Metabolomics analysis reveals altered metabolites in lean compared with obese adolescents and additional metabolic shifts associated with hyperinsulinaemia and insulin resistance in obese adolescents: a cross-sectional study

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Online Resource 2

Anthropometry

Body height was measured by a stadiometer (Ulmer stadiometer, Busse, Elchingen, Germany) and body weight by a digital scale (SECA model 704, Hamburg, Germany). BMI was calculated as kg/m^2. Sex- and age-independent BMI standard deviation score (SDS) was calculated using the WHO 2006-2007 growth reference. Pubertal stage was assessed using sex-specific cut-off points for sex hormones and growth charts for height.

Clinical assessment

Glucose (Abbott Architect, Abbott Diagnostics, Lake Forest, Ill) and insulin (Cobas E602, Roche Diagnostics, Indianapolis, IN) analyses were performed at the University Hospital, Uppsala, Sweden. Insulin data from four control subjects were missing and were therefore analysed by enzyme linked immunosorbent assay (ELISA; Mercodia). The HOMA-IR was calculated as fasting insulin (µIU/mL) × fasting glucose (mmol/mL)/22.5 (Matthews *et al.*, 1985). Matsuda Index was calculated as described previously (Matsuda and DeFrozo, 1999).

Sample and data retrieval

Plasma samples were retrieved from ULSCO cohort (Forslund *et al.*, 2014). Insulin and glucose concentrations, gender, status of puberty, age and BMI were retrieved from the database of ULSCO cohort (Forslund *et al.*, 2014).

Metabolic profiling

Ethylenediaminetetraacetic acid (EDTA) plasma samples were used for metabolomics analyses. Sample preparation was performed based on a previously described protocol (Röhnisch *et al.*, 2018; Tiziani *et al.*, 2008). In brief, Nanosep centrifugal filters with 3-kDa cut-off (Pall Life Science, Port Washington, NY) were washed 8 times with 500 µL water at 2,000 g and 36°C. Then 60 µL of plasma sample were filtered at 10,000 g, 4°C. For quantification of metabolites, 40 µL of plasma filtrate were mixed with 50 µL phosphate buffer (0.4 mol/L, pH 7.0), 15 µL D_2O, 55 µL Millipore water and 10 µL sodium-3-(trimethlsilyl)-2,2,3,3,-tetradeuteriopropionate (TSP, 5.8 mmol/L) (Cambridge Isotope Laboratories, Andover, MA) as an internal standard. Analyses were performed on a Bruker spectrometer operating at 600 MHz equipped with a cryogenically cooled probe and autosampler. The nuclear magnetic resonance (^1H NMR) spectra were obtained using the zgesgp pulse sequence (Bruker

Spectrospin Ltd) at 25°C with 512 scans at 65,356 data points over a spectral width of 17,942.58 Hz (acquisition time: 1.83 sec, relaxation delay 4 sec) (Hwang and Shaka, 1995; Röhnisch *et al.*, 2018). Forty-nine metabolites were identified based on previous literature (Nagana Gowda *et al.*, 2015), search in human metabolome database (HMDB), spiking with authentic standards and commercial library (NMR Suite Professional Software package, version 7.5, ChenomX Inc., Edmonton, Canada) as previously described (Röhnisch *et al.*, 2018). For the list of identified metabolites and their HMDB ID (Metabolomics Standard Initiative (MSI) identification, at least, level 2) see Online Resource 7.

The concentrations of the identified metabolites were calculated using an automated quantification algorithm (AQuA) capable of accounting for interfering signals (Röhnisch *et al.*, 2018). AQuA was modified by inclusion of EDTA and its calcium and magnesium complexes (Barton *et al.*, 2010) in the metabolite library, in order to account for interferences of EDTA signals with signals from other metabolites.

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