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Obesity

Weight Loss Predictability by Plasma Metabolic Signatures in Adults with Obesity and Morbid Obesity of the DiOGenes Study

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Objective: Aim is to predict successful weight loss by metabolic signatures at baseline and to identify which differences in metabolic status may underlie variations in weight loss success.
Methods: In DiOGenes, a randomized, controlled trial, weight loss was induced using a low-calorie diet (800 kcal) for 8 weeks. Men ($N = 236$) and women ($N = 431$) as well as groups with overweight/obesity and morbid obesity were studied separately. The relation between the metabolic status before weight loss and weight loss was assessed by stepwise regression on multiple data sets, including anthropometric parameters, NMR-based plasma metabolites, and LC-MS-based plasma lipid species.
Results: Maximally, 57% of the variation in weight loss success can be predicted by baseline parameters. The most powerful predictive models were obtained in subjects with morbid obesity. In these models, the metabolites most predictive for weight loss were acetoacetate, triacylglycerols, phosphatidylcholines, specific amino acids, and creatine and creatinine. This metabolic profile suggests that high energy metabolism activity results in higher amounts of weight loss.
Conclusions: Possible predictive (pre-diet) markers were found for amount of weight loss for specific subgroups.

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Introduction

Obesity has several adverse effects on health, which are related to an increase in the likelihood of development of various diseases including metabolic syndrome, cardiovascular disease, and cancer. Therefore, strategies to prevent and manage obesity are needed.

A dietary regimen that induces weight loss but maintains proper nutrition remains the most effective nutritional intervention. Since calorie restriction was shown to retard the aging process in the 1930s (1), calorie restriction has been widely studied. Over the years several beneficial outcomes of calorie restriction have been demonstrated that may be accomplished by the resulting induction of weight loss (2,3). These beneficial outcomes include the improvement of glucose metabolism by improving pancreatic functioning and β-cell responsiveness to glucose. Furthermore, the increased hepatic insulin sensitivity leads to reduced fasting plasma insulin levels and glycemia (4-6). In addition, the plasma lipid profile improves; plasma triacylglycerols and low-density cholesterol particles reduce while the level of circulating high-density cholesterol particles increases (5,7,8). Finally, blood pressure, metabolic rate (both resting metabolic rate and diet-induced thermogenesis), and oxidative damage are reduced (9-12).

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Nevertheless, these studies also showed that the successfulness of weight loss is not identical in every person as major variability is observed in the amount of weight loss induced by calorie restriction (2). Obviously, part of this variation can be explained by differences in compliance. However, another part of this variation remains unexplained. As described previously, a high variability in weight loss success despite a very strict dietary regiment using a low-calorie diet was also observed in the DiOGenes intervention study (13). DiOGenes is a Pan-European dietary intervention study investigating after a substantial weight loss the effects of dietary protein and glycemic index on weight (re)gain, metabolic, and cardiovascular risk factors in adults with overweight and (morbid) obesity (14). To achieve the "substantial weight loss," subjects underwent an energy restriction using a low-calorie diet for 8 weeks. During and after this hypocalorie diet period, a high inter-individual variation in weight loss success was observed, both in men and women as well as in subjects with overweight, obesity, and morbid obesity. Weight will change either due to decreased intake or increased energy expenditure. As the energy intake was strictly defined, about 800 kcal, in the Diogenes study, the variation in weight loss will mainly occur by inter-individual differences in energy expenditure (15). Which processes may be underlying these differences is currently unclear.

In the present study, we aim to predict successful weight loss by metabolic signatures at baseline. Metabolomics may reveal metabolic differences in individual energy expenditure.

This analysis will reveal which differences in metabolic status in the subjects may underlie variations in weight loss. Secondly, we investigate the metabolic changes during weight loss underlying the observed variation in inter-individual weight loss success upon an 8-week calorie restriction period. Differences in mechanisms underlying weight loss may be revealed by this analysis.

Methods

Dietary intervention study

This study is part of the European Framework project titled Diet, Obesity, and Genes (DiOGenes). Previously, Larsen et al. (14) and Moore et al. (16) provided a thorough description of the overall objective and goals of this dietary intervention. Briefly, after an initial weight loss phase for 8 weeks subjects were randomized for a subsequent 6-months weight maintenance phase. From eight European cities (Maastricht, Copenhagen, Cambridge, Heraklion, Potsdam, Pamplona, Sofia, and Prague), 932 Caucasian adults with overweight and (morbid) obesity were recruited to study the effects of dietary protein and glycemic index on weight (re)gain and metabolic and cardiovascular risk factors.

For the present study only the weight loss period was analyzed. Weight loss was induced by calorie restriction for 8 weeks using a low-calorie formula diet (LCD 3300 kJ/day, approximately 800 kcal; Modifast; Nutrition et Santé).

Subject selection

Additionally, Larsen et al. (14) provided an outline of inclusion and exclusion criteria for study participation. Participants of the study were weight stable (<3 kg change in body weight) during a 2-month period before initiating the study. In the current study, men and women were studied separately and of each the 5% most and least successful weight losers were excluded in order to avoid the inclusion of variation due to compliance. In total, 236 men and 431 women were included in the analyses. Based on initial statistical analysis and for biological reasons it was decided to separate these subjects into subjects with overweight and obesity ($27 \leq \text{BMI} < 40$ kg/m^2, from now on referred to as subjects with obesity) and morbid obesity (BMI ≥ 40 kg/m^2–MO). Clinical and genetic evidence indicates that subjects with obesity have a different metabolic status than subjects with normal weight and that subjects with obesity with body mass index (BMI) lower or higher than 40 kg/m^2 are metabolically different, e.g., MO coincides with affected thyroid hormone regulation, lung volumes, basal metabolic rate, oxygen consumption, and cardiovascular function (17-21). In addition, males and females were separated, because metabolic functioning is proven to be substantially different between men and women (22,23). Differences in the metabolomics profiles (as described by lipidomics and NMR profiles) between men and women and BMI lower or higher than 40 kg/m^2 were confirmed in the current data set by partial least squares discriminant analysis (Supporting Information Figure S1).

