Title

Metabolomics reveals determinants of weight loss during lifestyle intervention in obese children

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Abbreviated title

Determinants of weight loss in children

Abstract

The amount of weight loss in obese children during lifestyle intervention differs strongly between individuals. The metabolic processes underlying this variability are largely unknown. We hypothesize that metabolomics analyses of serum samples might help to identify metabolic predictors of weight loss. In this study, we investigated 80 obese children aged 6 to 15 years having completed the one-year lifestyle intervention program 'Obeldicks', 40 that achieved a substantial reduction of their body mass index standard deviation score (BMI-SDS) during this intervention (defined as BMI-SDS reduction ≥ 0.5), and 40 that did not improve their overweight status (BMI-SDS reduction < 0.1). Anthropometric and clinical parameters were measured and baseline fasting serum samples of all children were analyzed with a mass spectrometry-based metabolomics approach targeting 163 metabolites. Both univariate regression models and a multivariate least absolute shrinkage and selection operator (LASSO) approach identified lower serum concentrations of long-chain unsaturated phosphatidylcholines as well as smaller waist circumference as significant predictors of BMI-SDS reduction during intervention (p-values univariate models: 5.3E-03 to 1.0E-04). A permutation test showed that the LASSO model explained a significant part of BMI-SDS change (p = 4.6E-03). Our results suggest a role of phosphatidylcholine metabolism and abdominal obesity in body weight regulation. These findings might lead to a better understanding of the mechanisms behind the large inter-individual variation in response to lifestyle interventions, which is a prerequisite for the development of individualized intervention programs.

Key words: Childhood obesity; weight loss prediction; overweight reduction; metabolomics; BMI-SDS reduction; LASSO

1 Introduction

Lifestyle intervention programs based on physical activity, nutrition and behaviour modification lead to a moderate weight loss in overweight and obese children (Oude Luttikhuis et al., 2009; Reinehr, 2011). However, the degree of overweight reduction during such programs largely differs between individuals. Furthermore, not all participating children reduce their overweight to a degree that is sufficient for an improvement of cardiovascular risk factors (Reinehr and Andler, 2004; Reinehr et al., 2004; Ford et al., 2010). For instance, during the lifestyle intervention program 'Obeldicks', about twenty percent of the children achieved a body mass index standard deviation score (BMI-SDS) reduction of at least 0.5, which is associated with improvements of insulin sensitivity, blood lipid profile and blood pressure (Reinehr and Andler, 2004; Reinehr et al., 2004). A similar success rate was observed during other programs (Sabin et al., 2007; Ford et al., 2010).

The search for factors predicting a child's response to a lifestyle intervention is of great interest. With the knowledge of such factors, lifestyle based therapeutic options could be focused on the children that are likely to benefit most (Reinehr et al., 2003). In addition, a thorough understanding of the metabolic processes underlying the large inter-individual variability in weight loss is essential for the development of personalized intervention strategies.

So far, few determinants have been identified that reliably predict the response to lifestyle intervention. Both environmental and genetic factors are likely to play a role. Familial environment, socio-economic status and psychosocial factors affect a child's adaptation of behaviour changes (Reinehr, 2011). At the same time, weight change in response to hypo- or hypercaloric challenge has a considerable heritable component, as observed in twin studies (Bouchard et al., 1990; Bouchard et al., 1994). Also, genetic (Ghosh et al., 2011; Reinehr, 2011) and epigenetic (Campión et al., 2009) factors showed an association with the amount of weight loss in children. Furthermore, metabolic factors have been linked to weight loss in both adults and children, most prominently serum leptin concentration (Fleisch et al., 2007; Reinehr et al., 2009).

In the search for weight loss predictors, the potential of high-throughput -omics techniques such as metabolomics or transcriptomics has merely been exploited (Ghosh et al., 2011; Pathmasiri et al., 2012; Wang et al., 2012). Earlier metabolomics studies have shown that childhood obesity is associated with characteristic changes in the serum metabolome (Mihalik et al., 2012; Wahl et al., 2012). We therefore hypothesize that the serum metabolite profile might also be reflective of metabolic processes involved in weight loss regulation. In this study, we aimed to identify serum metabolites, anthropometric and clinical variables associated with weight

loss in obese children during the lifestyle intervention program "Obeldicks". Going a step further, we used a regularized regression approach, the least absolute shrinkage and selection operator (LASSO), to build a predictive model for BMI-SDS change (Δ BMI-SDS) during intervention.

2 Materials and Methods

2.1 Subjects

'Obeldicks' is a one-year weight loss program based on physical activity, nutritional education and behaviour therapy that includes individual psychological care of the child and his/her family. The program is tailored to obese children aged 6 to 15 years and is conducted at the outpatient clinic for obesity of the Vestische Kinder- und Jugendklinik Datteln, Germany. All participating children were born in Germany. Children with syndromal obesity, psychiatric or endocrine disorders including type 2 diabetes mellitus were excluded. A detailed description of the program can be found elsewhere (Reinehr et al., 2006). Written informed consent was obtained from all parents and all children from the age of 12 years. The study was approved by the Ethics Committee of the University of Witten/Herdecke.

Of the children who had completed the 'Obeldicks' program in 2008 or 2009, we randomly selected 40 children who had reduced their BMI-SDS substantially during their one-year participation, as defined by a BMI-SDS reduction of ≥ 0.5, and 40 with a BMI-SDS reduction of < 0.1 and a similar distribution of sex, pubertal stage and age. The cut-off at a BMI-SDS of 0.5 was chosen based on the finding of previous studies that this amount of BMI-SDS reduction leads to a considerable improvement of the cardiovascular risk profile (Reinehr et al., 2004; Ford et al., 2010). Compliance was given for all 80 children by participation in at least 90% of the meetings.

