

# BMJ Open

## Screening of gestational diabetes mellitus in early pregnancy by oral glucose tolerance test and novel biomarkers: study protocol for an international, prospective, multi-centre cohort trial.



Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-012115
Article Type:	Protocol
Date Submitted by the Author:	07-Apr-2016
Complete List of Authors:	Huhn, Evelyn; Frauenklinik Universitatsspital Basel, Obstetrics and Gynaecology Fischer, Thorsten; Paracelsus Medizinische Privatuniversitat, Obstetrics and Gynaecology Göbl, Christian; Medizinische Universität Wien, Obstetrics and Gynaecology, Devison of Obstetrics and feto-maternal Medicine Todesco-Bernasconi, Monya; Kantonsspital,, Obstetrics and Gynaecology Kreft, Martina; UniversitatsSpital Zurich, Obstetrics and Gynaecology Kunze, Mirjam; Universitatsklinikum Freiburg, Obstetrics and Gynaecology Schoetzau, Andreas; Universitatsspital Basel, Obstetrics and Gynaecology Eppel, Wolfgang; Medizinische Universität Wien, Obstetrics and Gynaecology, Devison of Obstetrics and feto-maternal Medicine Husslein, Peter; Medizinische Universität Wien, Obstetrics and Gynaecology, Devison of Obstetrics and feto-maternal Medicine Ochsenbein-Koelble, Nicole; UniversitatsSpital Zurich, Obstetrics and Gynaecology Zimmermann, Roland; UniversitatsSpital Zurich, Obstetrics and Gynaecology Bäz, Elke; Universitatsklinikum Freiburg Prömpeler, Heinrich; Universitatsklinikum Freiburg Bruder, Elisabeth; Universitatsspital Basel Institut fur Pathologie, Pathology Hahn, Sinuhe; Universitat Basel, Biomedicine, Laboratory of Perinatology Hoesli, Irene; University Hospital, University Basel, Obstetrics and Gynaecology
<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics, Obstetrics and gynaecology
Keywords:	gestational diabetes mellitus, diagnosis, screening, oral glucose tolerance test, glycosylated fibronectin, pregnancy

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

## Original Article

### 1 Screening of gestational diabetes mellitus in early pregnancy by 2 oral glucose tolerance test and novel biomarkers: study protocol 3 for an international, prospective, multi-centre cohort trial.

4  
5 Huhn E.A.<sup>1\*</sup>, Fischer T.<sup>2</sup>, Göbl C.S.<sup>3</sup>, Todesco Bernasconi M.<sup>4</sup>, Kreft M.<sup>5</sup>,  
6 Kunze M.<sup>6</sup>, Schoetzau A.<sup>1</sup>, Eppel W.<sup>3</sup>, Husslein P.<sup>3</sup>, Ochsenbein-Koelble  
7 N.<sup>5</sup>, Zimmermann R.<sup>5</sup>, Bätz E.<sup>6</sup>, Proempeler H.<sup>6</sup>, Bruder E.<sup>7</sup>, Hahn S.<sup>8</sup>,  
8 Hoesli I.<sup>1</sup>.

9  
10 <sup>1</sup> Department of Obstetrics and Gynaecology, University Hospital Basel,  
11 Switzerland

12 <sup>2</sup> Department of Obstetrics and Gynaecology, Salzburger  
13 Landeskrankenhaus, Paracelsus Medical University, Salzburg, Austria

14 <sup>3</sup> Department of Obstetrics and Gynaecology, Division of Obstetrics and  
15 feto-maternal Medicine, Medical University of Vienna, Austria

16 <sup>4</sup> Department of Obstetrics and Gynaecology, Cantonal Hospital Aarau,  
17 Switzerland

18 <sup>5</sup> Department of Obstetrics and Gynaecology, University Hospital Zurich,  
19 Switzerland

20 <sup>6</sup> Department of Obstetrics and Gynaecology, University Hospital Freiburg,  
21 Germany

22 <sup>7</sup> Department of Pathology, University Hospital Basel, Switzerland

1  
2  
3 1 <sup>8</sup> Department of Biomedicine, Laboratory of Perinatology, University Basel,  
4  
5 2 Switzerland  
6  
7  
8  
9  
10 4

11  
12 5 \*Correspondence to: Evelyn A. Huhn, Department of Obstetrics and  
13  
14 6 Gynaecology, University Hospital Basel, Spitalstrasse 21, 4031 Basel,  
15  
16 7 Switzerland  
17

18  
19 8 Phone: +41 61 556 51 44  
20

21 9 E-mail: evelyn.huhn@usb.ch  
22  
23  
24  
25

26 10 Trial was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) under NCT02035059 on  
27  
28 11 the 12<sup>th</sup> January 2014.  
29  
30  
31  
32

### 33 13 **Conflict of interest**

34  
35 The authors declare that there are no further financial or personal  
36  
37 relationships with other people or organizations that could inappropriately  
38  
39 influence the work reported or the conclusions, implications, or opinions  
40  
41 stated.  
42  
43  
44  
45  
46  
47

48 14 **KEY WORDS:** Gestational diabetes mellitus, diagnosis, screening, oral  
49  
50 15 glucose tolerance test, glycosylated fibronectin, pregnancy.  
51  
52  
53  
54

55 17 **Short title:** Screening of gestational diabetes mellitus in early pregnancy  
56  
57 18 by oral glucose tolerance test and biomarkers.  
58  
59  
60

1  
2  
3 1  
4  
5 2 Manuscript includes: 20 text pages (27 in total with front page,  
6  
7  
8 3 references)  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 ABSTRACT

4  
5 2 INTRODUCTION: As the accurate diagnosis and treatment of gestational  
6  
7 3 diabetes mellitus (GDM) is of increasing importance, new diagnostic  
8  
9 4 approaches for the assessment of GDM in early pregnancy were recently  
10  
11 5 suggested. We evaluate the diagnostic power of an "early" oral glucose  
12  
13 6 tolerance test (OGTT) 75g and a promising new biomarker, glycosylated  
14  
15 7 Fibronectin (glyFn), for GDM screening in a normal cohort.  
16  
17

18  
19 8 METHODS AND ANALYSIS: In a prospective cohort study, 531 singleton  
20  
21 9 pregnancies are recruited in six centres in Switzerland, Austria and  
22  
23 10 Germany. Women are screened for pre-existing diabetes mellitus and  
24  
25 11 GDM by an "early" OGTT 75g and/or the new biomarker, glyFn, at 12 to  
26  
27 12 15 weeks of gestation. Different screening strategies are compared to  
28  
29 13 evaluate the impact on detection of GDM by an OGTT 75g at 24 to 28  
30  
31 14 weeks of gestation as recommended by the International Association of  
32  
33 15 Diabetes and Pregnancy Study Groups (IADPSG). A new screening  
34  
35 16 algorithm is created by using multivariable risk estimation based on  
36  
37 17 "early" OGTT 75g and/or glyFn results, incorporating maternal risk factors.  
38  
39 18 Recruitment began in May 2014.  
40  
41

42  
43 19 ETHICS AND DISSEMINATION: This study received ethical approval from  
44  
45 20 the ethics committees in Basel, Zurich, Vienna, Salzburg and Freiburg. It  
46  
47 21 was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT02035059) on 12<sup>th</sup>  
48  
49 22 January 2014. Data will be presented at international conferences and  
50  
51 23 published in peer-reviewed journals.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 STRENGTHS AND LIMITATION OF THIS STUDY  
4

- 5 2 - This is an international, prospective, multi-centre cohort trial  
6  
7 3 recruiting at six centres in Switzerland, Austria and Germany.  
8  
9 4 - It is the first study to assess an "early" OGTT 75g and novel  
10  
11 5 biomarkers like glyFn for screening of gestational diabetes mellitus  
12  
13 6 in early pregnancy.  
14  
15 7 - The recruitment of 531 pregnant women are planned and we have  
16  
17 8 designed the study to be sufficiently powered to compare the  
18  
19 9 different early screening approaches with the detection of  
20  
21 10 gestational diabetes mellitus at 24 to 28 weeks of gestation.  
22  
23 11 - This study may be underpowered for the evaluation of neonatal  
24  
25 12 outcomes like LGA infants, neonatal hypoglycaemia, shoulder  
26  
27 13 dystocia or birth trauma (secondary outcomes).  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 INTRODUCTION  
4

5 2 Gestational diabetes mellitus (GDM) is defined as "any degree of glucose  
6  
7 3 intolerance that was first recognized during pregnancy"<sup>1</sup> regardless of  
8  
9 4 whether or not the condition predated the pregnancy or persisted after  
10  
11 5 pregnancy.<sup>2</sup> The increasing number of women with undiagnosed type 2  
12  
13 6 diabetes mellitus (T2DM) in pregnancy has led to the recommendation of  
14  
15 7 screening women with risk factors for pre-existing diabetes at the first  
16  
17 8 antenatal visit. GDM is still diagnosed in the late second or early third  
18  
19 9 trimester, because accurate diagnostic approaches for GDM assessment in  
20  
21 10 first trimester are still lacking.<sup>3</sup>  
22  
23  
24

25  
26 11 GDM is associated with adverse maternal and perinatal outcomes, such as  
27  
28 12 fetal overgrowth, shoulder dystocia, operative delivery, birth injury,  
29  
30 13 preeclampsia, haemorrhage and preterm delivery,<sup>4-6</sup> but also a seven fold  
31  
32 14 higher risk of the mother developing T2DM after pregnancy.<sup>7</sup> In addition,  
33  
34 15 the maternal metabolic milieu was also identified as a key determinant for  
35  
36 16 the susceptibility to obesity, metabolic syndrome and T2DM in the  
37  
38 17 offspring,<sup>8</sup> a phenomenon often described as "fetal programming".  
39  
40  
41

42 18 The current – but still widely discussed – standard of care in GDM  
43  
44 19 screening is the oral glucose tolerance test (OGTT) of 75g glucose  
45  
46 20 performed late at 24 to 28 weeks of gestation as recommended by the  
47  
48 21 International Association of Diabetes and Pregnancy Study Groups  
49  
50 22 (IADPSG).<sup>9</sup> The new screening thresholds are based on the results of a  
51  
52 23 large prospective cohort multicentre trial, the Hyperglycaemia and  
53  
54 24 Adverse Pregnancy Outcome (HAPO) study.<sup>5</sup> The aim of the HAPO study  
55  
56  
57  
58  
59  
60



1 was to associate the degree of maternal glycaemia with adverse perinatal  
2 outcome, such as large for gestational age infants (LGA), neonatal  
3 hypoglycaemia and caesarean section rates. The results showed no  
4 obvious threshold, but rather a continuous increase of these adverse  
5 outcomes across the range of glucose concentrations. The IADPSG criteria  
6 resulted in a considerable increase in GDM prevalence of 17.8%, a  
7 detection rate of 83% for adverse outcome and a positive predictive value  
8 of 16%.<sup>9</sup>

9 An early and rapid diagnosis of GDM even before 24 weeks of gestation is  
10 desirable. By targeted early intervention including physical activity,  
11 moderate diet or insulin/drug therapy starting in the first trimester, rates  
12 of macrosomia (birth weight > 4000 g) or large for gestational age  
13 (LGA=birth weight > 90<sup>th</sup> percentile) infants, operative vaginal delivery  
14 and perinatal morbidity could be possibly reduced. Moreover, there could  
15 be a long term downstream effect on the offspring, thereby leading to  
16 considerable savings in healthcare costs by possibly decreased prevalence  
17 of generational transmission of metabolic diseases.

18 We propose that an "early" OGTT combined with maternal history,  
19 maternal condition and promising new biomarkers such as glycosylated  
20 fibronectin (glyFn) could diagnose similarly GDM, even in first trimester.

21 Rasanen et al. published a study in September 2013 introducing glyFn as  
22 a new early GDM screening approach with an area under the curve (AUC)  
23 of 0.91 and a 95% confidence interval (CI) of 0.87-0.96, a positive  
24 predictive value of 63% and a negative predictive value of 95%. Although

1  
2  
3 1 some predictors of GDM have been studied retrospectively, no study to  
4  
5 2 date has considered the use of promising new biomarkers combined with  
6  
7  
8 3 an "early" OGTT and maternal risk factors evaluation in first trimester of  
9  
10 4 pregnancy.  
11

## 12 13 14 6 STUDY OBJECTIVES

### 15 16 17 7 Primary objective

18  
19 8 The use of the "early" OGTT 75g and/or the new biomarker, glyFn, as a  
20  
21 9 new screening approach in late first/early second trimester will be  
22  
23  
24 10 evaluated and compared to GDM diagnosis by OGTT 75g at 24 to 28  
25  
26 11 weeks of gestation in a normal cohort.  
27  
28

### 29 30 31 13 Secondary objectives

32  
33 14 1. A new screening algorithm will be created by using multivariable risk  
34  
35 15 estimation based on "early" OGTT 75g and/or glyFn results, incorporating  
36  
37 16 maternal risk factors.  
38

39  
40 17 2. The significance of the association between glyFn, "early" OGTT 75g  
41  
42 18 and maternal body mass index and/or clinical conditions including chronic  
43  
44 19 hypertension, pregnancy-induced hypertension or preeclampsia and fetal  
45  
46 20 conditions such as intrauterine growth restriction will be evaluated.  
47  
48

## 49 50 51 52 22 METHODS

### 53 54 55 23 Study settings/design

56  
57  
58  
59  
60

1 This is an international, prospective, multi-centre cohort trial conducted at  
2 one secondary and five tertiary referral centres in Switzerland, Austria and  
3 Germany. Study recruitment commenced primarily at the coordination  
4 centre at University Hospital Basel on 1<sup>st</sup> May 2014. All other centres  
5 started recruitment consecutively until the end of March 2016.  
6 Recruitment is expected to last until December 2017. The aim is to enroll  
7 531 women at 12 to 15 weeks of gestation with a minimum recruitment of  
8 50 women planned for each centre.

