Changes in urine and plasma metabolomic profiles after a lifestyle intervention program in obese prepubertal children

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BACKGROUND

Obesity is a major risk factor for metabolic and cardiovascular disorders, and its global prevalence has increased exponentially in the last decades. Excessive weight gain during early childhood increases long-term risk of disease (1); fortunately, reversing obesity in children reduces risk, improving quality of life (2).

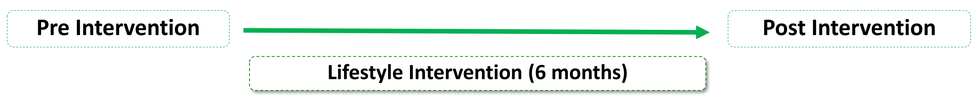
OBJECTIVE AND HYPOTHESES

We hypothesize that a lifestyle intervention in prepubertal children with obesity would result in differential metabolic signatures, in parallel to improvements in BMI.
The aim of this study is to determine the changes in the urine and plasma metabolomic profiles induced by the lifestyle intervention program.

METHODS

The study included 53 children with obesity attending the Hospital Sant Joan de Déu (Barcelona), and recruited in 2013-2014. The intervention consisted on nutritional education and physical activity recommendation for 6 months. Inclusion criteria was age 7-10 years; obesity defined as BMI greater than two-times the standard deviation for a given age and sex; prepubertal status throughout the study, with "Tanner I" in girls and "testicular volume" less than 4 ml in boys.

FIG 1: Longitudinal Prospective Study



Anthropometric measures: Weight, height, waist circumference and blood pressure.
BMI z-score was calculated according to the WHO tables.
Biochemical analysis: Fasting glucose and insulin, HbA1c, and ultrasensitive CRP.
Metabolomics profile: Plasma samples were analysed by liquid chromatography (Acquity UPLC BEH HILIC) coupled to a mass spectrometer (ESI+ mode, Agilent Technologies Q-TOF). Urine samples were analysed in a nuclear magnetic resonance (NMR) Bruker Avance III spectrometer.
Statistical Analysis: pre- and post-intervention parameters were analysed with two-tail paired *t*-test, with $p<0.05$ considered as significant. Principal Component Analysis (PCA) was implemented in R. Pathway enrichment analysis using the KEGG database was applied to characterize the principal factors obtained after the PCA.

RESULTS

Intervention Results

Of the 53 subjects enrolled, 5 did not complete the program, 6 of them had pubertal status at the post-intervention time point, 2 refused to provide the blood sample, 2 had altered CRP levels suggesting concomitant infection , and 3 samples could not be analyzed in the metabolomics platform for technical reasons. Therefore, we analysed samples from 35 subjects pre- and post-intervention. The intervention induced a significant decrease in BMI z score, waist circumference, and HbA1c levels (Table 1); fasting glycaemia was slightly increased post-intervention, and no improvement in insulinemia or HOMA-IR was observed.

Table 1. Pre an Post Lifestyle Intervention anthropometric and biochemical data

	Pre	Post	p value*
Subjects	n=35 (M=18; F=17)		
Age	8.9 ± 0.2		
SDS-BMI[a]	3.5 ± 0.8	3.1 ± 0.7	<0.0001
Waist Circumference (cm)	83.4 ± 9.3	81.6 ± 9.7	0.0009
Systolic Pressure (mmHg)	112 ± 8.2	110 ± 9.2	0.332
Fasting Glycemia (mg/dL)	85 ± 8.3	89 ± 6.1	0.001
Fasting Insulin (mg/dL)	13.2 ± 6.8	13.4 ± 6.8	0.419
HOMA-IR[b]	2.8 ± 1.5	2.9 ± 1.6	0.187
HbA1c (%)[c]	5.3 ± 0.2	5.2 ± 0.2	0.0002
CRP (mg/dL)[d]	3.5 ± 3.5	3.8 ± 3.1	0.768

All values are presented as mean ± SD; [a] SDS-BMI calculated according to AnthroPlus, WHO; [b] HOMA-IR: Homeostatic model assessment to quantify insulin resistance; [c] HbA1c: Glycated Haemoglobin; [d] CRP: C-reactive protein. * two-tail paired *t*-test.

