Supplementary Online Content

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eMethods

eTable 1. Numbers of HAPO FUS participants at each field center
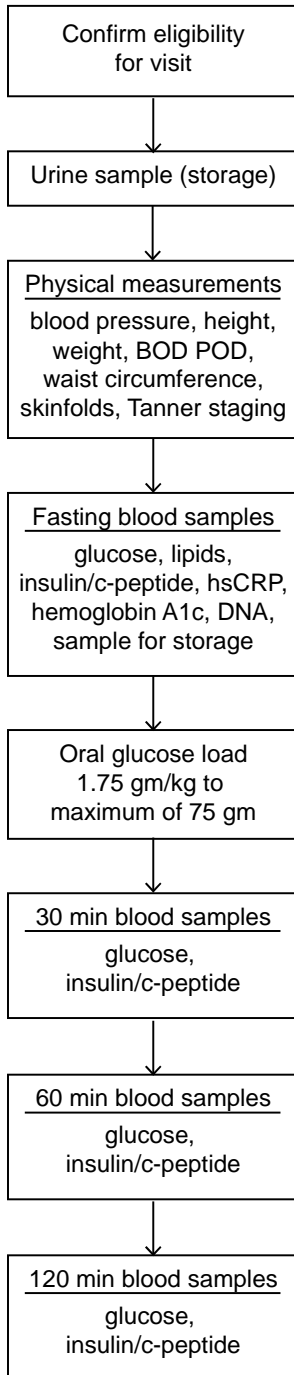
eTable 2. Race/ethnicity by field center for participating children

eTable 3. Race/ethnicity by field center for participating mothers
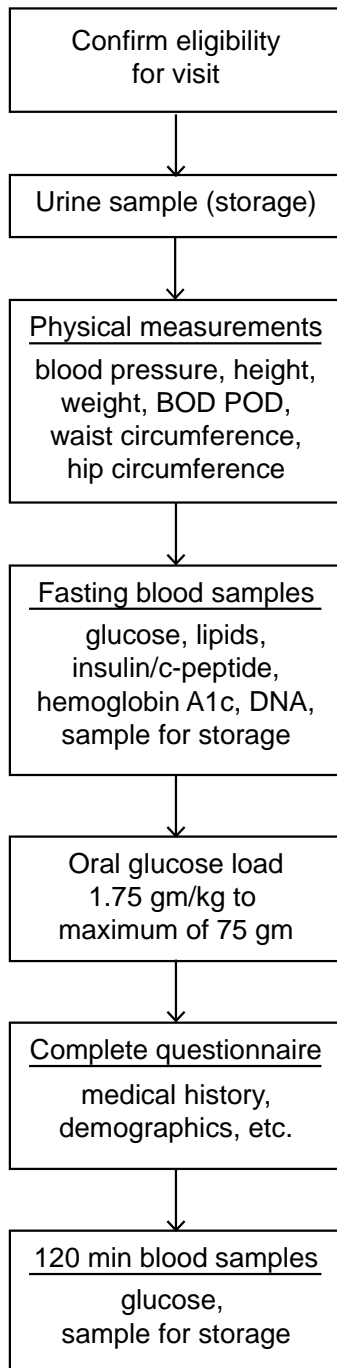
eTable 4. Characteristics of mothers of HAPO FUS child participants during HAPO pregnancy OGTT