#### 10 Recruitment and informed consent

11 Participants are identified at their first antenatal visit between 6 to 15  
12 weeks of gestation. The investigator or obstetrician in charge informs the  
13 women about all aspects pertaining to the trial. The informed consent  
14 includes permission for gathering data from medical records and the  
15 optional storage of blood for a maximum of 10 years for additional  
16 analyses related to the current study. Participants are informed that trial  
17 participation is voluntary and that they are free to withdraw without any  
18 effects on subsequent care. All members of the research team are aware  
19 of the guidelines for good clinical practice for obtaining consent.<sup>10</sup>

#### 21 Eligibility criteria

22 Inclusion criteria are:

- 23 - women at least 18 years of age and not under guardianship

1  
2  
3 1 - healthy singleton pregnancy after spontaneous conception or after  
4  
5 2 fertility treatment

6  
7 3 - 6-15 weeks of gestation

8  
9 4 - signed informed consent

10  
11 5

12 6 Exclusion criteria are:

13  
14 7 - previous bariatric surgery

15  
16 8 - known pre-existing diabetes mellitus or under treatment with metformin

17  
18 9 - known chronic infection like hepatitis or human immunodeficiency virus

19  
20 10 or chronic kidney, liver or heart disease

21  
22 11 - known maternal history of hypertensive diseases in a previous  
23  
24 12 pregnancy and now under prophylactic acetylsalicylate treatment

25  
26 13 - fetal genetic, chromosomal or intervention-requiring morphologic  
27  
28 14 abnormalities

29  
30 15 - the inability to read and/or understand the participant`s information  
31  
32 16 sheet

33  
34 17

35  
36 18 Study procedure

37  
38 19 All healthy pregnant patients with regular care at the participating

39  
40 20 hospitals are counselled and asked at 6 to 15 weeks of gestation to

41  
42 21 participate. At 11+0 to 13+6 weeks of gestation, all women have a first

43  
44 22 trimester ultrasound scan which is standard care at participating sites. The

45  
46 23 ultrasound scan is used to confirm gestational age, diagnose any major

47  
48 24 foetal abnormalities, and optionally measure foetal nuchal translucency

49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 thickness, which together with maternal free beta-chorionic gonadotropin  
4  
5 2 and pregnancy-associated plasma protein A, is used for screening for  
6  
7 3 chromosomal abnormalities. In addition, if informed consent had been  
8  
9 4 given during the first antenatal visit or at the time of the first trimester  
10  
11 5 scan, the maternal history and condition are assessed, and blood for  
12  
13 6 biomarker analysis and for the "early" OGTT 75g is drawn at the study  
14  
15 7 visit at 12 to 15 weeks of gestation. The "early" OGTT 75g is compared to  
16  
17 8 plasma glucose results obtained at 24 to 28 weeks of gestation after the  
18  
19 9 OGTT 75g. No additional visit is necessary beyond the further standard  
20  
21 10 routine antenatal care visits.  
22  
23  
24  
25  
26  
27  
28

## 29 GlyFn and "early" OGTT 75g

30  
31 13 All participants are instructed to fast for at least 10 hours. Two fasting  
32  
33 14 glucose samples are taken. One sample is collected for storage of two  
34  
35 15 aliquots (2x 1ml) at -80 °C for later analysis of glyFn and another sample  
36  
37 16 for the fasting glucose value. After intake of the 75g glucose load, blood  
38  
39 17 samples are drawn 60 and 120 minutes later for determination of glucose  
40  
41 18 levels. Plasma glucose is measured by an automated colorimetric-  
42  
43 19 enzymatic method (hexokinase/glucose-6-phosphate-dehydrogenase) on  
44  
45 20 a Hitachi/Roche-Modular P analyser. GlyFn will be analysed as previously  
46  
47 21 reported by Rasanen et al.<sup>11</sup> by DiabetOmics Inc., Beaverton, Oregon,  
48  
49 22 USA. The maternal glyFn and "early" OGTT 75g results are blinded to the  
50  
51 23 investigators.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 Un-blinding  
4

5 2 Values will be un-blinded if fasting glucose levels are  $\geq 7.0$ mmol/L or 2-  
6  
7 3 hour plasma glucose levels are  $\geq 11.1$ mmol/L, suggesting pre-existing  
8  
9 4 diabetes mellitus. The diagnosis of pre-existing diabetes mellitus needs to  
10  
11 5 be confirmed by an elevated glycosylated haemoglobin A1c (HbA1c) value  
12  
13 6  $\geq 6.5\%$ . Plasma glucose levels of  $\leq 2.5$ mmol/L are also abnormal and  
14  
15 7 requires further clarification. Women with confirmed pre-existing diabetes  
16  
17 8 mellitus are treated according to a standardised protocol in line with  
18  
19 9 current recommendations.  
20  
21  
22  
23

24 10  
25  
26 11 Study outcomes  
27

28 12 Diagnosis of GDM  
29

30  
31 13 All women who screen positive with OGTT 75 g at 24 to 28 weeks of  
32  
33 14 gestation are followed up by a nutritionist and a diabetic nurse in contact  
34  
35 15 with a diabetologist, and have frequent regular appointments in our  
36  
37 16 obstetrical outpatient clinic in 2 to 4 week intervals depending on clinical  
38  
39 17 condition, glucose values and ultrasound findings. Women who fail to  
40  
41 18 meet the target glucose values after 1 to 2 weeks of diet management are  
42  
43 19 treated with insulin according to the guidelines of the Swiss Society for  
44  
45 20 Endocrinology and Diabetology (SGED), the Austrian Diabetes Association  
46  
47 21 (ÖDG), the German Diabetes Association (DDG) and the German  
48  
49 22 Association of Gynaecology and Obstetrics (DGGG).<sup>12-14</sup> The glycaemic  
50  
51 23 targets, insulin therapy, dose adjustments, concomitant medication and/or  
52  
53 24 supplements are recorded.  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1  
4  
5 2 Pregnancy, delivery and neonatal outcome data  
6  
7 3 Maternal data such as preeclampsia, pregnancy induced hypertension,  
8  
9 4 rate of sonographic estimated polyhydramnios or macrosomia, delivery  
10  
11 5 outcome including delivery mode and indication, and neonatal outcome  
12  
13 6 data such as birth weight, preterm birth, 5 and 10 minute Apgar scores,  
14  
15 7 arterial umbilical cord pH  $\leq$  7.0, shoulder dystocia, birth trauma,  
16  
17 8 hypoglycaemia, jaundice, respiratory distress syndrome, congenital  
18  
19 9 anomalies and admission to intensive care unit are prospectively collected.  
20  
21  
22  
23

## 24 25 26 11 Statistics

### 27 28 12 Sample size justification

29  
30  
31 13 We aim to demonstrate that the recently reported biomarker, glyFn  
32  
33 14 and/or the "early" OGTT 75g at 12 to 15 weeks of gestation has sufficient  
34  
35 15 diagnostic power to evaluate women at risk of developing GDM compared  
36  
37 16 to the OGTT 75g at 24 to 28 weeks of gestation. The sample size  
38  
39 17 calculation is based on a test for the ROC (receiver operator  
40  
41 18 characteristic) curve of glyFn by Rasanen et al. 2013.<sup>11</sup> The OGTT 75g  
42  
43 19 screening test using the IADPSG criteria has not been tested in early  
44  
45 20 pregnancy so far. We assume that 0.5-2% of recruited women will be  
46  
47 21 diagnosed with pre-existing diabetes mellitus by the "early" OGTT 75g.  
48  
49 22 The prevalence of GDM is assumed to be 17.8% according to the HAPO  
50  
51 23 study.<sup>5</sup> A proposed true area under the curve of 0.9 with a lower boundary  
52  
53 24 of 0.8 would lead with a power of 90% and an  $\alpha$ -level of 5% to an  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 estimated sample size of 462 (66 women with GDM, 396 women without  
4  
5 2 GDM). Offsetting a dropout of 15%, this leads to a total sample size of  
6  
7 3 531. The power calculation was performed using MedCalc version 12.7  
8  
9 4 2013.<sup>15</sup>  
10  
11  
12  
13

#### 14 6 Data analysis plan

15  
16  
17 7 Descriptive statistics and graphical examination will be performed for all  
18  
19 8 primary and secondary study variables.

20  
21 9 Primary objective: In order to predict GDM, ROC curves with  
22  
23 10 corresponding AUCs will be calculated separately for glyFn and the "early"  
24  
25 11 OGTT 75g. GDM diagnosis by OGTT 75g at 24 to 28 weeks of gestation is  
26  
27 12 considered as routine method. AUCs will be estimated with a 95%  
28  
29 13 confidence interval (CI). It will be hypothesized that a 95% CI of the AUC  
30  
31 14 of glyFn > 0.8.  
32  
33  
34

35  
36 15 Secondary objectives: Logistic regression will allow combining glyFn and  
37  
38 16 the "early" OGTT in a multivariable risk model. Subsequent AUC will be  
39  
40 17 calculated with 95% CI. Results will be internally cross-validated to  
41  
42 18 prevent overoptimistic results. Optimal cut-off points to predict GDM will  
43  
44 19 be determined based on these ROC curve. Sensitivity, specificity, positive  
45  
46 20 and negative predictive value will also be estimated with 95% CI.  
47  
48 21 However other "machine learning" algorithms could be better than logistic  
49  
50 22 regression. Therefore, other popular procedures will be additionally  
51  
52 23 tested: Random Forest, penalized logistic regression (Lasso). Details are  
53  
54 24 described in Hastie et al..<sup>16</sup> Predictive performance will be internally cross-  
55  
56  
57  
58  
59  
60



1 validated and explored in examining AUC of the ROC and predicted vs.  
2 observed probabilities. Random forest and penalized logistic regression  
3 avoid overfitting (to certain extent) whereas logistic regression does not.  
4 Random forest will be chosen because of its popularity and good  
5 benchmark results. Lasso is known for its good interpretability. Logistic  
6 regression will probably show its inferiority compared to the other  
7 methods. Internal cross-validation will be done using the package "mlr"  
8 within R. Internal cross-validation (e.g. 10-fold) is a good possibility to  
9 estimate the fitting on a potential future dataset. Based on these results it  
10 will be decided whether a new prediction model for GDM will be proposed.  
11 In order to potentially improve the primary study variables, secondary  
12 study parameters such as changes in maternal body mass index and/or  
13 clinical conditions like chronic hypertension and pregnancy induced  
14 hypertension or preeclampsia and fetal conditions including intrauterine  
15 growth restriction will be added to the machine learning models.  
16 Statistical analyses and graphs will be performed using the current version  
17 of the statistical computation program R.<sup>17</sup>

#### 19 Data recording

20 Each participant receives an identification number to ensure confidentiality  
21 and the collected data is exchanged between centres using only the  
22 identification number. The name and birth date of each participant are  
23 stored with a different identification number in order to preserve the  
24 possibility to look for inconsistencies during the study.

1

2 Reporting of adverse events

3 This study is a low risk trial. Any (serious) adverse events (AE/SAE)  
4 related to the first additional "early" OGTT 75g or the blood sampling are  
5 recorded by the investigator using the specific AE/SAE sheet of the clinical  
6 report form (CRF). All SAE are reported to the responsible ethics  
7 committee within an appropriate time frame.

8

## 9 ETHICS AND DISSEMINATION

10 The study is conducted in accordance with the "Helsinki Declaration"  
11 1996. It was approved by each local institutional ethical board in Basel,  
12 Zurich, Freiburg, Salzburg and Vienna. Furthermore, written informed  
13 consent is obtained from each participant. All findings will be disseminated  
14 through presentations at national and international conferences and  
15 publications in peer-reviewed journals. The trial was registered under  
16 [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) under NCT02035059 on 12<sup>th</sup> January 2014.

17

## 18 OTHER STUDY MEASUREMENTS

### 19 Other biomarkers

20 An extensively studied biomarker for early GDM screening is  
21 adiponectin.<sup>18-20</sup> Adiponectin is an adipocyte-derived hormone and reflects  
22 whole body insulin sensitivity.<sup>21</sup> A recently published meta-analysis  
23 calculated a summary sensitivity of 60.3% and a specificity of 81.3% with  
24 an AUC of 0.79.<sup>19</sup> Maternal serum adiponectin concentration is measured

59

60

1 by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA)  
2 technique. Another potential biomarker is pregnancy-specific glycoprotein-  
3 1 (PSG-1).<sup>22</sup> PSG-1 had a detection rate of 74% with a false positive rate  
4 of 6% and an AUC of 0.81. PSG-1 is analysed as previously reported by  
5 Nagalla et al.<sup>22</sup> by DiabetOmics Inc., Beaverton, Oregon, USA.

## 6 7 Evaluation of insulin and HbA1c

8 At the early study visit at 12 to 15 weeks of gestation, insulin, c-peptide  
9 and HbA1c are additionally measured from the fasting blood sample in all  
10 women. HbA1c is measured by reversed-phase cation exchange  
11 chromatography (ADAMS HA-8160, Menarini Diagnostics Benelux,  
12 Zaventem, Belgium) or high performance liquid chromatography (HPLC)  
13 with Variant II, Bio-Rad (IFCC standardized and DCCT aligned with CV  
14 1.8% for HbA1c=5.6%). Insulin is measured by chemiluminescence  
15 immunoassay CLIA (Roche Modular E170, Basel, Switzerland). The  
16 homeostasis model assessment of insulin resistance (HOMA-IR) or the  
17 Quantitative Insulin Sensitivity Check Index (QUICKI) is used as an  
18 approximate of fasting (i.e. hepatic) insulin resistance.<sup>23,24</sup> Beta-cell  
19 function is assessed from fasting glucose and insulin levels according to  
20 Wallace et al. 2004.<sup>23</sup>

## 21 22 Measurement of Vitamin D

23 25 OH Vitamin D seems to have an association with glucose metabolism  
24 and influences insulin secretion and sensitivity in type 1 diabetes.<sup>25</sup> It

1  
2  
3 1 additionally leads to an immunologic reaction resulting into type 2  
4  
5 2 diabetes.<sup>25</sup> We would like to have actual values for 25 OH Vitamin D and it  
6  
7 3 will be evaluated in this cohort by CLIA (Liaison, DiaSorin, Saluggia,  
8  
9 4 Italy).

10  
11  
12  
13  
14 6 Influence of stress in early pregnancy on development of GDM

15  
16  
17 7 Psychological factors in early pregnancy might contribute to adverse  
18  
19 8 obstetric outcome.<sup>26</sup> This trial investigates the influence of perceived  
20  
21 9 stress, stressful life events and depression on the development of GDM.

22  
23  
24 10 The participants recruited in Basel are therefore asked to collect salivary  
25  
26 11 samples for cortisol directly at time of awakening, at 30 and at 60 minutes  
27  
28 12 after awakening. The saliva samples are stored at -20 °C until analysis.

29  
30  
31 13 After thawing, saliva samples are centrifuged. Cortisol levels are  
32  
33 14 determined employing a competitive solid phase time-resolved  
34  
35 15 fluorescence immunoassay with fluorometric end point detection

36  
37  
38 16 (DELFI<sup>®</sup>).<sup>27</sup> Copeptin is a more stable precursor hormone of arginine-  
39  
40 17 vasopressin and is found to be elevated in many diseases.<sup>28-31</sup> This effect

41  
42  
43 18 can be attributed to the response of the hypothalamic-pituitary-adrenal  
44  
45 19 axis to psychological stress. Copeptin will be measured in the fasting

46  
47  
48 20 blood sample with time-resolved amplified cryptate emission technology  
49  
50 21 (TRACE<sup>™</sup>) by BRAHMS Kryptor Compact immunoanalyzer by Thermo

51  
52 22 Scientific Brahms GmbH, Henningsdorf, Germany.

