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# Energy balance and health in pregnancy: a pre-pilot project for the LifeGene Study

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## Overview

From January 2008 through June 2009 we conducted a study in Umeå focusing on lifestyle behaviors and pregnancy with three main aims:

i) to validate a range of physical activity and diet assessment methods in pregnant and pre-pregnant women
ii) to assess the feasibility of assessing physical activity and diet during pregnancy and in young infants
iii) to study associations between lifestyle behaviors during pregnancy and markers of cardiometabolic health in the infant offspring and the mother at birth and 4 months post-partum.

## Participants & Methods

We studied a total of 108 women at the baseline visit, of whom 35 were pregnant and 73 were not pregnant (most of whom stated that they intended to have children in the future and are thus termed 'pre-pregnant'). All pre-pregnant women underwent a urine pregnancy test at the first visit. One woman was determined to be pregnant on this basis and did not continue with the protocol. Pregnant and pre-pregnant women were broadly matched on demographic characteristics. All women lived in the eastern area of the county of Västerbotten in Sweden. The age of the women at baseline was 20-35 yrs and their body mass indexes (BMI) (during the first trimester for the pregnant women) ranged from 18-46 kg/m$^2$ (see Table 1).

Women were recruited through advertisements in the local papers, other media advertising, and with the assistance of midwives through anti-natal clinics. Pregnant women were recruited in trimester one or two and studied in trimester three (28-32 weeks gestation).

All pregnant women had successful pregnancies, one of which involved twins. Information concerning the delivery was collected with the mothers' consent from the hospital birth registry. In the majority of women, whole placenta (n=26) and cord blood (n=27) were collected and immediately stored in a –80˚C freezer.

The mothers of the children who were born-into-cohort were asked to return to the research clinic with their babies 3-4 months post-partum. Of the 35 women who were invited, 33 agreed to participate in this stage of the protocol. One of the

women who declined to participate was obese and had a premature delivery involving infant health complications that continued after birth. It was for this reason that she chose not to participate in the final stage of the study. The second woman did not provide a reason for declining to participate in this stage of the study.