To study the metabolic status and changes underlying the observed variation in inter-individual weight loss success, subjects were classified according to their weight loss success. The weight loss success was expressed as the percentage change in BMI (weight/height^2). The percentage change in BMI upon the LCD phase was calculated as (BMI at CID2 − BMI at CID1)/BMI at CID1. CID1 indicates the time point before commencing the LCD phase and CID2 the time point at the end of the LCD phase. While the subjects with MO were studied as a whole due to the limited number of subjects, the subjects with obesity were divided in tertiles and accordingly classified as "most successful" (S+), "successful" (S), and "least successful" (S−).

Male and female subjects were considered separately. For each group, the number of subjects was (males and females respectively): S+ 67, 120; S 66, 118; S− 67, 120; MO 36, 73.

Blood sampling

Blood samples were taken after an overnight fasting period at CID1 (before commencing the LCD phase) and CID2 (at the end of the LCD phase) as outlined by Larsen et al. (14) for the analysis of blood metabolites.

Anthropometric parameters

Basal systolic and diastolic blood pressure (systolic BP and diastolic BP, respectively), waist circumference, and BMI, calculated from measured body weight and height, were determined.

Clinical parameters

Glucose, insulin, free fatty acids (FFA), triacylglycerols (total TG), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and total cholesterol were measured from fasting blood samples (14). Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as HOMA-IR = (glucose × insulin)/22.5.

NMR-based metabolite profiling

Eighteen low-molecular-weight metabolites (LMWM), i.e., glucose, lactate, tyrosine, proline, glycine, alanine, valine, isoleucine, leucine, creatinine, creatine, acetoacetate, *n*-acetyl, acetate, 3-OH-butyrate, hydroxyisobutyrate, oxo-isovaleric acid, and total triacylglycerols, were

quantified from the NMR spectra. Analysis was carried out on the NMR quantified metabolites (qNMR). The NMR acquisition protocol and the quantification protocols are given in the Supporting Information.

LC-MS-based lipid profiling

A total of 140 lipid species was quantified in plasma by means of liquid chromatography-mass spectrometry (LC-MS). A complete list of detected lipid species is given in Supporting Information Table S1. The full protocol for LC-MS based lipid profiling is given in the Supporting Information.

Data sets

The prediction of successful weight loss was assessed on multiple data sets measured at baseline. The first data set encompasses anthropometric and clinical parameters ($p = 13$). The second data set comprises NMR-based metabolites ($p = 18$). The third data set comprises LC-MS-based lipid species and chemically assessed total free fatty acid concentrations ($p = 141$). The final data set is the fusion of the previous three data sets ($p = 172$). The separate data sets represent measurements of increasing complexity and thereby we wanted to show the added value of metabolomics (above or alongside the more conventional markers).

Statistical analyses

In this study, both univariate and omics profile data have been collected and analyzed by using both univariate and multivariate approaches to arrive to a global overview of the data (24).

Univariate analyses: ANOVA have been used to assess differences between groups (omnibus test) followed by the Tukey's honest significant difference (HSD) procedure as a post hoc test. Normality of data was assessed with the Jarque–Brera test (25) and square rooting was used for correction. The non-parametric Kruskall–Wallis test (26) was applied when appropriate. P-values < 0.05 were considered significant. The Benjamini–Hochberg method (25) was applied to correct for multiple testing.

Multivariate analyses: Principal component analysis was applied using the standard algorithm as implemented in the Matlab Statistics Toolbox. Partial least squares discriminant analysis (PLS-DA) and multilevel PLS-DA (27) was performed applying a double cross-validation scheme as detailed in ref. 28. The area under the receiver curve (AUROC) was used as a measure of the discriminant ability of the model. Autoscaling and Pareto scaling (29) were used for lipidomics and NMR data, respectively.

Stepwise regression was performed as detailed in ref. 23 within a double-cross validation scheme (CV). The analysis was repeated 30 times with different assignments of samples into validation, calibration, and test sets. In the first cross validation loop, initial conditions of analysis were optimized by selecting one optimal initial variable to which other variables were added in further steps of stepwise variable selection (forward selection). In the stepwise variable selection, inclusion and exclusion criteria were automatically set to ensure a compromise between model complexity and interpretability. In the second cross-validation loop, performance of the regression model defined in the first CV loop was assessed by predicting the left-out data to generate a cross-validated R^2 index measuring the percentage of explained variation captured by the model which was used as a measure of the quality of the regression model.

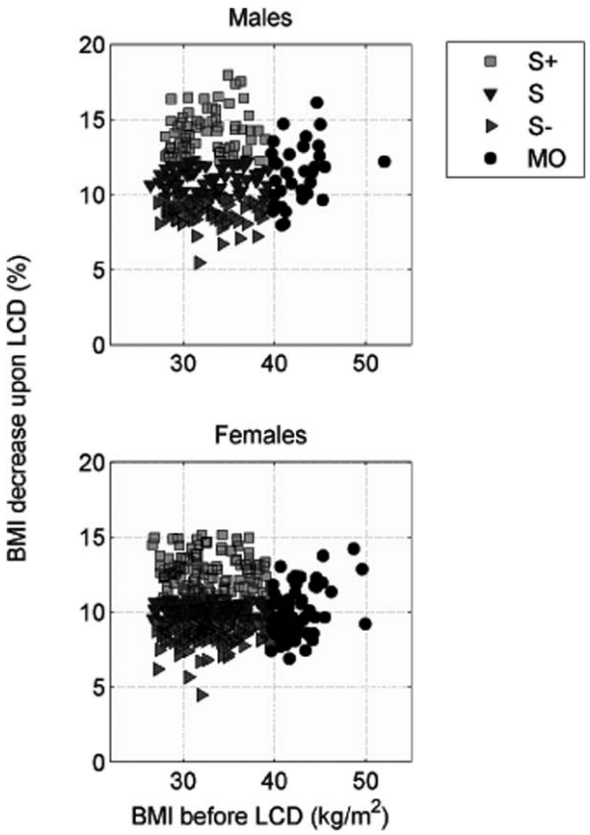


Figure 1 Classification of subjects according to gender, baseline BMI, and the percentage change in BMI upon the 8-week LCD phase. The subjects with overweight and obesity ($27 \leq BMI < 40$ kg/m^2) were divided in tertiles according to their percentage change in BMI upon the LCD phase and classified as most successful (S+, light gray squares), successful (S, black rectangles), and least successful (S−, dark gray rectangles). Subjects with morbid obesity (BMI$\geq$40 kg/m^2, black circles) were studied as a whole due to the limited number of subjects.

