SUPPLEMENTARY DATA FOR MANUSCRIPT

PREVIEW: Prevention of diabetes through lifestyle intervention in a multicentre study in Europe in children (10-17y). Design, methods, and baseline results

Running title: PREVIEW children study: methods and baseline results

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DETAILED DESCRIPTION OF STUDY PROTOCOL AND METHODS

1. Subjects

1.1 Inclusion/exclusion criteria

Inclusion criteria were 1) age 10-17 years, 2) overweight or obesity, defined as age- and sex-adjusted BMI≥25kg/m^2 [1], 3) IR (HOMA-IR≥2.0 for children Tanner stages ≥3 or any HOMA-IR for children Tanner stages 1-2), 4) written informed consent by both parents and children aged ≥12 years, 5) proficiency of the local language and 6) willingness to be randomized and adhere to the study protocol.

Exclusion criteria were 1) medical conditions that might compromise study outcomes or adherence (e.g. T2DM, malabsorption diseases, bariatric surgery, and chronic respiratory, neurological, musculoskeletal disorders), 2) medication use that potentially influenced body weight or glucose metabolism (e.g. metformin) ≤3 months prior to enrolment, 3) blood donation or transfusion ≤1 month prior to enrolment, 4) self-reported weight change ≥5% 2 months prior to screening, 5) special diets 2 months before screening, 6) severe food intolerance, and 7) psychological or behavioural problems leading to difficulty in complying with the protocol.

1.2 Enrollment

Children were pre-screened by telephone to assess inclusion and exclusion criteria, and subsequently they underwent a short screening at one of the intervention centres. Children that were found to be eligible at the screening and agreed to continue study participation, were enrolled for baseline measurements and randomization *(Figure 1)*.

2. Intervention and study protocol

2.1. Dietary intervention

All diets – regardless of intervention group – were aimed at weight stabilization in spite of growth, thus decreasing age- and sex-adjusted BMI z-score. Participants completed a four-day food record at the start of the study, after which a dietician calculated the basal metabolic rate for each child using the WHO formula[i]. All children received personalized sample menus constructed by dieticians, that were in line with their study allocation and energy needs with a maximum of 8700kJ/24 hours. During the first study phase, all children received sample menus with the same target macronutrient composition of 15/55/30En% protein/carbohydrate/fat. During the following phase of 96 weeks children received personalized sample menus adhering to the targeted macronutrient composition of their randomization arm (*Supplementary Table 1*). In order increase compliance menus were kept as simple as possible and no instructions were given on micronutrient composition and dietary fibre. In addition, children were provided with recipes which were in line with their randomization group and received dietary counselling at each study visit.

The consumption of sugar-sweetened beverages and energy-dense foods between meals were discouraged, and the intake of fruits and vegetables stimulated.

2.2. Physical activity

Because of natural variability in physical activity in different age categories during childhood, all children received instructions on both high-intensity (HI) and moderate-intensity (MI) PA of which they could choose exercises *(Supplementary Table 1)*. Sports in general were encouraged. During each study visit, children were counselled on physical activity.

3. **Measurements**

Measurements were performed during CIDs *(Supplementary Table 2)*.

3.1 Anthropometric measurements and BMI z-score calculation

Height was measured to 0.1cm using a wall-mounted stadiometer (De Grood Metaaltechniek, Nijmegen, the Netherlands) and weight to 0.1kg on a digital scale (Seca, Chino, CA, USA). Because mean BMI in childhood is influenced by periods of growth, age- and sex-adjusted BMI z-scores were calculated to assess BMI deviation in respect to the mean BMI. Since mean BMI has increased during the childhood obesity epidemic, it was decided to calculate BMI z-scores to an older reference cohort as this represented a child's true overweight status. As most of the cohort was Dutch, reference data of the Dutch National Growth Study of 1980 was used to calculate BMI z-scores (Growth Analyser VE, Rotterdam, the Netherlands). Inter-cohort testing showed no difference in height between Dutch, Spanish and British children, making this reference cohort suitable for all children in the study.

3.2 Body composition

Body composition was measured using air-displacement plethysmography by the BodPod (Life Measurement Instrument, Concord, CA, USA) or bio-impedance measurements (BIA, Tanita SC-330, Tanita Corp, Tokyo, Japan), after which fat mass (FM), fat free mass (FFM) and fat mass percentage (FM%) were calculated. Subsequently, fat mass index (FMI) was calculated as *fat mass (kg) / height (m)^2*, and fat free mass index (FFMI) as *fat free mass (kg) / height (m)^2*.

