**Clinical and biochemical factors associated with pre-eclampsia in obese and lean women.**

**Appendix A**

**Methodology**

Of the 7 additional biomarkers assessed in this analysis, 3 (total cholesterol, HDL-cholesterol and triglycerides) were measured using Beckman Unicel Syncron DxC (Beckman Coulter, Inc., Brea, CA 92821 USA). Measurements were not repeated for participants with previously available results for these analytes, which were performed using COBAS (Roche Diagnostics Ltd., CH-6343 Rotkreuz, Switzerland).1 Both methods used the same reactions. The LDL-cholesterol was calculated using the Friedewald Formula.

The remaining three additional biomarkers (adiponectin, insulin and placental growth hormone) were measured using the same platform used for measurement of the 47 previously reported biomarkers2 with the methodology below.

Mouse derived recombinant Fab(s), generated by unique phage display technology (Alere, San Diego), were used in several immunoassay formats for the measurement of biomarker concentrations. These reagents were optimized for use in Luminex xMap technology (Alere, San Diego) or micro-titer plate ELISAs. Once optimized for format, a small subset of clinical patient samples were used to determine the final reagents (pairings or single recombinant Fabs) to use in the full cohort screening.

Luminex assays utilize magnetic beads that have a unique ratio of two dyes that are used to distinguish the identity of each bead, thereby enabling the multiplexing of assays within the same well. The Luminex sandwich assay format uses a recombinant Fab conjugated to a magnetic bead as the capture and a biotin-conjugated recombinant Fab as the assay detection. Recombinant Fab conjugated magnetic beads are added to the plate and washed. Sample is then incubated with the beads, followed by incubation with the detection antibody. The plate is washed, incubated with strepavidin labeled phycoerythrin, washed and then read using a Luminex 200 reader. The Luminex competitive assay format uses a recombinant Fab conjugated to the bead and a biotin-conjugated antigen as the assay detection. Recombinant Fab conjugated magnetic beads are added to the plate and washed. The sample and detection reagent are premixed, added simultaneously and then incubated. The final steps are as described for the Luminex sandwich assay.

The micro-titer ELISA assays use a streptavidin coated plate and biotin or fluorescein conjugated recombinant Fabs. The ELISA sandwich assay uses a biotin-conjugated recombinant Fab as the capture and a fluorescein-conjugated recombinant Fab as the detection antibody. Capture antibody is coated on the plate, incubated, washed and sample added. After sample incubation, the plate is washed and then incubated with detection antibody. Following washing, the plate is incubated with anti fluorescein antibody conjugated to alkaline phosphatase, washed, fluorescent substrate added and then read using a Tecan infinite F200 reader.

The ELISA competitive assay uses a biotin-conjugated antigen as the capture and a fluorescein-conjugated recombinant Fab as the detection antibody. The plate is coated with capture antibody, incubated and washed. Addition of sample is immediately followed by addition of the detection antibody and incubated. The final steps are the same as the ELISA sandwich.

Each assay uses an 8 point dose curve prepared gravimetrically in EDTA plasma or buffer.

**References**

1. Fyfe EM, Rivers KS, Thompson JM, Thiyagarajan KP, Groom KM, Dekker GA, et al. Elevated maternal lipids in early pregnancy are not associated with risk of intrapartum caesarean in overweight and obese nulliparous women. BMC Pregnancy Childbirth. 2013;13:143

2. Kenny LC, Black MA, Poston L, Taylor R, Myers JE, Baker PN, et al. Early pregnancy prediction of preeclampsia in nulliparous women, combining clinical risk and biomarkers: the Screening for Pregnancy Endpoints (SCOPE) international cohort study. Hypertension. 2014;64(3):644-52.