This supplementary material has been provided by the authors to give readers additional information about their work.
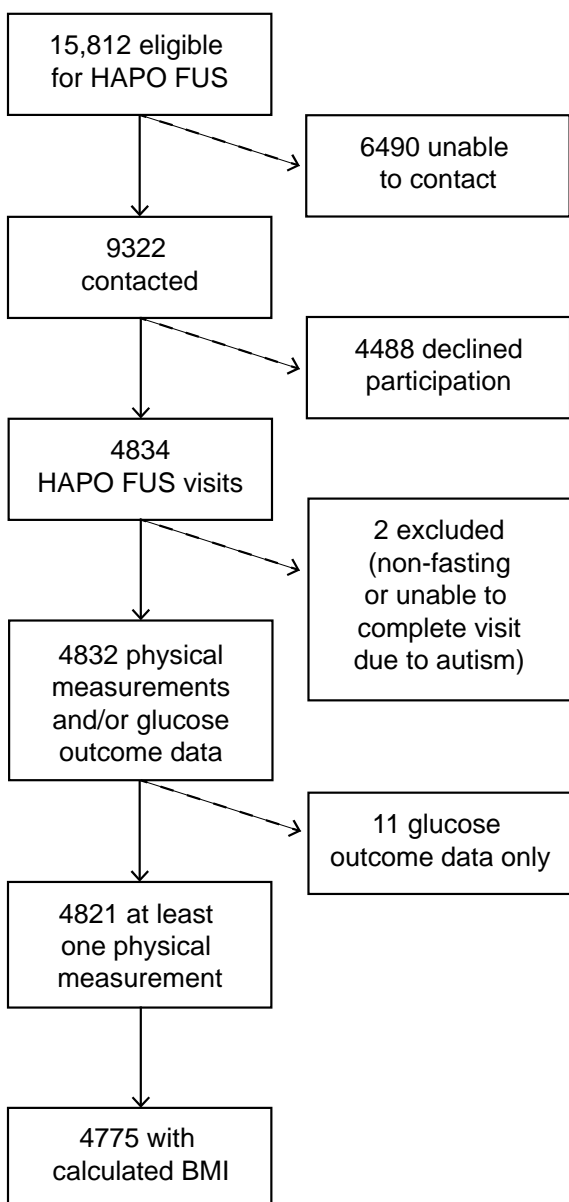
eFigure 1. Flowchart describing the HAPO follow-up study visit for children including urine sample, physical measurements and 2-hr OGTT

```
┌─────────────────────────┐
│    Confirm eligibility   │
│        for visit         │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│   Urine sample (storage) │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│  Physical measurements   │
│  blood pressure, height, │
│     weight, BOD POD,     │
│   waist circumference,   │
│ skinfolds, Tanner staging│
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│   Fasting blood samples  │
│     glucose, lipids,     │
│  insulin/c-peptide, hsCRP,│
│   hemoglobin A1c, DNA,   │
│    sample for storage    │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│     Oral glucose load    │
│       1.75 gm/kg to      │
│    maximum of 75 gm      │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│   30 min blood samples   │
│         glucose,         │
│     insulin/c-peptide    │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│   60 min blood samples   │
│         glucose,         │
│     insulin/c-peptide    │
└─────────────────────────┘
             │
             ▼
┌─────────────────────────┐
│   120 min blood samples  │
│         glucose,         │
│     insulin/c-peptide    │
└─────────────────────────┘
```

eFigure 2. Flowchart describing the HAPO follow-up study visit for mothers including urine sample, physical measurements, questionnaire and 2-hr OGTT
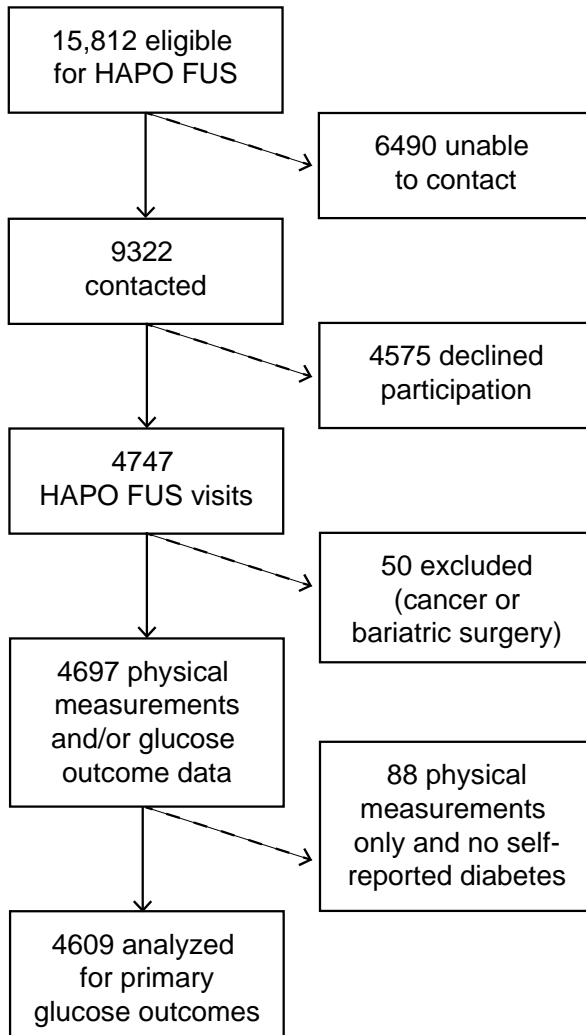
```
┌─────────────────────────┐
│   Confirm eligibility    │
│        for visit         │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  Urine sample (storage)  │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  Physical measurements   │
│                          │
│  blood pressure, height, │
│      weight, BOD POD,    │
│    waist circumference,  │
│     hip circumference    │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  Fasting blood samples   │
│                          │
│     glucose, lipids,     │
│    insulin/c-peptide,    │
│    hemoglobin A1c, DNA,  │
│     sample for storage   │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│    Oral glucose load     │
│       1.75 gm/kg to      │
│    maximum of 75 gm      │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  Complete questionnaire  │
│                          │
│     medical history,     │
│     demographics, etc.   │
└─────────────────────────┘
            │
            ▼
┌─────────────────────────┐
│  120 min blood samples   │
│                          │
│         glucose,         │
│     sample for storage   │
└─────────────────────────┘
```