2.2 Anthropometric measures

Body height was measured to the nearest centimetre using a rigid stadiometer. Undressed body weight was measured to the nearest 0.1 kilogram (kg) using a calibrated balance scale. Body mass index (BMI) was

calculated as body weight divided by squared body height in m^2. BMI percentiles as well as BMI-SDS were calculated according to Cole's LMS-method (Cole, 1990), applied to German reference data (Kromeyer-Hauschild et al., 2001). All children's BMI was above the 97th percentile.

Waist circumference was measured half-way between lower rib and iliac crest (Kromeyer-Hauschild et al., 2008). Pubertal stage was assessed according to Marshall and Tanner (1969; 1970) and categorized into three stages based on pubic hair and genital stages: *prepubertal* = boys / girls with pubic hair stage I and gonadal / breast stage I; *pubertal/postpubertal* = boys / girls with pubic hair stage ≥ II and gonadal / breast stage ≥ II and boys with change of voice and girls with menarche. Systolic and diastolic blood pressure was measured twice according to a validated protocol and the two measurements were averaged (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents, 2004).

2.3 Sampling and biochemical measurements

Blood samples were taken at 8 a.m. after overnight fasting for at least 10 hours. Following coagulation at room temperature, blood samples were centrifuged for 10 min at 8000 rpm and aliquoted. Biochemical measurements were conducted directly on the fresh serum samples. Triglyceride, total cholesterol and glucose concentrations were determined with a colorimetric test using the Vitro^{TM} analyzer (Ortho Clinical Diagnostics, Neckargemuend, Germany). Low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol were measured with an enzymatic test using the LDL-C and HDL-C-Plus^{TM} assays (Roche Diagnostics, Mannheim, Germany), respectively. Insulin concentrations were determined with a microparticle-enhanced immunometric assay (MEIA^{TM}, Abbott, Wiesbaden, Germany). Intra- and interassay coefficients of variation were < 5% for all tests. As a measure of insulin resistance, the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as serum insulin (mU/l) * serum glucose (mmol/l) / 22.5 (Matthews et al., 1985). This index has been validated in healthy children (Gungor et al., 2004). Aliquoted serum samples were stored at -80 °C and thawed only once at room temperature for the metabolomics assay.

2.4 Targeted metabolomics

For the quantification of 163 metabolites, the Absolute*IDQ*^{TM} kit p150 (Biocrates Life Sciences AG, Innsbruck, Austria) was used, following the instructions described in the manufacturer`s manual. Liquid handling of serum

samples was performed with a Hamilton Microlab STAR^{TM} robot (Hamilton Bonaduz AG, Bonaduz, Switzerland). Samples were analyzed on an API4000 LC/MS/MS system (AB Sciex Deutschland GmbH, Darmstadt, Germany). The whole procedure has been described in detail elsewhere (Illig et al., 2010; Römisch-Margl et al., 2011).

Measurements took place in two batches. To ensure data quality, metabolites that failed in two or more of the following criteria for measurement stability were excluded from the analysis: (i) The concentration of the metabolite should be above the limit of detection specified by the manufacturer in at least 60% of the samples. (ii) The Pearson's correlation coefficient of the metabolite concentrations in 43 samples that were measured on both batches should be at least 0.5. (iii) For each batch, the coefficient of variation for the metabolite concentration in a reference sample that was measured five times should not be higher than 0.2. In total, 130 metabolites passed the quality control. Most of the 33 excluded metabolites were characterized by concentrations below or marginally above the limit of detection. Potential batch effects were corrected by multiplying all values by a metabolite- and batch-specific correction factor, calculated as the overall geometric mean divided by the batch-specific geometric mean of metabolite concentrations of the 43 repeatedly measured samples.

2.5 Statistical Analysis

2.5.1 Baseline comparisons

Baseline differences in anthropometric variables between children with and without substantial BMI-SDS reduction were assessed using Wilcoxon rank-sum tests and chi-squared tests for continuous and binary traits, respectively. Age and BMI-SDS distributions in the two groups of children were additionally compared using Kolmogorov-Smirnov tests. Changes in anthropometric and clinical variables during the intervention were investigated using Wilcoxon signed-rank tests.

2.5.2 Univariate regression models

To identify pre-intervention variables associated with successful weight loss, two approaches were applied. First, univariate regression models were fit for each of the pre-intervention metabolites, anthropometric or clinical

variables (in total 144 variables) with the binary outcome "Substantial BMI-SDS reduction" and the continuous outcome Δ BMI-SDS. Second, Δ BMI-SDS was further examined by a multivariate LASSO regression approach described below. Missing values (20 in waist circumference and two in LDL and HDL cholesterol concentration) were assumed to be missing completely at random, and therefore all analyses could be performed with the available observations only.

Univariate logistic regression models with the outcome "Substantial BMI-SDS reduction" were adjusted for sex and baseline age, pubertal stage and BMI-SDS. To correct for multiple testing, the false discovery rate was controlled at 5% using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). Assuming an increased power when replacing dichotomized by continuous Δ BMI-SDS as outcome, linear regression models were used to identify pre-intervention variables associated with the continuous outcome Δ BMI-SDS. Since the distribution of the outcome Δ BMI-SDS, per design, did not follow a normal distribution (Fig. S1 in the Online Resource), empirical p-values obtained from a permutation test rather than p-values based on asymptotic theory are reported (Moore et al., 2003). The idea behind permutation tests is that the distribution of a test statistic obtained with randomly resampled outcome vectors resembles its distribution under the null hypothesis that there is no effect. The proportion of resampling folds where the test statistic is at least as extreme as the test statistic of the original data, can therefore be interpreted as a p-value. Here, we used 10,000 random permutations of the outcome vector. Permutation p-values were subjected to Benjamini-Hochberg correction.

