae of  
386 women with pre-eclampsia, *Placenta* 23(4) (2002) 303-10.
- 387 [39] J.C. Shin, J.H. Lee, D.E. Yang, H.B. Moon, J.G. Rha, S.P. Kim, Expression of insulin-  
388 like growth factor-II and insulin-like growth factor binding protein-1 in the placental basal  
389 plate from pre-eclamptic pregnancies, *Int J Gynaecol Obstet* 81(3) (2003) 273-80.
- 390 [40] P.A. Crossey, C.C. Pillai, J.P. Miell, Altered placental development and intrauterine  
391 growth restriction in IGF binding protein-1 transgenic mice, *J Clin Invest* 110(3) (2002) 411-  
392 8.
- 393 [41] S. Sifakis, R. Akolekar, D. Kappou, N. Mantas, K.H. Nicolaides, Maternal serum  
394 insulin-like growth factor-I at 11-13 weeks in preeclampsia, *Prenat Diagn* 30(11) (2010)  
395 1026-31.
- 396 [42] S. Sifakis, R. Akolekar, D. Kappou, N. Mantas, K.H. Nicolaides, Maternal serum  
397 insulin-like growth factor-binding protein-1 (IGFBP-1) at 11-13 weeks in pre-eclampsia,  
398 *Prenat Diagn* 31(2) (2011) 196-201.
- 399 [43] S. Sifakis, R. Akolekar, D. Kappou, N. Mantas, K.H. Nicolaides, Maternal serum  
400 insulin-like growth factor-binding protein-3 (IGFBP-3) at 11-13 weeks in preeclampsia, *J*  
401 *Hum Hypertens* 26(4) (2012) 253-8.
- 402 [44] M. Ingec, H.G. Gursoy, L. Yildiz, Y. Kumtepe, S. Kadanali, Serum levels of insulin,  
403 IGF-1, and IGFBP-1 in pre-eclampsia and eclampsia, *Int J Gynaecol Obstet* 84(3) (2004)  
404 214-9.
- 405 [45] Y. Kocyigit, G. Bayhan, A. Atamer, Y. Atamer, Serum levels of leptin, insulin-like  
406 growth factor-I and insulin-like growth factor binding protein-3 in women with pre-  
407 eclampsia, and their relationship to insulin resistance, *Gynecol Endocrinol* 18(6) (2004) 341-  
408 8.
- 409 [46] L.J. Vatten, T.I. Nilsen, A. Juul, S. Jeansson, P.A. Jenum, A. Eskild, Changes in  
410 circulating level of IGF-I and IGF-binding protein-1 from the first to second trimester as  
411 predictors of preeclampsia, *Eur J Endocrinol* 158(1) (2008) 101-5.
- 412 [47] R. Hietala, P. Pohja-Nylander, E.M. Rutanen, T. Laatikainen, Serum insulin-like growth  
413 factor binding protein-1 at 16 weeks and subsequent preeclampsia, *Obstet Gynecol* 95(2)  
414 (2000) 185-9.
- 415 [48] N. Anim-Nyame, F.A. Hills, S.R. Sooranna, P.J. Steer, M.R. Johnson, A longitudinal  
416 study of maternal plasma insulin-like growth factor binding protein-1 concentrations during  
417 normal pregnancy and pregnancies complicated by pre-eclampsia, *Hum Reprod* 15(10)  
418 (2000) 2215-9.

419 [49] E. Ekholm, R. Erkkola, J. Hartiala, Second trimester ambulatory blood pressure in  
420 nulliparous pregnancy: a useful screening test for pre-eclampsia?, *Br J Obstet Gynaecol*  
421 101(9) (1994) 828.

422 [50] S. Caritis, B. Sibai, J. Hauth, M. Lindheimer, P. VanDorsten, M. Klebanoff, E. Thom,  
423 M. Landon, R. Paul, M. Miodovnik, P. Meis, G. Thurnau, M. Dombrowski, D. McNellis, J.  
424 Roberts, Predictors of pre-eclampsia in women at high risk. National Institute of Child Health  
425 and Human Development Network of Maternal-Fetal Medicine Units, *Am J Obstet Gynecol*  
426 179(4) (1998) 946-51.

427 [51] N. Onwudiwe, C.K. Yu, L.C. Poon, I. Spiliopoulos, K.H. Nicolaides, Prediction of pre-  
428 eclampsia by a combination of maternal history, uterine artery Doppler and mean arterial  
429 pressure, *Ultrasound Obstet Gynecol* 32(7) (2008) 877-83.

430 [52] G. Pete, Y. Hu, M. Walsh, J. Sowers, J.C. Dunbar, Insulin-like growth factor-I decreases  
431 mean blood pressure and selectively increases regional blood flow in normal rats, *Proc Soc*  
432 *Exp Biol Med* 213(2) (1996) 187-92.

433 [53] A. Tivesten, E. Bollano, I. Andersson, S. Fitzgerald, K. Caidahl, K. Sjogren, O. Skott,  
434 J.L. Liu, R. Mobini, O.G. Isaksson, J.O. Jansson, C. Ohlsson, G. Bergstrom, J. Isgaard, Liver-  
435 derived insulin-like growth factor-I is involved in the regulation of blood pressure in mice,  
436 *Endocrinology* 143(11) (2002) 4235-42.

437 [54] M. Galderisi, G. Vitale, G. Lupoli, M. Barbieri, G. Varricchio, C. Carella, O. de Divitiis,  
438 G. Paolisso, Inverse association between free insulin-like growth factor-1 and isovolumic  
439 relaxation in arterial systemic hypertension, *Hypertension* 38(4) (2001) 840-5.

440 [55] J. Diez, Insulin-like growth factor I in essential hypertension, *Kidney Int* 55(2) (1999)  
441 744-59.

442  
443  
444  
445  
446  
447  
448  
449  
450  
451  
452  
453  
454  
455  
456  
457  
458  
459  
460

461 **Figure Legends**

462

463 **Fig. 1. Serum GH-V, IGF-1, IGF-2, IGFBP-1, IGFBP-3 concentrations and IGF-**  
464 **1/IGFBP-3 ratio.**

465 Data are shown as Tukey box-whisker plots (median, 25th centile, 75th centile and range).

466 Outliers are presented as hollow symbols. \* $p < 0.05$ .

467

468

469

470