eFigure 3. Flowchart describing enrollment for children participating in the HAPO follow-up study (FUS)

```
┌──────────────────┐
│ 15,812 eligible  │
│ for HAPO FUS     │────────────┐
└──────────────────┘            │    ┌──────────────────┐
        │                       └───▶│ 6490 unable      │
        │                            │ to contact       │
        ▼                            └──────────────────┘
┌──────────────────┐
│ 9322             │
│ contacted        │────────────┐
└──────────────────┘            │    ┌──────────────────┐
        │                       └───▶│ 4488 declined    │
        │                            │ participation    │
        ▼                            └──────────────────┘
┌──────────────────┐
│ 4834             │
│ HAPO FUS visits  │────────────┐
└──────────────────┘            │    ┌──────────────────┐
        │                       │    │ 2 excluded       │
        │                       └───▶│ (non-fasting     │
        │                            │ or unable to     │
        │                            │ complete visit   │
        ▼                            │ due to autism)   │
┌──────────────────┐                 └──────────────────┘
│ 4832 physical    │
│ measurements     │
│ and/or glucose   │────────────┐
│ outcome data     │            │    ┌──────────────────┐
└──────────────────┘            └───▶│ 11 glucose       │
        │                            │ outcome data only│
        │                            └──────────────────┘
        ▼
┌──────────────────┐
│ 4821 at least    │
│ one physical     │
│ measurement      │
└──────────────────┘
        │
        ▼
┌──────────────────┐
│ 4775 with        │
│ calculated BMI   │
└──────────────────┘
```

Multiple attempts were made to contact all 15,812 eligible mother/child pairs. Of these 9322 were contacted and 4834 children completed at least one component of the HAPO Follow-Up Study visit. Data were excluded from 2 children (1 due to non-fasting status and 1 due to inability to complete the study visit due to autism) leaving 4832 children with physical measurements and/or glucose outcome data. Of these, 4821 had at least one physical measurement and were analyzed for the primary anthropometric outcomes. Of the 4821, 4775 had data available for calculating BMI.

eFigure 4. Flowchart describing enrollment for mothers participating in the HAPO follow-up study (FUS)

```
┌─────────────────┐
│ 15,812 eligible │
│ for HAPO FUS    │────────────┐
└─────────────────┘            │      ┌─────────────────┐
        │                      └─────→│ 6490 unable     │
        │                             │ to contact      │
        ↓                             └─────────────────┘
┌─────────────────┐
│ 9322            │
│ contacted       │────────────┐
└─────────────────┘            │      ┌─────────────────┐
        │                      └─────→│ 4575 declined   │
        │                             │ participation   │
        ↓                             └─────────────────┘
┌─────────────────┐
│ 4747            │
│ HAPO FUS visits │────────────┐
└─────────────────┘            │      ┌─────────────────┐
        │                      └─────→│ 50 excluded     │
        │                             │ (cancer or      │
        │                             │ bariatric       │
        │                             │ surgery)        │
        ↓                             └─────────────────┘
┌─────────────────┐
│ 4697 physical   │
│ measurements    │
│ and/or glucose  │            ┌─────────────────┐
│ outcome data    │───────────→│ 88 physical     │
└─────────────────┘            │ measurements    │
        │                      │ only and no self-│
        │                      │ reported diabetes│
        ↓                      └─────────────────┘
┌─────────────────┐
│ 4609 analyzed   │
│ for primary     │
│ glucose outcomes│
└─────────────────┘
```

Multiple attempts were made to contact all 15,812 eligible mother/child pairs. Of these 9322 were contacted and 4747 mothers completed at least one component of the HAPO Follow-Up Study visit. Data were excluded from 50 mothers (49 due to prior bariatric surgery, 1 due to ongoing cancer treatment) leaving 4697 mothers for analyses. Of these, 4609 mothers had glucose measurements or self-reported T2DM on treatment and were analyzed for primary glucose outcomes in mothers.