Table A.1. List of biomarkers measured at 14-16 weeks’ gestation and the assay method.

|  |  |  |  |
| --- | --- | --- | --- |
| **Biomarker** | **Assay method** | **Included in analysis** | **Convert to MoM** |
| Adam-9 (Disintegrin and metalloproteinase domain-containing protein 9) | Luminex Sandwich | Exclude LOD a |  |
| Adiponectin b | Luminex Competitive | Yes |  |
| Angiogenin | Luminex Competitive | Yes |  |
| Arginase-1 | Luminex Sandwich | Yes |  |
| Arginase-2 | Luminex Sandwich | Yes |  |
| Atrial natriuretic peptide (ANP)-propeptide | Luminex Sandwich | Yes |  |
| Big Endothelin-1 | Luminex Sandwich | Exclude LOD a |  |
| Brain natriuretic peptide (BNP) | Luminex Sandwich | Yes | Yes |
| C-Met | Luminex Sandwich | Yes |  |
| C-reactive protein (CRP) | Luminex Competitive | Yes |  |
| C-X-C motif chemokine 10 (CXCL 10) | Luminex Sandwich | Yes |  |
| Carboxypeptidase A4 (CPA-4) precursor | Luminex Sandwich | Yes |  |
| Caspase-3 | Luminex Sandwich | Yes |  |
| Chemokine (C-C motif) ligand 23 (CCL23) | Luminex Sandwich | Yes |  |
| Cholesterol (total) b | Enzymatic Colorimetric | Yes | Yes |
| Cystatin C | ELISA Competitive | Yes |  |
| Elafin | Luminex Competitive | Yes |  |
| Endoglin | ELISA Sandwich | Yes |  |
| Endothelial cell-selective adhesion molecule (ESAM-1) | Luminex Sandwich | Yes |  |
| Ephrin-receptor-2 | Luminex Sandwich | Exclude LOD a |  |
| Factor inhibiting hypoxia inducible factor 1α (FIH) | Luminex Sandwich | Yes |  |
| Fas cell surface death receptor (FAS) | Luminex Sandwich | Yes | Yes |
| Fas ligand (Fas L)-soluble | Luminex Sandwich | Exclude LOD a |  |
| Fetal Haemoglobin (HbF) | ELISA Sandwich | Yes |  |
| HDL-cholesterol b | Enzymatic Colorimetric | Yes |  |
| Insulin b | Luminex Sandwich | Exclude LOD a |  |
| Intercellular adhesion molecule-1 (ICAM-1) | Luminex Competitive | Yes |  |
| Interleukin 1 receptor antagonist (IL-1ra) | Luminex Sandwich | Yes |  |
| Kunitz type protease inhibitor 2 (HAI-2) | Luminex Sandwich | Yes |  |
| LDL-cholesterol b | Calculated (Friedewald Formula) | Yes | Yes |
| Leptin | Luminex Sandwich | Yes |  |
| Leptin receptor | Luminex Sandwich | Yes |  |
| Macrophage migration inhibitory factor (MIF) | Luminex Sandwich | Yes |  |
| Matrix metalloproteinase-9 (MMP-9) | Luminex Sandwich | Yes |  |
| Nephrin | Luminex Sandwich | Yes | Yes |
| Neutrophil gelatinase-associated lipocalin (NGAL) | ELISA Sandwich | Yes |  |
| Pentraxin-3 | Luminex Sandwich | Exclude LOD a |  |
| Periostin | Luminex Sandwich | Yes |  |
| Placental growth factor (PlGF) | Luminex Sandwich | Yes | Yes |
| Placental growth hormone (PlGH) b | Luminex Sandwich | Yes | Yes |
| Plasminogen activator inhibitor 1 (PAI-1) | Luminex Sandwich | Exclude LOD a |  |
| Plasminogen activator inhibitor 2 (PAI-2) | Luminex Sandwich | Yes | Yes |
| Podocalyxin | Luminex Sandwich | Exclude LOD a |  |
| Pregnancy associated plasma protein A (PAPP-A) | Luminex Sandwich | Yes | Yes |
| Procalcitonin (PCT) | Luminex Sandwich | Exclude LOD a |  |
| ST2 | Luminex Sandwich | Yes |  |
| TIMP metallopeptidase inhibitor 1 (TIMP-1) | Luminex Competitive | Yes |  |
| Transforming growth factor (TGF) β receptor 2 | Luminex Sandwich | Yes |  |
| Triglycerides b | Enzymatic Colorimetric | Yes | Yes |
| Tumour necrosis factor receptor 1a (TNFR1a) | Luminex Sandwich | Yes |  |
| Vascular endothelial growth factor C (VEGF-C) | Luminex Sandwich | Yes |  |
| Vascular endothelial growth factor receptor 1 (VEGFR1) | Luminex Sandwich | Yes |  |
| Visfatin | Luminex Sandwich | Exclude LOD a |  |
| WAP four disulfide core domain protein 2 (HE4) | Luminex Sandwich | Yes |  |