**Table 1**. Participant characteristics at Visit 1

| | Pre-pregnant women (n=73) | | | | Pregnant women (n=35) | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean | s.d. | Range | | Mean | s.d. | Range | |
| | | | min | max | | | min | max |
| ***Variable*** | | | | | | | | |
| Age (yrs) | 28.6 | 4.4 | 20.0 | 36.0 | 30.4 | 2.9 | 21.8 | 35 |
| Height (m) | 1.67 | 0.74 | 1.47 | 1.83 | 1.67 | 0.60 | 1.54 | 1.77 |
| Weight (kg)† | 77.4 | 20.3 | 51.3 | 143.9 | 68.2 | 15.5 | 55 | 132 |
| BMI ($kg/m^2$)† | 27.6 | 6.7 | 18.6 | 44.9 | 24.6 | 5.0 | 20.4 | 45.7 |
| Waist (cm) | 84.8 | 16.6 | 63.8 | 128.0 | n/a | - | - | - |
| DXA total adipose mass (kg) | 28.8 | 14.5 | 8.2 | 72.6 | n/a | - | - | - |
| DXA total lean mass (kg) | 46.4 | 6.0 | 35.5 | 59.8 | n/a | - | - | - |
| DXA total adipose mass (%) | 37.6 | 10.1 | 14.9 | 60.9 | n/a | - | - | - |
| DLW total adipose mass (kg) | 30.3 | 15.5 | 11.1 | 75.0 | 25.2 | 10.9 | 14.4 | 67.3 |
| DLW total lean mass (kg) | 47.3 | 6.5 | 35.7 | 69.9 | 50.9 | 4.8 | 43.6 | 65.7 |
| DLW % adipose mass | 36.9 | 9.3 | 18.8 | 58.5 | 32.1 | 7.0 | 20.5 | 50.6 |
| Diastolic blood pressure (mmHg) | 74 | 8 | 58 | 96 | 68 | 6 | 53 | 85 |
| Systolic blood pressure (mmHg) | 112 | 11 | 87 | 136 | 108 | 10 | 91 | 138 |
| Fasting glucose (capillary) (mmol/l) | 5.74 | 0.67 | 4.5 | 8.9 | 5.15 | 0.53 | 4.1 | 6.9 |
| Fasting glucose (mmol/l) | 4.54 | 0.50 | 3.8 | 7.3 | 4.02 | 0.41 | 3.3 | 5.3 |
| 30min glucose (mmol/l) | 6.60 | 1.15 | 4.6 | 9.6 | 6.27 | 0.97 | 4.2 | 8.2 |
| 1hr glucose (mmol/l) | 6.08 | 1.79 | 2.6 | 10.6 | 6.23 | 1.36 | 3.6 | 9.3 |
| 2hr glucose (mmol/l) | 5.15 | 1.13 | 2.4 | 8.6 | 5.0 | 1.13 | 2.9 | 7.3 |
| Fasting insulin (mmol/l) | 7.25 | 7.13 | 1.6 | 51 | 10.25 | 9.17 | 1.6 | 55 |
| $HbA_{1c}$ (%) | 4.0 | 0.4 | 3.1 | 6.0 | 3.9 | 0.2 | 3.3 | 4.4 |
| Leucocytes ($10^9$/l) | 5.9 | 1.6 | 3.6 | 10.9 | 9.4 | 1.8 | 6.2 | 12.8 |
| Apo A1 (g/l) | 1.41 | 0.27 | 0.85 | 2.39 | 1.95 | 0.23 | 1.46 | 2.36 |
| Apo B (g/l) | 0.79 | 0.16 | 0.48 | 1.37 | 1.25 | 0.26 | 0.53 | 1.79 |
| Apo B/Apo A1 | 0.58 | 0.19 | 0.31 | 1.16 | 0.65 | 0.17 | 0.23 | 1.00 |
| TG (mmol/l) | 0.88 | 0.42 | 0.25 | 2.38 | 1.80 | 0.53 | 0.31 | 3.41 |
| Cholesterol (mmol/l) | 4.4 | 0.7 | 3.1 | 6.8 | 6.3 | 1.0 | 4.1 | 8.6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| HDL (mmol/l) | 1.5 | 0.4 | 0.8 | 2.4 | 2.0 | 0.4 | 1.2 | 2.8 |
| LDL (mmol/l) | 2.6 | 0.7 | 1.4 | 5.2 | 3.5 | 1.0 | 0.9 | 5.3 |
| LDL/HDL | 1.9 | 0.9 | 0.6 | 4.8 | 1.8 | 0.6 | 0.4 | 3.1 |
| Resting energy expenditure (kJ/kg/min) | DPU | DPU | DPU | DPU | DPU | DPU | DPU | DPU |
| Total energy expenditure (TEE)/ Total energy intake (TEI) (kCal/day) | 2687 | 471 | 1849 | 4998 | 2672 | 281 | 2228 | 3470 |
| Physical activity: | | | | | | | | |
| GENEA (g or counts/day) | DPU | DPU | DPU | DPU | DPU | DPU | DPU | DPU |
| ActiWatch-mini (counts/day) | DPU | DPU | DPU | DPU | DPU | DPU | DPU | DPU |
| ActiHeart (kCal/day) | DPU | DPU | DPU | DPU | DPU | DPU | DPU | DPU |
| DLW (kCal/day) | 853 | 312 | 67 | 1931 | 898 | 209 | 533 | 1365 |
| Maximal aerobic capacity ($O^2$ ml/kg/min) | DPU | DPU | DPU | DPU | DPU | DPU | DPU | DPU |

†Measured during the first trimester. Blood measures were made in venous blood unless otherwise indicated. DPU = data processing underway

A written and verbal description of all aspects of the study protocol was provided to the participants, following which written informed consent was obtained. The study was approved by the research ethics committee of Umeå University Hospital.