3.3 Parameters of glucose metabolism, lipids, inflammation, and liver parameters

Fasting blood glucose concentrations, total cholesterol, high-density lipoprotein (LDL) cholesterol, low-density lipoprotein (HDL) cholesterol, triacylglycerides (TAG), C-reactive protein (CRP), alanine transaminase (ALT), and aspartate transaminase (AST) concentrations were measured with the COBAS 800 modular analyser (Roche, Woerden, the Netherlands). Fasting insulin and HbA1c concentrations were measured with the fully automated HPLC Variant II 155 (Bio-Rad Laboratories, Veenendaal, the Netherlands) and C-peptide concentration with Immulite XPI (Siemens, Eindhoven, the Netherlands). All laboratory measurements were performed in the Maastricht University

Medical Centre laboratory. Insulin sensitivity was assessed by HOMA-IR, a commonly used marker for IR in children because its relatively non-invasive nature (*glucose (mmol/L) * insulin (mU/L) / 22,5*)[2]. In the absence of consensus on a HOMA-IR cut-off point for IR, we defined children as insulin resistant when HOMA-IR≥2.0. Because HOMA-IR is physiologically lower in early pubertal stages while these children still may be at risk of HOMA-IR increase during puberty, all HOMA-IR values were accepted in children at Tanner stages 1-2[3].

3.4 Blood pressure and heart rate

Blood pressure and heart rate were measured on the right arm, using the Mobil-O-Graph (I.E.M., GmbH, Stolberg, Germany) and a cuff that corresponded with upper arm circumference.

3.5 Food intake

Children completed a 4-day food record on paper or through a food diary app to assess food intake and compliance to the study protocol. Food records were analysed at each site for energy intake, macronutrient composition, micronutrients, dietary fibre, GI and glycaemic load (GL). For the latter, local GI data for individual food items were used. As a biomarker, 24h urinary nitrogen was obtained to calculate protein intake in a subcohort at UM.

3.6 Physical activity

PA was measured by 7-day accelerometry (Actigraph GT3X accelerometer, Actigraph Corp, USA). Wear time validation was performed with a minimum of 4 days >10 hours including 1 weekend-day.

3.7 Sleep

Self-assessed sleep parameters was assessed by the Pittsburgh Sleep Quality Index (PSQI) and Epworth Sleepiness Scale (ESS)[4,5]. In addition, a subcohort at UM underwent a polysomnography to obtain information on total sleeping time (TST), Rapid Eye Movement (REM) sleep, slow wave sleep (SWS), wake after sleep onset (WASO), and quality of sleep (QS, SWS+REM)/TST).

4. **Primary and secondary endpoints**

The primary endpoint of the PREVIEW children study was change in HOMA-IR, corrected for puberty, after two years of intervention. Secondary endpoints were changes in HbA1c, BMI z-score, FM%, FMI, cardiovascular risk factors, inflammation and liver transaminases, and their associations with HOMA-IR change. Further endpoints included changes in PA and dietary restraint. In a subgroup changes in sleep architecture and their associations with HOMA-IR changes were studied.

5. **Data management**

Data was stored in a central project database at the University of Copenhagen[ii]. Anthropometric data was entered in case report forms in the online Open Clinica database. Questionnaires were entered in an Questionnaire Delivery Platform (QDP, NetUnion, Lausanne, Switzerland) or on paper after which the questionnaire was entered in QDP by PREVIEW researchers. Laboratory analyses were centrally performed and entered in a database at UM. Accelerometry data was collected at each site and analysed at SU. Data cleaning was performed by independent researchers at UM and aberrant values checked with a paediatrician.

6. Statistical analyses

6.1 Power calculation

Considering an estimated 25% drop-out, α of 0.05 and sample size of 100, a power of 0.96 will be achieved (G*power, Universität Düsseldorf, Düsseldorf, Germany).

6.2 Analysis for baseline results

Baseline analyses in this paper were performed using the Statistical Package for the Social Sciences (SPSS) 24.0 (SPSS Inc, IBM Corporation, Armonk, NY, USA). Normal distribution was tested with the Shapiro-Wilk test and outliers were assessed and removed if necessary. ANOVA or Mann-Whitney-U test were used to assess differences in baseline characteristics between the two intervention groups, depending on normality of data. Associations between parameters were assessed with Pearson's or Spearman's correlation coefficients, which were corrected for relevant variables. A p-value <0.05 was considered statistically significant.