2.5.3 LASSO regression

Δ BMI-SDS was further investigated using a multivariate approach. In contrast to univariate modeling, multivariate approaches consider interdependencies between variables, allowing for the formation of predictive models and the assessment of their prediction accuracy. Due to the fact that the number of variables ($p = 144$) is larger than the number of subjects ($n = 80$), a classical multivariate regression model could not be fit to the data at hand including all 144 variables (Hastie et al., 2009). Therefore, we chose a regularized regression approach, the LASSO (Tibshirani, 1996), using the R package *glmnet* (Friedman et al., 2010). Briefly, a penalization term is added to the least squares criterion, yielding coefficient estimates shrunk towards zero, dependent on the size of a penalization parameter λ. We favored this precise approach over other supervised statistical learning approaches for its intrinsic variable selection property: The most predictive variables are selected into the model, while the coefficients of the remaining variables are shrunk to zero. The coefficients of the selected variables can be interpreted as effect strengths (Hastie et al., 2009).

To obtain prediction accuracy measures that are unbiased estimates of the true measures in independent data, we chose a nested cross-validation (CV) approach (Varma and Simon, 2006) in order to tune the penalization parameter λ in the inner CV loop and estimate the prediction accuracy of the model in the outer 10-fold CV loop (Ambroise and McLachlan, 2002) (Fig. S2 in the Online Resource). This procedure was repeated randomly 10 times to improve its stability (Braga-Neto and Dougherty, 2004).

As measures of prediction accuracy, we calculated the R^2 and Q^2 values, defined as 1 minus the residual sum of squares divided by the total sum of squares, for the total data set, and within CV, respectively. Although these values cannot, unlike in unregularized regression models, be interpreted as the percentage of total variance of the outcome explained by the model, they might serve as goodness-of-fit measures with respect to the fit of the present dataset and to the prediction of independent data, respectively. A permutation test with 10,000 permutations was applied to assess model significance (Radmacher et al., 2002), regarding permutation-based p-values < 0.05 as significant. The precise CV and permutation scheme is illustrated in Fig. S2 in the Online Resource.

To visualize how variables selected by LASSO regression represent groups of variables showing also a univariate association with BMI-SDS reduction, the matrix of pairwise Pearson's correlation coefficients was subjected to agglomerative hierarchical clustering using the R package *Heatplus* (Ploner, 2011). Cluster distance was defined through complete linkage and distance between pairs of variables defined as $(1-\rho)/2$, where ρ is the Pearson's correlation coefficient. All calculations were performed using R, version 2.14.2 (R Development Core Team, 2012).

3 Results

3.1 Study characteristics at baseline and changes upon lifestyle intervention

By design, baseline age, sex, and pubertal stage, but also weight, BMI and BMI-SDS distribution did not differ significantly between the 40 children who substantially reduced their BMI-SDS (Δ BMI-SDS ≤ -0.5) and the 40 who did not (Δ BMI-SDS > 0.1) (Table 1; Fig. S3 in the Online Resource).

During the intervention, Δ BMI-SDS ranged from -1.49 to +0.49 and differed significantly between children with and without substantial BMI-SDS reduction, with a mean (sd) Δ BMI-SDS of -0.68 (0.27) and +0.07 (0.15), respectively (p = 1.4E-14).

Children with substantial BMI-SDS reduction significantly improved their waist circumference (-6.0 (15.2) cm, *p* = 5.8E-03) as well as their metabolic risk profile (fasting insulin -5.3 (9.3) mU/l, p = 2.2E-04; HOMA-IR -0.5 (4.9) mU/l*mmol/l, p = 4.8E-04; HDL +3.9 (10.2) mg/dl, p = 4.8E-02; triglycerides -17.9 (34.4) mg/dl, p = 5.3E-03; systolic blood pressure -7.6 (19.5) mmHg, p = 2.3-E-03). In contrast, children without substantial BMI-SDS reduction mostly did not (Table S1 in the Online Resource).

3.2 Pre-intervention variables associated with weight loss

In total, 144 pre-intervention variables, including 130 metabolites and 14 anthropometric or clinical traits, were subjected to univariate logistic regression with the binary outcome "Substantial BMI-SDS reduction". None of the variables reached significance after correction for multiple testing.

Next, linear regression models were fit with the continuous outcome Δ BMI-SDS. 18 variables showed a significant positive association with Δ BMI-SDS after correction for multiple testing (permutation p-values ranging from 5.3E-03 to 1.0E-04) (Fig. 1, Table S2 in the Online Resource). These variables included waist circumference, arginine and LPC a C18:0 serum concentrations, as well as serum concentrations of 13 diacyl PCs and two acyl-alkyl PCs, which were all long-chained and unsaturated. Most of these variables were also nominally associated with substantial BMI-SDS reduction (Fig. 1). By trend, a positive association was observed for all measured diacyl PCs (Table S2 in the Online Resource). None of the baseline clinical traits (blood pressure, blood lipid and insulin resistance parameters) was significantly associated with Δ BMI-SDS after correction for multiple testing.

3.3 Prediction of weight loss

In order to investigate associations between the 144 pre-intervention variables and Δ BMI-SDS in a multivariate manner, thereby building a predictive model for Δ BMI-SDS and assessing its predictive potential, we employed a regularized regression approach, the LASSO.

