

Supplemental Material and Methods

Human maternal and offspring anthropometric measurements

Maternal body weight was measured at T0 and then weekly until 36 weeks of gestation (T1) using a digital scale to the nearest kg (Tanita Corp., Tokyo, Japan). The weight measurement near delivery (38/39th week assessment) was performed by the gynecologist at the last meeting. Maternal weight gain was defined as the difference between self-reported pre-pregnancy weight and the weight measured close to delivery. The Institute of Medicine recommendations ¹ were used to classify excessive weight gain. Height was measured once at the beginning using a metal stadiometer to the nearest 0.1 cm.

Mid-arm circumferences were measured once a month on the right side to the nearest 0.1 cm with a non-extensible, flexible tape.

Skinfold thickness was measured at T0 and T1 by an observer using a Harpenden skinfold caliper (John Bull British Indicators Ltd., Harpenden UK) with a constant pressure of 10g/mm². The procedure was carefully standardized and each measurement was made in triplicate on the right side of the body and the results were averaged. Three points were measured to the nearest 0.2 mm at the triceps, thigh, and suprailiac. Body fat percentage was calculated using triceps, thigh, and suprailiac skinfold thickness according to Jackson *et al.* ². The upper arm fat estimate and upper arm fat-free mass estimate were calculated according to Rolland-Cachera *et al.* ³.

Questionnaires: Socio-demographic variables and other covariates such as pre-pregnancy weight, parity or smoking habits were determined by questionnaires ^{4,5}.

Nutritional intake: Nutritional data for each woman were collected from 7-day weighed food diary at baseline (T0) and mid-term (24 weeks of gestation) to provide a detailed description of their food and fluid intake as well as supplement use, if applicable. Participants were given

comprehensive verbal and written instructions to report how food/fluid was cooked or prepared, brand names, and quantities; electronic portable scales were provided. Data from the diary were entered and processed using a commercially available dietary analysis software (DGExpert software) to calculate the daily amount of food and nutrition substances. A single researcher (ecotrophologist) analyzed the dietary data in order to avoid variability in the interpretation of these data. Based on the results, individual dietary counselling took place for the intervention group.

Fetal outcome: After delivery, infant weight was measured using an electronic baby scale to the nearest 1g and the height was measured crown-heel in the recumbent position to the nearest 1mm by midwives. Further information about the birth, medical history, and anthropometric status of the infant was extracted from the hospital records. Weight and height at birth (O1), after 3 days (O2), 1 month (O3, 1.1 ± 0.2 month), 4 months (O4, 3.4 ± 0.6 month) and 6 months (O5, 6.3 ± 0.6 month) were obtained from the medical examination records. Infants from both groups were breast-fed and the feeding regime was in accordance with the national breast-feeding recommendations ⁶.

Quantification of gene expression

White adipose tissue (WAT) samples were taken at postnatal day 21 and frozen at -80 °C. Total RNA was isolated using TRI-Reagent[®] (Sigma-Aldrich) according to the manufacturer's guidelines. RNA quantity and purity was determined by measuring UV absorption with a NanoDrop spectrophotometer (Nano Quant infinite M200 Pro). Quantitative changes in mRNA expression were determined by qRT-PCR as previously described⁷, using the 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The following genes related to fat/glucose metabolism were evaluated: mRNA levels of Ppar γ (peroxisome

proliferator-activated receptor γ) were measured in WAT using the following primers: 5'-CCCAATGGTTGCTGATTACAAA-3' forward and 5'-GCCTGTTGTAGAGCTGGGTCTT-3' reverse; probe ACCTGAAGCTCCAAGAATACCAAAGTGCGA, and Glut4 expression in WAT was measured using the following primers: 5'-GATACCTCTACATCATCCGGAACCT-3' forward and 5'-AAGGACATTGGACGCTCTCTCT-3' reverse; probe CTGCCCCGAAAGAGTCTAAAGCGCCTG. Expression levels of the genes of interest were normalized to GUSB using the following primer: 5'-CGCTGAGAGTAATCGGAAACAA-3' forward and 5'-CGCAAAATAAAGGCCGAAGT-3' reverse; probe ATCTTCACTCGCCAGAGACAGCCCA)

Western blot analysis

Frozen dissected white adipose tissue was homogenized in lysis buffer as previously described⁷. Blots were probed with the following antibodies: polyclonal rabbit antimouse Akt (Cell Signaling; no. 9272, 1:1000), monoclonal rabbit anti-phosphorylated(p) AKT (Cell Signaling; no. 4058), Polyclonal rabbit-anti-HPRT (Abcam, no. ab10479) served as a loading control. Anti-mouse IgG, HRP-linked (no. 7076), and anti-rabbit IgG, HRP-linked (no. 7074) were used as secondary antibodies.

References

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