53  
54  
55 23

1  
2  
3 1 Three self-administered questionnaires are obtained at the study visit at  
4  
5 2 12 to 15 weeks of gestation:  
6  
7  
8  
9

10 4 Questionnaire on perceived stress

11  
12 5 The perceived stress scale is a 10-items inventory for measuring the  
13  
14 6 perception of stress.<sup>32</sup> It is widely used and has been validated in  
15  
16 7 pregnancy.<sup>33</sup>  
17  
18  
19

20  
21  
22 9 Questionnaire on stressful life events

23  
24 10 The Holmes and Rahe Stressful Life Events Scale (SLE)<sup>34</sup> is an 43-items  
25  
26 11 instrument for evaluation of stressful experiences in the past 12 months  
27  
28 12 prior to the answering the questionnaire.  
29  
30  
31

32  
33 14 Questionnaire on depression

34  
35 15 The ten-items Edinburgh Postnatal Depression Screen (EPDS)<sup>35</sup> is used to  
36  
37 16 assess symptoms of depression during the past seven days. It has been  
38  
39 17 widely validated in pregnancy<sup>36,37</sup> and it is part of the standard evaluation  
40  
41 18 of pregnant women attending routine prenatal visits at the University  
42  
43 19 Hospital in Basel. Scores  $\geq 13$  indicate at least probable minor depression  
44  
45 20 and scores  $\geq 15$  indicated probable major depression. Pregnant women  
46  
47 21 with scores  $\geq 13$  are routinely offered psychological or psychiatric  
48  
49 22 counselling during pregnancy.  
50  
51  
52  
53  
54

55  
56  
57 24 Histologic examination of the placenta  
58  
59  
60

1  
2  
3 1 The fetal nutrients supply is regulated by maternal-foetal glucose and lipid  
4 concentration, placental blood flow and trophoblastic nutrient  
5  
6 2 concentration, placental blood flow and trophoblastic nutrient  
7  
8 3 transporters.<sup>38</sup> The placenta reacts with adaptive changes in structure and  
9  
10 4 function to a hyperglycaemic milieu.<sup>39</sup> These changes of the placenta will  
11  
12 5 be assessed depending on the maternal glycaemic control by standard  
13  
14 6 pathology examination.<sup>40</sup>  
15  
16  
17  
18  
19

## 20 8 DISCUSSION

21  
22 9 To our knowledge, this is the first large European cohort study that  
23  
24 10 prospectively evaluates the promising new biomarker, glyFn, and the  
25  
26 11 "early" OGTT 75g by comparing the impact of different GDM screening  
27  
28 12 strategies with IADPSG criteria at 24 to 28 weeks of gestation. Our  
29  
30 13 hypothesis is that glyFn and/or the "early" OGTT 75g should at least result  
31  
32 14 in an AUC of 0.8 compared to the OGTT 75g in later pregnancy. A  
33  
34 15 multivariable prediction model incorporating risk factors, glyFn and/or  
35  
36 16 "early" OGTT values might have a good predictive accuracy for the  
37  
38 17 development of GDM and could facilitate early universal screening or help  
39  
40 18 improve risk stratification for GDM. Rasanen et al.<sup>11</sup> could show that glyFn  
41  
42 19 was independent of maternal age, parity, gestational age, time of sample  
43  
44 20 collection and the administration of the OGTT at 24 to 28 weeks of  
45  
46 21 gestation. Our trial might clarify whether glyFn is depending on maternal  
47  
48 22 body mass index or clinical conditions such as chronic hypertension,  
49  
50 23 pregnancy induced hypertension or the development of preeclampsia or  
51  
52 24 intrauterine growth restriction. GlyFn can be analyzed out of a dried blood  
53  
54  
55  
56  
57  
58  
59  
60

1 stain and the resulting test is affordable, which would help especially  
2 underdeveloped countries that suffer disparities in diabetes care, to  
3 benefit from a cheap screening tool for GDM.<sup>41</sup>

4 One limitation of our trial is that it is not powered for the evaluation of  
5 neonatal outcomes like LGA infants, neonatal hypoglycaemia, shoulder  
6 dystocia or birth trauma (these are planned secondary outcomes). The  
7 study would need to be significantly larger to detect effects on these  
8 neonatal outcomes. If this trial were to show positive diagnostic power for  
9 the new screening algorithm, a large multi-centre study may be required  
10 to be sufficiently powered to determine the algorithm's effect on rate of  
11 LGA infants or neonatal hypoglycaemia as proposed in the HAPO study.<sup>5</sup>

12 The results of our study may have a major impact on future screening  
13 approaches for GDM by development of a simple, cost effective and for  
14 the pregnant women comfortable screening method for GDM in first  
15 trimester.

16

### 17 **List of abbreviations**

18 AE, adverse events; AUC, area under the curve; BMI, body mass index;  
19 CRF, clinical report form; GDM, gestational diabetes mellitus; GlyFn,  
20 glycosylated Fibronectin; HOMA-IR, homeostasis model assessment of  
21 insulin resistance; IADPSG, International Association of Diabetes and  
22 Pregnancy Study Groups; LGA, large for gestational age; OGTT, oral  
23 glucose tolerance test; QUICKI, quantitative insulin sensitivity check  
24 index; ROC, receiver operator characteristic; SAE, serious adverse events.

1

## 2 **Disclosure of interests**

3 The authors declare that there are no further financial or personal  
4 relationships with other people or organizations that could inappropriately  
5 influence the work reported or the conclusions, implications, or opinions  
6 stated.

7

## 8 **Contribution to authorship**

9 EH is principal investigator, study protocol author, obtained ethical  
10 approval and drafted this manuscript. IH is the sponsor, assisted with the  
11 original study protocol and revised the manuscript. SH and EBR designed  
12 the study together with EAH and revised the manuscript. AS performed  
13 the power calculation and statistical planning. TF, CSG, WE, PH, MK, NO,  
14 EBAE, HP, MT, MK, RZ made important contributions and critically  
15 reviewed the content. All authors have given final approval of the version  
16 to be published.

17

## 18 **Acknowledgements**

19 The authors would like to thank DiabetOmics Inc., Beaverton, Oregon,  
20 USA for their support in the analysis of glyFn and the help preparing this  
21 manuscript. We also would like to thank Dorothy Huang for critical  
22 proofreading in English and Cristina Granado and Doris Mueller Borer for  
23 their precious assistance in performing the study visits and in completing

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60



1 the data acquisition. Special thanks to all families who participate in this  
2 study.

### 4 **Details of ethics approval**

5 The study was approved by each local institutional review board in Basel  
6 (Ethikkommission Nordostschweiz (EKNZ)), Zurich, Freiburg, Salzburg and  
7 Vienna. The trial was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) under  
8 NCT02035059 on 12<sup>th</sup> January 2014.

### 10 **Funding**

11 This study is supported by the Diabetes Society Basel, Switzerland and the  
12 Gottfried and Julia Bangerter-Rhyner-Foundation, Bern, Switzerland.

13

1  
2  
3 1 REFERENCES:

- 4 1. Report of the Expert Committee on the Diagnosis and Classification  
5 of Diabetes Mellitus. *Diabetes Care*. 1997;20(7):1183-1197.  
6 <http://www.ncbi.nlm.nih.gov/pubmed/9203460>. Accessed February  
7 23, 2015.
- 8 2. Classification and Diagnosis of Diabetes. *Diabetes Care*.  
9 2014;38(Supplement\_1):S8-S16. doi:10.2337/dc15-S005.
- 10 3. Aziz NL, Abdelwahab S, Moussa M, Georgy M. Maternal fructosamine  
11 and glycosylated haemoglobin in the prediction of gestational glucose  
12 intolerance. *Clin Exp Obstet Gynecol*. 1992;19(4):235-241.  
13 <http://www.ncbi.nlm.nih.gov/pubmed/1294344>. Accessed January  
14 27, 2016.
- 15 4. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson  
16 JS. Effect of treatment of gestational diabetes mellitus on pregnancy  
17 outcomes. *N Engl J Med*. 2005;352(24):2477-2486.  
18 doi:10.1056/NEJMoa042973.
- 19 5. Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse  
20 pregnancy outcomes. *N Engl J Med*. 2008;358(19):1991-2002.  
21 doi:10.1056/NEJMoa0707943.
- 22 6. Casey BM, Lucas MJ, McIntire DD, Leveno KJ. Pregnancy outcomes in  
23 women with gestational diabetes compared with the general obstetric  
24 population. *Obstet Gynecol*. 1997;90(6):869-873.  
25 <http://www.ncbi.nlm.nih.gov/pubmed/9397092>. Accessed January  
26 27, 2016.
- 27 7. Bellamy L, Casas J-P, Hingorani AD, Williams D. Type 2 diabetes  
28 mellitus after gestational diabetes: a systematic review and meta-  
29 analysis. *Lancet*. 2009;373:1773-1779.  
30 [http://www.scopus.com/inward/record.url?eid=2-s2.0-  
31 65649084159&partnerID=40&md5=9b8db7211090a8100741c6e532  
32 63fa08](http://www.scopus.com/inward/record.url?eid=2-s2.0-65649084159&partnerID=40&md5=9b8db7211090a8100741c6e53263fa08).
- 33 8. Osgood ND, Dyck RF, Grassmann WK. The inter- and  
34 intragenerational impact of gestational diabetes on the epidemic of  
35 type 2 diabetes. *Am J Public Health*. 2011;101(1):173-179.  
36 doi:10.2105/AJPH.2009.186890.
- 37 9. Metzger BE, Gabbe SG, Persson B, et al. International association of  
38 diabetes and pregnancy study groups recommendations on the  
39 diagnosis and classification of hyperglycemia in pregnancy. *Diabetes  
40 Care*. 2010;33(3):676-682. doi:10.2337/dc09-1848.
- 41 10. ICH harmonized tripartite guideline: Guideline for Good Clinical  
42 Practice. *J Postgrad Med*. 47(1):45-50.  
43 <http://www.ncbi.nlm.nih.gov/pubmed/11590294>. Accessed October  
44 6, 2015.
- 45 11. Rasanen JP, Snyder CK, Rao P V, et al. Glycosylated fibronectin as a

- 1 first-trimester biomarker for prediction of gestational diabetes.  
2 *Obstet Gynecol.* 2013;122(3):586-594.  
3 doi:10.1097/AOG.0b013e3182a0c88b.
- 4 12. Lehmann R, Troendle A, Brändle M. [New insights into diagnosis and  
5 management of gestational diabetes mellitus: recommendations of  
6 the Swiss Society for Endocrinology and Diabetes]. *Ther Umsch.*  
7 2009;66(10):695-706. doi:10.1024/0040-5930.66.10.695.
- 8 13. Kleinwechter H, Schäfer-Graf U, Bühner C, et al. Gestational diabetes  
9 mellitus (GDM) diagnosis, therapy and follow-up care: Practice  
10 Guideline of the German Diabetes Association(DDG) and the German  
11 Association for Gynaecology and Obstetrics (DGGG). *Exp Clin*  
12 *Endocrinol Diabetes.* 2014;122(7):395-405. doi:10.1055/s-0034-  
13 1366412.
- 14 14. Kautzky-Willer A, Bancher-Todesca D, Pollak A, Repa A, Lechleitner  
15 M, Weitgasser R. [Gestational diabetes mellitus]. *Wien Klin*  
16 *Wochenschr.* 2012;124 Suppl :58-65. doi:10.1007/s00508-012-  
17 0265-3.
- 18 15. Hanley JA, McNeil BJ. The meaning and use of the area under a  
19 receiver operating characteristic (ROC) curve. *Radiology.*  
20 1982;143(1):29-36. doi:10.1148/radiology.143.1.7063747.
- 21 16. Hastie T, Tibshirani R and FJ. *The Elements of Statistical Learning:*  
22 *Prediction, Inference and Data Mining.* Springer Verlag; 2009.
- 23 17. R Development Core Team R. R: A Language and Environment for  
24 Statistical Computing. *R Found Stat Comput.* 2014;1:409.  
25 doi:10.1007/978-3-540-74686-7.
- 26 18. Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaides KH.  
27 Prediction of gestational diabetes mellitus by maternal factors and  
28 biomarkers at 11 to 13 weeks. *Prenat Diagn.* 2011;31(2):135-141.  
29 doi:10.1002/pd.2636.
- 30 19. Iliodromiti S, Sassarini J, Kelsey TW, Lindsay RS, Sattar N, Nelson  
31 SM. Accuracy of circulating adiponectin for predicting gestational  
32 diabetes: a systematic review and meta-analysis. *Diabetologia.*  
33 January 2016. doi:10.1007/s00125-015-3855-6.
- 34 20. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational  
35 diabetes. *Lancet Diabetes Endocrinol.* 2014;2(6):488-499.  
36 doi:10.1016/S2213-8587(13)70176-1.
- 37 21. Yadav A, Kataria MA, Saini V, Yadav A. Role of leptin and adiponectin  
38 in insulin resistance. *Clin Chim Acta.* 2013;417:80-84.  
39 doi:10.1016/j.cca.2012.12.007.
- 40 22. Nagalla SR, Snyder CK, Michaels JE, et al. Maternal serum  
41 biomarkers for risk assessment in gestational diabetes. A potential  
42 universal screening test to predict GDM status. *Indian J Endocrinol*  
43 *Metab.* 19(1):155-159. doi:10.4103/2230-8210.140226.