Biomarkers previously reported in Kenny et al2 except adiponectin, cholesterol (total), HDL-cholesterol insulin, LDL-cholesterol, PlGH, triglycerides (b).

a Excluded as the majority of measurements for the biomarker were below the limit of detection (LOD) of the assay.

Table A.2. Concentration of biomarkers measured in plasma from 14-16 weeks' gestation by PET status in lean and obese women.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **LEAN** | |  | **OBESE** | |  |
|  | **Non-PE (n=3001)** | **PE (n=105)** |  | **Non-PE (n=757)** | **PE (n=77)** |  |
| **Biomarkers, units** | **Median (IQR)** | **Median (IQR)** | **p a** | **Median (IQR)** | **Median (IQR)** | **p a** |
| Adiponectin, ng/ml (n=3905) | 4548 (3467-5809) | 4322 (3446-5527) | 0.95 | 3782 (2892-4965) | 3369 (2550-4737) | 0.03 |
| Angiogenin, ng/ml (n=3906) | 6595 (4970-9214) | 6857 (4907-9227) | 0.90 | 7497 (5652-10611) | 7713 (6044-10455) | 0.33 |
| Arginase-1, ng/ml (n=3906) | 0.6 (0.3-1.0) | 0.7 (0.3-1.0) | 0.38 | 0.6 (0.3-0.9) | 0.6 (0.3-0.8) | 0.18 |
| Arginase-2, ng/ml (n=3907) | 7.3 (5.2-10.0) | 7.2 (5.5-9.1) | 0.52 | 7.0 (5.0-9.3) | 7.5 (5.1-9.7) | 0.98 |
| Atrial natriuretic peptide (ANP)-propeptide, ng/ml (n=3906) | 0.5 (0.2-0.9) | 0.4 (0.2-0.9) | 0.80 | 0.4 (0.2-0.8) | 0.3 (0.2-0.6) | 0.05 |
| Brain natriuretic peptide (BNP) b, pg/ml (n=3907) | 0.06 (0.04-0.08) | 0.05 (0.04-0.08) | 0.36 | 0.05 (0.03-0.07) | 0.05 (0.03-0.07) | 0.03 |
| Carboxypeptidase A4 (CPA-4) precursor, ng/ml (n=3906) | 2.3 (1.8-2.9) | 2.5 (1.8-3.0) | 0.18 | 2.3 (1.8-2.8) | 2.2 (1.7-2.8) | 0.10 |
| Caspase-3, ng/ml (n=3907) | 2.3 (1.3-4.0) | 2.5 (1.6-3.9) | 0.29 | 2.5 (1.5-4.2) | 2.1 (1.2-3.8) | 0.49 |
| Chemokine (C-C motif) ligand 23 (CCL23), ng/ml (n=3905) | 0.3 (0.2-0.3) | 0.3 (0.2-0.3) | 0.27 | 0.3 (0.2-0.4) | 0.3 (0.2-0.4) | 0.75 |
| Cholesterol (total) b, mg/dl (n=3930) | 202 (182-223) | 200 (178-236) | 0.44 | 211 (190-237) | 218 (190-241) | 0.23 |
| C-Met, ng/ml (n=3905) | 111 (87-134) | 113 (86-128) | 0.82 | 108 (88-131) | 99 (78-127) | 0.06 |
| C-reactive protein (CRP), ng/ml | 8006 | 9468 | 0.26 | 19103 | 21098 | 0.11 |
| (n=3906) | (4269-14128) | (5341-15444) |  | (10924-32290) | (12541-29807) |  |
