Distinct lipid profiles predict improved glycemic control in obese, nondiabetic patients after a low-caloric diet intervention: the Diet, Obesity and Genes randomized trial

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ABSTRACT

Background: An aim of weight loss is to reduce the risk of type 2 diabetes (T2D) in obese subjects. However, the relation with long-term glycemic improvement remains unknown.

Objective: We evaluated the changes in lipid composition during weight loss and their association with long-term glycemic improvement.

Design: We investigated the plasma lipidome of 383 obese, non-diabetic patients within a randomized, controlled dietary intervention in 8 European countries at baseline, after an 8-wk low-caloric diet (LCD) (800–1000 kcal/d), and after 6 mo of weight maintenance.

Results: After weight loss, a lipid signature identified 2 groups of patients who were comparable at baseline but who differed in their capacities to lose weight and improve glycemic control. Six months after the LCD, one group had significant glycemic improvement [homeostasis model assessment of insulin resistance (HOMA-IR) mean change: -0.92; 95% CI: -1.17, -0.67)]. The other group showed no improvement in glycemic control (HOMA-IR mean change: -0.26; 95% CI: -0.64, 0.13). These differences were sustained for ≥1 y after the LCD. The same conclusions were obtained with other endpoints (Matsuda index and fasting insulin and glucose concentrations). Significant differences between the 2 groups were shown in leptin gene expression in adipose tissue biopsies. Significant differences were also observed in weight-related endpoints (body mass index, weight, and fat mass). The lipid signature allowed prediction of which subjects would be considered to be insulin resistant after 6 mo of weight maintenance [validation’s receiver operating characteristic (ROC) area under the curve (AUC): 71%; 95% CI: 62%, 81%]. This model outperformed a clinical data–only model (validation’s ROC AUC: 61%; 95% CI: 50%, 71%; P = 0.01).

Conclusions: In this study, we report a lipid signature of LCD success (for weight and glycemic outcome) in obese, nondiabetic patients. Lipid changes during an 8-wk LCD allowed us to predict insulin-resistant patients after 6 mo of weight maintenance. The determination of lipid composition during an LCD enables the identification of nonresponders and may help clinicians manage metabolic outcomes with further intervention, thereby improving the long-term outcome and preventing T2D. This trial was registered at clinicaltrials.gov as NCT00390637.

Keywords: adipose tissue, dietary intervention, insulin resistance, lipidemia, obesity, weight loss

INTRODUCTION

Obesity is a major risk factor for a number of comorbidities including cardiovascular disease, dyslipidemia, hypertension, insulin resistance, type 2 diabetes (T2D),¹ and cancer (1–3). Excess fat mass is associated with insulin resistance, but the underlying mechanisms are not fully understood.

Caloric restriction reduces fat mass and also affects adipose tissue function. Adipose tissue is important in energy homeostasis and responds dynamically to caloric intake (4–6) by changing the lipolysis rate and adipose tissue triacylglycerol turnover. Both glyceroneogenesis and de novo lipogenesis respond to dietary energy intake.

Multiple studies have shown that weight loss through energy-restricted dietary interventions improved dysfunctions involved in the metabolic syndrome (7, 8). Nevertheless, a large interindividual variability was observed regarding the capacity to lose and maintain weight (9). In clinical practice, a dietary intervention is done with the use of a caloric-restricted diet and is followed by a weight-maintenance (stabilization) phase. However, the long-term outcomes of these dietary interventions are not predictable because of the lack of knowledge including the complexity of adipose tissue adaptation that results from the interventions. Although initial BMI has some predictive value for intervention success (10), patients with

¹Supported by the European Commission, Food Quality and Safety Priority of the Sixth Framework Program (FP6-2005-513946) and the Nestlé Institute of Health Sciences. This is a free access article, distributed under terms (http://www.nutrition.org/publications/guidelines-and-policies/license/) that permit unrestricted noncommercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

²The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Abbreviations used: CID, clinical intervention day; Diogenes, Diet, Obesity and Genes; FDR, false discovery rate; LCD, low-calorie diet; mRNA, messenger RNA; PC, principal component; PCA, principal component analysis; PC1, first principal component; ROC, receiver operating characteristic; T2D, type 2 diabetes.