Three out of the 144 variables were selected into the predictive model (see Material and Methods), namely waist circumference, PC aa C36:5, and PC aa C32:2. Fig. 2 shows coefficient paths and variable stability for these variables. The strongest effect and highest stability, that is, the highest selection frequency across the CV folds, was observed for PC aa C36:5 ($\beta = 0.0152$, selection frequency 100%). Of note, LASSO coefficients are not comparable with the coefficients of the univariate linear regression models due to the shrinkage behavior of the LASSO (see Materials and Methods).

In terms of prediction accuracy, the model had R^2 and Q^2 values of 0.267 and 0.116, respectively (Fig. 3). The significance of the prediction was assessed using a permutation test with the null hypothesis stating that a Q^2 value of 0.116 would be observed by chance. The corresponding p-value was 4.6E-03 so that this hypothesis was rejected. Thus, we were able to show that our predictive model comprising three metabolic variables explains a significant part of Δ BMI-SDS in obese children during one-year lifestyle intervention.

The three variables selected into the LASSO model were also univariately associated with Δ BMI-SDS (Fig. 1), with the exception of PC aa C32:2, for which a univariate association was observed only by trend. The selected variables represented groups of correlated variables significantly associated with Δ BMI-SDS in the univariate regression analysis, as can be seen from the correlation and clustering results (Fig. 4).

4 Discussion

Applying a targeted metabolomics approach combined with clinical and anthropometric measurements, we investigated pre-intervention factors determining response to lifestyle intervention in obese children. The factors that showed the strongest association as well as the most stable predictive potential for weight loss were serum concentrations of diacyl phosphatidylcholines (PCs), and waist circumference.

4.1 Phosphatidylcholines and weight loss

Children with substantial BMI-SDS reduction had lower pre-intervention serum concentrations in several PC species compared to children without substantial BMI-SDS reduction. PCs are produced in most mammalian cells via the cytidine diphosphate (CDP)-choline pathway (DeLong et al., 1999). In the liver, 30% of PC synthesis occurs via the phosphatidylethanolamine methyltransferase (PEMT) pathway (Li and Vance, 2008). The enzyme PEMT methylates phosphatidylethanolamine to produce PCs, which constitutes the only endogenous pathway of choline synthesis. The PC species derived from both pathways differ in chain length and degree of saturation (DeLong et al., 1999).

The long-chain unsaturated PCs C34:1, C34:3, C36:2, C36:3, C36:5, C38:5 and C40.6 were negatively associated with BMI-SDS reduction in this study and have recently been shown to be down-regulated in livers of PEMT-/- mice (Jacobs et al., 2010). Also, total serum PC concentration was reduced in PEMT-/- mice. Most interestingly, PEMT-/- mice were protected from high-fat diet-induced obesity, having an increased energy expenditure and normal peripheral insulin sensitivity. These effects were prevented by choline supplementation. Thus, they are attributable to reduced choline availability upon diminished choline *de novo* production via PEMT, and an increased consumption of choline by increased compensatory PC production via the CDP-choline pathway (Jacobs et al., 2010). A protective effect of low plasma choline levels on body mass has also been observed in a human population-based study (Konstantinova et al., 2008). Low choline levels could increase energy expenditure via several mechanisms, one being the attenuation of acetylcholine signaling in the brain (Gautam et al., 2006; Jacobs et al., 2010).

We therefore hypothesize that the PC signature that we observed in children with substantial weight loss may reflect a reduced PEMT activity. Once these children change their nutritional habits, and thereby reduce the dietary intake of choline, they might have a greater potential to reduce their weight. This assumption is supported

by a dietary intervention study in overweight adults, where a PC species that is likely PEMT-derived was negatively associated with body fat reduction (Smilowitz et al., 2009).

4.2 Abdominal adipose tissue and weight loss

Waist circumference is an established marker of abdominal obesity in children (Taylor et al., 2000; Schwandt et al., 2008). In this study, a higher waist circumference was inversely associated with BMI-SDS reduction. This observation is consistent with the negative link between markers of abdominal fat mass and weight loss success as well as improvement of insulin sensitivity observed upon lifestyle intervention in adults (Teixeira et al., 2004; Thamer et al., 2007). However, the opposite association has been reported (Wabitsch et al., 1992; Carmichael et al., 1998).

There is biological evidence for a role of abdominal adipose tissue in weight regulation. It is well recognized that abdominal adipose tissue is an endocrine organ that contributes to the subclinical inflammation associated with obesity by secreting a range of bioactive molecules called adipokines (Wajchenberg, 2000). Of note, an increasing number of studies in both children (Fleisch et al., 2007; Reinehr et al., 2009; Murer et al., 2011) and adults (Verdich et al., 2001; Shih et al., 2006) showed higher serum levels of the adipokine leptin to be associated with weight gain or poor response to lifestyle intervention. Although leptin exerts anorexigenic functions, suppressing food intake and increasing energy expenditure, these negative associations might be explained by the presence of leptin resistance or central leptin insufficiency (Kalra, 2008; Reinehr et al., 2009).

Further, high baseline levels of the adipokine adiponectin predicted weight gain over four years in adults (Hivert et al., 2011) and promoter methylation of the *tumor necrosis factor-α* (TNF-α) gene, which positively regulated circulating TNF-α concentration, was negatively associated with weight loss success (Campión et al., 2009).

A further line of evidence connects abdominal obesity with resistance to weight loss during lifestyle intervention via the central action of insulin. Abdominal adipose tissue has been reported to associate with cerebral insulin resistance (Tschritter et al., 2009), which was related to impaired body fat loss during lifestyle intervention (Tschritter et al., 2012).