| C-X-C motif chemokine 10 (CXCL 10), ng/ml (n=3907) | 0.2 (0.1-0.2) | 0.2 (0.1-0.2) | 1.00 | 0.2 (0.1-0.2) | 0.2 (0.1-0.2) | 0.64 |
| Cystatin C, ng/ml (n=3906) | 1813 (1454-2233) | 1860 (1559-2375) | 0.04 | 2167 (1693-2806) | 2155 (1663-2818) | 0.63 |
| Elafin, ng/ml (n=3906) | 121 (88-163) | 138 (93-193) | 0.21 | 128 (99-172) | 131 (96-167) | 0.67 |
| Endoglin, ng/ml (n=3904) | 16.9 (13.3-21.8) | 18.8 (14.4-25.5) | 0.02 | 12.9 (9.8-16.9) | 13.6 (9.3-19.5) | 0.34 |
| Endothelial cell-selective adhesion molecule (ESAM-1), ng/ml (n=3907) | 3.9 (3.1-4.7) | 4.1 (3.3-4.7) | 0.12 | 4.0 (3.1-4.7) | 4.2 (3.2-4.8) | 0.23 |
| Factor inhibiting hypoxia inducible factor 1α (FIH), ng/ml (n=3906) | 0.1 (0.1-0.2) | 0.1 (0.1-0.2) | 0.80 | 0.2 (0.1-0.3) | 0.1 (0.1-0.2) | 0.21 |
| Fas cell surface death receptor (FAS) b, ng/ml (n=3907) | 1.2 (0.8-1.7) | 1.2 (0.9-1.7) | 0.49 | 1.3 (0.9-1.8) | 1.2 (0.9-1.9) | 0.56 |
| Fetal Haemoglobin (HbF), ng/ml (n=3906) | 58 (34-91) | 60 (38-91) | 0.89 | 58 (33-89) | 47 (30-73) | 0.06 |
| HDL-cholesterol b, mg/dl (n=3929) | 74 (64-85) | 71 (63-85) | 0.30 | 66 (57-76) | 63 (51-75) | 0.03 |
| Insulin, µIU/ml (n=3905) | 16.4 (9.6-28.2) | 15.7 (9.7-27.4) | 0.80 | 14.8 (9.7-25.9) | 20.2 (10.5-31.5) | 0.06 |
| Intercellular adhesion molecule-1 (ICAM-1), ng/ml (n=3906) | 631 (507-797) | 655 (519-852) | 0.48 | 643 (531-796) | 694 (577-812) | 0.12 |
| Interleukin 1 receptor antagonist (IL-1ra), ng/ml (n=3907) | 0.01 (0.01-0.01) | 0.01 (0.01-0.01) | 0.79 | 0.02 (0.01-0.02) | 0.02 (0.01-0.03) | 0.13 |
| Kunitz type protease inhibitor 2 (HAI-2), ng/ml (n=3907) | 0.2 (0.1-0.3) | 0.2 (0.1-0.2) | 0.95 | 0.2 (0.1-0.3) | 0.2 (0.1-0.2) | 0.39 |
| LDL-cholesterol b, mg/dl (n=3925) | 101 (84-121) | 104 (85-128) | 0.26 | 115 (96-133) | 114 (93-148) | 0.10 |
| Leptin, ng/ml (n=3907) | 7.2 (5.5-13.6) | 7.8 (5.5-14.2) | 0.34 | 14.1 (7.2-19.8) | 14.7 (8.7-20.3) | 0.68 |
| Leptin receptor, ng/ml (n=3907) | 156 (119-191) | 160 (112-205) | 0.92 | 119 (91-152) | 108 (79-143) | 0.51 |
| Macrophage migration inhibitory factor (MIF), ng/ml (n=3907) | 9.2 (7.9-11.4) | 9.4 (8.5-11.7) | 0.15 | 9.3 (8-11.7) | 8.5 (7.6-10.7) | 0.09 |