Software: All calculations where performed using built-in and in-house written Matlab routines (MATLAB 7.11, The MathWorks Inc., Natick, MA, 2010).

Results

Anthropometric, clinical, and metabolic differences at baseline

To test whether men and women with a different weight loss success and a different initial BMI were already different at baseline, the anthropometric, clinical, and metabolic data were compared between the four different groups (groups with MO obesity-most successful (S+), obesity-successful (S), and obesity-least successful (S−) (see Figures 1A,B).

Compared to subjects with MO, subjects with obesity (S, S+, and S− together) were substantially different in both anthropometric (as expected based on grouping: weight, BMI, and waist circumference) and clinical variables (Table 1). Male subjects with MO showed

TABLE 1 Anthropometric and clinical variables in subjects with obesity and morbid obesity

	Obesity				Morbid Obesity		
	Total	S+	S	S−	Total MO	*P*-value	Significance
Men							
n	200	67	66	67	36		
Age (year)	43 ± 0	43 ± 1	43 ± 1	43 ± 1	41 ± 1	0.456	
Height (m)	1.78 ± 0.01	1.78 ± 0.01	1.80 ± 0.01	1.77 ± 0.01	1.77 ± 0.01	0.254	
Weight (kg)	104.2 ± 1.0	103.5 ± 1.6	105.7 ± 1.7	103.3 ± 1.8	133.4 ± 12.2	<0.001	def
BMI (kg/m^2)	32.7 ± 0.2	32.6 ± 0.4	32.7 ± 0.4	32.7 ± 0.4	42.5 ± 0.4	<0.001	def
Waist circumference (cm)	110.28 ± 0.66	110.20 ± 1.02	110.66 ± 1.26	109.97 ± 1.14	133.03 ± 1.23	<0.001	def
Systolic BP (mmHg)	130.7 ± 0.9	131.1 ± 1.4	129.4 ± 1.8	131.7 ± 1.4	135.1 ± 2.0	0.187	
Diastolic BP (mmHg)	80.8 ± 0.7	81.1 ± 1.2	81.1 ± 1.5	80.1 ± 1.2	83.2 ± 1.7	0.550	
Fasting glucose (mmol/L)	5.2 ± 0.0	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	0.805	
Fasting insulin (μIU/L)	12.78 ± 0.52	12.28 ± 0.79	12.68 ± 0.80	13.37 ± 1.09	14.54 ± 1.03	0.178	
HOMA-IR	3.0 ± 0.1	3.0 ± 0.2	3.0 ± 0.2	3.0 ± 0.2	3.4 ± 0.2	0.197	
Fasting triacylglycerols (mmol/L)	1.56 ± 0.05	1.55 ± 0.09	1.64 ± 0.09	1.49 ± 0.08	1.79 ± 0.13	0.166	
Fasting free fatty acids (μmol/L)	543.4 ± 31.4	585.9 ± 45.0	557.5 ± 29.1	486.8 ± 20.1	643.7 ± 63.9	0.028	f
Fasting cholesterol (mmol/L)	5.04 ± 0.08	5.02 ± 0.14	4.86 ± 0.13	5.22 ± 0.14	5.20 ± 0.20	0.375	
Fasting LDL (mmol/L)	3.22 ± 0.07	3.21 ± 0.12	3.07 ± 0.11	3.38 ± 0.12	3.36 ± 0.16	0.318	
Fasting HDL (mmol/L)	1.08 ± 0.02	1.06 ± 0.03	1.05 ± 0.03	1.12 ± 0.04	1.04 ± 0.04	0.505	
Women							
n	358	120	118	120	73		
Age (year)	41 ± 0	40 ± 1	42 ± 0	40 ± 1	41 ± 1	0.033	a
Height (m)	1.66 ± 0.00	1.66 ± 0.01	1.67 ± 0.01	1.66 ± 0.01	1.65 ± 0.01	0.718	
Weight (kg)	90.3 ± 0.6	89.7 ± 1.1	91.8 ± 1.1	89.5 ± 1.0	115.8 ± 1.4	<0.001	def
BMI (kg/m^2)	32.7 ± 0.2	32.7 ± 0.3	32.9 ± 0.3	32.6 ± 0.3	42.2 ± 0.3	<0.001	def
Waist circumference (cm)	100.89 ± 0.51	99.92 ± 0.87	101.23 ± 0.90	101.53 ± 0.88	116.81 ± 1.32	<0.001	def
Systolic BP (mmHg)	120.6 ± 0.7	119.1 ± 1.1	122.0 ± 1.3	120.7 ± 1.5	127.6 ± 1.7	0.001	df
Diastolic BP (mmHg)	74.8 ± 0.5	74.4 ± 0.8	77.3 ± 0.9	72.7 ± 1.0	80.4 ± 1.2	<0.001	dcf
Fasting glucose (mmol/L)	5.0 ± 0.0	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	0.327	
Fasting insulin (μIU/L)	9.44 ± 0.32	8.87 ± 0.45	10.17 ± 0.70	9.25 ± 0.46	12.80 ± 0.83	<0.001	def
HOMA-IR	2.1 ± 0.1	2.0 ± 0.1	2.3 ± 0.2	2.1 ± 0.1	3.0 ± 0.2	<0.001	def
Fasting triacylglycerols (mmol/L)	1.21 ± 0.03	1.16 ± 0.05	1.22 ± 0.05	1.24 ± 0.05	1.36 ± 0.07	0.052	d
Fasting free fatty acids (μmol/L)	697.0 ± 29.6	709.1 ± 28.8	682.5 ± 28.4	699.5 ± 31.6	783.3 ± 42.8	0.165	
Fasting cholesterol (mmol/L)	4.81 ± 0.05	4.82 ± 0.10	4.66 ± 0.08	4.96 ± 0.08	4.97 ± 0.11	0.038	e
Fasting LDL (mmol/L)	2.97 ± 0.05	2.96 ± 0.08	2.80 ± 0.07	3.14 ± 0.08	3.10 ± 0.09	0.009	ce
Fasting HDL (mmol/L)	1.30 ± 0.02	1.30 ± 0.03	1.31 ± 0.03	1.28 ± 0.03	1.25 ± 0.03	0.502	