Together, these findings concerning adipokines corroborate a complex role of abdominal fat in weight regulation and might contribute to the explanation why higher waist circumference is associated with poorer weight loss success during lifestyle intervention in our study. Adipokine measurement was not subject of our study, so it could not be investigated whether the observed association was mediated by these factors.

4.3 Predictive potential of the LASSO model and comparison to other studies

Widely used multivariate approaches in metabolomics data analysis are Partial Least Squares (PLS) related methods. They have, however, the disadvantage, that variable effect strengths are not readily obtained and sparse models containing only a few important predictor variables for assessment in future studies cannot be derived easily. We therefore chose to use a LASSO regression approach, which provides, besides measures of prediction accuracy for the whole model, measures of effect strength and variable stability for the selected variables. Using this approach, we obtained a model comprising three pre-intervention variables that explained a significant part of Δ BMI-SDS. Although no hard cut-offs exist for R^2 and Q^2 values in this regularized regression setting, the prediction accuracy of the presented model seemed rather moderate (R^2 = 0.267, Q^2 = 0.116). A recent investigation of urinary metabolite traits predictive of substantial BMI change in a 3-week treatment camp for adolescents reported higher values of prediction accuracy (Pathmasiri et al., 2012). A direct comparison is difficult since their study differed from ours in terms of statistical methods, length and characteristics of intervention as well as metabolomics technique and investigated biofluids. Overweight change over the course of one year in an outpatient intervention program might be more strongly influenced by environmental and psychosocial factors and therefore be less predictable by the here investigated metabolic variables. Also, Pathmasiri et al. included post-intervention metabolite levels in their prediction model, which we did not, aiming to obtain a model with prognostic applicability. Results of both studies require external validation in larger independent data sets.

Other studies searching for metabolic predictors of weight loss success investigated single parameters and found better insulin sensitivity (i.e. lower HOMA-IR, lower fasting insulin or absence of type 2 diabetes) (Harden et al., 2007; Madsen et al., 2009; Ford et al., 2010) as well as lower serum triglyceride levels (Harden et al., 2007; Madsen et al., 2009) as predictors of weight loss. In our study, these parameters were not identified as significant predictors. However, HOMA-IR and serum triglycerides showed a borderline significant negative association with Δ BMI-SDS.

4.4 Strengths and limitations

This is one of the first studies applying a metabolomics approach to identify metabolic predictors of overweight reduction in obese children upon lifestyle intervention. In addition to the univariate identification of pre-intervention variables associated with overweight reduction, we used a carefully validated LASSO approach to build a predictive model for BMI-SDS change.

As a limitation of this study, we investigated a small group of children. Larger studies might allow for the development of sex-, age- and maturity-specific predictive models. The underlying study population did not represent a random group of obese children. Therefore, the predictive potential of the variables on which the children were matched (sex, age, and pubertal stage) could not be assessed (Sabin et al., 2007; Danielsson et al., 2012). Moreover, weight loss success is not only determined by compliance regarding participation at meetings, but also by implementation of the recommendations into daily life. This might be strongly influenced by environmental and psychosocial factors, which were not obtained in this study. Furthermore, our analysis was limited to changes in BMI-SDS as outcome. Further investigations should aim at identifying predictors for secondary outcomes such as changes in body fat distribution and insulin sensitivity. In addition, studies investigating metabolite changes during lifestyle intervention might give additional information about the mechanisms underlying weight change.

5 Conclusions

Our results confirm a role of phosphatidylcholine metabolism for human energy regulation and success in overweight reduction as has previously been observed in animal studies. They further corroborate the connection between abdominal obesity and impaired overweight reduction. These are both important aspects for understanding the large inter-individual variation in response to lifestyle interventions, which is a prerequisite for the development of individualized intervention programs.

6 Acknowledgement

This work was supported by the following grants from the German Federal Ministry of Education and Research (BMBF): Grant numbers 01GS0820 and 01GS0823 of the National Genome Research Network (NGFNplus), grant number 01GI0839 of the German Competence Network Obesity (consortium LARGE), grant number 0315494A of the Systems Biology of Metabotypes project (SysMBo), and grant number 03IS206IB of the Gani_Med project to WRM and the German Center for Diabetes Research (DZD e.V.). It was further supported by funding from the University of Witten/Herdecke and from the Helmholtz Zentrum München. I.K. and C.F. were supported by the European Union within the ERC grant LatentCauses. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

We offer our sincere thanks to the participants of the study as well as their parents. We are grateful to Petra Nicklowitz for conducting the biochemical measurements. We thank Julia Scarpa, Werner Römisch-Margl, Katharina Sckell and Arsin Sabunchi for metabolomics measurements performed at the Helmholtz Zentrum München, Genome Analysis Center, Metabolomics Core Facility, Neuherberg, Germany.