| Matrix metalloproteinase-9 (MMP-9), ng/ml (n=3906) | 34 (26-45) | 36 (27-44) | 0.44 | 37 (29-52) | 37 (28-47) | 0.59 |
| Nephrin b, ng/ml (n=3907) | 0.1 (0.1-0.2) | 0.1 (0.1-0.3) | 0.83 | 0.1 (0.1-0.2) | 0.1 (0.1-0.2) | 0.92 |
| Neutrophil gelatinase-associated lipocalin (NGAL), ng/ml (n=3907) | 40 (30-52) | 40 (28-54) | 0.95 | 43 (32-56) | 44 (32-62) | 0.70 |
| Periostin, ng/ml (n=3907) | 7.3 (5.7-9.0) | 7.2 (5.9-8.9) | 0.71 | 6.1 (4.7-7.6) | 5.9 (4.3-8.2) | 0.76 |
| Placental growth factor (PlGF) b, ng/ml (n=3905) | 0.02 (0.01-0.03) | 0.01 (0.01-0.03) | 0.06 | 0.02 (0.01-0.03) | 0.01 (0.01-0.02) | <0.001 |
| Plasminogen activator inhibitor 2 (PAI-2) b, ng/ml (n=3907) | 7.5 (5.8-9.9) | 8.0 (6.4-10.3) | 0.14 | 7.0 (5.6-9.0) | 7.1 (5.6-9.1) | 0.61 |
| Pregnancy associated plasma protein A (PAPP-A) b, ng/ml (n=3907) | 76 (46-136) | 88 (53-139) | 0.10 | 55 (33-97) | 50 (31-96) | 0.28 |
| ST2, ng/ml (n=3907) | 2.4 (1.6-3.6) | 2.7 (1.7-4.0) | 0.26 | 2.2 (1.4-3.6) | 2.3 (1.4-3.5) | 0.95 |
| TIMP metallopeptidase inhibitor 1 (TIMP-1), ug/ml (n=3906) | 89 (71-116) | 91 (72-120) | 0.53 | 96 (77-126) | 102 (80-140) | 0.38 |
| Transforming growth factor (TGF) β receptor 2, ng/ml (n=3907) | 1.2 (0.9-1.6) | 1.3 (0.9-1.6) | 0.37 | 1.4 (1.1-1.8) | 1.4 (1.0-1.9) | 0.64 |
| Triglycerides b, mg/dl (n=3930) | 116 (93-145) | 120 (93-157) | 0.29 | 143 (114-183) | 163 (126-196) | 0.10 |
| Tumour necrosis factor receptor 1a (TNFR1a), ng/ml (n=3907) | 4.2 (3.1-5.5) | 4.1 (3.3-5.5) | 0.68 | 4.3 (3.2-5.4) | 4.3 (3.4-5.9) | 0.52 |
| Vascular endothelial growth factor C (VEGF-C), ng/ml (n=3907) | 13.1 (11.1-14.8) | 13.5 (11.4-15.0) | 0.27 | 13.1 (11.1-14.7) | 13.6 (11.2-15.1) | 0.80 |
| Vascular endothelial growth factor receptor 1 (VEGFR1), ng/ml (n=3907) | 0.4 (0.2-0.6) | 0.4 (0.3-0.7) | 0.06 | 0.3 (0.2-0.4) | 0.3 (0.2-0.4) | 0.91 |
| WAP four disulfide core domain protein 2 (HE4), ng/ml (n=3906) | 11.2 (10.0-12.3) | 11.3 (10.3-12.4) | 0.73 | 10.9 (9.8-12) | 10.6 (9.3-11.8) | 0.25 |

a Biomarkers were log transformed and a t-test was then used for comparison; b Plasma concentrations are shown. Multiples of median (MoM) for gestational age were used for comparison and analysis.