- 1  
2  
3 1 23. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA  
4 2 modeling. *Diabetes Care*. 2004;27(6):1487-1495.  
5 3 <http://www.ncbi.nlm.nih.gov/pubmed/15161807>. Accessed August  
6 4 17, 2015.
- 7  
8 5 24. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity  
9 6 check index: a simple, accurate method for assessing insulin  
10 7 sensitivity in humans. *J Clin Endocrinol Metab*. 2000;85(7):2402-  
11 8 2410. doi:10.1210/jcem.85.7.6661.
- 12 9 25. Tai K, Need AG, Horowitz M, Chapman IM. Vitamin D, glucose,  
13 10 insulin, and insulin sensitivity. *Nutrition*. 2008;24(3):279-285.  
14 11 doi:10.1016/j.nut.2007.11.006.
- 15 12 26. Alder J, Fink N, Bitzer J, Hösli I, Holzgreve W. Depression and  
16 13 anxiety during pregnancy: a risk factor for obstetric, fetal and  
17 14 neonatal outcome? A critical review of the literature. *J Matern Fetal*  
18 15 *Neonatal Med*. 2007;20(3):189-209.  
19 16 doi:10.1080/14767050701209560.
- 20 17 27. Dressendörfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ.  
21 18 Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in  
22 19 an immunoassay for salivary cortisol measurement. *J Steroid*  
23 20 *Biochem Mol Biol*. 1992;43(7):683-692.  
24 21 <http://www.ncbi.nlm.nih.gov/pubmed/1472460>. Accessed January  
25 22 21, 2016.
- 26 23 28. Christ-Crain M, Fenske W. Copeptin in the diagnosis of vasopressin-  
27 24 dependent disorders of fluid homeostasis. *Nat Rev Endocrinol*.  
28 25 2016;12(3):168-176. doi:10.1038/nrendo.2015.224.
- 29 26 29. Reinstadler SJ, Klug G, Feistritz H-J, Metzler B, Mair J. Copeptin  
30 27 testing in acute myocardial infarction: ready for routine use? *Dis*  
31 28 *Markers*. 2015;2015:614145. doi:10.1155/2015/614145.
- 32 29 30. Zhang R, Liu J, Zhang Y, Liu Q, Li T, Cheng L. Association Between  
33 30 Circulating Copeptin Level and Mortality Risk in Patients with  
34 31 Intracerebral Hemorrhage: a Systemic Review and Meta-Analysis.  
35 32 *Mol Neurobiol*. January 2016. doi:10.1007/s12035-015-9626-z.
- 36 33 31. Viasus D, Del Rio-Pertuz G, Simonetti AF, et al. Biomarkers for  
37 34 predicting short-term mortality in community-acquired pneumonia: A  
38 35 systematic review and meta-analysis. *J Infect*. January 2016.  
39 36 doi:10.1016/j.jinf.2016.01.002.
- 40 37 32. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived  
41 38 stress. *J Health Soc Behav*. 1983;24(4):385-396.  
42 39 <http://www.ncbi.nlm.nih.gov/pubmed/6668417>. Accessed February  
43 40 9, 2015.
- 44 41 33. Silveira ML, Whitcomb BW, Pekow P, et al. Perceived psychosocial  
45 42 stress and glucose intolerance among pregnant Hispanic women.  
46 43 *Diabetes Metab*. 2014;40(6):466-475.  
47 44 doi:10.1016/j.diabet.2014.05.002.

- 1  
2  
3 1 34. Holmes TH, Rahe RH. The Social Readjustment Rating Scale. *J*  
4 2 *Psychosom Res.* 1967;11(2):213-218.  
5 3 <http://www.ncbi.nlm.nih.gov/pubmed/6059863>. Accessed October  
6 4 28, 2015.
- 7  
8 5 35. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression.  
9 6 Development of the 10-item Edinburgh Postnatal Depression Scale.  
10 7 *Br J Psychiatry.* 1987;150:782-786.  
11 8 <http://www.ncbi.nlm.nih.gov/pubmed/3651732>. Accessed March 16,  
12 9 2015.
- 13  
14 10 36. Byrn M, Penckofer S. The relationship between gestational diabetes  
15 11 and antenatal depression. *J Obstet Gynecol Neonatal Nurs.*  
16 12 44(2):246-255. doi:10.1111/1552-6909.12554.
- 17  
18 13 37. Ertel KA, Silveira M, Pekow P, et al. Prenatal depressive symptoms  
19 14 and abnormalities of glucose tolerance during pregnancy among  
20 15 Hispanic women. *Arch Womens Ment Health.* 2014;17(1):65-72.  
21 16 doi:10.1007/s00737-013-0379-2.
- 22  
23 17 38. Desoye G, Gauster M, Wadsack C. Placental transport in pregnancy  
24 18 pathologies. *Am J Clin Nutr.* 2011;94(6 Suppl):1896S - 1902S.  
25 19 doi:10.3945/ajcn.110.000851.
- 26  
27 20 39. Jarmuzek P, Wielgos M, Bomba-Opon D. Placental pathologic  
28 21 changes in gestational diabetes mellitus. *Neuro Endocrinol Lett.*  
29 22 2015;36(2):101-105.  
30 23 <http://www.ncbi.nlm.nih.gov/pubmed/26071574>. Accessed  
31 24 December 4, 2015.
- 32  
33 25 40. Bernischke K, Burton GJ BR. *Pathology of the Human Placenta.* Vol  
34 26 Sixth. 2012.
- 35  
36 27 41. Rasanen J, Quinn MJ, Laurie A, et al. Maternal serum glycosylated  
37 28 fibronectin as a point-of-care biomarker for assessment of  
38 29 preeclampsia. *Am J Obstet Gynecol.* 2015;212(1):82.e1-e9.  
39 30 doi:10.1016/j.ajog.2014.07.052.
- 40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description
<b>Administrative information</b>		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
	3	Date and version identifier
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
<b>Introduction</b>		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)



**Methods: Participants, interventions, and outcomes**

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

**Methods: Assignment of interventions (for controlled trials)**

## Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
---------------------	-----	--

1			
2	Allocation	16b	Mechanism of implementing the allocation sequence (eg, central
3	concealment		telephone; sequentially numbered, opaque, sealed envelopes),
4	mechanism		describing any steps to conceal the sequence until interventions are
5			assigned
6			
7	Implementation	16c	Who will generate the allocation sequence, who will enrol participants,
8			and who will assign participants to interventions
9			
10	Blinding	17a	Who will be blinded after assignment to interventions (eg, trial
11	(masking)		participants, care providers, outcome assessors, data analysts), and
12			how
13			
14		17b	If blinded, circumstances under which unblinding is permissible, and
15			procedure for revealing a participant's allocated intervention during
16			the trial
17			
18			

### Methods: Data collection, management, and analysis

20			
21	Data collection	18a	Plans for assessment and collection of outcome, baseline, and other
22	methods		trial data, including any related processes to promote data quality (eg,
23			duplicate measurements, training of assessors) and a description of
24			study instruments (eg, questionnaires, laboratory tests) along with
25			their reliability and validity, if known. Reference to where data
26			collection forms can be found, if not in the protocol
27			
28			
29		18b	Plans to promote participant retention and complete follow-up,
30			including list of any outcome data to be collected for participants who
31			discontinue or deviate from intervention protocols
32			
33	Data	19	Plans for data entry, coding, security, and storage, including any
34	management		related processes to promote data quality (eg, double data entry;
35			range checks for data values). Reference to where details of data
36			management procedures can be found, if not in the protocol
37			
38			
39	Statistical	20a	Statistical methods for analysing primary and secondary outcomes.
40	methods		Reference to where other details of the statistical analysis plan can be
41			found, if not in the protocol
42			
43		20b	Methods for any additional analyses (eg, subgroup and adjusted
44			analyses)
45			
46		20c	Definition of analysis population relating to protocol non-adherence
47			(eg, as randomised analysis), and any statistical methods to handle
48			missing data (eg, multiple imputation)
49			

### Methods: Monitoring

50			
51			
52	Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role
53			and reporting structure; statement of whether it is independent from
54			the sponsor and competing interests; and reference to where further
55			details about its charter can be found, if not in the protocol.
56			Alternatively, an explanation of why a DMC is not needed
57			
58			
59			
60			



1		21b	Description of any interim analyses and stopping guidelines, including
2			who will have access to these interim results and make the final
3			decision to terminate the trial
4			
5			
6	Harms	22	Plans for collecting, assessing, reporting, and managing solicited and
7			spontaneously reported adverse events and other unintended effects
8			of trial interventions or trial conduct
9			
10	Auditing	23	Frequency and procedures for auditing trial conduct, if any, and
11			whether the process will be independent from investigators and the
12			sponsor
13			

### Ethics and dissemination

14			
15			
16			
17	Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
18			
19			
20	Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
21			
22			
23			
24			
25			
26	Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
27			
28			
29		26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
30			
31	Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
32			
33			
34			
35			
36	Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
37			
38			
39	Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
40			
41			
42			
43	Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
44			
45			
46	Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
47			
48			
49			
50			
51			
52		31b	Authorship eligibility guidelines and any intended use of professional writers
53			
54			
55		31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
56			
57			
58			
59			
60			

## Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

---

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.

# BMJ Open

## Screening of gestational diabetes mellitus in early pregnancy by oral glucose tolerance test and glycosylated fibronectin: study protocol for an international, prospective, multi-centre cohort trial.



Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2016-012115.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Jul-2016
Complete List of Authors:	Huhn, Evelyn; Frauenklinik Universitatsspital Basel, Obstetrics and Gynaecology Fischer, Thorsten; Paracelsus Medizinische Privatuniversitat, Obstetrics and Gynaecology Göbl, Christian; Medizinische Universität Wien, Obstetrics and Gynaecology, Division of Obstetrics and feto-maternal Medicine Todesco-Bernasconi, Monya; Kantonsspital,, Obstetrics and Gynaecology Kreft, Martina; UniversitatsSpital Zurich, Obstetrics and Gynaecology Kunze, Mirjam; Universitatsklinikum Freiburg, Obstetrics and Gynaecology Schoetzau, Andreas; Universitatsspital Basel, Obstetrics and Gynaecology Dölzlmüller, Eva; Paracelsus Medizinische Privatuniversitat, Obstetrics and Gynaecology Eppel, Wolfgang; Medizinische Universität Wien, Obstetrics and Gynaecology, Division of Obstetrics and feto-maternal Medicine Husslein, Peter; Medizinische Universität Wien, Obstetrics and Gynaecology, Division of Obstetrics and feto-maternal Medicine Ochsenbein-Koelble, Nicole; UniversitatsSpital Zurich, Obstetrics and Gynaecology Zimmermann, Roland; UniversitatsSpital Zurich, Obstetrics and Gynaecology Bäz, Elke; Universitatsklinikum Freiburg Prömpeler, Heinrich; Universitatsklinikum Freiburg Bruder, Elisabeth; Universitatsspital Basel Institut fur Pathologie, Pathology Hahn, Sinuhe; Universität Basel, Biomedicine, Laboratory of Perinatology Hoesli, Irene; University Hospital, University Basel, Obstetrics and Gynaecology
<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Diagnostics, Obstetrics and gynaecology
Keywords:	gestational diabetes mellitus, diagnosis, screening, oral glucose tolerance test, glycosylated fibronectin, pregnancy

SCHOLARONE™  
Manuscripts

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

For peer review only

BMJ Open: first published as 10.1136/bmjopen-2016-012115 on 12 October 2016. Downloaded from <http://bmjopen.bmj.com/> on April 23, 2024 by guest. Protected by copyright.

## Original Article

### 1 Screening of gestational diabetes mellitus in early pregnancy by 2 oral glucose tolerance test and glycosylated fibronectin: study 3 protocol for an international, prospective, multi-centre cohort 4 trial.

5  
6 Huhn E.A.<sup>1\*</sup>, Fischer T.<sup>2</sup>, Göbl C.S.<sup>3</sup>, Todesco Bernasconi M.<sup>4</sup>, Kreft M.<sup>5</sup>,  
7 Kunze M.<sup>6</sup>, Schoetzau A.<sup>1</sup>, Dölzlmüller E.<sup>2</sup>, Eppel W.<sup>3</sup>, Husslein P.<sup>3</sup>,  
8 Ochsenbein-Koelble N.<sup>5</sup>, Zimmermann R.<sup>5</sup>, Bänz E.<sup>6</sup>, Proempeler H.<sup>6</sup>,  
9 Bruder E.<sup>7</sup>, Hahn S.<sup>8</sup>, Hoesli I.<sup>1</sup>.

10  
11 <sup>1</sup> Department of Obstetrics and Gynaecology, University Hospital Basel,  
12 Switzerland

13 <sup>2</sup> Department of Obstetrics and Gynaecology, Salzburger  
14 Landeskrankenhaus, Paracelsus Medical University, Salzburg, Austria

15 <sup>3</sup> Department of Obstetrics and Gynaecology, Division of Obstetrics and  
16 feto-maternal Medicine, Medical University of Vienna, Austria

17 <sup>4</sup> Department of Obstetrics and Gynaecology, Cantonal Hospital Aarau,  
18 Switzerland

19 <sup>5</sup> Department of Obstetrics and Gynaecology, University Hospital Zurich,  
20 Switzerland

21 <sup>6</sup> Department of Obstetrics and Gynaecology, University Hospital Freiburg,  
22 Germany

23 <sup>7</sup> Department of Pathology, University Hospital Basel, Switzerland

1  
2  
3 1 <sup>8</sup> Department of Biomedicine, Laboratory of Perinatology, University Basel,  
4  
5 2 Switzerland  
6  
7  
8  
9  
10 4

11  
12 5 \*Correspondence to: Evelyn A. Huhn, Department of Obstetrics and  
13  
14 6 Gynaecology, University Hospital Basel, Spitalstrasse 21, 4031 Basel,  
15  
16 7 Switzerland  
17

18  
19 8 Phone: +41 61 556 51 44  
20

21 9 E-mail: evelyn.huhn@usb.ch  
22  
23

24  
25  
26 10 Trial was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) under NCT02035059 on  
27  
28 11 the 12<sup>th</sup> January 2014.  
29  
30  
31 12

### 32 33 13 **Conflict of interest**

34  
35  
36 The authors declare that there are no further financial or personal  
37  
38 relationships with other people or organizations that could inappropriately  
39  
40 influence the work reported or the conclusions, implications, or opinions  
41  
42 stated.  
43  
44  
45  
46  
47

48 14 **KEY WORDS:** Gestational diabetes mellitus, diagnosis, screening, oral  
49  
50 15 glucose tolerance test, glycosylated fibronectin, pregnancy.  
51  
52 16

53  
54  
55 17 **Short title:** Screening of gestational diabetes mellitus in early pregnancy  
56  
57 18 by oral glucose tolerance test and glycosylated fibronectin.  
58  
59  
60

1  
2  
3 1  
4  
5 2 Manuscript includes: 21 text pages (31 in total with front page,  
6  
7 3 references)  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 ABSTRACT  
4

5 2 INTRODUCTION: As the accurate diagnosis and treatment of gestational  
6  
7 3 diabetes mellitus (GDM) is of increasing importance, new diagnostic  
8  
9 4 approaches for the assessment of GDM in early pregnancy were recently  
10  
11 5 suggested. We evaluate the diagnostic power of an "early" oral glucose  
12  
13 6 tolerance test (OGTT) 75g and glycosylated Fibronectin (glyFn) for GDM  
14  
15 7 screening in a normal cohort.  
16  
17