Drop-out analyses, consisting of ANOVA or Mann-Whitney-U tests depending on normality of data, were performed to assess differences in anthropometrics, body composition and glucose metabolism between children that did and did not complete questionnaires.

6.3 Future analyses for comparing the two intervention groups

For comparing the two intervention groups in future analyses, the two dietary arms will be compared using intention-to-treat analyses. Changes over time in HOMA-IR and other outcome measures will be assessed using repeated measurement analyses, and multiple regression analyses will be used to identify the contribution of different variables to HOMA-IR change. For comparisons between the two groups, a factorial ANOVA with repeated measures will be used.

7. Ethical considerations

Medical Ethics Committees at each study site approved the PREVIEW study protocol and amendments. The study protocol was compliant with the Declaration of Helsinki and the ICH-GCP and registered on ClinicalTrials.gov (number NCT01777893). All study data was handled according to local regulations and the European Directive

95/46/CE. Research staff was GCP trained and UM staff was also trained in clinical paediatrics. Signed informed consent was obtained of parents and children ≥12 years.

DISCUSSION POINTS

1. *HOMA-IR was significantly higher in pubertal children with morbid obesity compared to prepubertal children with morbid obesity and all children with overweight*

We found that pubertal children with morbid obesity had significantly higher HOMA-IR levels than pubertal children with overweight/obesity, identifying this group of children as having a particularly high risk for T2DM development *(Supplementary Figure 1)*. This finding confirms earlier studies in which especially children with morbid obesity showed high HOMA-IR at the end of puberty, instead of decreasing HOMA-IR towards the end of puberty as is the pattern in lean children[6,7]. Mechanistically, elevated IR in subjects with morbid obesity might be a direct result of increased ectopic fat storage, which results in increased free fatty acid (FFA) concentrations and inflammation, leading to reduced muscle glucose uptake and thereby maintenance of peripheral IR[6]. High HOMA-IR in late puberty in children with morbid obesity in this and previous studies, demonstrates that these children especially are at high risk for β-cell exhaustion and T2DM development[3,6,7].

2. *Fasting blood glucose concentrations were negatively associated with Baecke Sport*

Baecke Sport and fasting blood glucose concentrations were inversely related, independently of sex, Tanner stage, BMI z-score and FM% (*Supplementary Table 3*). This finding might suggest that higher self-reported PA was associated with better regulated blood glucose concentrations. During exercise, metabolism shifts from predominant reliance on free fatty acids (FFA) in rest to carbohydrate oxidation. As glycogen stores in the muscle become deplete, insulin sensitivity of the muscle increases, thereby increasing fasting glucose uptake and muscle insulin sensitivity. Additionally, muscle contractions increase GLUT4 transporter protein translocation and thus enhanced muscle glucose uptake, even in IR[ii,iii]. However, glucose metabolism was not associated with accelerometry counts, and Baecke scores and accelerometry data were not interrelated.

3. *Fasting blood glucose concentrations were positively related to sleepiness*

The positive association between fasting blood glucose concentrations and ESS daytime sleepiness scores indicates that children that experienced more sleepiness had higher fasting blood glucose concentrations *(Supplementary Tables 4)*. This is consistent with an earlier study[iii]. Obesity is associated with higher apnoea-hypopnea indexes and intermittent nocturnal hypoxemia, both of which are independently associated with sleepiness and IR. In addition,

sleeping time declines during puberty. However, it should be noted that all fasting blood glucose concentrations in this cohort were within normal ranges.

4. Missing data regarding lifestyle factors

For some questionnaires and food records, numbers of returned data are relatively low. This is caused by refusal to answer questionnaires, incomplete questionnaires or because questionnaires were not returned. For all questionnaires, a certain number of items have to be filled in to correctly calculate scores, incomplete questionnaires therefore sometimes led to exclusion of the questionnaire for that child for analyses. Food records were often incompletely filled out or not returned at all. Food records ≥2 days of adequate food composition were used for analyses, food records with fewer days or severely inadequately filled out records were excluded for this study. Drop-out analyses found no differences in children that completed the PA and TFEQ questionnaires and children that did not complete these questionnaires. Children that answered sleep questionnaires had a significantly higher FFMI and fasting glucose concentrations and lower FM% than children that did not return the sleep questionnaires. These factors will be taken into consideration in future analyses.