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Figure Legends

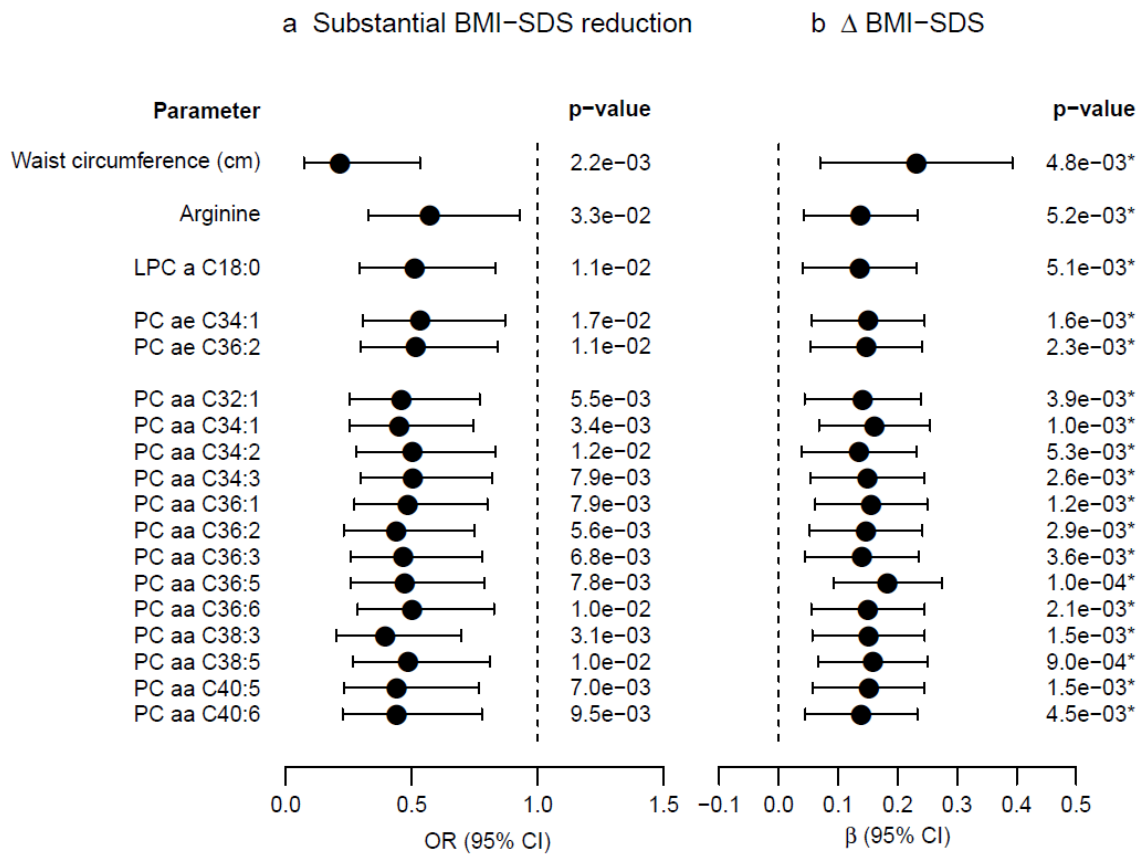


Fig. 1 Pre-intervention variables associated with overweight reduction. Effects on (**a**) the binary outcome "Substantial BMI-SDS reduction" and (**b**) continuous Δ BMI-SDS are shown for the 18 variables significantly associated with Δ BMI-SDS after correction for multiple testing. (**a**) Odds ratios (OR) with 95% confidence interval (CI). (**b**) β estimates with 95% CI and permutation-based p-values. All effects are derived from univariate regression models adjusted for sex and baseline age, pubertal stage and BMI-SDS. The unit of variables is µmol/l, if not indicated otherwise. *Significant after correction for multiple testing. BMI-SDS, body mass index standard deviation score; Cx:y, acyl-group with chain length x and y double bonds; LPC a, lysophosphatidylcholine with acyl chain; PC aa, diacyl phosphatidylcholine; PC ae, acyl-alkyl phosphatidylcholine