All values are means ± SEM.
P-value of 0.05 was considered significant: a: S+ vs. S, b: S+ vs. S−, c: S vs. S−, d: S+ vs. MO, e: S vs. MO, f: S− vs. MO.
MO, subjects with morbid obesity; S+, most successful; S, successful; S−, least successful; BP, blood pressure; HOMA-IR, homeostatic model assessment for insulin resistance; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol

higher levels of fasting free fatty acids in plasma when compared to S− men with obesity. Female subjects with MO showed higher systolic as well as diastolic BP levels compared to obesity subjects with S+ and S−, higher fasting insulin, and HOMA-IR levels compared to all three obesity categories and higher fasting cholesterol and LDL levels compared to S subjects. In contrast, no differences in fasting glucose and HDL cholesterol between the men and women with obesity and MO were observed.

Between the groups with obesity also differences in clinical data were observed, but only in women. The S category women showed significantly higher diastolic blood pressure levels compared to S− women and significantly lower fasting LDL cholesterol levels compared to S− women (Table 1).

Only a subset of metabolites showed significant differences predominantly between subjects with obesity and MO. In women, branched-chain amino acids (BCAA), leucine, valine, and isoleucine were significantly lower in subjects with obesity compared to MO, but not between the subjects with MO and the three groups with obesity individually (Figure 2 and Supporting Information Table S1). Also several other amino acids (alanine,

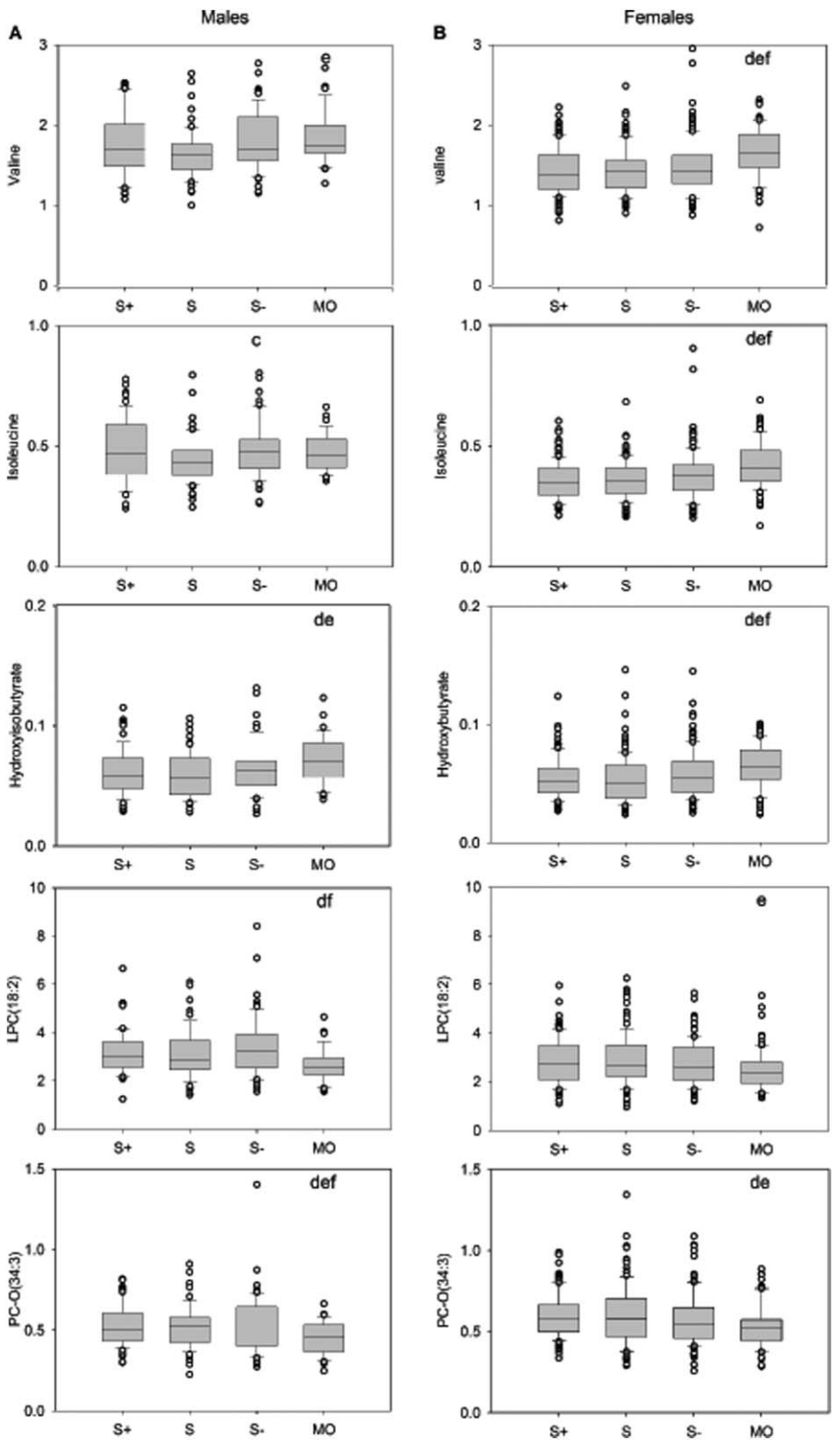


Figure 2 Box plots of significant differences at baseline. Fasting valine, leucine, hydroxyisobutyrate, LPC (18:2), and PC-O (34:3) levels in (**A**) males and (**B**) females. *P*-value of 0.05 was considered significant: a: S+ vs. S, b: S+ vs. S−, c: S vs. S−, d: S+ vs. MO, e: S vs. MO, f: S− vs. MO. MO, subjects with morbid obesity; S+, most successful; S, successful; S−, least successful.