18 8 METHODS AND ANALYSIS: In a prospective cohort study, 748 singleton  
19  
20 9 pregnancies are recruited in six centres in Switzerland, Austria and  
21  
22 10 Germany. Women are screened for pre-existing diabetes mellitus and  
23  
24 11 GDM by an "early" OGTT 75g and/or the new biomarker, glyFn, at 12 to  
25  
26 12 15 weeks of gestation. Different screening strategies are compared to  
27  
28 13 evaluate the impact on detection of GDM by an OGTT 75g at 24 to 28  
29  
30 14 weeks of gestation as recommended by the International Association of  
31  
32 15 Diabetes and Pregnancy Study Groups (IADPSG). A new screening  
33  
34 16 algorithm is created by using multivariable risk estimation based on  
35  
36 17 "early" OGTT 75g and/or glyFn results, incorporating maternal risk factors.  
37  
38 18 Recruitment began in May 2014.  
39  
40  
41  
42  
43  
44

45 19 ETHICS AND DISSEMINATION: This study received ethical approval from  
46  
47 20 the ethics committees in Basel, Zurich, Vienna, Salzburg and Freiburg. It  
48  
49 21 was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) (NCT02035059) on 12<sup>th</sup>  
50  
51 22 January 2014. Data will be presented at international conferences and  
52  
53 23 published in peer-reviewed journals.  
54  
55  
56  
57  
58  
59  
60



1  
2  
3 1 STRENGTHS AND LIMITATION OF THIS STUDY  
4

- 5 2 - This is an international, prospective, multi-centre cohort trial  
6  
7 3 recruiting at six centres in Switzerland, Austria and Germany.  
8  
9 4 - It is the first study to assess an "early" OGTT 75g and novel  
10  
11 5 biomarkers like glyFn for screening of gestational diabetes mellitus  
12  
13 6 in early pregnancy.  
14  
15 7 - The recruitment of 748 pregnant women is planned. We have  
16  
17 8 designed the study to be sufficiently powered to compare the  
18  
19 9 different early screening approaches with the detection of  
20  
21 10 gestational diabetes mellitus at 24 to 28 weeks of gestation.  
22  
23 11 - This study may be underpowered for the evaluation of neonatal  
24  
25 12 outcomes like LGA infants, neonatal hypoglycaemia, shoulder  
26  
27 13 dystocia or birth trauma (secondary outcomes).  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 1 INTRODUCTION

2 Gestational diabetes mellitus (GDM) is defined as diabetes diagnosed  
3 during pregnancy that is not clearly overt diabetes.<sup>1</sup> The increasing  
4 number of women with undiagnosed type 2 diabetes mellitus (T2DM) in  
5 pregnancy has led to the recommendation of screening women with risk  
6 factors for pre-existing diabetes at the first antenatal visit. GDM is still  
7 diagnosed in the late second or early third trimester, because accurate  
8 diagnostic approaches for GDM assessment in first trimester are still  
9 lacking.<sup>2</sup>

10 GDM is associated with adverse maternal and perinatal outcomes, such as  
11 fetal overgrowth, shoulder dystocia, operative delivery, birth injury,  
12 preeclampsia, haemorrhage and preterm delivery,<sup>3-5</sup> but also a seven fold  
13 higher risk of the mother developing T2DM after pregnancy.<sup>6</sup> In addition,  
14 the maternal metabolic milieu was also identified as a key determinant for  
15 the susceptibility to obesity, metabolic syndrome and T2DM in the  
16 offspring,<sup>7</sup> a phenomenon often described as "fetal programming".

17 The current – but still widely discussed – standard of care in GDM  
18 screening is the oral glucose tolerance test (OGTT) of 75g glucose  
19 performed late at 24 to 28 weeks of gestation as recommended by the  
20 International Association of Diabetes and Pregnancy Study Groups  
21 (IADPSG).<sup>8</sup> The new screening thresholds are based on the results of a  
22 large prospective cohort multicentre trial, the Hyperglycaemia and  
23 Adverse Pregnancy Outcome (HAPO) study.<sup>4</sup> The aim of the HAPO study  
24 was to associate the degree of maternal glycaemia with adverse perinatal

1 outcome, such as large for gestational age infants (LGA), neonatal  
2 hypoglycaemia and caesarean section rates. The results showed no  
3 obvious threshold, but rather a continuous increase of these adverse  
4 outcomes across the range of glucose concentrations. The IADPSG criteria  
5 resulted in a considerable increase in GDM prevalence of 17.8%, a  
6 detection rate of 83% for adverse outcome and a positive predictive value  
7 of 16%.<sup>8</sup>

8 An early and rapid diagnosis of GDM even before 24 weeks of gestation is  
9 desirable. By targeted early intervention including physical activity,  
10 moderate diet or insulin/drug therapy starting in the first trimester, rates  
11 of macrosomia (birth weight > 4000 g) or large for gestational age  
12 (LGA=birth weight > 90<sup>th</sup> percentile) infants, operative vaginal delivery  
13 and perinatal morbidity could be possibly reduced. Moreover, there could  
14 be a long term downstream effect on the offspring, thereby leading to  
15 considerable savings in healthcare costs by possibly decreased prevalence  
16 of generational transmission of metabolic diseases. But further research is  
17 necessary to evaluate the effects of an early intervention on short and  
18 long term outcomes for mother and child.

19 We propose that an "early" OGTT combined with maternal history,  
20 maternal condition and promising new biomarkers such as glycosylated  
21 fibronectin (glyFn) could diagnose similarly GDM, even in first trimester.  
22 Rasanen et al. published a study in September 2013 introducing glyFn as  
23 a new early GDM screening approach with an area under the curve (AUC)  
24 of 0.91 and a 95% confidence interval (CI) of 0.87-0.96, a positive

1  
2  
3 1 predictive value of 63% and a negative predictive value of 95%. Although  
4  
5 2 some predictors of GDM have been studied retrospectively, no study to  
6  
7 3 date has considered the use of promising new biomarkers combined with  
8  
9  
10 4 an "early" OGTT and maternal risk factors evaluation in first trimester of  
11  
12 5 pregnancy.  
13  
14  
15 6

## 17 7 STUDY OBJECTIVES

### 19 8 Primary objective

21 9 The use of the "early" OGTT 75g and/or the new biomarker, glyFn, as a  
22 10 new screening approach in late first/early second trimester will be  
23 11 evaluated and compared to GDM diagnosis by OGTT 75g at 24 to 28  
24 12 weeks of gestation.  
25  
26  
27  
28  
29  
30  
31 13

### 33 14 Secondary objectives

- 35 15 1. A new screening algorithm will be created by using multivariable risk  
36 16 estimation based on "early" OGTT 75g and/or glyFn results, incorporating  
37 17 maternal risk factors.  
38 18 2. The significance of the association between glyFn, "early" OGTT 75g  
39 19 and maternal body mass index and/or clinical conditions including chronic  
40 20 hypertension, pregnancy-induced hypertension or preeclampsia and fetal  
41 21 conditions such as intrauterine growth restriction will be evaluated.  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55 23

## 56 24 METHODS

1 This study protocol was developed on the basis of Standard Protocol  
2 Items: Recommendations for Interventional Trials (SPIRIT) (see the  
3 supplementary "SPIRIT checklist" for further details).

#### 4 5 Study settings/design

6 This is an international, prospective, multi-centre cohort trial conducted at  
7 one secondary and five tertiary referral centres in Switzerland, Austria and  
8 Germany. Study recruitment commenced primarily at the coordination  
9 centre at University Hospital Basel on 1<sup>st</sup> May 2014. All other centres  
10 started recruitment consecutively until the end of March 2016.  
11 Recruitment is expected to last until December 2017 (see Figure 1 for  
12 details about expected time frame). The aim is to enrol 748 women at 12  
13 to 15 weeks of gestation with a minimum recruitment of 50 women  
14 planned for each centre dependent on size and time of recruitment  
15 (Aarau: n=50, Basel: n=358, Freiburg: n=60, Salzburg: n=100, Vienna:  
16 n=100, Zurich: n=80). Fifty percent of eligible women are expected to  
17 accept participation. The study was approved by each local institutional  
18 ethical board of Basel, Zurich, Freiburg, Salzburg and Vienna. The trial  
19 was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) under NCT02035059 on 12<sup>th</sup>  
20 January 2014.

#### 21 22 Recruitment and informed consent

23 Participants are identified at their first antenatal visit between 6 to 15  
24 weeks of gestation. The investigator or obstetrician in charge informs the

1 women about all aspects pertaining to the trial. The informed consent  
2 includes permission for gathering data from medical records and the  
3 optional storage of blood for a maximum of 10 years for additional  
4 analyses related to the current study. Participants are informed that trial  
5 participation is voluntary and that they are free to withdraw without any  
6 effects on subsequent care. All members of the research team are aware  
7 of the guidelines for good clinical practice for obtaining consent.<sup>9</sup>

8

9 Eligibility criteria

10 Inclusion criteria are:

- 11 - women at least 18 years of age and not under guardianship
- 12 - healthy singleton pregnancy after spontaneous conception or after
- 13 fertility treatment
- 14 - 6-15 weeks of gestation
- 15 - signed informed consent

16

17 Exclusion criteria are:

- 18 - previous bariatric surgery
- 19 - known pre-existing diabetes mellitus or under treatment with metformin
- 20 - known chronic infection like hepatitis or human immunodeficiency virus
- 21 or chronic kidney, liver or heart disease
- 22 - known maternal history of hypertensive diseases in a previous
- 23 pregnancy and now under prophylactic acetylsalicylate treatment

1  
2  
3 1 - fetal genetic, chromosomal or intervention-requiring morphologic  
4  
5 2 abnormalities

6  
7  
8 3 - the inability to read and/or understand the participant`s information  
9  
10 4 sheet

11  
12 5

## 13 14 6 Study procedure

15  
16  
17 7 All healthy pregnant patients with regular care at the participating  
18  
19 8 hospitals are counselled and asked at 6 to 15 weeks of gestation to  
20  
21 9 participate. At 11+0 to 13+6 weeks of gestation, all women have a first  
22  
23 10 trimester ultrasound scan which is standard care at participating sites. The  
24  
25 11 ultrasound scan is used to confirm gestational age, diagnose any major  
26  
27 12 fetal abnormalities, and optionally measure fetal nuchal translucency  
28  
29 13 thickness, which together with maternal free beta-chorionic gonadotropin  
30  
31 14 and pregnancy-associated plasma protein A, is used for screening for  
32  
33 15 chromosomal abnormalities. In addition, if informed consent had been  
34  
35 16 given during the first antenatal visit or at the time of the first trimester  
36  
37 17 scan, the maternal history and condition are assessed, and blood for  
38  
39 18 biomarker analysis and for the "early" OGTT 75g is drawn at the study  
40  
41 19 visit at 12 to 15 weeks of gestation. The "early" OGTT 75g is compared to  
42  
43 20 plasma glucose results obtained at 24 to 28 weeks of gestation after the  
44  
45 21 OGTT 75g. No additional visit is necessary beyond the further standard  
46  
47 22 routine antenatal care visits.

48  
49  
50  
51  
52  
53 23

54  
55  
56  
57 24 GlyFn and "early" OGTT 75g  
58  
59  
60

1 All participants are instructed to fast for at least 10 hours. Two fasting  
2 glucose samples are taken. One sample is collected for storage of two  
3 aliquots (2x 1ml) at -80 °C for later analysis of glyFn and another sample  
4 for the fasting plasma glucose (FPG) value. After intake of the 75g glucose  
5 load, blood samples are drawn 60 and 120 minutes later for determination  
6 of glucose levels. Plasma glucose is measured by an automated  
7 colorimetric-enzymatic method (hexokinase/glucose-6-phosphate-  
8 dehydrogenase) on a Hitachi/Roche-Modular P analyser. GlyFn will be  
9 analysed as previously reported by Rasanen et al.<sup>10</sup> (monoclonal glyFn  
10 antibody) by DiabetOmics Inc., Beaverton, Oregon, USA. The maternal  
11 glyFn and "early" OGTT 75g results are blinded to the investigators.

### 12 13 Un-blinding

14 Values will be un-blinded if FPG levels are  $\geq 7.0$ mmol/L or 2-hour plasma  
15 glucose levels are  $\geq 11.1$ mmol/L, suggesting pre-existing diabetes  
16 mellitus.<sup>11</sup> The diagnosis of pre-existing diabetes mellitus needs to be  
17 confirmed by an elevated glycosylated haemoglobin A1c (HbA1c) value  $\geq$   
18 6.5%. Plasma glucose levels of  $\leq 2.5$ mmol/L are also abnormal and  
19 requires further clarification. Women with confirmed pre-existing diabetes  
20 mellitus are treated according to a standardised protocol in line with  
21 current recommendations.

### 22 23 Study outcomes

### 24 Diagnosis of GDM



1 GDM is diagnosed if at least one value of the 75g OGTT at 24 to 28 weeks  
2 of gestation exceeds the recommended IADPSG threshold: FPG of  $\geq 5.1$   
3 mmol/L (92 mg/dL), 1-hour glucose of  $\geq 10.0$  mmol/L (180 mg/dL) and  
4 2-hour glucose of  $\geq 8.5$  mmol/L (153 mg/dL). All women who screen  
5 positive are followed up by a nutritionist and a diabetic nurse in contact  
6 with a diabetologist, and have frequent regular appointments in our  
7 obstetrical outpatient clinic in 2 to 4 week intervals depending on clinical  
8 condition, glucose values and ultrasound findings. Women who fail to  
9 meet the target glucose values after 1 to 2 weeks of diet management are  
10 treated with insulin according to the guidelines of the Swiss Society for  
11 Endocrinology and Diabetology (SGED), the Austrian Diabetes Association  
12 (ÖDG), the German Diabetes Association (DDG) and the German  
13 Association of Gynaecology and Obstetrics (DGGG).<sup>12-14</sup> The glycaemic  
14 targets, insulin therapy, dose adjustments, concomitant medication and/or  
15 supplements are recorded.

16  
17 Pregnancy, delivery and neonatal outcome data

18 Maternal data such as preeclampsia (blood pressure BP  $\geq 140/90$  mmHg >  
19 20 weeks of gestation with proteinuria), pregnancy induced hypertension  
20 (BP  $\geq 140/90$  mmHg > 20 weeks of gestation, rate of sonographic  
21 estimated polyhydramnios (amniotic fluid index  $\geq 25$  cm) or macrosomia  
22 (estimated birth weight  $\geq 90$ . Percentile), delivery outcome including  
23 delivery mode (spontaneous vaginal, forceps, vacuum, planned caesarean  
24 section or during labour) and indication, and neonatal outcome data such

1 as birth weight, rate of LGA (birth weight  $\geq$  90. Percentile), preterm birth  
2  $\leq$  37 completed weeks of gestation, 5 and 10 minute Apgar scores,  
3 arterial umbilical cord pH  $\leq$  7.0, shoulder dystocia, birth trauma, neonatal  
4 hypoglycaemia (glucose value of  $<$  2.5 mmol/L in infants born  $\geq$  34 weeks  
5 of gestation), jaundice (transcutaneous bilirubin  $>$  95. Percentile or need  
6 of phototherapy at any time after delivery), respiratory distress syndrome,  
7 congenital anomalies and admission to intensive care unit are  
8 prospectively collected.