**Visit 1 and birth**

Women were invited to attend the clinical research facility at Umeå University Hospital on the morning following a 10hr overnight fast.

*Anthropometry, blood pressure and resting energy expenditure (REE)*

Height and weight were measured using a calibrated wall-mounted stadiometer and digital scale (Tanita Corporation, Tokyo, Japan), respectively. Height was measured to the nearest 0.5 cm and weight to the nearest 0.1 kg. Blood pressure was measured in triplicate at 1 minute intervals with a mercury-gauge sphygmomanometer using an appropriately sized cuff on the right arm with the participant seated. Participants were then asked to lie still and quietly on a bed without sleeping for 30 minutes, during which REE was measured continuously via indirect calorimetry using a ventilated hood (Deltatrac II, Datex-Ohmeda, Inc., WI, USA).

*Dual X-ray absorptiometry (DXA)*

Pre-pregnant participants underwent a full-body scan for the assessment of adipose and lean tissue mass and bone density and mass using a Lunar Prodigy DXA scanner (GE Healthcare, Diegem, Belgium). The volunteer wore light clothing free from metallic parts and was instructed to lie down on the DXA scanning table while the scanner passed over the body. DXA scans were not performed in pregnant women to avoid exposing the fetus to the radiation dose inherent in the DXA method. In pregnant and pre-pregnant women, body composition was also calculated using the doubly labeled water (DLW) method, as previously described in detail (1).

*Blood sampling and Oral glucose tolerance test (OGTT)*

Diabetes was one of the exclusion criteria for this study; therefore, for safety purposes, capillary plasma glucose was assessed from a finger stick sample on a Hemocue Glucose 201 Analyzer (Hemocue, Inc., CA, USA). One very obese pre-pregnant woman had a capillary glucose level of 8.9 mmol/l and the OGTT was thus not performed. The woman was referred to her primary care provider for follow-up. None of the women had very low glucose concentrations. Venous blood samples were drawn into different storage media (EDTA-plasma and serum), immediately placed on ice, processed, and sent to the hospital clinical biochemistry laboratory for analyses. Additional blood samples were stored at -80°C for later analyses. During the OGTT, blood was drawn fasting and at 30, 60, and 120 mins following a 75g oral glucose load according to the criteria of the World Health Organization (WHO, 1999).

*Exercise stress test*

Participants were fitted with an Actiheart monitor (Cambridge Neurotechnology, Papworth, UK) above the left breast, configured in inter-beat-interval mode. Real-time assessments of exercise heart rate were obtained using a Polar RS800 heart rate monitor to ensure women did not exceed an age-predicted heart rate of 90% (220-age in years). The exercise stress test comprised an eight minute step test with a gradually increasing step rate. The step rate was dictated by an audio-visual metronome displayed on a computer screen positioned in front of the participant during the test. Using this test, the work-rate increases progressively in a curvilinear fashion until i) completion of the test, ii) the participant indicates that they wish to stop, or iii) the participant's heart rate approaches 90% of their age-predicted maximum. On completion of the step test, participants were instructed to sit quietly for two minutes during which heart rate recovery was recorded. As we have shown previously (2) there is no added benefit to individually calibrating accelerometers; therefore these were not used at this stage of the protocol. We have previously demonstrated the validity of this method for individual calibrating heart rate against energy expenditure in non-pregnant individuals (2).

*Questionnaires*

Participants responded to a range of questionnaires (summarized in Table 2) focusing mainly on dietary intake and physical activity. Additional data was also collected relating to sociodemographic factors.