TABLE 2 Multiple regression used to explain the variation in the successfulness in the percentage change in BMI upon low-calorie diet (expressed in variation explained (R^2), see *Methods*)

	Men				Women			
	Obesity		Morbid obesity		Obesity		Morbid obesity	
	R^2	p	R^2	p	R^2	p	R^2	p
Clinical data	0.03	6	0.20	7	0.06	9	0.16	7
NMR data	0.11	13	0.57	10	0.08	8	0.28	7
LC-MS data	0.21	14	0.44	7	0.13	13	0.36	12
C + N + L	0.28	15	0.53	9	0.14	13	0.38	10

p depicts the amount of parameters the regression model is built upon to explain the variation.
Men and women and subjects with obesity and morbid obesity were analyzed separately. Furthermore, multiple data sets were used: (1) anthropometric and clinical parameters (C, $p = 13$), (2) NMR-based metabolites (N, $p = 18$), (3) LC-MS-based lipid species and chemically assed total free fatty acid concentrations (L, $p = 141$), and (4) the amalgamation of the previous three data sets (C + N + L, $p = 172$).

proline, and tyrosine) were lower in women with obesity compared to MO. Hydroxyisobutyrate was significantly different both in men and women. Moreover, phosphatidylcholine species, such as LPC (18:2), PC-O (34:3), PC-O (38:7), PC-O (40:6), PC (38:3), PC (40:4), and PC (40:8), showed significant differences between subjects with MO and obesity both in men and women. Next to the differences at baseline between MO and obesity, only isoleucine was significantly lower in S men compared to S− men and PC-O (42:6) exhibited significantly higher levels in S− men compared to S+ men. Moreover, no discrimination within the groups with obesity was possible using multivariate PLS-DA, which was possible for groups with obesity vs. the group with MO (both in men and women) (Supporting Information Figure S1).

Metabolic profiles predicting weight loss

To find metabolic profiles predicting weight loss (percentage change BMI) for subjects with obesity and MO, stepwise regression was used on all data sets (anthropometric, clinical, metabolite) and their combination (Table 2). The resulting models were able to predict maximally 57% of the variation in the percentage BMI change upon LCD in men and maximally 38% of the variation observed in women (Table 2). The strongest models in men and women were built on 10 metabolites (Tables 2 and 3 and Figure 3). Both models of subjects with MO are built on energy metabolism-related metabolites, including a ketone body (acetoacetate), so-called building blocks (i.e., acetate, *n*-acetyl, and hydroxyisobutyrate), triacylglycerols (total triacylglycerols and TG (48:4)), phosphatidylcholines (PC(O-34:1), and PC(O-44:5)), amino acids (valine, tyrosine, alanine, and proline), and finally creatine and creatinine. The models with the highest explained variation were based on the combination of all data types (anthropometric, clinical data NMR, LC-MS lipids). Generally, the regression models for subjects with MO (both in men and women) result in a higher explained variation than the models built on subjects with obesity. To test the specificity of the models of MO, these models were applied on percentage change in BMI in subjects with obesity, resulting in an explanation of 3% of the variation in both men and women. Building multiple regression models for the complete group of subjects (with obesity and MO) resulted only in a predicting of 12% of the variation in women and 19% of the variation in men.

Metabolomics analysis to reveal weight loss mechanisms

To investigate the response of different groups with obesity and MO to the LCD period, we analyzed the metabolic changes between the groups (Supporting Information Table S2 and S3).

TABLE 3 Contributing parameters of the two strongest stepwise regression models of morbid obesity

Morbid obesity					
Men—NMR data			Women—C + N + L data		
R^2	p	Directionality	R^2	p	Directionality
0.57	*n*-Acetyl	0.41	0.38	Acetate	−0.23
	Creatinine	−0.15		Creatinine	−0.21
	Acetoacetate	0.30		Creatine	−0.15
	Valine	−0.59		Systolic BP	−0.21
	Tyrosine	0.34		Acetoacetate	−0.24
	Triacylglycerols	−0.14		SM (d18:1/16:1)	0.20
	Proline	−0.30		*n*-Acetyl	0.14
	Alanine	0.36		TG (48:4)	−0.15
	Creatine	−0.14		PC-O (34:1)	0.08
	Hydroxyisobutyrate	0.08		PC-O (44:5)	0.08

Stepwise regression was used to predict the successfulness in the percentage change in BMI (expressed in variation explained (R^2), see *Methods*). p are the parameters on which the stepwise regression model was built, and the directionality (regression coefficients) indicates the direction of their contribution.
It is shown that both models are built on energy metabolism-related parameters, including a keton body (acetoacetate), so-called building blocks (i.e., acetate, *n*-acetyl and hydroxyisobutyrate), triacylglycerols (total triacylglycerols and TG (48:4)), phosphatidylcholines (PC(O-34:1) and PC(O-44:5)), amino acids (valine, tyrosine, alanine and proline), and finally creatine and creatinine.