## 9 10 Statistics

### 11 Sample size justification

12 We aim to demonstrate that the recently reported biomarker, glyFn  
13 and/or the "early" OGTT 75g at 12 to 15 weeks of gestation has sufficient  
14 diagnostic power to evaluate women at risk of developing GDM compared  
15 to the OGTT 75g at 24 to 28 weeks of gestation. The ROC (receiver  
16 operator characteristic) curve of glyFn has a reported area under the  
17 curve (AUC) of 0.91 (95% confidence interval (CI) 0.87-0.96).<sup>15</sup> The  
18 OGTT 75g screening test using the IADPSG criteria has not been tested in  
19 early pregnancy so far. The FPG value in early pregnancy has an AUC of  
20 0.61 (95% CI: 0.54-0.68) compared to IADPSG criteria in later pregnancy  
21 in a retrospective study.<sup>16</sup> We assume – according to unpublished data  
22 from the centre in Basel – that around 0.6% of recruited women will be  
23 diagnosed with pre-existing diabetes mellitus by the "early" OGTT 75g.  
24 The prevalence of GDM is assumed to be around 10.9% according to a

1 current IADPSG screening study from various Swiss laboratories.<sup>17</sup> The  
2 new screening approach should have a proposed true AUC of 0.9 with a  
3 lower boundary of 0.8 (95% CI>0.8) which would lead with a power of  
4 90% and an  $\alpha$ -level of 5% to an estimated sample size of 650 (65 women  
5 with GDM, 585 women without GDM). This power calculation is valid for  
6 OGTT, glyFn or combined markers. It ensures that the AUC is estimated  
7 with a good precision regardless of the chosen biomarker or any  
8 combination. Offsetting a dropout of 15%, this leads to a total sample size  
9 of 748. The dropout rate is expected to be equally distributed between  
10 centres. A sample size review will be performed after the first 300  
11 recruitments. The power calculation was performed using MedCalc version  
12 15.11 2015.<sup>18</sup>

#### 14 Statistical analysis plan

15 Descriptive statistics and graphical examination will be performed for all  
16 primary and secondary study variables.

17 Primary objective: In order to predict GDM, ROC curves with  
18 corresponding AUCs will be calculated separately for glyFn and the "early"  
19 OGTT 75g. GDM diagnosis by OGTT 75g at 24 to 28 weeks of gestation is  
20 considered as routine method. AUCs will be estimated with a 95%  
21 confidence interval (CI). It will be hypothesized that a 95% CI of the AUC  
22 of glyFn alone or in combination with FPG, post-load glucose values is >  
23 0.8.

1  
2  
3 1 Secondary objectives: Logistic regression will allow combining glyFn, the  
4  
5 2 "early" OGTT and maternal risk factors in a multivariable risk model.  
6  
7 3 Subsequent AUC will be calculated with 95% CI. Results will be internally  
8  
9 4 cross-validated to prevent overoptimistic results. Optimal cut-off points to  
10  
11 5 predict GDM will be determined based on these ROC curve. Sensitivity,  
12  
13 6 specificity, positive and negative predictive value will also be estimated  
14  
15 7 with 95% CI. However other "machine learning" algorithms could be  
16  
17 8 better than logistic regression. Therefore, other popular procedures will be  
18  
19 9 additionally tested: Random Forest, penalized logistic regression (Lasso).  
20  
21 10 Details are described in Hastie et al.<sup>19</sup> Predictive performance will be  
22  
23 11 internally cross-validated and explored in examining AUC of the ROC and  
24  
25 12 predicted vs. observed probabilities. Random forest and penalized logistic  
26  
27 13 regression avoid overfitting (to certain extent) whereas logistic regression  
28  
29 14 does not. Random forest will be chosen because of its popularity and good  
30  
31 15 benchmark results. Lasso is known for its good interpretability. Logistic  
32  
33 16 regression will probably show its inferiority compared to the other  
34  
35 17 methods. Internal cross-validation will be done using the package "mlr"  
36  
37 18 within R. Internal cross-validation (e.g. 10-fold) is a good possibility to  
38  
39 19 estimate the fitting on a potential future dataset. Based on these results it  
40  
41 20 will be decided whether a new prediction model for GDM will be proposed.  
42  
43 21 In order to potentially improve the primary study variables, secondary  
44  
45 22 study parameters such as changes in maternal body mass index and/or  
46  
47 23 clinical conditions like chronic hypertension and pregnancy induced  
48  
49 24 hypertension or preeclampsia and fetal conditions including intrauterine  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1 growth restriction will be added to the machine learning models.  
2 Statistical analyses and graphs will be performed using the current version  
3 of the statistical computation program R.<sup>20</sup>

#### 4 5 Data recording

6 Each participant receives an identification number to ensure confidentiality  
7 and the collected data is exchanged between centres using only the  
8 identification number. The name and birth date of each participant are  
9 stored with a different identification number in order to preserve the  
10 possibility to look for inconsistencies during the study.

#### 11 12 Reporting of adverse events

13 Any (serious) adverse events (AE/SAE) are recorded by the investigator  
14 using the specific AE/SAE sheet of the clinical report form (CRF). All SAE  
15 are reported to the responsible ethics committee within an appropriate  
16 time frame.

#### 17 18 OTHER STUDY MEASUREMENTS

##### 19 Other biomarkers

20 An extensively studied biomarker for early GDM screening is  
21 adiponectin.<sup>21-23</sup> Adiponectin is an adipocyte-derived hormone and reflects  
22 whole body insulin sensitivity.<sup>24</sup> A recently published metaanalysis  
23 calculated a summary sensitivity of 60.3% and a specificity of 81.3% with  
24 an AUC of 0.79.<sup>22</sup> Maternal serum adiponectin concentration is measured

1 by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA)  
2 technique. Another potential biomarker is pregnancy-specific glycoprotein-  
3 1 (PSG-1).<sup>25</sup> PSG-1 had a detection rate of 74% with a false positive rate  
4 of 6% and an AUC of 0.81. PSG-1 is analysed as previously reported by  
5 Nagalla et al.<sup>25</sup> by DiabetOmics Inc., Beaverton, Oregon, USA.

#### 6 7 Evaluation of insulin and HbA1c

8 At the early study visit at 12 to 15 weeks of gestation, insulin, c-peptide  
9 and HbA1c are additionally measured from the fasting blood sample in all  
10 women. HbA1c is measured by reversed-phase cation exchange  
11 chromatography (ADAMS HA-8160, Menarini Diagnostics Benelux,  
12 Zaventem, Belgium) or high performance liquid chromatography (HPLC)  
13 with Variant II, Bio-Rad (IFCC standardized and DCCT aligned with CV  
14 1.8% for HbA1c=5.6%). Insulin is measured by chemiluminescence  
15 immunoassay CLIA (Roche Modular E170, Basel, Switzerland). The  
16 homeostasis model assessment of insulin resistance (HOMA-IR) or the  
17 Quantitative Insulin Sensitivity Check Index (QUICKI) is used as an  
18 approximate of fasting (i.e. hepatic) insulin resistance.<sup>26,27</sup> Beta-cell  
19 function is assessed from fasting glucose and insulin levels according to  
20 Wallace et al. 2004.<sup>26</sup>

#### 21 22 Measurement of Vitamin D

23 Vitamin D deficiency is associated with inhibited insulin secretion, insulin  
24 resistance, and  $\beta$ -cell dysfunction in the pancreas in T2DM.<sup>28,29</sup>

1  
2  
3 1 Additionally, Vitamin D has immunomodulatory properties which protects  
4  
5 2 against the development of T1DM. Supplementation of 1,25-OH Vitamin D  
6  
7 3 seems to alter T cells composition and reduces cytokine-induced apoptosis  
8  
9 4 of pancreatic islet cells.<sup>30,31</sup> We would like to have actual values for 25-OH  
10  
11 5 Vitamin D and it will be evaluated in this cohort by CLIA (Liaison,  
12  
13 6 DiaSorin, Saluggia, Italy).  
14  
15  
16  
17  
18

19 8 Influence of stress in early pregnancy on development of GDM

20  
21 9 Psychological factors in early pregnancy might contribute to adverse  
22  
23 10 obstetric outcome.<sup>32</sup> This trial investigates the influence of perceived  
24  
25 11 stress, stressful life events and depression on the development of GDM.  
26  
27 12 The participants recruited in Basel are therefore asked to collect salivary  
28  
29 13 samples for cortisol directly at time of awakening, at 30 and at 60 minutes  
30  
31 14 after awakening. The saliva samples are stored at -20 °C until analysis.  
32  
33 15 After thawing, saliva samples are centrifuged. Cortisol levels are  
34  
35 16 determined employing a competitive solid phase time-resolved  
36  
37 17 fluorescence immunoassay with fluorometric end point detection  
38  
39 18 (DELFI<sup>®</sup>).<sup>33</sup> Copeptin is a more stable precursor hormone of arginine-  
40  
41 19 vasopressin and is found to be elevated in many diseases.<sup>34-37</sup> This effect  
42  
43 20 can be attributed to the response of the hypothalamic-pituitary-adrenal  
44  
45 21 axis to psychological stress. Copeptin will be measured in the fasting  
46  
47 22 blood sample with time-resolved amplified cryptate emission technology  
48  
49 23 (TRACE<sup>™</sup>) by BRAHMS Kryptor Compact immunoanalyzer by Thermo  
50  
51 24 Scientific Brahms GmbH, Henningsdorf, Germany.  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1  
4  
5 2  
6  
7  
8 3  
9  
10 4  
11  
12 5  
13  
14 6  
15  
16 7  
17  
18 8  
19  
20 9  
21  
22 10  
23  
24 11  
25  
26 12  
27  
28 13  
29  
30 14  
31  
32 15  
33  
34 16  
35  
36 17  
37  
38 18  
39  
40 19  
41  
42 20  
43  
44 21  
45  
46 22  
47  
48 23  
49  
50 24  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Three self-administered questionnaires are obtained at the study visit at 12 to 15 weeks of gestation:

#### Questionnaire on perceived stress

The perceived stress scale is a 10-items inventory for measuring the perception of stress.<sup>38</sup> It is widely used and has been validated in pregnancy.<sup>39</sup>

#### Questionnaire on stressful life events

The Holmes and Rahe Stressful Life Events Scale (SLE)<sup>40</sup> is an 43-items instrument for evaluation of stressful experiences in the past 12 months prior to the answering the questionnaire.

#### Questionnaire on depression

The ten-items Edinburgh Postnatal Depression Screen (EPDS)<sup>41</sup> is used to assess symptoms of depression during the past seven days. It has been widely validated in pregnancy<sup>42,43</sup> and it is part of the standard evaluation of pregnant women attending routine prenatal visits at the University Hospital in Basel. Scores  $\geq 13$  indicate at least probable minor depression and scores  $\geq 15$  indicated probable major depression. Pregnant women with scores  $\geq 13$  are routinely offered psychological or psychiatric counselling during pregnancy.



1  
2  
3 1 Histologic examination of the placenta  
4  
5 2 The fetal nutrients supply is regulated by maternal-fetal glucose and lipid  
6  
7 3 concentration, placental blood flow and trophoblastic nutrient  
8  
9 4 transporters.<sup>44</sup> The placenta reacts with adaptive changes in structure and  
10  
11 5 function to a hyperglycaemic milieu.<sup>45</sup> These changes of the placenta will  
12  
13 6 be assessed depending on the maternal glycaemic control by standard  
14  
15 7 pathology examination.<sup>46</sup>  
16  
17  
18  
19  
20  
21  
22

## 23 DISCUSSION

24 To our knowledge, this is the first large European cohort study that  
25  
26 11 prospectively evaluates the promising new biomarker, glyFn, and the  
27  
28 12 “early” OGTT 75g by comparing the impact of different GDM screening  
29  
30 13 strategies with IADPSG criteria at 24 to 28 weeks of gestation. The  
31  
32 14 IADPSG criteria have not been tested prospectively in early pregnancy,  
33  
34 15 despite the suggestion of the IADPSG consensus panel in 2010<sup>8</sup> to take a  
35  
36 16 FPG value  $\geq 5.1$  mmol/L as a cut-off value for GDM. This recommendation  
37  
38 17 was based on a retrospective study observing that high first-trimester FPG  
39  
40 18 in early pregnancy was associated with adverse pregnancy outcome.<sup>47</sup> We  
41  
42 19 propose that glyFn with FPG alone or in combination with post-load  
43  
44 20 glucose values should at least result in an AUC of 0.8 compared to the  
45  
46 21 OGTT 75g in later pregnancy. Rasanen et al.<sup>15</sup> could show that glyFn was  
47  
48 22 independent of maternal age, parity, gestational age, time of sample  
49  
50 23 collection and the administration of the OGTT at 24 to 28 weeks of  
51  
52 24 gestation. The current trial might clarify whether glyFn is depending on  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 maternal BMI or clinical conditions such as chronic hypertension,  
4  
5 2 pregnancy induced hypertension or the development of preeclampsia or  
6  
7 3 intrauterine growth restriction.  
8  
9

10 4 The screening approach combining glyFn +/- FPG could overcome some  
11  
12 5 problems of the OGTT. Firstly, an OGTT is time consuming. GlyFn and FPG  
13  
14 6 alone can be drawn in a fasting state of the women in early morning and  
15  
16 7 the women do not have to wait further. Secondly, OGTT is inconvenient to  
17  
18 8 administer and some women suffer intolerance to the glucose load  
19  
20 9 resulting in nausea and vomiting. No glucose administration would be  
21  
22 10 necessary with a screening method combining glyFn and FPG alone.  
23  
24 11 Thirdly, we propose that glyFn might overcome the problem of low  
25  
26 12 reproducibility as the OGTT. Like HbA1c, glyFn might also assess long  
27  
28 13 term serum glucose concentration. But this is hypothetical and needs to  
29  
30 14 be proven. Additionally, we suppose that a multivariable prediction model  
31  
32 15 incorporating risk factors i.e. maternal age and/or BMI, together with  
33  
34 16 glyFn and/or FPG, post-load glucose values might improve risk  
35  
36 17 stratification in early pregnancy and could possibly decrease the required  
37  
38 18 OGTTs later in pregnancy.  
39  
40  
41  
42  
43  
44

45 19 In the current study, the diagnostic power of glyFn will be evaluated using  
46  
47 20 serum samples. But glyFn can be analysed additionally out of a dried  
48  
49 21 blood stain. The resulting test is affordable (ie. estimated costs in India  
50  
51 22 are 2-3 USD/dried blood spot, in Europe 20-30 USD/serum sample), which  
52  
53 23 would help especially developing countries that suffer particularly from  
54  
55 24 problems with the implementation of the IADPSG recommendations,<sup>48</sup> to  
56  
57  
58  
59  
60

1 benefit from a possibly simple and cheap screening tool for GDM. The  
2 analysis of glyFn in dried blood is not part of this trial and needs to be  
3 validated separately in future studies.

