Electronic supplementary material

Methods

Cohort and patient characteristics

Inclusion and exclusion criteria for respective cohorts:

Leipzig Schoolchildren cohort: representative cohort of urban central German paediatric population *n*=2,675; inclusion criteria for subsample: healthy, lean children, free of medications, free of co-morbidities; children were selected and stratified for sex and pubertal stage.

Leipzig Atherobesity Childhood cohort: inclusion criteria: healthy children, aged 7 to 18 years, free of medications, free of co-morbidities, no weight reduction within past 6 months.

Lifestyle intervention cohort: inclusion criteria: Age 8 to 16 years, BMI >97th centile, free of medications, free of co-morbidities. Children with endocrine disorders, familial hyperlipidaemia or syndromal obesity were excluded from the study.

Bariatric surgery intervention cohort: Individuals fulfilled the following inclusion criteria: (1) absence of any acute or chronic inflammatory disease as determined by a leucocyte count >7,000 Gpt/l, C-reactive protein (CrP) >5.0 mg/dl or clinical signs of infection; (2) undetectable antibodies against glutamic acid decarboxylase; (3) no clinical evidence of either cardiovascular or peripheral artery disease; (4) no thyroid dysfunction; (5) no alcohol or drug abuse; and (6) no pregnancy. BMI was calculated as weight divided by squared height. Hip circumference was measured over the buttocks; waist circumference was measured at the midpoint between the lower ribs and iliac crest.

Exercise intervention cohort: All individuals fulfilled the following inclusion criteria: (1) fasting plasma glucose <6.0 mmol/l; (2) HbA_{1c} <6.0%; and (3) stable weight, defined as the absence of fluctuations of >2% of body weight for at least 3 months. In addition, the following exclusion criteria have been defined: (1) medical and family history of type 1 or type 2 diabetes; (2) medical history of hypertension or systolic blood pressure (SBP) >140 mmHg and diastolic blood pressure (DBP) >85 mmHg; (3) any acute or chronic inflammatory disease as determined by a leucocyte

count >8000 Gpt/l, CrP >5.0 mg/dl or clinical signs of infection; (4) clinical evidence of either cardiovascular or peripheral artery disease; (5) any type of malignant disease; (6) thyroid dysfunction; (7) Cushing's disease or hypercortisolism; (8) alcohol or drug abuse; (9) pregnancy; and (10) concomitant medication except contraceptives.

Determination of NAMPT enzymatic activity

For preparation of lysates, 1×10^7 cells were resuspended in 100 µl 0.01 mol/l $NaHPO_4$ buffer, pH 7.4, and frozen at –80°C for 24 h. Cell lysates were then centrifuged at 23,000*g* and 0°C for 90 min to remove cell debris. To precipitate DNA, protamine sulphate solution (1% in $NaHPO_4$ buffer) was added to the supernatant fraction (70 µl/ml) and samples were incubated for 15 min on ice. After centrifugation at 23,000 *g* and 0°C for 30 min aliquots of the supernatant fraction were stored at –80°C. For each measurement of supernatant fraction, 6 ml of cell culture supernatant fraction were concentrated using Amicon 4Ultra columns with a molecular weight cut-off at 50 kDa (Millipore, Billerica, MA, USA). Ten microlitres of the cell lysates and concentrated supernatant fraction were transferred into 50 µl of a reaction mix containing 50 mmol/l Tris (pH 7.4), 2 mmol/l ATP, 5 mmol/l $MgCl_2$, 0.5 mmol/l 5-phosphoribosyl-1-pyrophosphate, and 5 µmol/l [carbonyl-^{14}C]nicotinamide (American Radiolabeled Chemicals, St Louis; MO, USA). Reactions were incubated for 2 h at 37°C. The reaction was terminated by transfer of 2×50 µl into tubes containing 2 ml of acetone. Production of labelled nicotinamide mononucleotide from [^{14}C]nicotinamide was analysed by a precipitation-filtration assay. Therefore, the reaction mixture was pipetted onto acetone-pre-soaked glass microfibre filters (GF/A Ø 24 mm; Whatman, Maidstone, UK). After rinsing with 3×1 ml acetone, filters were dried, transferred into vials with 6 ml scintillation cocktail (Betaplate Scint, PerkinElmer, Waltham, MA, USA) and radioactivity of [^{14}C]NMN was quantified in a liquid scintillation counter (Wallac 1409 DSA, PerkinElmer). After subtraction of blank values, NAMPT activity was normalised to total protein amount.