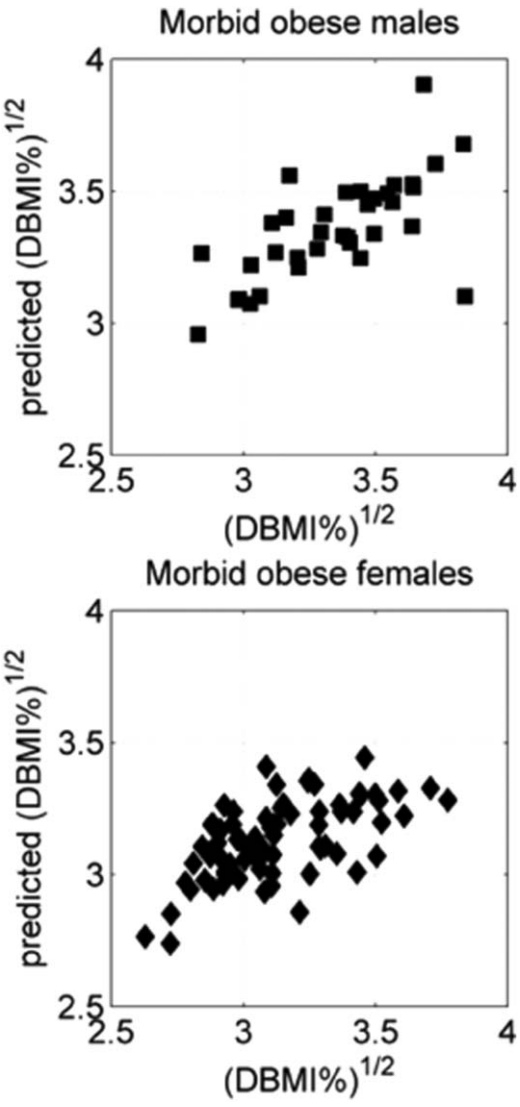


Figure 3 Predictive power of the multiple regression models in males and females with morbid obesity. A high inter-individual variation in percentage BMI change upon 8-week LCD was observed. Stepwise regression was used to predict the successfulness in the percentage change in BMI (expressed in variation explained R^2, see *Methods*). This figure shows the predictive power of the two strongest stepwise regression models by comparing the actual measured percentage change in BMI (*X*-axis) to the predicted change (*Y*-axis).

Differences in changes between subjects with MO and obesity were observed. LCD reduced fasting glucose less strongly in men with MO when compared to S+ and S men with obesity. In addition, HDL cholesterol was significantly more reduced in men with MO compared to S+ men (Figure 4).

Moreover, between the groups with obesity significant differences in response were seen. For example, in women, S+ subjects showed a stronger decrease in cholesterol and LDL cholesterol compared to S and S− subjects (Figure 4). Furthermore, both in men and women, most triacylglycerol species containing 53 or less carbon atoms showed the highest reduction in S+, an average reduction in S, and the lowest reduction in S− subjects, and thus showed a so-called success rate-dependent decline. This success rate-dependent decline upon the LCD was also observed in other lipid species; generally, LPC, PC, and SM species show a similar response pattern. Additionally, success rate-dependent responses were seen for acetoacetate and 3-OH-butyrate in both men and women and creatine showed a success rate-dependent decline in women.

A PLS-DA analysis was performed to test whether metabolism in general is changed differently between the groups. PLS-DA analyses on the combination of NMR and LC-MS data (comparing the changes in metabolites) were able to discriminate the success categories of subjects with obesity from each other (both in men and women, Supporting Information Figure S2). The male models were more based on lipid species (especially sphingomyelins and phosphatidylcholines), whereas several amino acids were included in the models for females, especially in the models separating subjects with obesity from subjects with MO.

Discussion

Weight loss, for instance induced by LCD, improves glucose metabolism, insulin sensitivity, and the plasma lipid profile (4-8). However, successfulness of weight loss is highly variable. Part of this variability can be explained by compliance, but another part remains unexplained. We aim to predict the percentage change in BMI upon an 8-week LCD period using parameters at baseline and thereby explain possible inter-individual weight loss variation within a cohort that received an identical calorie restriction regime. We question the metabolic changes underlying the high inter-individual variation in weight loss success, in order to understand the mechanisms. For this purpose, anthropometric characteristics and plasma metabolite and lipid profiles were assessed before and after a period of weight loss by LCD. To the best of our knowledge this is the first study examining a large set of metabolomics data in predicting weight loss in humans.

The highest explained variation is obtained by statistical prediction models built on the combination of clinical parameters, NMR-based metabolites, and LC-MS-based lipid species, demonstrating the added values of metabolomics technologies alongside the classical technologies (i.e., anthropometric and clinical chemistry determinants). The predictive models of subjects with morbid obesity result in higher explained variations than the models predicting percentage change in BMI in subjects with obesity and were shown to be specific for the sub-population with morbid obesity. The parameters constituting the model for morbid obesity in both men and women were mainly related to energy metabolism (e.g., acetoacetate, triacylglycerols, phosphatidylcholines, amino acids, and creatine and creatinine). We postulate the following hypothesis: weight loss success in subjects with obesity and morbid obesity is dependent on their energy metabolism functioning at baseline. This relation is stronger in subjects with morbid obesity as the model can predict the weight loss better. It is obvious that in subjects with morbid obesity energy needs are higher due to a higher body weight. Also, this hypothesis implies that extension of the data sets with energy metabolism-related compounds such as organic acids might further improve the explained variation of the models. Studying these contributing parameters reveals mechanisms that may underlie the inter-individual variability in energy metabolism at baseline. For instance, tyrosine levels contributed positively to successful weight loss. Tyrosine is among others a precursor of thyroid hormone, which is involved in the regulation of the rate of