FIGURES

Supplementary table 1. Description of the PREVIEW intervention in children

	HPLGI	MPMGI
Dietary intervention	**High protein (25 En%)** **Moderate carbohydrate (45 En%)** **Low GI (≤50) diet** Food items with increased use[a]: • Whole-grain cereals with low GI • Pasta • Low-fat dairy products • Poultry • Fish • Legumes	**Moderate protein (15 En%)** **Higher carbohydrate (55 En%)** **moderate GI (≥56) diet** Food items with increased use[a]: • Whole-grain cereals with moderate/high GI (e.g. bread) • Potatoes, sweet potatoes, couscous, rice • Bananas
Physical activity intervention[b]	High-intensity physical activity: ≥75 minutes per week of high intensity physical activity, such as vigorous bicycling, jogging >8km/h and strenuous ball games and moderate-intensity physical activity: ≥150 minutes per week of moderate intensity activity, such as moderate bicycling, brisk walking (4-6km/h), and swimming	

GI = GIycaemic Index; HPLGI = high-protein low-GI diet; MPMGI = moderate-protein moderate-GI diet; En% = Energy percentage.

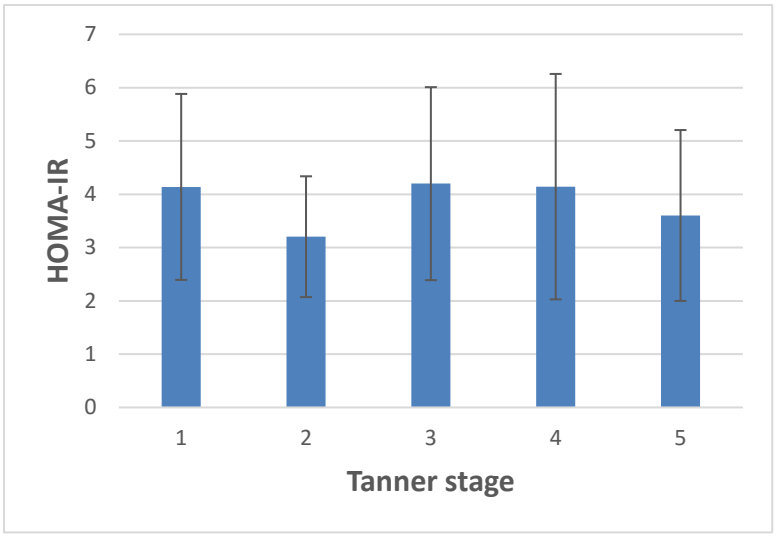
[a.] Increased use relative to the other intervention group

[b.] Both groups received instructions for both PA intensities

Supplementary table 2. Overview of data collection at different Clinical Investigation Days (CID) in the PREVIEW children intervention

Data collection	Assessment time-points (week)					
	0 CID1	8 CID2	26 CID3	52 CID4	78 CID5	104 CID6
Randomization	X					
General information • Age (y) • Tanner G/M stage	X	X	X	X	X	X
Anthropometric characteristics • Body weight (kg) • Height (cm) • Sitting height (subgroup) • BMI (kg/m2) • BMI z-score (SD) • IOTF class • Waist circumference (cm) • Hip circumference (cm) • Thigh circumference (cm)	X	X	X	X	X	X
Body composition • Fat free mass index (FFMI, kg/m2) • Fat mass index (FMI, kg/m2) • Fat mass (%)	X	X	X	X	X	X
Parameters of glucose metabolism • Fasting glucose, fasting insulin, HOMA-IR, HbA1c, C-peptide	X	X	X	X	X	X
Lipids • Total cholesterol, HDL-cholesterol, LDL-cholesterol, TAG	X	X	X	X	X	X
Inflammation • CRP	X	X	X	X	X	X
Liver parameters • AST, ALT	X	X	X	X	X	X
Blood pressure and heart rate • Systolic and diastolic blood pressure • Heart rate	X	X	X	X	X	X
Physical activity • 7-day accelerometry • Baecke questionnaire	X	X	X	X		X
Food intake behaviour • 4-day food record • TFEQ questionnaire • VAS appetite scores	X		X	X		X
Protein intake • Urinary nitrogen (subgroup)	X			X		X
Sleep assessment • Polysomnography (subgroup)	X			X		X
Sleep questionnaires • PSQI sleep questionnaire • ESS Sleep questionnaire	X	X	X	X		X