4 Cost effectiveness analyses of IADPSG criteria using decision analysis  
5 models showed that the one step screening with OGTT 75g might be cost  
6 effective when post-delivery care would reduce the development of T2DM  
7 in the mothers.<sup>49,50</sup> Another study reported cost effectiveness if  
8 preeclampsia would decrease > 0.55% and caesarean delivery rate would  
9 fall > 2.7%.<sup>51</sup> A new screening in first trimester could be cost-effective if  
10 the method would reduce firstly the 1- and -2h blood sampling and/or  
11 secondly would decrease laboratory workload by avoiding a second  
12 screening in 24 to 28 weeks of gestation. Additionally, the new found  
13 screening approach could result in the identification of women with overt  
14 diabetes or/and GDM in first trimester. Aim of an early GDM diagnosis is  
15 the start of a timely intervention with diet, exercise or – if necessary –  
16 insulin therapy in early second trimester. Earlier treatment potentially  
17 should result in reduction of neonatal and maternal morbidities i.e.  
18 physical exercise reduces total maternal weight gain and the rate of  
19 GDM.<sup>52</sup> But still there is a paucity of randomized-controlled interventional  
20 trials that diagnosis and treatment of GDM < 24 weeks of gestation  
21 improve pregnancy outcomes. Until the efficacy of early treatment is not  
22 studied and verified thoroughly, a cost effectiveness analysis will be of  
23 restricted value, but a cost analysis could be performed assuming different  
24 outcome scenarios.

1 One limitation of our trial is that it is not powered for the evaluation of  
2 neonatal outcomes like LGA infants, neonatal hypoglycaemia, shoulder  
3 dystocia or birth trauma (these are planned secondary outcomes). The  
4 study would need to be significantly larger to detect effects on these  
5 neonatal outcomes. If this trial were to show positive diagnostic power for  
6 the new screening algorithm, a large multi-centre study may be required  
7 to be sufficiently powered to determine the algorithm's effect on rate of  
8 LGA infants or neonatal hypoglycaemia as proposed in the HAPO study.<sup>4</sup>

9 Finally, the glyFn assay is not yet commercially available and will be  
10 performed by DiabetOmics Inc., Beaverton, Oregon, USA. For a widespread  
11 implementation of the finally proposed screening approach, a  
12 standardisation between laboratories will be necessary first.

13 The results of our study may have a major impact on future screening  
14 approaches for GDM by development of a potentially simple, cost effective  
15 and for the pregnant women comfortable screening method for GDM in  
16 first trimester.

17

### 18 **List of abbreviations**

19 AE, adverse events; AUC, area under the curve; BMI, body mass index;  
20 CI, confidence interval; CRF, clinical report form; GDM, gestational  
21 diabetes mellitus; GlyFn, glycosylated Fibronectin; HbA1c, glycosylated  
22 haemoglobin A1c; HOMA-IR, homeostasis model assessment of insulin  
23 resistance; IADPSG, International Association of Diabetes and Pregnancy  
24 Study Groups; LGA, large for gestational age; OGTT, oral glucose

1 tolerance test; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes  
2 mellitus; QUICKI, quantitative insulin sensitivity check index; ROC,  
3 receiver operator characteristic; SAE, serious adverse events.  
4

### 5 **Disclosure of interests**

6 The authors declare that there are no further financial or personal  
7 relationships with other people or organizations that could inappropriately  
8 influence the work reported or the conclusions, implications, or opinions  
9 stated.  
10

### 11 **Contribution to authorship**

12 EH is principal investigator, study protocol author, obtained ethical  
13 approval and drafted this manuscript. IH is the sponsor, assisted with the  
14 original study protocol and revised the manuscript. SH and EBR designed  
15 the study together with EAH and revised the manuscript. AS performed  
16 the power calculation and statistical planning. TF, CSG, WE, PH, MK, ED,  
17 NO, EBAE, HP, MT, MK, RZ made important contributions and critically  
18 reviewed the content. All authors have given final approval of the version  
19 to be published.  
20

### 21 **Acknowledgements**

22 The authors would like to thank DiabetOmics Inc., Beaverton, Oregon,  
23 USA for their support in the analysis of glyFn and the preparation of a  
24 previous version of this manuscript. We also would like to thank Dorothy

1  
2  
3 1 Huang for critical proofreading in English and Cristina Granado and Doris  
4  
5 2 Mueller Borer for their precious assistance in performing the study visits  
6  
7 3 and in data acquisition. Special thanks to all families who participate in  
8  
9 4 this study.  
10  
11  
12  
13

## 14 **Details of ethics approval**

15  
16  
17 7 The study was approved by each local institutional review board in Basel  
18  
19 8 (Ethikkommission Nordostschweiz (EKNZ)), Zurich, Freiburg, Salzburg and  
20  
21 9 Vienna. The trial was registered under [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) under  
22  
23 10 NCT02035059 on 12<sup>th</sup> January 2014.  
24  
25  
26  
27  
28

## 29 **Funding**

30  
31 13 This study is supported by the Diabetes Society Basel, Switzerland and the  
32  
33 14 Gottfried and Julia Bangerter-Rhyner-Foundation, Bern, Switzerland.  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

1  
2  
3 1 REFERENCES:

- 4 1. American Diabetes Association. (2) Classification and diagnosis of  
5 diabetes. *Diabetes Care*. 2015;38 Suppl:S8-S16. doi:10.2337/dc15-  
6 S005.  
7  
8 2. Aziz NL, Abdelwahab S, Moussa M, Georgy M. Maternal fructosamine  
9 and glycosylated haemoglobin in the prediction of gestational glucose  
10 intolerance. *Clin Exp Obstet Gynecol*. 1992;19(4):235-241.  
11 <http://www.ncbi.nlm.nih.gov/pubmed/1294344>. Accessed January  
12 27, 2016.  
13  
14 3. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson  
15 JS. Effect of treatment of gestational diabetes mellitus on pregnancy  
16 outcomes. *N Engl J Med*. 2005;352(24):2477-2486.  
17 doi:10.1056/NEJMoa042973.  
18  
19 4. Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse  
20 pregnancy outcomes. *N Engl J Med*. 2008;358(19):1991-2002.  
21 doi:10.1056/NEJMoa0707943.  
22  
23 5. Casey BM, Lucas MJ, McIntire DD, Leveno KJ. Pregnancy outcomes in  
24 women with gestational diabetes compared with the general obstetric  
25 population. *Obstet Gynecol*. 1997;90(6):869-873.  
26 <http://www.ncbi.nlm.nih.gov/pubmed/9397092>. Accessed January  
27 27, 2016.  
28  
29 6. Bellamy L, Casas J-P, Hingorani AD, Williams D. Type 2 diabetes  
30 mellitus after gestational diabetes: a systematic review and meta-  
31 analysis. *Lancet*. 2009;373:1773-1779.  
32 [http://www.scopus.com/inward/record.url?eid=2-s2.0-  
33 65649084159&partnerID=40&md5=9b8db7211090a8100741c6e532  
34 63fa08](http://www.scopus.com/inward/record.url?eid=2-s2.0-65649084159&partnerID=40&md5=9b8db7211090a8100741c6e53263fa08).  
35  
36 7. Osgood ND, Dyck RF, Grassmann WK. The inter- and  
37 intragenerational impact of gestational diabetes on the epidemic of  
38 type 2 diabetes. *Am J Public Health*. 2011;101(1):173-179.  
39 doi:10.2105/AJPH.2009.186890.  
40  
41 8. Metzger BE, Gabbe SG, Persson B, et al. International association of  
42 diabetes and pregnancy study groups recommendations on the  
43 diagnosis and classification of hyperglycemia in pregnancy. *Diabetes  
44 Care*. 2010;33(3):676-682. doi:10.2337/dc09-1848.  
45  
46 9. ICH harmonized tripartite guideline: Guideline for Good Clinical  
47 Practice. *J Postgrad Med*. 47(1):45-50.  
48 <http://www.ncbi.nlm.nih.gov/pubmed/11590294>. Accessed October  
49 6, 2015.  
50  
51 10. Rasanen J, Quinn MJ, Laurie A, et al. Maternal serum glycosylated  
52 fibronectin as a point-of-care biomarker for assessment of  
53 preeclampsia. *Am J Obstet Gynecol*. 2015;212(1):82.e1-e9.  
54 doi:10.1016/j.ajog.2014.07.052.  
55  
56  
57  
58  
59  
60



- 1  
2  
3 1 11. World Health Organization. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia.*; 2006.  
4 2  
5  
6 3 12. Lehmann R, Troendle A, Brändle M. [New insights into diagnosis and  
7 4 management of gestational diabetes mellitus: recommendations of  
8 5 the Swiss Society for Endocrinology and Diabetes]. *Ther Umsch.*  
9 6 2009;66(10):695-706. doi:10.1024/0040-5930.66.10.695.  
10  
11 7 13. Kleinwechter H, Schäfer-Graf U, Bühner C, et al. Gestational diabetes  
12 8 mellitus (GDM) diagnosis, therapy and follow-up care: Practice  
13 9 Guideline of the German Diabetes Association(DDG) and the German  
14 10 Association for Gynaecology and Obstetrics (DGGG). *Exp Clin*  
15 11 *Endocrinol Diabetes.* 2014;122(7):395-405. doi:10.1055/s-0034-  
16 12 1366412.  
17  
18 13 14. Kautzky-Willer A, Bancher-Todesca D, Pollak A, Repa A, Lechleitner  
19 14 M, Weitgasser R. [Gestational diabetes mellitus]. *Wien Klin*  
20 15 *Wochenschr.* 2012;124 Suppl :58-65. doi:10.1007/s00508-012-  
21 16 0265-3.  
22  
23 17 15. Rasanen JP, Snyder CK, Rao P V, et al. Glycosylated fibronectin as a  
24 18 first-trimester biomarker for prediction of gestational diabetes.  
25 19 *Obstet Gynecol.* 2013;122(3):586-594.  
26 20 doi:10.1097/AOG.0b013e3182a0c88b.  
27  
28 21 16. Corrado F1, D'Anna R, Cannata ML, Interdonato ML, Pintaudi B DBA.  
29 22 Correspondence between first-trimester fasting glycaemia, and oral  
30 23 glucose tolerance test in gestational diabetes diagnosis. *Diabetes*  
31 24 *Metab.* 2012;38(5):458-461. doi:0.1016/j.diabet.2012.03.006.  
32  
33 25 17. Ryser Rüetschi J, Jornayvaz FR, Rivest R, Huhn EA, Irion O, Boulvain  
34 26 M. Fasting glycaemia to simplify screening for gestational diabetes.  
35 27 *BJOG.* January 2016. doi:10.1111/1471-0528.13857.  
36  
37 28 18. Hanley JA, McNeil BJ. The meaning and use of the area under a  
38 29 receiver operating characteristic (ROC) curve. *Radiology.*  
39 30 1982;143(1):29-36. doi:10.1148/radiology.143.1.7063747.  
40  
41 31 19. Hastie T, Tibshirani R and FJ. *The Elements of Statistical Learning:*  
42 32 *Prediction, Inference and Data Mining.* Springer Verlag; 2009.  
43  
44 33 20. R Development Core Team R. R: A Language and Environment for  
45 34 Statistical Computing. *R Found Stat Comput.* 2014;1:409.  
46 35 doi:10.1007/978-3-540-74686-7.  
47  
48 36 21. Nanda S, Savvidou M, Syngelaki A, Akolekar R, Nicolaidis KH.  
49 37 Prediction of gestational diabetes mellitus by maternal factors and  
50 38 biomarkers at 11 to 13 weeks. *Prenat Diagn.* 2011;31(2):135-141.  
51 39 doi:10.1002/pd.2636.  
52  
53 40 22. Iliodromiti S, Sassarini J, Kelsey TW, Lindsay RS, Sattar N, Nelson  
54 41 SM. Accuracy of circulating adiponectin for predicting gestational  
55 42 diabetes: a systematic review and meta-analysis. *Diabetologia.*  
56 43 January 2016. doi:10.1007/s00125-015-3855-6.  
57  
58  
59  
60



- 1  
2  
3 1 23. Fasshauer M, Blüher M, Stumvoll M. Adipokines in gestational  
4 2 diabetes. *Lancet Diabetes Endocrinol*. 2014;2(6):488-499.  
5 3 doi:10.1016/S2213-8587(13)70176-1.  
6  
7 4 24. Yadav A, Kataria MA, Saini V, Yadav A. Role of leptin and adiponectin  
8 5 in insulin resistance. *Clin Chim Acta*. 2013;417:80-84.  
9 6 doi:10.1016/j.cca.2012.12.007.  
10  
11 7 25. Nagalla SR, Snyder CK, Michaels JE, et al. Maternal serum  
12 8 biomarkers for risk assessment in gestational diabetes. A potential  
13 9 universal screening test to predict GDM status. *Indian J Endocrinol  
14 10 Metab*. 19(1):155-159. doi:10.4103/2230-8210.140226.  
15  
16 11 26. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA  
17 12 modeling. *Diabetes Care*. 2004;27(6):1487-1495.  
18 13 <http://www.ncbi.nlm.nih.gov/pubmed/15161807>. Accessed August  
19 14 17, 2015.  
20  
21 15 27. Katz A, Nambi SS, Mather K, et al. Quantitative insulin sensitivity  
22 16 check index: a simple, accurate method for assessing insulin  
23 17 sensitivity in humans. *J Clin Endocrinol Metab*. 2000;85(7):2402-  
24 18 2410. doi:10.1210/jcem.85.7.6661.  
25  
26 19 28. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency  
27 20 inhibits pancreatic secretion of insulin. *Science*.  
28 21 1980;209(4458):823-825.  
29 22 <http://www.ncbi.nlm.nih.gov/pubmed/6250216>. Accessed July 6,  
30 23 2016.  
31  
32 33 29. Maestro B, Molero S, Bajo S, Dávila N, Calle C. Transcriptional  
34 25 activation of the human insulin receptor gene by 1,25-  
35 26 dihydroxyvitamin D(3). *Cell Biochem Funct*. 2002;20(3):227-232.  
36 27 doi:10.1002/cbf.951.  
37  
38 28 30. Takiishi T, Ding L, Baeke F, et al. Dietary supplementation with high  
39 29 doses of regular vitamin D3 safely reduces diabetes incidence in NOD  
40 30 mice when given early and long term. *Diabetes*. 2014;63(6):2026-  
41 31 2036. doi:10.2337/db13-1559.  
42  
43 32 31. Riachy R, Vandewalle B, Moerman E, et al. 1,25-Dihydroxyvitamin  
44 33 D3 protects human pancreatic islets against cytokine-induced  
45 34 apoptosis via down-regulation of the Fas receptor. *Apoptosis*.  
46 35 2006;11(2):151-159. doi:10.1007/s10495-006-3558-z.  
47  
48 36 32. Alder J, Fink N, Bitzer J, Hösli I, Holzgreve W. Depression and  
49 37 anxiety during pregnancy: a risk factor for obstetric, fetal and  
50 38 neonatal outcome? A critical review of the literature. *J Matern Fetal  
51 39 Neonatal Med*. 2007;20(3):189-209.  
52 40 doi:10.1080/14767050701209560.  
53  
54 41 33. Dressendörfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ.  
55 42 Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in  
56 43 an immunoassay for salivary cortisol measurement. *J Steroid  
57 44 Biochem Mol Biol*. 1992;43(7):683-692.