Urine Metabolome (NMR)

Untargeted NMR identified thirty-three metabolites in the urine. Data were log-transformed and normalized to creatinine levels. The Significance Analysis of Microarray/Metabolites method (SAM) was applied to identify biomarkers, addressing the false discovery rate. Trimethylamineoxide (TMAO) was the only metabolite that significantly differed after the intervention (0.72 ± 0.19 vs 0.33 ± 0.07, FDR q=0.019, Figure 2). TMAO is a major cardiovascular risk biomarker and precursor of atherogenesis (3).

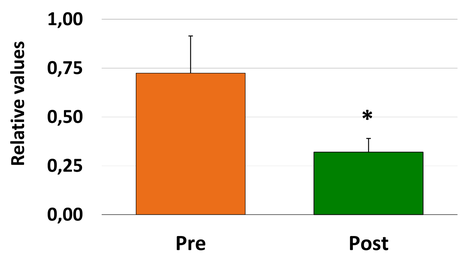


FIG 2. TMAO values pre- and post-lifestyle intervention. Data are mean ± sem. * FDR q<0.05 after SAM analysis.

Plasma Metabolome (LC-MS)

The metabolomics analysis identified 2566 features, and PCA was applied to consolidate them into 15 principal factors (Fig.3). Factor 1 was the only significantly different factor between pre- and post-intervention after adjusting for multiple comparisons ($p<0.001$, Fig.4). Pathway enrichment analysis identified Sphingolipid metabolism as the main contributor to Factor 1 ($q=1.86\times10^{-11}$), with the intervention decreasing levels of the metabolites belonging to this pathway (Table 2).

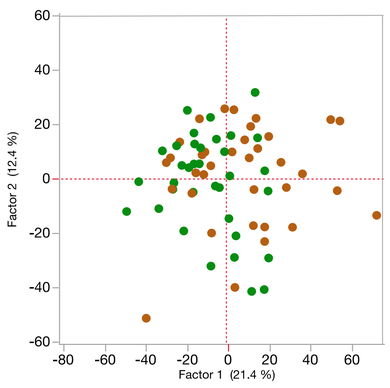


FIG 3. Score plot of Factors 1 and 2. Orange and green dots correspond to pre- and post-intervention subjects, respectively.

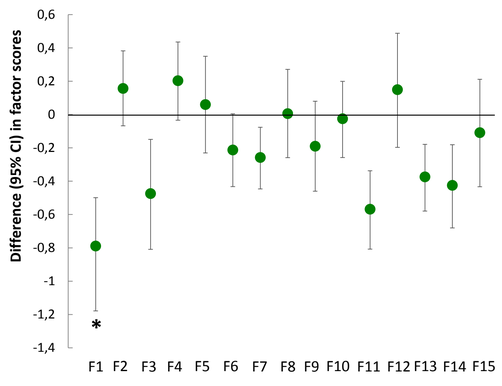


FIG 4. Comparison of 15 factor scores obtained from the PCA. Data are the mean difference of post- minus pre-intervention factor scores. * p<0.0001, Bonferroni's correction was applied to adjust for multiple comparisons after a two-tail paired *t*-test.

Table 2. Identified metabolites in Factor 1 belonging to the Sphingolipid metabolism pathway.

	Fold Change (log)	p value[a]
1. Galabiosylceramide	-0.7	0.0001
2. Lactosylceramide	-0.5	0.0007
3. Ceramides N-acylsphingosine	-0,6	0.0002
4. Gluosylceramide	-0.5	0.0005
5. Galactosylceramide	-0.5	0.0005
6. Sphingomyelin	-0.4	0.0008
7. 3-0 Sulfogalactosylceramide	-0.4	0.0015

[a] two-tail paired *t*-test.

CONCLUSIONS

- The 6-month lifestyle intervention in pre-pubertal children achieved a significant reduction in BMI z-score, waist circumference, and HbA1c levels.
- The intervention reduced urine TMAO levels, a major cardiovascular risk factor.
- A plasma sphingolipid metabolism signature was associated with the intervention. This signature was characterized by reduced levels of a number of ceramides, including proinflammatory signals (4).

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