CID = Clinical Investigation Day; Tanner G/M stage = Tanner stage for genitals (boys[8]) or mammae (girls[9]); BMI = Body Mass Index; IOTF = International Obesity Task Force overweight class[1]; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance[2]; HDL-cholesterol = high density lipoprotein-cholesterol; LDL-cholesterol = low density lipoprotein-cholesterol; TAG = triacylglycerides; CRP = c-reactive protein; AST = aspartate transaminase; ALT = alanine transaminase; TFEQ = Three Factor Eating Questionnaire[10]; PSQI = Pittsburgh Sleep Quality Index[4]; ESS = Epworth Sleep Scale questionnaire[5]



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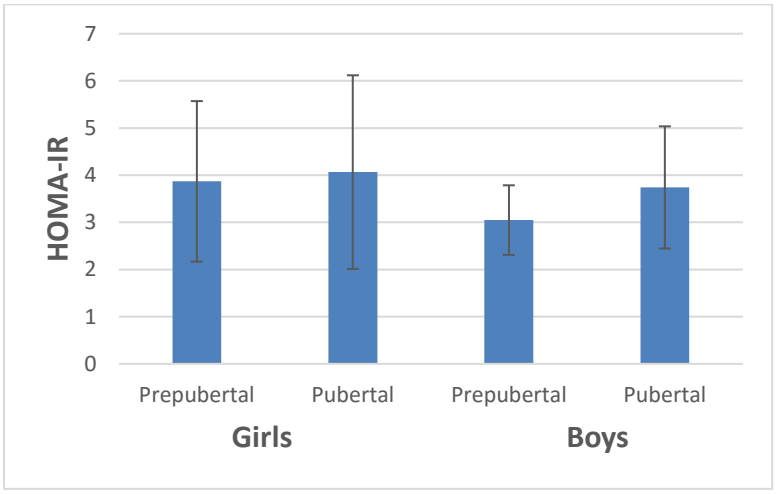

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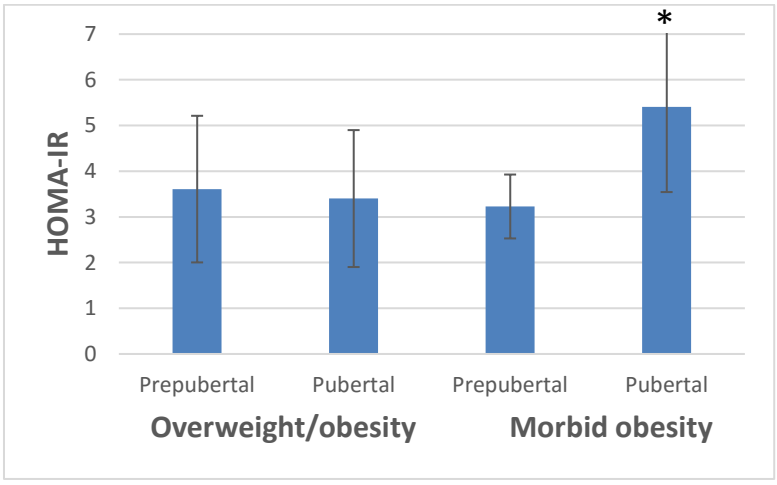

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226 **Supplementary figure 3. HOMA-IR for different puberty stages**

227 HOMA-IR at different puberty stages in children with HOMA-IR ≥2.0 (n=94), presented as mean±SD.
228 A) HOMA-IR in children in different Tanner stages. Mean HOMA-IR was not different between children in the
229 different puberty stages. B) HOMA-IR in prepubertal and pubertal boys and girls. No differences in HOMA-IR were
230 found between the groups. C) HOMA-IR in prepubertal and pubertal children with overweight/obesity and morbid
231 obesity. Pubertal children with morbid obesity had significant higher mean HOMA-IR compared to the children in
232 the other groups. HOMA-IR = Homeostatic Model Assessment of Insulin Resistance[2]. Prepubertal: Tanner G/M stage
233 1-2. Pubertal: Tanner G/M stage 3-5. * p<0.01
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Supplementary table 3. Correlation coefficients for physical activity, food intake behaviour, and sleep with parameters of glucose metabolism, corrected for sex, Tanner stage, BMI z-score, and FM%