Received May 11, 2016. Accepted for publication June 27, 2016.
First published online August 10, 2016; doi: 10.3945/ajcn.116.137646.
similar starting BMIs have different responses. The rate of weight loss alone is also a poor predictor and monitor for sustained weight maintenance (11). More-objective molecular biomarkers are scarce (12). In addition, the long-term relation between weight loss, the improvement of lipid profiles, and glycemic control remains to be explored.

In this report, we investigated changes in lipidemia in overweight, non-diabetic subjects from the Diogenes (Diet, Obesity and Genes) study (clinicaltrials.gov; NCT00390637), which is one of the largest dietary intervention studies. We studied how changes in lipid profiles after a low-caloric diet would be associated with long-term improvements (after 6 mo of weight maintenance) in both weight and glycemic control. We also investigated whether a comprehensive lipidomic approach would enable the prediction of insulin-resistant subjects despite the subjects having completed a weight-loss and weight-maintenance intervention and whether such a model would perform significantly better than a model that was based only on easily accessible clinical variables.

METHODS

Study design

The Diogenes study was a multicenter, randomized, controlled dietary intervention study that involved 8 European countries (13, 14). A flowchart that describes the intervention and the sample size at each phase is shown in Figure 1. Briefly, 938 overweight or obese, non-diabetic adults [BMI (in kg/m²) between 27 and 45 and blood fasting glucose concentrations <6.1 mmol/L] underwent an 8-wk weight-loss phase with the use of a complete meal-replacement low-calorie diet (LCD). The LCD provided 800 kcal/d (Modifast; Nutrition et Sante´ France). Participants could eat ≤400 g vegetables (corresponding to a maximal addition of 200 kcal/d).

FIGURE 1 Diogenes study. CID, clinical intervention day; Diogenes, Diet, Obesity and Genes; HP-HGI, high protein content and high glycemic index; HP-LGI, high protein content and low glycemic index; LCD, low-calorie diet; LP-HGI, low protein content and high glycemic index; LP-LGI, low protein content and low glycemic index; WMD, weight-maintenance diet.
Participants who achieved >8% weight loss (n = 773) were randomly assigned to consume a weight-maintenance diet for 26 wk. The diets corresponded to a 2-by-2 factorial design as follows: diets with a high or low content of protein and a high or low glycemic index or a control diet according to local food customs (15). All diets had a moderate fat content (25–30% of total energy consumed) and were consumed ad libitum. Food intake was monitored through food diaries (validated by a nutritionist). Of 773 participants, 548 subjects (71%) completed the 6-mo weight-maintenance intervention. Such a dropout rate (29%) has been discussed in details previously (14) and was in line with reports from other dietary studies (16, 17).

Two centers (Netherlands and Denmark) provided most of the food to participants during the weight-maintenance intervention (following recommendations from dietitians and in accordance with the participant’s randomized diet). These 2 centers, which were referred to as shop centers, extended the weight-maintenance protocol up to 1 y. The remaining centers provided participants with dietary instructions for the 6-mo period. Additional details are presented in Moore et al. (15).

The analysis described here included 383 participants who completed the entire intervention (LCD and ≥6 mo of weight maintenance) and had available plasma samples. Plasma lipids were profiled at baseline [clinical intervention day (CID) 1], after 8-wk of LCD (CID2), and after 6 mo of weight maintenance (CID3).

Ethics

Local ethics committees approved the study, written informed consent was obtained from each patient, and the study was carried out in accordance with the principles of the Declaration of Helsinki.

Lipidomic analysis

Liquid chromatography–mass spectrometry data generation has been described previously (18). A mixture of internal standards and calibration standards was added to each sample and was followed by liquid–liquid extraction with a (2:1) mixture of dichloromethane and methanol according to the procedure described in detail in Hu et al. (19). The organic phase that contained most of the lipids was removed and prepared for further analysis. Extracted lipids were separated on a Ascentis Express C8 2.1 9 150-mm (2.7-μm particle size) column (Sigma-Aldrich) with the use of an Acquity UPLC system (Waters) and analyzed with the use of quadrupole time-of-flight mass spectrometry (Agilent Technologies). Experimental conditions during the chromatographic analysis and detection by mass spectrometry were similar to the protocol described by Hu et al. (19). In total, 125 intact lipids including triacylglycerides, phosphocholines sphingomyelins, cholesterol esters, cholesterol, and diacylglycerides were measured.