During the baseline visit the participants completed five questionnaires. Two of these were short physical activity questionnaires (IPAQ and RPAQ). The participants also completed the Short Diet Questionnaire (developed by Christel Larsson at the department of Food and Nutrition at Umeå University). This one page questionnaire focuses on quantifying amounts of macronutrient-containing foods consumed, which can subsequently be used to estimate macronutrient and energy intake. Participants also completed the questionnaire used in the Umeå EPIC Study, which includes a 64-item FFQ, a Swedish modification of the EPIC physical activity questionnaire and the SF-36. The diet and physical activity questionnaires listed above were the focus of the DLW validation component of this study. A fifth questionnaire focused on assessing body self-image was also included to enable collaborators at the Department of Obstetrics and Gynecology in Umeå (I Mogren and M Persson) to study the qualitative experiences of pregnant research participants. This, in combination with the SF-36, was included to help determine how obesity in young pregnant and pre-pregnant women affects self-rated health and quality of life. These qualitative data will be analyzed in collaboration with I Mogren and M Persson. One additional diet questionnaire (Selma) was administered in pregnant women only and was included in the DLW validation aspects of this study. To ensure that the time-frame of subjective and objective estimates of energy intake/expenditure overlapped, questionnaires were administered at home during the DLW monitoring period.

**Table 2**. A brief description of each questionnaire used at the baseline visit.

| Questionnaire | Brief description |
|---|---|
| Västerbotten Intervention Programme / Swedish EPIC questionnaire | An extensive questionnaire monitoring sociodemographic factors, diet, physical activity, alcohol consumption, self-rated physical and psychic health. (includes a 64-item FFQ and a Swedish modification of the EPIC Physical Activity Questionnaire) |
| IPAQ (International Physical Activity Questionnaire) | A short, validated, questionnaire monitoring habitual physical activity levels during the last seven days to estimate health related physical activity levels. |
| RPAQ (Recent Physical Activity Questionnaire) | A validated questionnaire on habitual physical activity levels during the past four weeks estimating total and physical energy expenditure. |
| Brief physical activity questionnaire | A questionnaire with 6 questions on work and spare time physical activity plus duration of sleep, estimating habitual physical activity levels (Johansson, IJO, 2008). |
| Short Diet Questionnaire | A condensed dietary questionnaire (one page) to be validate against the DLW analysis within this study (designed by C Larsson). |
| Self-rated body image questionnaire | 36 questions focusing on self-rated body image, aiming to capture psychopathological tendencies. |
| Selma | An extensive questionnaire focusing on diet, health and lifestyle designed for pregnant women. |

*Physical activity monitoring*

During the 10 days following their visit to the clinical research facility, detailed assessments of total energy expenditure (TEE), resting energy expenditure (REE), physical activity energy expenditure (PAEE) and total energy intake (TEI) during free-living conditions were obtained using objective methods. These methods were Actiheart (combined heart rate and movement sensing), Unilever GENEA Monitor (high resolution [40 Hz] triaxial accelerometry), and Actiwatch Mini (uniaxial accelerometry) (see Figure 1). The Actiheart monitor was worn on the chest (as described above), whereas the GENEA and Actiwatch Mini monitors were worn on the wrists. The GENEA monitors were on loan from UniLever Corporate Research (Bedfordshire, UK). The analysis of data from the activity monitors will be done with the help of scientists from Unilever (M Catt), NIH (K Chen) and Cambridge (S Brage and U Ekelund).

Figure 1. The Actiwatch Mini monitor

*Doubly labeled water (DLW)*

The DLW technique is the gold standard for the assessment of habitual energy expenditure and energy intake. Physical activity energy expenditure (PAEE) was calculated by subtracting BMR (estimated using the method described by Weir, 1949) from total energy expenditure (TEE). On arrival at the clinical research facility, participants were asked to void into a container (pre-dose sample). Prior to the completion of the testing session, participants were given a body weight-dependent oral dose of stable isotopes (0.07 g $^{2}H_2O$ and 0.174 g $H_2{}^{18}O$ per kg body weight). In addition to the pre-dose sample ten additional urine samples were collected, one for each of the 10 days following the day of dosing. The date and time of day when a sample was collected was noted by the participant in a log. Urine samples were stored in plastic urine vials at +4˚C until the collection was returned. Samples were subsequently stored at –20˚C pending analysis. Isotopic enrichments of dose and urine samples were analyzed at Human Nutrition Research, University of Cambridge, Cambridge, UK. Analysis of DLW data will be undertaken in collaboration with scientists from Human Nutrition Research (A Wright) and elsewhere.