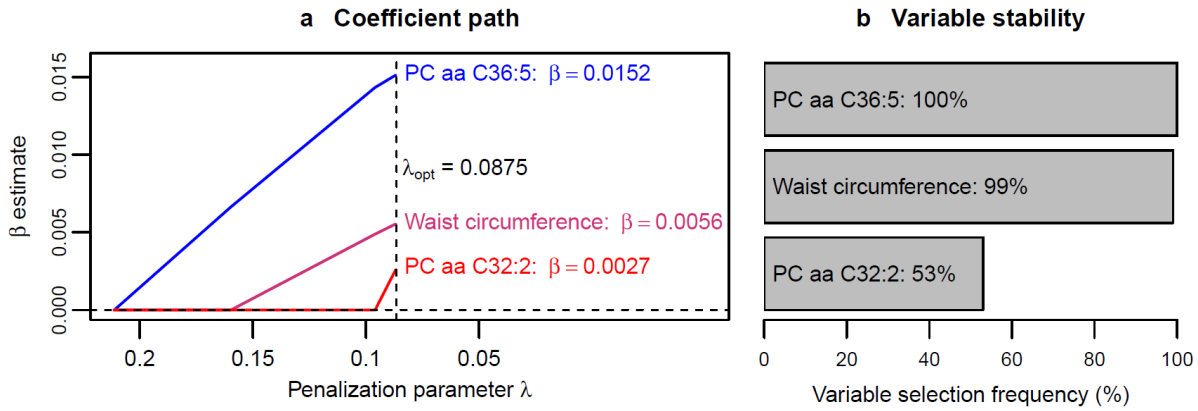
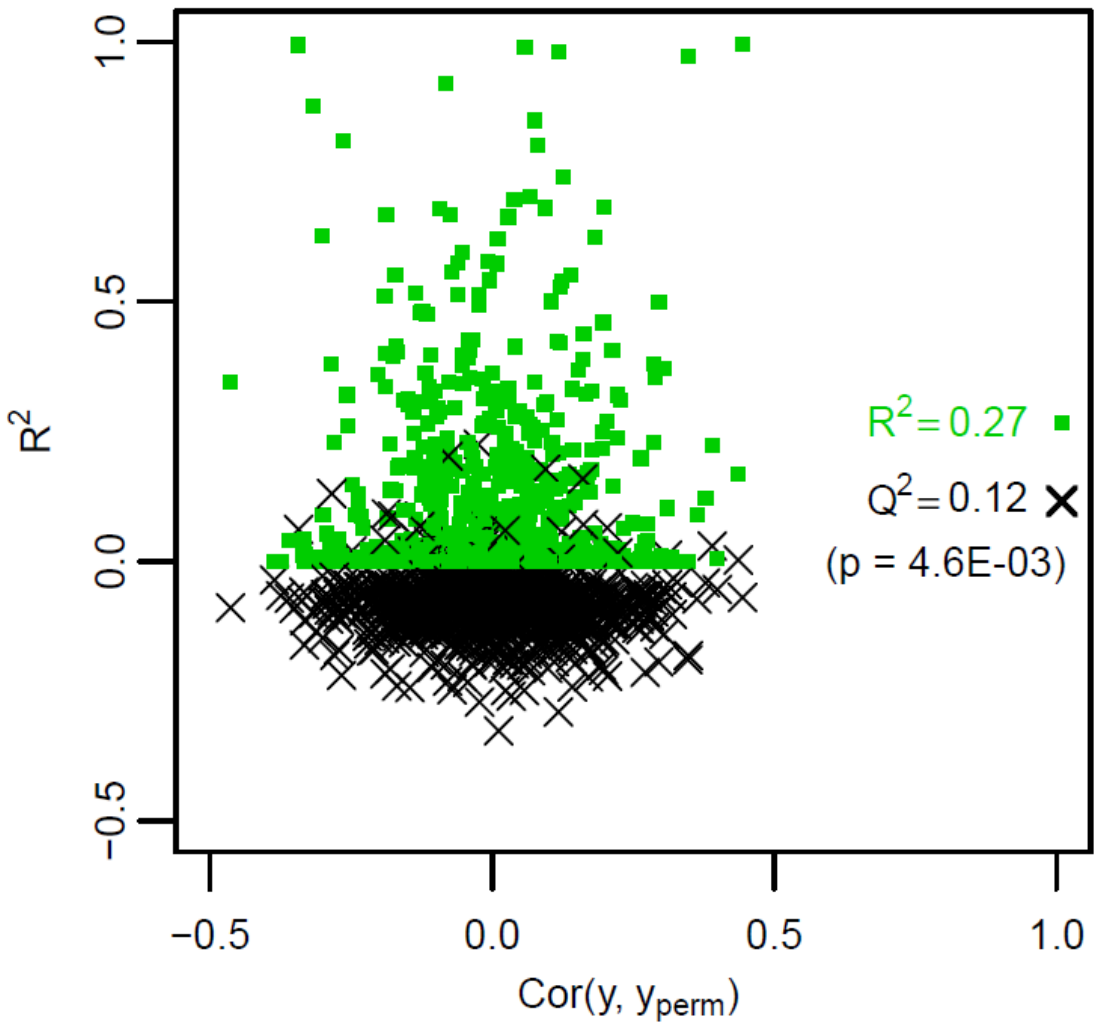


Fig. 2 LASSO regression results. Pre-intervention variables selected as predictors for Δ BMI-SDS. (**a**) Coefficient paths truncated at the optimal penalization parameter λ_{opt} = 0.0875 (vertical dashed line). β estimates are plotted against a sequence of the penalization parameter λ ranging from the λ threshold, beyond which no variables are retained in the model, to λ_{opt}, β estimates are displayed for λ_{opt}. (**b**) Variable stability, defined as the frequency with which a variable was selected by the LASSO approach across the 100 outer cross-validation loops, for the chosen variables. Cx:y, acyl-group with chain length x and y double bonds; PC aa, diacyl phosphatidylcholine



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553 **Fig. 3** Permutation test results for the LASSO approach. Data for the first 1000 permutations are shown. R^2
554 (green squares) and Q^2 (black crosses) values are plotted against the Pearson's correlation between original and
555 permuted outcome vector. R^2 is limited to ≥ 0, whereas Q^2 is not. At correlation = 1, R^2 and Q^2 values of the
556 original data are plotted. Permutation-based p-value for Q^2 is given, which is defined as the proportion of
557 permutation folds where the Q^2 value was larger than the Q^2 value of the original data. Cor, Pearson's correlation
558 coefficient; perm, permutation