A differential expression analysis was performed with the use of paired t test (2 sided with unequal variance). P values were adjusted for multiple testing with the use of the Benjamini-Hochberg procedure (20). Differentially expressed lipids were combined into a signature by performing a principal component analysis (PCA) on their expression fold changes between CID1 and CID2. Expression fold changes were scaled to a mean of zero and unit variance before the PCA.

Clinical endpoints

The following clinical endpoints were included in the analysis: BMI and weight; fat mass and fat-free mass were measured with the use of dual-energy X-ray absorptiometry. The following glycemic control measures were analyzed: HOMA-IR, fasting glucose and insulin, and the Matsuda index, which is a measure of insulin sensitivity derived from 2-h oral glucose tolerance tests (21). Total lipid concentrations (cholesterol, triglycerides, HDL, and derived LDL determined with the use of Friedwald’s formula) were also analyzed with blood biochemistry.

Transcriptomics analysis

Abdominal subcutaneous adipose tissue biopsies were obtained by needle aspiration under local anesthesia after an overnight fast at baseline, at the end of the LCD, and at the end of weight maintenance. Biopsy samples were stored at −80°C until total RNA extraction, and leptin messenger RNA (mRNA) concentrations were assessed with the use of high-throughput real-time polymerase chain reaction (22). Data are available from the Gene Expression Omnibus (series GSE60946, http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE60946). Analyses focused on 93 subjects who had available lipidomic and transcriptomic data. The change in leptin expression was assessed with the use of a 2-sided t test.

Statistical analysis

Changes in clinical endpoints were tested with the use of linear mixed-effect models. The lipid signature, sex, and age were modeled as fixed effects; the center was modeled as a random effect. P values were adjusted for multiple testing (20). Prediction analyses were aimed at predicting subjects who were classified as insulin resistant after weight maintenance (i.e., at CID3) with the use of data that were available either at CID1 or during the LCD (CID1 and CID2). To ensure the robustness of the models, the following definitions of insulin resistance at CID3 were used: Matsuda index <3 and <2.5 (21) and HOMA-IR ≥3 and ≥2.5 (23). The Matsuda index was derived from oral-glucose-tolerance test (21), whereas the HOMA-IR (23) was derived from fasting concentrations of glucose and insulin. Both variables have been shown to correlate well with euglycemic insulin clamps and to adapt for the assessment of insulin-resistance in nondiabetic subjects (21, 24–26). For each insulin-resistance definition, the following 2 clinical models were constructed: one model used baseline characteristics, and the other model used the percentage of change during the LCD for the same clinical variables. None of these models used variables at CID3. Clinical models included the following variables: age, sex, BMI, lipid concentrations from blood biochemistry (total triglyceride, total cholesterol, derived LDL, and HDL), and either the Matsuda index or HOMA-IR (according to the insulin-resistance definition that had been used). A third combined clinical and lipidomic model was created by adding the lipid signature during the LCD to the most-predictive clinical model. Models were trained on the shop centers (Denmark and Netherlands), and the predictive performance was evaluated on the remaining centers (independent validation set). Such
a setting provided an independent replication of the predictive models. Only the prediction performance on the validation set is reported; the performance on the training set is, by definition, significantly higher and should not be used for evaluating the utility of a model. Models were fitted with the use of logistic regression and receiver operating characteristic (ROC) curves, and a model evaluation was performed with the use of the pROC package (27). A statistical comparison between ROC curves was performed with the use of Delong’s test (28). The specificity and sensitivity of a model were determined with the use of the Youden index (29). All analyses were performed with the use of R software (v3.0.0, www.r-project.org).

RESULTS

Baseline characteristics and overall clinical outcome

Patients’ baseline characteristics are described in Table 1. The study included 383 of 548 patients (70%) who completed the Diogenes intervention (14) (Figure 1). Patient inclusion for lipidomic analyses was defined by the availability of plasma samples at the 3 CIDs. Patients included in the lipidomic analyses were representative of the full cohort, and no sampling bias was detected (for both baseline characteristics and the change in clinical outcomes).