- 1  
2  
3 1 <http://www.ncbi.nlm.nih.gov/pubmed/1472460>. Accessed January  
4 2 21, 2016.
- 5  
6 3 34. Christ-Crain M, Fenske W. Copeptin in the diagnosis of vasopressin-  
7 4 dependent disorders of fluid homeostasis. *Nat Rev Endocrinol*.  
8 5 2016;12(3):168-176. doi:10.1038/nrendo.2015.224.
- 9  
10 6 35. Reinstadler SJ, Klug G, Feistritz H-J, Metzler B, Mair J. Copeptin  
11 7 testing in acute myocardial infarction: ready for routine use? *Dis*  
12 8 *Markers*. 2015;2015:614145. doi:10.1155/2015/614145.
- 13  
14 9 36. Zhang R, Liu J, Zhang Y, Liu Q, Li T, Cheng L. Association Between  
15 10 Circulating Copeptin Level and Mortality Risk in Patients with  
16 11 Intracerebral Hemorrhage: a Systemic Review and Meta-Analysis.  
17 12 *Mol Neurobiol*. January 2016. doi:10.1007/s12035-015-9626-z.
- 18  
19 13 37. Viasus D, Del Rio-Pertuz G, Simonetti AF, et al. Biomarkers for  
20 14 predicting short-term mortality in community-acquired pneumonia: A  
21 15 systematic review and meta-analysis. *J Infect*. January 2016.  
22 16 doi:10.1016/j.jinf.2016.01.002.
- 23  
24 17 38. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived  
25 18 stress. *J Health Soc Behav*. 1983;24(4):385-396.  
26 19 <http://www.ncbi.nlm.nih.gov/pubmed/6668417>. Accessed February  
27 20 9, 2015.
- 28  
29  
30 21 39. Silveira ML, Whitcomb BW, Pekow P, et al. Perceived psychosocial  
31 22 stress and glucose intolerance among pregnant Hispanic women.  
32 23 *Diabetes Metab*. 2014;40(6):466-475.  
33 24 doi:10.1016/j.diabet.2014.05.002.
- 34  
35 25 40. Holmes TH, Rahe RH. The Social Readjustment Rating Scale. *J*  
36 26 *Psychosom Res*. 1967;11(2):213-218.  
37 27 <http://www.ncbi.nlm.nih.gov/pubmed/6059863>. Accessed October  
38 28 28, 2015.
- 39  
40 29 41. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression.  
41 30 Development of the 10-item Edinburgh Postnatal Depression Scale.  
42 31 *Br J Psychiatry*. 1987;150:782-786.  
43 32 <http://www.ncbi.nlm.nih.gov/pubmed/3651732>. Accessed March 16,  
44 33 2015.
- 45  
46 34 42. Byrn M, Penckofer S. The relationship between gestational diabetes  
47 35 and antenatal depression. *J Obstet Gynecol Neonatal Nurs*.  
48 36 44(2):246-255. doi:10.1111/1552-6909.12554.
- 49  
50 37 43. Ertel KA, Silveira M, Pekow P, et al. Prenatal depressive symptoms  
51 38 and abnormalities of glucose tolerance during pregnancy among  
52 39 Hispanic women. *Arch Womens Ment Health*. 2014;17(1):65-72.  
53 40 doi:10.1007/s00737-013-0379-2.
- 54  
55  
56 41 44. Desoye G, Gauster M, Wadsack C. Placental transport in pregnancy  
57 42 pathologies. *Am J Clin Nutr*. 2011;94(6 Suppl):1896S - 1902S.  
58 43 doi:10.3945/ajcn.110.000851.
- 59  
60

- 1  
2  
3 1 45. Jarmuzek P, Wielgos M, Bomba-Opon D. Placental pathologic  
4 2 changes in gestational diabetes mellitus. *Neuro Endocrinol Lett.*  
5 3 2015;36(2):101-105.  
6 4 <http://www.ncbi.nlm.nih.gov/pubmed/26071574>. Accessed  
7 5 December 4, 2015.
- 8  
9 6 46. Bernischke K, Burton GJ BR. *Pathology of the Human Placenta*. Vol  
10 7 Sixth. 2012.
- 11 8 47. Riskin-Mashiah S, Younes G, Damti A, Auslender R. First-trimester  
12 9 fasting hyperglycemia and adverse pregnancy outcomes. *Diabetes*  
13 10 *Care*. 2009;32(9):1639-1643. doi:10.2337/dc09-0688.
- 14 11 48. Nielsen KK, de Courten M, Kapur A. The urgent need for universally  
15 12 applicable simple screening procedures and diagnostic criteria for  
16 13 gestational diabetes mellitus--lessons from projects funded by the  
17 14 World Diabetes Foundation. *Glob Health Action*. 2012;5.  
18 15 doi:10.3402/gha.v5i0.17277.
- 19 16 49. Werner EF, Pettker CM, Zuckerwise L, et al. Screening for Gestational  
20 17 Diabetes Mellitus: Are the Criteria Proposed by the International  
21 18 Association of the Diabetes and Pregnancy Study Groups Cost-  
22 19 Effective? *Diabetes Care*. 2012;35:529-535. doi:10.2337/dc11-1643.
- 23 20 50. Marseille E, Lohse N, Jiwani A, et al. The cost-effectiveness of  
24 21 gestational diabetes screening including prevention of type 2  
25 22 diabetes: application of a new model in India and Israel. *J Matern*  
26 23 *Fetal Neonatal Med*. 2013;26(8):802-810.  
27 24 doi:10.3109/14767058.2013.765845.
- 28 25 51. Mission JF, Ohno MS, Cheng YW, Caughey AB. Gestational diabetes  
29 26 screening with the new IADPSG guidelines: A cost-effectiveness  
30 27 analysis. *Am J Obstet Gynecol*. 2012;207.  
31 28 doi:10.1016/j.ajog.2012.06.048.
- 32 29 52. Sanabria-Martínez G, García-Hermoso A, Poyatos-León R, Álvarez-  
33 30 Bueno C, Sánchez-López M, Martínez-Vizcaíno V. Effectiveness of  
34 31 physical activity interventions on preventing gestational diabetes  
35 32 mellitus and excessive maternal weight gain: a meta-analysis. *BJOG*.  
36 33 2015;122(9):1167-1174. doi:10.1111/1471-0528.13429.

34

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

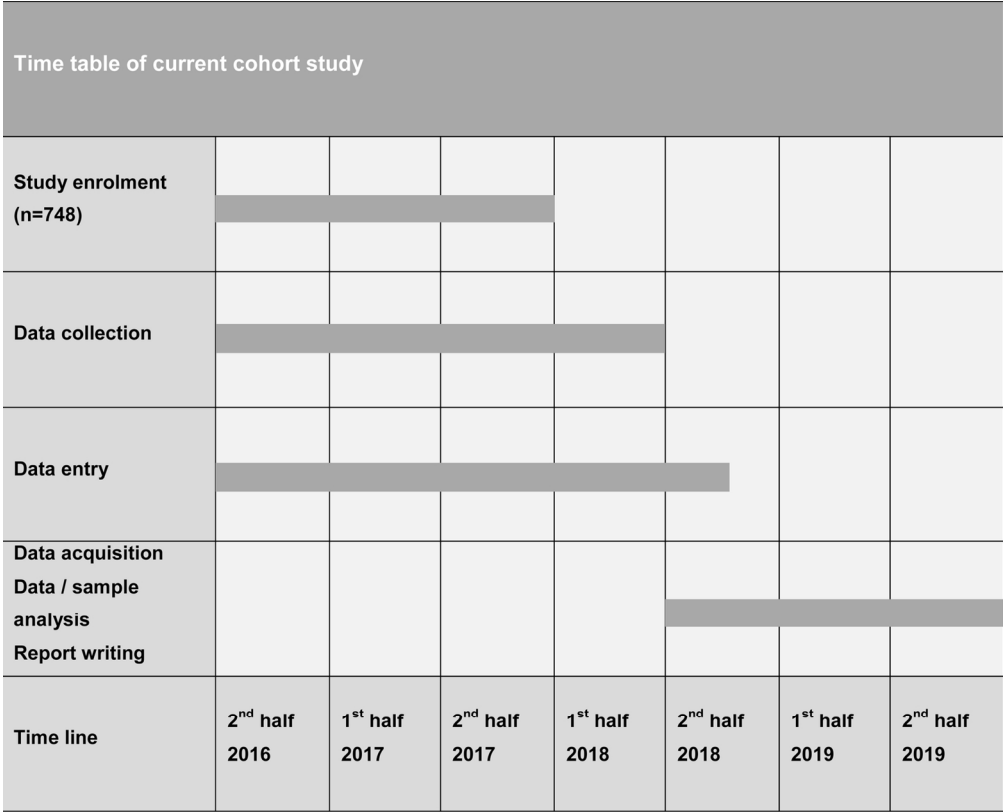


Figure 1: Expected time frame

141x115mm (300 x 300 DPI)

W Only



STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents\*

Section/item	Item No	Description
<b>Administrative information</b>		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym → <a href="#">title page</a>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry → <a href="#">page 2</a>
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier → only in original protocol
Funding	4	Sources and types of financial, material, and other support → <a href="#">page 2</a>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors → <a href="#">page 2</a>
	5b	Name and contact information for the trial sponsor → <a href="#">page 2/21</a>
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
<b>Introduction</b>		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention → <a href="#">page 5-7 (Introduction)</a>
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses → <a href="#">page 7 (Study objectives)</a>

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

Trial design 8 Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) → [see Title](#)

### Methods: Participants, interventions, and outcomes

Study setting 9 Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained → [page 7/8 \(Study settings/design\)](#)

Eligibility criteria 10 Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) → [page 8/9 \(Eligibility criteria\)](#)

Interventions 11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered → [page 9/10 \(Study procedure\)](#)

11b Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) → [not applicable](#)

11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) → [not applicable](#)

11d Relevant concomitant care and interventions that are permitted or prohibited during the trial → [not applicable](#)

Outcomes 12 Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended → [page 11/12 \(Study outcomes\)](#)

Participant timeline 13 Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) → [Figure 1](#)

Sample size 14 Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations → [page 12/13 \(Sample size justification\)](#)

Recruitment 15 Strategies for achieving adequate participant enrolment to reach target sample size

**Methods: Assignment of interventions (for controlled trials) → [not applicable](#)**



## Allocation:

- 1  
2  
3  
4 Sequence generation 16a Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
- 5  
6  
7  
8  
9  
10  
11  
12 Allocation concealment mechanism 16b Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
- 13  
14  
15  
16  
17  
18 Implementation 16c Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
- 19  
20  
21 Blinding (masking) 17a Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how → not applicable
- 22  
23  
24  
25  
26  
27 17b If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial → see section "Un-blinding"
- 28  
29  
30

**Methods: Data collection, management, and analysis**

- 31  
32  
33 Data collection methods 18a Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
- 34  
35  
36  
37  
38  
39  
40  
41 18b Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
- 42  
43  
44  
45 Data management 19 Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
- 46  
47  
48  
49  
50  
51 Statistical methods 20a Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol → page 13-15 (Study analysis plan)
- 52  
53  
54  
55  
56  
57 20b Methods for any additional analyses (eg, subgroup and adjusted analyses) → page 15-19 (Other study measurements)
- 58  
59  
60

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60
- 20c Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

### Methods: Monitoring

- Data monitoring 21a Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
- 21b Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
- Harms 22 Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct → see Reporting of adverse events
- Auditing 23 Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

### Ethics and dissemination

- Research ethics approval 24 Plans for seeking research ethics committee/institutional review board (REC/IRB) approval → page 8 (Study settings/design)
- Protocol amendments 25 Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
- Consent or assent 26a Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
- 26b Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
- Confidentiality 27 How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial → page 15 (Data recording)
- Declaration of interests 28 Financial and other competing interests for principal investigators for the overall trial and each study site → (page 2 (Conflict of interest))
- Access to data 29 Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators



1			
2	Ancillary and	30	Provisions, if any, for ancillary and post-trial care, and for
3	post-trial care		compensation to those who suffer harm from trial participation
4			
5	Dissemination	31a	Plans for investigators and sponsor to communicate trial results to
6	policy		participants, healthcare professionals, the public, and other relevant
7			groups (eg, via publication, reporting in results databases, or other
8			data sharing arrangements), including any publication restrictions →
9			not applicable
10			
11		31b	Authorship eligibility guidelines and any intended use of professional
12			writers
13			
14		31c	Plans, if any, for granting public access to the full protocol, participant-
15			level dataset, and statistical code
16			
17			
18			
19	<b>Appendices</b>		
20			
21	Informed consent	32	Model consent form and other related documentation given to
22	materials		participants and authorised surrogates
23			
24	Biological	33	Plans for collection, laboratory evaluation, and storage of biological
25	specimens		specimens for genetic or molecular analysis in the current trial and for
26			future use in ancillary studies, if applicable
27			
28			

\*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](https://creativecommons.org/licenses/by-nc-nd/3.0/)" license.