eMethods

Multiple Imputation for Missing Tanner Stage

The HAPO Follow-Up Study visit protocol specified evaluation of Tanner Stage 1, 2/3 or 4/5 by trained medical study staff according to breast/areolar development or by asking about menstruation for girls and measurement of testicular volume using a Prader orchidometer for boys. For girls, 2008 trained assessments were completed and 381 (19.0%), 852 (42.5%) and 774 (38.5%) were Stage 1, 2/3 and 4/5, respectively. For boys, 1570 trained assessments were completed and 565 (36.0%), 726 (46.2%) and 279 (17.8%) were Stage 1, 2/3 and 4/5, respectively. Due to refusal of either the HAPO child or the HAPO child's mother, trained assessments were not collected for 359/2367 (15.2%) of girls and 895/2465 (36.3%) of boys. For all HAPO FUS child participants for whom a back-up blood sample was available, serum hormone-binding globulin (SHBG) was measured using an immunofluorometric assay. In addition, testosterone was measured for boys and estradiol was measured for girls. Testosterone and estradiol levels were measured by the Brigham Research Assay Core Laboratory using liquid chromatography (LC) tandem mass spectrometry (MS) at the Brigham and Women's Hospital [1-2]. The testosterone LC-MS/MS assay has a sensitivity of 0.5 ng/mL and estradiol LC-MS/MS assay 1 pg/mL. SHBG and testosterone were successfully measured in 2199 (89.2%) and 2230 (90.5%) of 2465 boys, respectively. SHBG and estradiol were successfully measured in 2090 (88.3%) and 2131 (90.0%) of 2367 girls, respectively.

Statistical models including adjustment for trained assessment of Tanner stage were performed using multiple imputation. All variables to be used for statistical analyses as well as sex steroid measurements were identified, and then 25 rounds of multiple imputation were performed to impute all missing observations across all variables. Imputations were performed separately for

boys and girls. Missing data assumptions 'missing at random' and 'missing not at random' were both explored. Estimated associations varied little for these assumptions, hence results were reported under the simpler 'missing at random' assumption.

[1] Jasuja GK, Travison TG, Davda M, Murabito JM, Basaria S, Zhang A, Kushnir MM, Rockwood AL, Meikle W, Pencina MJ, Coviello A, Rose AJ, D'Agostino R, Ramachandran SV, Bhasin S. (2013) Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham Heart Study. The Journals of Gerontology: Series A. 68(6): 733-740.

[2] Bhasin S, Pencina M, Jasuja GK, Travison TG, Coviello A, Orwoll E, Wang PY, Nielson C, Wu F, Tajar A, Labrie F, Vesper H, Zhang A, Ulloor J, Singh R, D'Agostino R, Vasan RS. (2011) Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham Heart Study and applied to three geographically distinct cohorts. Journal of Clinical Endocrinology and Metabolism. 96(8):2430-2439.

eTable 1. Numbers of HAPO FUS participants at each field center

Field Center	Total Eligible	Number (%) of Child Participants	Number (%) of Mother Participants
Bangkok, Thailand	2179	247 (11.3)	247 (11.3)
Barbados, West Indies	1827	618 (33.8)	584 (32.0)
Belfast, UK	1548	704 (45.5)	701 (45.3)
Bellflower, CA, USA	1774	535 (30.1)	514 (29.0)
Chicago, IL, USA	688	296 (43.0)	293 (42.6)
Cleveland, OH, USA	697	259 (37.2)	249 (35.7)
Hong Kong, China	1523	736 (48.3)	734 (48.2)
Manchester, UK	2086	522 (25.0)	520 (24.9)
Petah-Tiqva, Israel	1638	515 (31.4)	460 (28.1)
Toronto, Canada	1852	400 (21.6)	395 (21.3)
Total	15,812	4832 (30.6)	4697 (29.7)

eTable 2. Race/ethnicity by field center for participating children

Field Center	N	N (%)				
		White non-Hispanic	Hispanic	Black non-Hispanic	Asian	Other
Bangkok	247	0	0	0	247 (100.0)	0
Barbados	618	0	0	618 (100.0)	0	0
Belfast	704	700 (99.4)	0	0	0	4 (0.6)
Bellflower	535	30 (5.6)	444 (83.0)	46 (8.6)	12 (2.3)	3 (0.6)
Chicago	296	240 (81.1)	29 (9.8)	10 (3.4)	15 (5.1)	2 (0.7)
Cleveland	259	164 (63.3)	34 (13.1)	58 (22.4)	1 (0.4)	2 (0.8)
Hong Kong	736	0	0	0	709 (96.3)	27 (3.7)
Manchester	522	296 (56.7)	0	33 (6.3)	154 (29.5)	39 (7.5)
Petah-Tiqva	515	515 (100.0)	0	0	0	0
Toronto	400	342 (85.5)	0	10 (2.5)	38 (9.5)	10 (2.5)