Mean changes after the 2 interventions (LCD and weight maintenance) are also shown in Table 1. After the LCD, significant improvements occurred both in weight and glycemic control. After 6 mo of weight maintenance, weight changed by $-10.59$ kg (95% CI: $-11.28$, $-9.91$ kg), the Matsuda index changed by $+1.68$ (95% CI: $1.33, 2.02$), and the HOMA-IR changed by $-0.57$ (95% CI: $-0.81, -0.33$). Pearson’s correlation between changes in BMI and the Matsuda index was $-0.17$ (95% CI: $-0.27, -0.07$). This coefficient suggested rather modest improvements in both weight loss and glycemic control. In addition, a significant inter-individual variability was observed (BMI: CV: 63.28%; Matsuda index CV: 187.93%). Finally, patients with moderate weight loss were able to exhibit strong glycemic control improvements. These observations prompted the need for stratification analyses to distinguish between subjects who had strong weight and glycemic improvements from those who had lesser improvements.

Analyses of differentially expressed lipids and derivation of single lipid signature

One hundred twenty-five lipid species were identified in plasma of 383 patients at all CIDs. Seventy-nine of the lipids were shown to be differentially expressed. Figure 2A shows the correlation between these lipids and displays the following 2 clear blocks of species: one block was mostly composed of triacylglycerols, and the other block was mostly composed of phosphatidylcholines and sphingomyelins.

We summarized the log 2-fold-changes during the LCD of the 79 identified lipids with the use of a PCA. The first principal component (PC1) explained 33.8% of the variance, whereas other components explained $<15\%$ of the variance. Figure 2B shows that triacylglycerols were the lipids with the strongest influence on the PC1.

Associations between lipid signature and clinical outcomes after LCD and weight maintenance

We assessed whether this LCD lipid signature would be associated with weight and glycemic improvements after the 8-wk LCD. And indeed, the signature was significantly associated with BMI, weight, and all glycemic variables [false discovery rates (FDRs) $<5\%$]. A marginal association was observed with fat mass (FDR: 5.3%). The statistical analysis with the use of the first 5 principal components (PCs) of the PCA showed that only PC1 was significantly associated with clinical outcomes (data not shown).

We assessed whether this LCD lipid signature would be associated with long-term outcomes (after weight maintenance). The lipid signature was significantly associated with changes in weight-related outcomes (except for the change in fat-free mass) and glycemic control after 6 mo of weight

### TABLE 1

Clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Changes after LCD relative to baseline</th>
<th>Changes after weight maintenance relative to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>42 ± 6</td>
<td>$-3.84 ± 1.07$</td>
<td>$-3.63 ± 2.30$</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>34.30 ± 4.91</td>
<td>$-8.31 ± 4.67$</td>
<td>$-8.49 ± 6.39$</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>100.22 ± 17.67</td>
<td>$-11.24 ± 3.40$</td>
<td>$-10.59 ± 6.73$</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>40.19 ± 11.44</td>
<td>$-2.96 ± 4.30$</td>
<td>$-2.17 ± 3.53$</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>59.89 ± 12.95</td>
<td>$-2.00 ± 1.14$</td>
<td>$-0.57 ± 2.21$</td>
</tr>
<tr>
<td>Matsuda index</td>
<td>5.42 ± 3.25</td>
<td>$-3.37 ± 3.48$</td>
<td>$1.68 ± 3.15$</td>
</tr>
<tr>
<td>HOMA-IR, SI units</td>
<td>2.85 ± 1.78</td>
<td>$-2.65 ± 1.01$</td>
<td>$-0.57 ± 2.21$</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.04 ± 0.60</td>
<td>$-0.26 ± 0.52$</td>
<td>$-0.11 ± 0.57$</td>
</tr>
<tr>
<td>Fasting insulin, μIU/mL</td>
<td>10.74 ± 6.36</td>
<td>$-3.44 ± 6.23$</td>
<td>$-2.00 ± 7.42$</td>
</tr>
<tr>
<td>Fasting total cholesterol, mmol/L</td>
<td>4.84 ± 1.02</td>
<td>$-0.69 ± 0.76$</td>
<td>$0.07 ± 0.81$</td>
</tr>
<tr>
<td>Triglyceride, mmol/L</td>
<td>1.38 ± 0.65</td>
<td>$-0.32 ± 0.58$</td>
<td>$-0.14 ± 0.55$</td>
</tr>
<tr>
<td>Fasting LDL, mmol/L</td>
<td>3.00 ± 0.89</td>
<td>$-0.49 ± 0.64$</td>
<td>$-0.03 ± 0.66$</td>
</tr>
<tr>
<td>Fasting HDL, mmol/L</td>
<td>1.21 ± 0.34</td>
<td>$-0.06 ± 0.23$</td>
<td>$0.15 ± 0.24$</td>
</tr>
</tbody>
</table>

All values are means ± SDs. Clinical characteristics are shown for the 383 subjects at baseline (clinical intervention day 1) as well as changes after the LCD (clinical intervention day 2 – clinical intervention day 1) and changes after weight maintenance (clinical intervention day 3 – clinical intervention day 1). LCD, low-calorie diet.
maintenance. These results suggest that changes in lipid composition during an LCD may predict clinical changes after weight maintenance.