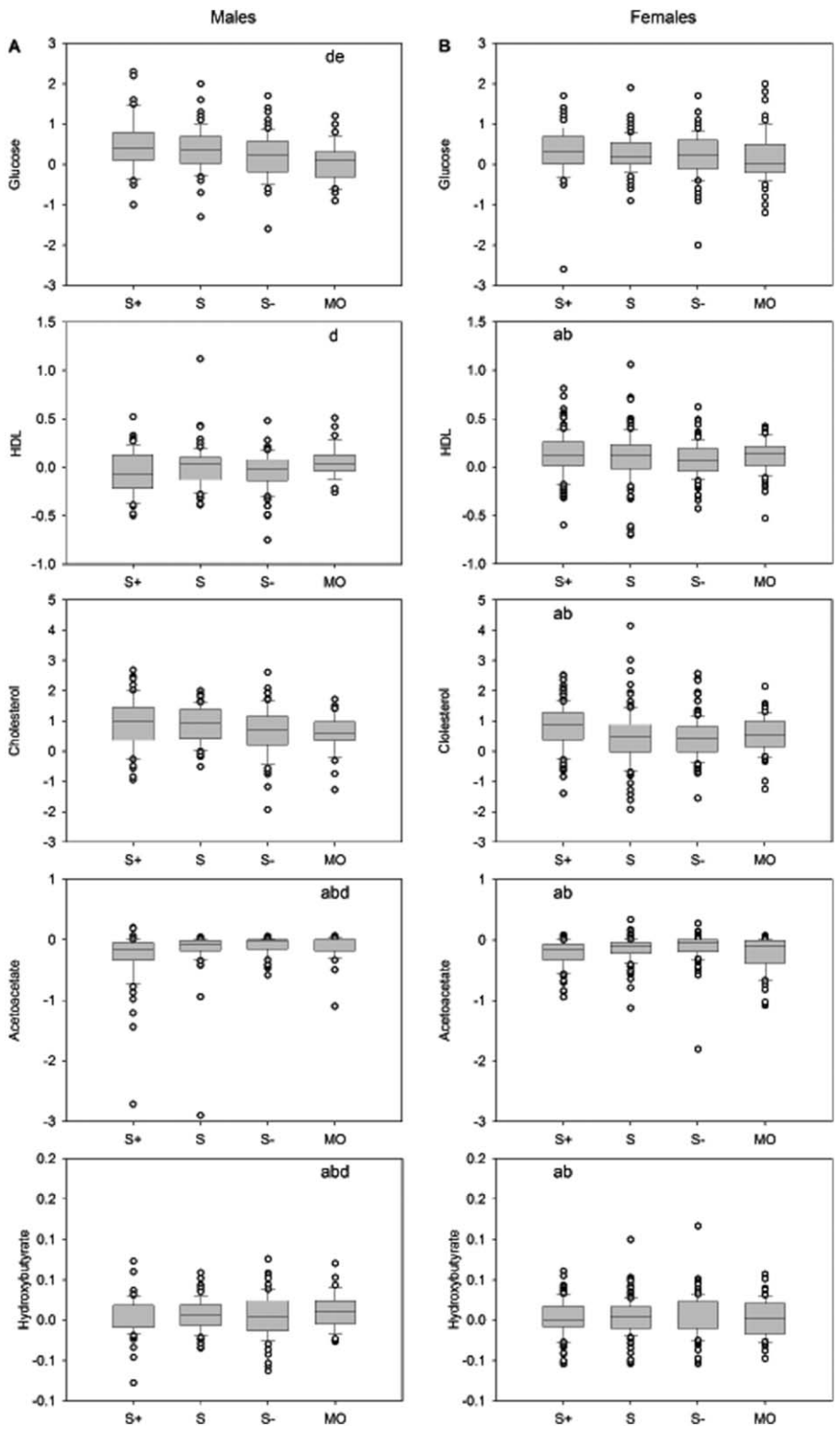


Figure 4 Box plots of significant changes upon 8-week LCD. Changes in glucose, HDL, cholesterol, acetoacetate, and hydroxybutyrate are shown in (**A**) males and (**B**) females. *P*-value of 0.05 was considered significant: a: S+ vs. S, b: S+ vs. S−, c: S vs. S−, d: S+ vs. MO, e: S vs. MO, f: S− vs. MO. MO, subjects with morbid obesity; S+, most successful; S, successful; S−, least successful.

metabolism (30). In addition, the total level of triacylglycerols and the level of a specific triacylglycerol, TG(48:4), were negatively impacting on weight loss success. High levels of TGs in the bloodstream can be caused by an higher intake of fat (31,32) or a lower fat turnover. Furthermore, phosphatidylcholines PC(O-34:1) and PC(O-44:5) and sphingomyelin (d18:1/16:1) also contributed positively to weight loss success. In general, phosphatidylcholine and sphingomyelin species are common lipids in circulating HDL particles (33), indicating a higher reverse cholesterol transport. Acetate, which is a building block for production of fatty acids (34), was also negatively related to weight loss. Creatine and creatinine levels were also negatively related to weight loss, these metabolites are positively associated to skeletal muscle mass (35). We therefore hypothesize that successful weight loss is related to a better preservation of muscle mass, which is connected to more energy usage from other sources (e.g., adipose tissue). Finally, valine, which is a branched-chain amino acid (BCAA), contributed negatively to weight loss success. BCAA levels are increased in subjects with obesity and contribute to insulin resistance (36). Despite the confirmation of higher levels of BCAA in subjects with morbid obesity compared to subjects with obesity in the current study, this study reveals less explained variation of BCAAs in explaining weight loss than do other metabolites such as *n*-acetyl, creatinine, and acetoacetate. This metabolic profile in fasting plasma, as estimation of energy expenditure, may have clinical application since current clinical methods are not easily applicable (37,38). Taken together, these results imply that relatively healthy subjects with morbid obesity (i.e., high in tyrosine, PC and SM levels, and low in TG, acetate, and BCAA levels) are most successful in losing weight upon an 8-week LCD period.

A second goal of the study was to further unravel the metabolic efficiency of weight loss. Therefore, the change in metabolic profile change during the LCD was studied. This analysis corroborated the metabolic profile at baseline; we saw a reduction of several lipids, cholesterol, and the increase in the ketone bodies acetoacetate and 3-OH-butyrate in the most successful group. These observations can be explained by a higher lipid turnover during the weight loss period in the successful group which corresponds to a higher energy metabolism at baseline (see earlier).

In conclusion, maximally 57% of the variation in weight loss success can be predicted by baseline parameters. The most powerful predictive models were obtained in subjects with morbid obesity. Parameters constituting these models imply that weight loss success in subjects with morbid obesity positively depends on the energy metabolism status in these subjects at baseline, implying that the subjects with morbid obesity with a high energy metabolism (as seen in components related to lipid turnover and physical activity) are more successful in losing weight upon 8-week LCD and that this difference in energy metabolism status becomes more pronounced and relevant for weight loss with increasing BMI. Being able to predict the amount of weight loss in advance of a LCD period might stimulate subjects to adhere to the strict dietary regimen. Furthermore, prediction of weight loss success allows more personalized weight loss advice. For instance, weight loss success may relate to the individual's energy expenditure efficiency of specific macronutrient types (fat, carbohydrate, and/or protein) and related intake. Finally, since predictions were based on anthropometric characteristics and plasma metabolite profiles at baseline, which are rather easily obtainable, these predictive models have potential for clinical application. O