eTable 3. Race/ethnicity by field center for participating mothers

Field Center	N	White non-Hispanic	Hispanic	Black non-Hispanic	Asian	Other
		N (%)				
Bangkok	247	0	0	0	247 (100.0)	0
Barbados	584	0	0	584 (100.0)	0	0
Belfast	701	697 (99.4)	0	0	0	4 (0.6)
Bellflower	514	29 (5.6)	427 (83.1)	43 (8.4)	12 (2.3)	3 (0.6)
Chicago	293	237 (80.9)	29 (9.9)	10 (3.4)	15 (5.1)	2 (0.7)
Cleveland	249	159 (63.9)	32 (12.9)	55 (22.1)	1 (0.4)	2 (0.8)
Hong Kong	734	0	0	0	707 (96.3)	27 (3.7)
Manchester	520	294 (56.5)	0	33 (6.3)	154 (29.6)	39 (7.5)
Petah-Tiqva	460	460 (100.0)	0	0	0	0
Toronto	395	337 (85.3)	0	10 (2.5)	38 (9.6)	10 (2.5)

eTable 4. Characteristics of mothers of HAPO FUS child participants during HAPO pregnancy OGTT

Characteristics – Mothers of HAPO FUS Children During HAPO Pregnancy	Gestational Diabetes Mellitus*	No Gestational Diabetes Mellitus	Overall	Child Non-Participants**
	N=683 (14.1%)	N=4149 (85.9%)	N=4832	N=10980 (16.4% GDM)
	Mean (SD)	Mean (SD)	Mean (SD)	
Age at OGTT (yrs)	31.9 (5.3)	29.6 (5.6)	29.9 (5.7)	29.1 (5.3)
Gestational Age at OGTT (wks)	27.9 (1.7)	27.6 (1.7)	27.7 (1.7)	27.7 (1.5)
Height (cm)	161.1 (7.0)	161.9 (6.7)	161.8 (6.8)	161.5 (6.3)
Weight (kg)	77.8 (16.0)	71.1 (13.6)	72.0 (14.2)	71.9 (13.4)
Body Mass Index (BMI) (kg/m^2)	29.9 (5.5)	27.1 (4.7)	27.5 (4.9)	27.5 (4.8)
Mean Arterial Pressure (mmHg)	83.5 (7.8)	80.0 (7.9)	80.5 (8.0)	80.7 (7.8)
Fasting Plasma Glucose (mg/dL)	89.0 (7.6)	79.7 (5.4)	81.0 (6.6)	81.2 (6.5)
1-hr Plasma Glucose (mg/dL)	173.0 (28.8)	126.6 (24.9)	133.1 (30.2)	133.0 (29.9)
2-hr Plasma Glucose (mg/dL)	137.1 (26.7)	106.0 (19.1)	110.4 (23.0)	110.6 (22.7)
	N (%)	N (%)	N (%)	(%)
Race/Ethnicity				
White, Non-Hispanic	277 (40.6)	2010 (48.4)	2287 (47.3)	(46.8)
Hispanic	113 (16.5)	394 (9.5)	507 (10.5)	(9.6)
Black, Non-Hispanic	81 (11.9)	694 (16.7)	775 (16.0)	(15.5)
Asian	196 (28.7)	980 (23.6)	1176 (24.3)	(25.7)
Other	16 (2.3)	71 (1.7)	87 (1.8)	(2.5)
Any Prenatal Alcohol Use	43 (6.3)	202 (4.9)	245 (5.1)	(6.7)
Any Prenatal Smoking	59 (8.6)	347 (8.4)	406 (8.4)	(8.3)
Parity (any prior delivery ≥ 20 weeks)	392 (57.4)	2093 (50.4)	2485 (51.4)	(49.7)
Family History of Diabetes	207 (30.3)	870 (21.0)	1077 (22.3)	(21.4)

To convert the values for glucose to mmol/L, multiply by 0.05551.

*GDM=gestational diabetes mellitus defined by IADPSG criteria (1 or more glucose values from a 75g OGTT equals or exceeds FPG 92 mg/dL, 1-hr 180 mg/dL, 2-hr 153 mg/dL)

**Summary statistics for non-participants are weighted by the number of participants at each field center