**Patient stratification on the basis of lipidomic signature**

To enable a visualization of the previously detailed results, we compared an arbitrary dichotomization of the signature (into positive and negative values) with a clustering approach ($k$-means clustering). These 2 approaches led to very similar clustering. Thus, we kept the simple dichotomization, which allowed us to define 2 groups of subjects and facilitated the visualization of their clinical differences.

The 2 groups had very distinct evolutions of their weight and glycemic variables over the intervention (see mean changes per group in Table 2). Figure 3 shows that the group with negative coefficients (PC $<$0, depicted by solid lines in Figure 3) had consistent and significantly fewer weight and glycemic improvements than were shown in the other group (PC $\geq$0, depicted by dashed lines in Figure 3). Specifically, patients from the PC $<$0 group showed no significant improvement in glycemic control; after 6 mo of weight maintenance, their mean HOMA-IR change was $-0.26$ (95% CI: $-0.64$, 0.13). Conversely, patients from the PC $\geq$0 group showed a significant improvement in glycemic control (mean HOMA-IR changes: $-0.92$; 95% CI: $-1.17$, $-0.67$). These observations were supported with results from other glycemic endpoints (Table 2).

For simplicity, in the rest of the article, we refer to the PC $<$0 group as nonresponders and to the PC $\geq$0 group as responders. At baseline, the 2 groups had comparable weight and glycemic control (Table 2); therefore, differences observed during the LCD and after weight maintenance could not be imputed to initial differences.

**Changes in leptin gene expression from adipose tissue support stratification**

To test whether the observed stratification would be consistent with well-studied weight-loss biomarkers, we assessed leptin gene expression in adipose tissue biopsies from a subset of subjects ($n = 93$). Figure 3 shows the clear difference in leptin expression over the full study. After weight maintenance, we observed a significant downregulation of leptin expression in responders ($P = 1.98 \times 10^{-7}$). By contrast, nonresponders did not show significant changes ($P = 0.78$). Differences between the 2 groups were significant ($P = 0.0016$). These findings were consistent with observed changes in weight and body composition. At baseline (CID1), no significant difference was shown between the 2 groups ($P = 0.42$), which indicated that there were no differences in leptin production or sensitivity.

**Association with long-term outcomes (1 y after LCD)**

Two centers (Denmark and Netherlands) extended the weight-maintenance diet for an additional 26 wk, which provided a fourth set of clinical data. The number of retained patients was 54 of 75 (72%) for Denmark and 47 of 74 (64%) for the Netherlands. A significant association (FDR <5%) was observed between the LCD lipid signature and changes in weight and glycemic outcomes 1 y after the LCD. These observations provided additional support to differences observed after 6 mo of weight maintenance.
### Table 2: Clinical characteristics of the 2 patient subgroups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonresponders</th>
<th>Responders</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>34.87±1.18</td>
<td>23.64±1.12</td>
<td>0.0400</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>100.14±6.78</td>
<td>88.11±6.78</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fat mass, kg</strong></td>
<td>38.51±12.64</td>
<td>31.45±12.16</td>
<td>0.0292</td>
</tr>
<tr>
<td><strong>Fat-free mass, kg</strong></td>
<td>58.60±12.64</td>
<td>51.17±12.16</td>
<td>0.0292</td>
</tr>
<tr>
<td><strong>Fasting insulin, mIU/mL</strong></td>
<td>10.42±5.86</td>
<td>5.18±3.63</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fasting total cholesterol, mmol/L</strong></td>
<td>2.94±0.60</td>
<td>2.36±0.60</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fasting HDL, mmol/L</strong></td>
<td>1.26±0.18</td>
<td>1.12±0.20</td>
<td>0.0125</td>
</tr>
</tbody>
</table>