		Glucose (mmol/L)	**Insulin** (pmol/L)	**HOMA-IR**	**HbA1c** (mmol/l)	**C-peptide** (nmol/L)
Physical activity						
Baecke Work	*r*	0.190	0.069	0.114	0.242	0.094
Baecke Sport	*r*	-0.223*	-0.105	-0.157	-0.142	-0.140
Baecke Leisure	*r*	-0.118	-0.028	0.173	0.032	-0.046
Baecke total score	*r*	-0.096	0.059	0.073	0.068	-0.139
Counts (cpm)	*r*	-0.068	-0.088	-0.083	0.231	0.157
Food intake behaviour						
TFEQ cognitive restraint of hunger	*r*	-0.164	0.018	-0.067	0.010	-0.105
TFEQ disinhibition	*r*	0.072	-0.112	-0.071	0.019	-0.150
TFEQ hunger	*r*	-0.039	0.024	0.015	0.149	-0.037
Sleep questionnaires						
PSQI	*r*	-0.162	-0.216	-0.209	-0.361	-0.202
ESS	*r*	0.280*	-0.002	0.020	0.258	0.041
Sleep assessment						
TST (min)	*r*	-0,065	0,082	0,115	-0,02	-0,063
SWS (min)	*r*	-0,093	-0,004	-0,026	-0,089	-0,050
REM (min)	*r*	-0,171	-0,031	-0,047	-0,002	-0,171
SE (%)	*r*	-0,039	0,047	0,103	0,037	0,042
QS (%)	*r*	-0,163	0,046	-0,014	-0,075	-0,024
WASO (min)	*r*	0,034	-0,139	-0,163	-0,164	-0,179

HOMA-IR = Homeostatic Model Assessment of Insulin Resistance[2]; TFEQ = Three Factor Eating Questionnaire[10]; PSQI = Pittsburgh Sleep Quality Index[4]; ESS = Epworth Sleep Scale questionnaire[5]; TST = total sleep time; REM = Rapid Eye Movement Sleep; SWS = Slow Wave Sleep; WASO = Wake after Sleep Onset; QS = Quality of Sleep ((REM + SWS) / TST). * $p<0.05$

244 **Supplementary table 4. Drop-out analyses for physical activity**
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	Physical activity measurements complete (n=107)	missing (n=19)
	mean ± SD	mean ± SD
Female n (%)	64 (59.8%)	10 (52.6%)
Age (yr)	13.7 ± 2.3	12.8 1.8
Tanner G/M stage	3 (2 - 5)	2 (1 - 2)
High protein n (%)	59 (55.1%)	9 (47.7%)
Anthropometric characteristics		
Height (m)	1.61 ± 0.1	1.57 ± 0.11
Weight (kg)	78.5 ± 19,70	75.4 ± 20.1
BMI (kg/m2)	29.73 ± 5,01	30.09 ± 4.21
BMI z-score	3.01 ± 0,64	3.22 ± 0.75
IOTF class	2 (1 - 2)	3 (2 - 5)
Body composition		
Fat free mass index (kg/m2)	17.5 ± 2.8	17.6 ± 2.7
Fat mass index (kg/m2)	12.3 ± 4.1	12.2 ± 3.3
Fat mass (%)	40.4 ± 8.5	41.1 ± 8.7
Parameters of glucose metabolism		
Glucose (mmol/L)	4.6 ± 0.7	4.6 ± 0.9
Insulin (pmol/L)	108.4 ± 77.8	116.3 ± 50.5
HOMA-IR	3.46 ± 2.4	5.60 ± 1.51
HbA1c (mmol/l)	32.7 ± 3.5	33.5 ± 3.3
C-peptide (nmol/L)	0.9 ± 0.3	0.9 ± 0.3

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247 Data presented as mean ± SD or median (interquartile range). Tanner
248 International Obesity Task Force overweight class[1]; HOMA-IR = Homeo
249 Pittsburgh Sleep Quality Index[4]; ESS = Epworth Sleep Scale questionna
250 = Wake after Sleep Onset; QS = Quality of Sleep ((REM + SWS) / T
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