**Visit 2**

A total of 33 mothers and their babies (N=35) attended the University Department of Pediatrics and the clinical research facility at 4 months post-partum. At this visit, detailed measurements were undertaken in the mother and child focusing on the same key areas as the mothers' pregnancy examination. Table 3 provides an overview of the measurements obtained.

**Table 3.** Overview of data collected during visit 2 (data presently being processed)

| | Mothers (n=33) | Infants (n=34) |
|---|---|---|
| Age (yrs) | ✓ | ✓ |
| Head circumference (cm) | ✗ | ✓ |
| Height/length (m; cm) | ✓ | ✓ |
| Weight (kg; g) | ✓ | ✓ |
| BMI (kg/m$^2$) | ✓ | ✗ |
| Waist (cm) | ✓ | ✗ |
| DXA total adipose mass (kg) | ✓ | ✗ |
| DXA total lean mass (kg) | ✓ | ✗ |
| DXA total adipose mass (%) | ✓ | ✗ |
| PeaPod total adipose mass (kg) | ✗ | ✓ |
| PeaPod total lean mass (kg) | ✗ | ✓ |
| PeaPod total adipose mass (%) | ✗ | ✓ |
| Diastolic blood pressure (mmHg) | ✓ | ✗ |
| Systolic blood pressure (mmHg) | ✓ | ✗ |
| Fasting glucose (capillary) (mmol/l) | ✓ | ✓ |
| Fasting glucose (mmol/l) | ✓ | ✓ |
| Fasting insulin (mmol/l) | ✓ | ✓ |
| $HbA_{1c}$ (%) | ✓ | ✓ |
| Leucocytes ($10^9$/l) | ✓ | ✓ |
| Apo A1 (g/l) | ✓ | ✓ |
| Apo B (g/l) | ✓ | ✓ |
| Apo B/Apo A1 | ✓ | ✓ |
| TG (mmol/l) | ✓ | ✓ |
| Cholesterol (mmol/l) | ✓ | ✓ |
| HDL (mmol/l) | ✓ | ✓ |
| LDL (mmol/l) | ✓ | ✓ |
| LDL/HDL | ✓ | ✓ |
| Homocystein (µmol/l) | ✗ | ✓ |
| Feces sample | ✓ | ✓ |
| Physical activity (g or counts/day) | ✓ | ✓ |
| Sleeping bouts (total/daytime/nighttime) | ✗ | ✓ |
| Sleeping duration (total/daytime/nighttime min) | ✗ | ✓ |
| Breast feeding bouts (total/daytime/nighttime) | ✗ | ✓ |
| Breast feeding duration (total/daytime/nighttime min) | ✗ | ✓ |

*Anthropometry and blood pressure*

Height, weight and blood pressures were measured in the mother using the methods described for Visit 1. In the babies, body weight was measured to the nearest 1 g using a digital scale (Seca, ErgoNordic AB, Bromma, Sweden), body length to the nearest 0.1 cm using a measuring board (CMS weighting equipment Ltd, London, UK) and head circumference to the nearest 0.1 cm with a non-stretchable nylon tape.

*Body composition*

The mothers underwent a DXA scan (Lunar Prodigy, GE Healthcare, Diegem, Belgium) using the methods described for Visit 1 (for pre-pregnant women). Body composition was assessed in the infants (without clothing or diaper) using air displacement plethysmography (PeaPod, Life Measurement Inc., CA, USA) (see figure 2a).

*Blood and fecal sampling*

Mothers were fasted for 10 hrs and babies for 2 hrs prior to blood sampling. Fasting blood samples were obtained from the mother using the methods described above for Visit 1. In the infant, anesthetic cream (Emla, AstraZeneca) was applied by a research nurse to the back of the hand 1 hour prior to the blood draw. Approximately 2-3 ml venous blood was drawn from the anesthetized region of the hand into serum tubes, immediately placed on ice, processed, and sent to the hospital clinical biochemistry laboratory for analyses. Additional samples were stored for later analyses.