#### Changes after weight maintenance relative to baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonresponders</th>
<th>Responders</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>−3.53±2.25</td>
<td>2.18±3.63</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>−10.15±5.68</td>
<td>−8.95±5.32</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fat mass, kg</strong></td>
<td>−2.24±1.49</td>
<td>−1.12±0.60</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fat-free mass, kg</strong></td>
<td>−1.59±0.85</td>
<td>−0.91±0.46</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fasting insulin, mIU/mL</strong></td>
<td>−1.05±0.58</td>
<td>−0.36±0.22</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fasting total cholesterol, mmol/L</strong></td>
<td>−0.11±0.06</td>
<td>−0.62±0.32</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fasting HDL, mmol/L</strong></td>
<td>−0.20±0.12</td>
<td>−0.15±0.06</td>
<td>0.0125</td>
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#### Changes after LCD relative to baseline

<table>
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<tr>
<td><strong>Weight, kg</strong></td>
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<td>0.0125</td>
</tr>
<tr>
<td><strong>Fat mass, kg</strong></td>
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<td>−1.12±0.60</td>
<td>0.0125</td>
</tr>
<tr>
<td><strong>Fat-free mass, kg</strong></td>
<td>−1.59±0.85</td>
<td>−0.91±0.46</td>
<td>0.0125</td>
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<tr>
<td><strong>Fasting insulin, mIU/mL</strong></td>
<td>−1.05±0.58</td>
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<tr>
<td><strong>Fasting total cholesterol, mmol/L</strong></td>
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<td><strong>Fasting HDL, mmol/L</strong></td>
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</tr>
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### Association with change in total lipid concentrations

We tested whether the LCD lipidomic signature was associated with routine biochemistry tests of total HDL, triglycerides, cholesterol, and LDL. As expected, changes in total lipid concentrations during the LCD were correlated strongly with the LCD lipidomic signature (Table 2). After 6 mo of weight maintenance, only changes in total HDL and triglycerides remained associated with the signature (FDRs <5%) (Table 2); responders had a higher increase in HDL and a stronger reduction of triglycerides.

At baseline, the 2 groups significantly differed in total triglycerides (FDR <5%) (Table 2) with responders having significantly higher concentrations than nonresponders. Therefore, the stronger triglyceride reduction in responders after the LCD was not surprising. However, Figure 3 shows clear differences in triglyceride profiles between responders and nonresponders.

After the LCD, nonresponders had marginal improvements (mean triglyceride change: −0.06 mmol/L; 95% CI: −0.11, 0.00 mmol/L; P = 0.54). However, these small improvements did not last. After weight maintenance, triglyceride concentrations for nonresponders were comparable to their initial baseline concentrations.

Statistical analyses of total triglyceride and cholesterol baseline concentrations showed that these variables were not associated with weight or glycemic outcomes (neither after the LCD or after weight maintenance). Joint analysis of these variables with the lipid signature excluded total lipid concentrations as potential confounding factors of the signature. Therefore, despite some baseline differences between responders and nonresponders in total lipid concentrations, the differences were not sufficient to predict clinical outcomes and could not be used as a substitute of a more-comprehensive lipid characterization.

### LDL lipid signature and prediction of insulin-resistant patients after weight maintenance

These analyses were aimed at predicting which subjects would be considered insulin resistant despite the weight-loss and maintenance interventions. Predictions were first performed with models that were based on clinical biochemistry variables (i.e., without the need to perform comprehensive lipidomic analyses). Two types of clinical models were constructed. One model used information at baseline (i.e., the CID1 clinical model), and the other model used changes during the LCD (i.e., the LCD clinical model). Then, a third model was evaluated and combined the best clinical model with the lipid signature.

We first defined insulin-resistant patients as those with a Matsuda index <3 at CID3. In this setting, an LCD clinical model was not significantly better than a random prediction [on the validation set, the AUC was 51% (95% CI: 35%, 67%)] (Figure 4A). Such a low performance was explained by the nonsignificant correlations between the LCD clinical variables and insulin-resistance outcome.

By contrast, the CID1 clinical model provided a marginally better performance [AUC: 65% (95% CI: 53%, 77%)] with specificity and sensitivity equal to 50.90% and 75.34%, respectively]. The combined CID1 clinical and lipidomic model provided a significantly better performance [AUC: 72% (95% CI: 61%, 83%)] with specificity and sensitivity equal to 62.40% and 73.19%, respectively]. This combined model outperformed the
clinical model (Delong’s $P = 0.032$), thereby showing the clinical utility of the model that combined the clinical variables with the lipid signature.