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References

1. McCay CM, Crowell MF, Maynard LA. The effect of retarded growth upon the length of life span and upon the ultimate body size. *J Nutr* 1935;63-79.
2. Mustajoki P, Pekkarinen T. Very low energy diets in the treatment of obesity. *Obes Rev* 2001;2:61-72.
3. Redman LM, Heilbronn LK, Martin CK, et al. Effect of calorie restriction with or without exercise on body composition and fat distribution. *J Clin Endocrinol Metab* 2007;92:865-872.
4. Escalante-Pulido M, Escalante-Herrera A, Milke-Najar ME, Alpizar-Salazar M. Effects of weight loss on insulin secretion and in vivo insulin sensitivity in obese diabetic and non-diabetic subjects. *Diabetes Nutr Metab* 2003;16:277-283.
5. Henry RR, Gumbiner B. Benefits and limitations of very-low-calorie diet therapy in obese NIDDM. *Diabetes Care* 1991;14:802-823.
6. Larson-Meyer DE, Heilbronn LK, Redman LM, et al. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care* 2006;29:1337-1344.
7. Uusitupa MI, Laakso M, Sarlund H, Majander H, Takala J, Penttila I. Effects of a very-low-calorie diet on metabolic control and cardiovascular risk factors in the treatment of obese non-insulin-dependent diabetics. *Am J Clin Nutr* 1990;51:768-773.
8. Lefevre M, Redman LM, Heilbronn LK, et al. Caloric restriction alone and with exercise improves CVD risk in healthy non-obese individuals. *Atherosclerosis* 2009; 203:206-213.
9. Heilbronn LK, de Jonge L, Frisard MI, et al. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *JAMA* 2006;295:1539-1548.
10. Martin CK, Heilbronn LK, de Jonge L, et al. Effect of calorie restriction on resting metabolic rate and spontaneous physical activity. *Obesity (Silver Spring)* 2007;15: 2964-2973.
11. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2006;113:898-918.
12. Choi KM, Han KA, Ahn HJ, et al. The effects of caloric restriction on fetuin-A and cardiovascular risk factors in rats and humans: a randomized controlled trial. *Clin Endocrinol (Oxf)* 2013;79:356–363.
13. Larsen TM, Dalskov SM, van Baak M, et al. Diets with high or low protein content and glycemic index for weight-loss maintenance. *N Engl J Med* 2010;363:2102-2113.
14. Larsen TM, Dalskov S, van Baak M, et al. The Diet, Obesity and Genes (Diogenes) Dietary Study in eight European countries—a comprehensive design for long-term intervention. *Obes Rev* 2010;11:76-91.
15. Vermorel M, Lazzer S, Bitar A, et al. Contributing factors and variability of energy expenditure in non-obese, obese, and post-obese adolescents. *Reprod Nutr Dev* 2005;45:129-142.
16. Moore CS, Lindroos AK, Kreutzer M, et al. Dietary strategy to manipulate ad libitum macronutrient intake, and glycaemic index, across eight European countries in the Diogenes Study. *Obes Rev* 2010;11:67-75.
17. Rotondi M, Magri F, Chiovato L. Thyroid and obesity: not a one-way interaction. *J Clin Endocrinol Metab* 2011;96:344-346.
18. Bedell GN, Wilson WR, Seebohm PM. Pulmonary function in obese persons. *J Clin Invest* 1958;37:1049-1060.
19. Alexander JK, Amad KH, Cole VW. Observations on some clinical features of extreme obesity, with particular referece to cardiorespiratory effects. *Am J Med* 1962;32:512-524.
20. Kress JP, Pohlman AS, Alverdy J, Hall JB. The impact of morbid obesity on oxygen cost of breathing (VO(2RESP)) at rest. *Am J Respir Crit Care Med* 1999; 160:883-886.
21. de Divitiis O, Fazio S, Petitto M, Maddalena G, Contaldo F, Mancini M. Obesity and cardiac function. *Circulation* 1981;64:477-482.
22. Seidell JC, Cigolini M, Charzewska J, et al. Fat distribution and gender differences in serum lipids in men and women from four European communities. *Atherosclerosis* 1991;87:203-210.
23. Szymanska E, Bouwman J, Strassburg K, et al. Gender-dependent associations of metabolite profiles and body fat distribution in a healthy population with central obesity: towards metabolomics diagnostics. *Omics* 2012;16:652-667.

24. Saccenti E, Hoefsloot HCJ, Smilde AK Westerhuis JA, Hendriks MMWB. Reflections on univariate and multivariate analysis of metabolomics data. *Metabolomics* 2014;10:361-374.
25. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Statist Soc B* 1995;57:289-300.
26. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. *J Am Stat Assoc* 1952;47:583-621.
27. van Velzen EJ, Westerhuis JA, van Duynhoven JP, et al. Multilevel data analysis of a crossover designed human nutritional intervention study. *J Proteome Res* 2008;7:4483-4491.
28. Szymanska E, Saccenti E, Smilde AK, Westerhuis JA. Double-check: validation of diagnostic statistics for PLS-DA models in metabolomics studies. *Metabolomics* 2012;8:3-16.
29. van den Berg RA, Hoefsloot HC, Westerhuis JA, Smilde AK, van der Werf MJ. Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC Genomics* 2006;7:142.
30. Cannon B, Nedergaard J. Thyroid hormones: igniting brown fat via the brain. *Nat Med* 2010;16:965-967.
31. Jones A. Triglycerides and cardiovascular risk. *Heart* 2013;99:1-2.
32. Kuksis A, Myher JJ, Geher K, et al. Gas chromatographic profiles of plasma total lipids as indicators of dietary history. Correlation with Fat Intake Based on 24-H Dietary Recall. *J Chromatogr* 1991;564:11-26.
33. Wiesner P, Leidl K, Boettcher A, Schmitz G, Liebisch G. Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry. *J Lipid Res* 2009;50:574-585.
34. Hellerstein MK, Schwarz JM, Neese RA. Regulation of hepatic de novo lipogenesis in humans. *Annu Rev Nutr* 1996;16:523-557.
35. Wyss M, Kaddurah-Daouk R. Creatine and creatinine metabolism. *Physiol Rev* 2000;80:1107-1213.
36. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 2012;15:606-614.
37. Bosy-Westphal A, Braun W, Schautz B, Muller MJ. Issues in characterizing resting energy expenditure in obesity and after weight loss. *Front Physiol* 2013;4:47.
38. Kross EK, Sena M, Schmidt K, Stapleton RD. A comparison of predictive equations of energy expenditure and measured energy expenditure in critically ill patients. *J Crit Care* 2012;27:321.e5-321.12.