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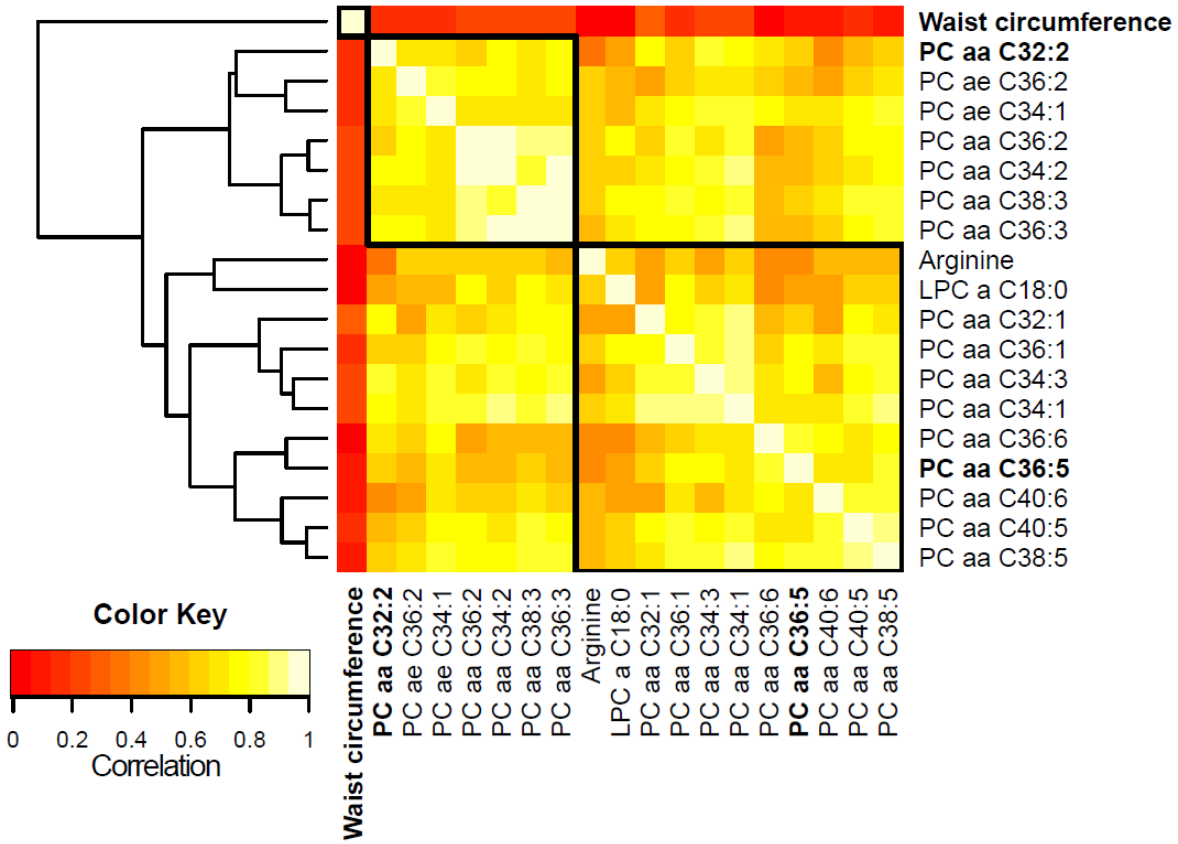

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562 **Fig. 4** Correlation among variables associated with overweight reduction. Heatmap of the matrix of pairwise
563 Pearson's correlation coefficients and hierarchical clustering dendrogram are shown. Variables selected in the
564 LASSO model are written in bold font. Dendrogram was cut vertically at correlation = 0.4, resulting clusters are
565 framed. Cx:y, acyl-group with chain length x and y double bonds; LPC a, lysophosphatidylcholine with acyl
566 chain; PC aa, diacyl phosphatidylcholine; PC ae, acyl-alkyl phosphatidylcholine

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Tables

Table 1 Baseline characteristics of the study population

Variable	Children with substantial overweight reduction (n = 40)	Children without substantial overweight reduction (n = 40)	p-value[a]
Age (years)	10.9 (2.3)	10.9 (2.0)	0.969
Sex (% male)	50	55	0.751
Pubertal stage (% prepubertal)	52.5	50	1.000
Weight (kg)	64.1 (16.3)	66.3 (18.8)	0.641
BMI (kg/m^2)	27.3 (3.3)	28.0 (4.6)	0.749
BMI-SDS	2.35 (0.43)	2.37 (0.45)	0.837
Waist circumference (cm)	83.8 (10.5)	92.4 (12.7)	0.009

Data are shown as mean (standard deviation) if not indicated otherwise. [a]p-values were derived from Wilcoxon rank-sum test and chi-squared test for continuous and binary variables, respectively. "With substantial BMI-SDS reduction" was defined as BMI-SDS reduction ≥ 0.5, "without substantial BMI-SDS reduction" as BMI-SDS reduction < 0.1. BMI, body mass index; BMI-SDS, BMI standard deviation score.

Online Resource

Fig. S1 Distribution of the continuous outcome variable „Change in body mass index standard deviation score (BMI-SDS) during the intervention“ (Δ BMI-SDS). (**a**) Histogram. (**b**) Normal quantile-quantile plot. The distribution is not normal according to Shapiro-Wilk test (p-value = 0.0019)

Fig. S2 Repeated nested cross-validation and permutation scheme. CV, cross-validation; MSEP, mean squared error of prediction

Fig. S3 Boxplots of **(a)** age and **(b)** BMI-SDS before the intervention in children with and without substantial weight loss during the intervention. P-values from Kolmogorov-Smirnov tests are shown. Age and BMI-SDS distribution did not differ significantly between children with and without substantial weight loss

Table S1 Anthropometric and clinical traits at baseline and at the end of the 1-year lifestyle intervention

Table S2 Results of univariate regression analyses. 144 baseline metabolites, anthropometric and clinical traits were subjected to logistic regression with the outcome "Substantial BMI-SDS reduction" (body mass index standard deviation score (BMI-SDS) reduction during the intervention ≥ 0.5 vs. < 0.1), adjusted for sex and baseline age, pubertal stage and BMI-SDS. Mean (standard deviation) of baseline values in the two groups of children are shown in columns 2 and 3; Odds Ratio (OR) with 95% Confidence Interval (CI), p-value and Benjamini-Hochberg-corrected p-value are reported in columns 4-6. Similarly, linear regression models were fit with the continuous outcome "Change in BMI-SDS during the intervention" (Δ BMI-SDS). β coefficient with 95% CI, Wald test-derived p-value, permutation-based p-value and Benjamini-Hochberg-corrected permutation-based p-value are reported in columns 7-11. Associations with corrected p-value < 0.05 were regarded as significant.