Analyses were repeated with the use of an insulin-resistance definition that was based on the HOMA-IR (insulin resistance defined as HOMA-IR $\geq 3$). Results were comparable to those obtained with a Matsuda-index definition (Figure 4B), whereby the combined model had AUC: 71% (95% CI: 62%, 81%), with specificity and sensitivity equal to 68.11% and 62.23%, respectively. This combined model outperformed the best clinical model ($P = 0.01$). Note that, in this setting, the best clinical model was only marginally better than random [AUC: 61% (95% CI: 50%, 71%), with specificity and sensitivity equal to 68.88% and 45.79%, respectively].

Analyses were also performed with the use of more-stringent cutoffs (Matsuda index $<2.5$ and HOMA-IR $\geq 2.5$), and the same conclusions were reached. This showed that our results were not biased because of the choice of a glycemic variable or its cutoff when defining insulin resistance.

**Figure 3** Mean (95% CI) differences between responders and nonresponders over weight-loss and weight-maintenance interventions. (A–F) Changes in BMI, the Matsuda index, total triglyceride concentrations, fat mass, HOMA-IR, and leptin gene expression, respectively. Each graph shows values at a given CID for the 2 patient subgroups. Groups were defined only on the basis of the sign of their low-calorie diet lipid signature. Dashed lines correspond to subjects with positive signature scores. Solid lines correspond to subjects with negative signature scores. Because of their clinical outcomes and for simplicity, patients denoted by dashed lines (positive signature scores) are referred to as responders; other patients are referred to as nonresponders. For panels A–E, the sample sizes were 205 nonresponders and 178 responders. For panel F, the sample sizes were 58 nonresponders and 35 responders. In all panels, $P$ values were obtained from an ANOVA that tested differences at a given CID between the 2 groups. ANOVAs were adjusted for sex, age, and participating center. A significant time-by-group interaction was observed for changes in the Matsuda index, HOMA-IR, triglyceride, and leptin concentrations (false discovery rates $<5$%). CID, clinical intervention day.

**DISCUSSION**

In this study, we aimed to understand the relation between altered lipid metabolism and long-term effects on glucose homeostasis. We investigated changes in lipidemia during weight loss and weight maintenance and the association of specific lipids with weight and glycemic control outcomes.

Differentially expressed lipids could be summarized into a signature, which stratified 2 response groups. One group (responders) significantly reduced weight and improved glycemic control, whereas the other group (nonresponders) showed significantly less improvement in weight and no improvement in glycemic control. Because all patients included in this analysis lost $>8\%$ of their initial body weights after the LCD, the results challenge the paradigm that weight loss in obese patients is correlated with improved T2D risk (30).

Differences in lipid profiles and their associations with metabolic outcomes were stable $\leq 1$ y after the end of the LCD, which suggested metabolic adaptation rather than short-term dietary effects. Responders had a significant that a decrease in leptin mRNA, whereas nonresponders showed no significant changes.
Leptin is downregulated after weight loss (22, 31, 32), which corresponds to a long-term adaptation after an LCD (32). Leptin also plays a role in improving insulin sensitivity in obese patients (5, 31). Hence, the clinical characteristics of the patient groups and their concentrations of leptin mRNA were consistent with current knowledge about the clinical and metabolic impacts of leptin.

Analyses of blood total lipids indicated differences in triglyceride metabolism between the 2 groups. Responders had significantly higher baseline total triglyceride concentrations than nonresponders did (1.58 compared with 1.22 mmol/L, respectively), but both groups were considered to be within the normal range (<1.69 mmol/L). Although the 2 groups had similar starting BMI and glycemia, responders likely had more flexibility to improve metabolically because of the slightly higher triglyceridemia, whereas nonresponders were already close to the recommended optimal range of 1.13 mmol/L (33). This result may raise the question of whether the current threshold on hypertriglyceridemia should be revised to more-stringent concentrations.

These observations from blood total lipids were consistent with the lipidomic results. An investigation of the individual triacylglycerol species revealed that responders showed a consistent decrease during the LCD that was maintained after weight maintenance, as was expected (data not shown). By contrast, nonresponders displayed a significantly lesser decrease for most triacylglycerol species. However, a significant increase during the LCD was observed for several triacylglycerols such as 55:2, 55:3, 52:2, 52:3, 54:3, 54:4, 56:6, and 57:2. Such an increase was only seen during the LCD because CID3’s concentrations were equivalent to starting baseline (CID1) concentrations. These results show the importance of studying triacylglycerol species instead of total triglycerides.