Fecal samples from the mother and child were collected prior to their second visit into plastic tube collection kit specifically designed for this purpose. Approximately one tablespoon of feces was collected and stored in -20˚C until the samples were returned, upon which the samples were stored in -80˚C pending analysis. These samples were collected at the request of collaborators at the Department of Pediatrics in Umeå (M Domellöf) to study the relation of gut flora and obesity. These data will be analyzed in collaboration with M Domellöf and others.

*Questionnaires*

The mothers responded to a range of questionnaires (summarized in Table 4) focusing mainly on their baby's health and sleeping and feeding patterns. Detailed information on the baby's feeding habits was noted by the parents in a log during five days following visit 2. Time and duration of each feeding occasion was noted and whether the baby was breast fed or given supplement. If the baby was fed supplement the amount was noted in the log. If breast fed, the mother was also asked to note if both breasts were used and to term each occasion 'feeding' or 'cozy relaxation', the latter to indicate that the baby was held against the breast but not feeding. Time and duration of each nap was noted during the same period in a separate log. The mother was also asked to indicate if anything above the ordinary happened during this five day period which might have interfered with normal feeding or sleeping patterns (e.g. illness or travel). In addition, the parents also

answered several questions regarding the child's health (e.g. illnesses, allergies, medications, vaccinations, and exposure to parental smoking or pets).

**Table 4**. A brief description of each questionnaire used at the mother/infant follow-up stage of the study

| Questionnaire | Brief description |
|---|---|
| Feeding pattern | Detailed log of the infant's feeding habits during a five day monitoring period. Contains information if the infant is breast fed or given supplement, and time and duration of each feeding occasion |
| Sleeping pattern | Detailed log of time and duration of each sleep bout during a five day monitoring period |
| General health | General questions relating to the infant's health (e.g. illness, vaccinations, allergies, and parental smoking) |

*Physical activity monitoring*

During the ten days following the follow-up examination, the mothers wore a triaxial accelerometer (GENEA monitor, UniLever Ltd, Bedford, UK) on the same hand as during the first visit (see methods for Visit 1). In the babies, two GENEA monitors were worn continuously for 48 hrs. One monitor was securely fitted to the right ankle using a hospital non-removable band. A second GENEA monitor was securely fastened to the baby's torso using a specially adapted garment which fitted snuggly over the diaper (see figure 2b). Mother's were instructed to remove the over-garment when changing the diaper and to refit the over-garment immediately thereafter. In order to distinguish the child's own movements from those when it was being moved around by someone else, the ankle monitor time-synchronized movement data was subtracted from the movement data recorded on the torso monitor. The synchronization of the mother's and baby's monitors also allows a direct assessment of the time the mother and child were in physical contact, and (theoretically) for assessing the duration of specific behaviors such as breast feeding. The monitoring period in the baby overlapped completely with the mother's monitoring period. The baby's sleeping and feeding patterns were also documented by the mother in a diary during a five day activity monitoring period. Table 3 summarizes the data collected during visit 2.

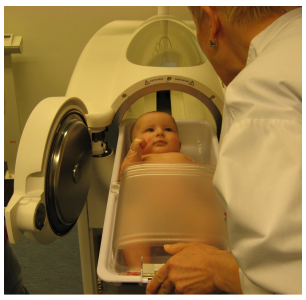

Figure 2a. PeaPod measurement.

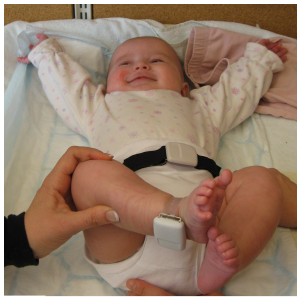

Figure 2b. Positioning of the GENEA-monitors on the infant.

## References


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