In addition, total triglycerides and total cholesterol, as obtained from classical blood biochemistry, could not explain differences in clinical outcomes, which indicated that the comprehensive elucidation of lipid species remains needed for prediction purposes. And indeed, a clinical model that was based on commonly accessible variables, including total blood lipids, offered only a moderate performance of the prediction of insulin-resistance status after weight maintenance. By contrast, a combined lipidomic and clinical model outperformed the clinical model. On a validation data set, the combined model increased the prediction performance by +10% (an increase of the ROC AUC from 61% to 71%), which represented strong improvements in both specificity and sensitivity.

Our results are strengthened by the fact that the Diogenes study is the largest weight-maintenance trial of its kind. It combines extensive clinical data, long-term weight-maintenance follow-up (both at 6 mo and 1 y), and gene expression from adipose tissue biopsies. Our conclusions originated from the consistency across multiple clinical endpoints, and results were fully reproduced when splitting the cohort into distinct discovery and validation sets. Our next study will investigate biological mechanisms that underlie the identified stratification to more-deeply elucidate differences in triacylglycerol speciation.

In conclusion, our study shows the importance of the stratification of patient populations after an LCD intervention and suggests that determining the lipid profile during weight loss could provide a powerful tool to identify responders to an LCD.

**FIGURE 4** Prediction of insulin-resistant subjects at CID3. (A) Prediction of subjects with a Matsuda index <3 at CID. (B) Prediction of subjects with a HOMA-IR $\geq$3 at CID3. ROC AUCs (95% CIs) are indicated. ROC curves for which the 95% CIs encompass 50% were not significantly better than those shown by a random predictor (diagonal lines). Indicated $P$ values were obtained from Delong’s test and compared the combined model (lipidomic and clinical) with the best clinical model. CID, clinical intervention day; LCD, low-calorie diet; PC1, first principal component; ROC, receiver operating characteristic.
The results challenge current practices of assessing total lipids (cholesterol and triglycerides) rather than investigating lipid composition. Mechanistic studies are needed to elucidate the physiological and molecular differences between the 2 patient groups. Finally, to our knowledge, our study is the first to link changes in the lipid composition during weight-management interventions in nondiabetic, obese patients with long-term weight and glycemic outcomes as well as to propose new predictive models that outperform clinical models for the prediction of insulin-resistant subjects. These findings may open new avenues in clinical research to study mechanisms of insulin resistance in the prevention of delay of T2D in obese patients, thereby providing new tools to clinicians to better monitor patients in weight-loss interventions.

We thank Hélène Ruffieux, Jérôme Carayol, and Yariv Levy from the Nestlé Institute of Health Sciences for statistical discussions. We also thank David Savage for fruitful scientific discussion and feedback; Jim Kaput from the Nestlé Institute of Health Sciences for useful feedback; and Nik Papageorgiou for proofreading the manuscript.

The authors’ responsibilities were as follows—AV, JH, and MM: designed the lipidomic data analyses; AV and MM: performed the statistical analyses, interpreted the results, wrote the manuscript, and had primary responsibility for the final content of the manuscript; WHMS and AA: designed the Diogenes study; and all authors: provided input into the writing of the manuscript and read and approved the final manuscript. AV, JH, and MM are full-time employees at the Nestlé Institute of Health Sciences SA. WHMS reported having research support from several food companies such as Nestlé, DSM, S-Biotek, Twinlab, and Vivus Inc.; grants from Arla Foods, the Danish companies such as GSK, Novartis, and Novo Nordisk. WHMS is a medical officer at the Nestlé Institute of Health Sciences for useful feedback; and Nik Papageorgiou for fruitful scientific discussion and feedback; Jim Kaput from the Nestlé Institute of Health Sciences for statistical discussions. We also thank Küras, László, and Royalties for the book first published in Danish as Verdens Bestste Diæt Plan (Kosmos Uitgevers, Utrecht/Antwerpen), in Spanish as Plan DIÓGENES para el control del peso. La dieta personalizada inteligente (Editorial Evergráficás, Léon), and in English as World’s Best Diet (Penguin, Australia).

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