OBJECTIVE—Nonalcoholic fatty liver (NAFL) is thought to contribute to insulin resistance and its metabolic complications. However, some individuals with NAFL remain insulin sensitive. Mechanisms involved in the susceptibility to develop insulin resistance in humans with NAFL are largely unknown. We investigated circulating markers and mechanisms of a metabolically benign and malignant NAFL by applying a metabolomic approach.

RESEARCH DESIGN AND METHODS—A total of 265 metabolites were analyzed before and after a 9-month lifestyle intervention in plasma from 20 insulin-sensitive and 20 insulin-resistant subjects with NAFL. The relevant plasma metabolites were then tested for relationships with insulin sensitivity in 17 subjects without NAFL and in plasma from 29 subjects with liver tissue samples.

RESULTS—The best separation of the insulin-sensitive from the insulin-resistant NAFL group was achieved by a metabolite pattern including the branched-chain amino acids leucine and isoleucine, ornithine, the acylcarnitines C3:0-, C16:0-, and C18:0-carnitine, and lysophosphatidylcholines (lyso-PC) C16:0 (area under the ROC curve, 0.77 [P = 0.0023] at baseline and 0.80 [P = 0.00019] at follow-up). Among the individual metabolites, predominantly higher levels of lyso-PC C16:0, both at baseline (P = 0.0039) and at follow-up (P = 0.001), were found in the insulin-sensitive compared with the insulin-resistant subjects. In the non-NAFL groups, no differences in lyso-PC C16:0 levels were found between the insulin-sensitive and insulin-resistant subjects, and these relationships were replicated in plasma from subjects with liver tissue samples.

CONCLUSIONS—From a plasma metabolomic pattern, particularly lyso-PCs are able to separate metabolically benign from malignant NAFL in humans and may highlight important pathways in the pathogenesis of fatty liver–induced insulin resistance.

Circulating Lysophosphatidylcholines Are Markers of a Metabolically Benign Nonalcoholic Fatty Liver
**RESEARCH DESIGN AND METHODS**

**Subjects**
Caucasians from the southern part of Germany participated in the ongoing Tübingen Lifestyle Intervention Program (12, 13). Individuals were included in the study when they fulfilled at least one of the following criteria: a family history of type 2 diabetes, a BMI >27 kg/m², and a previous diagnosis of impaired glucose tolerance and/or of gestational diabetes. They were considered healthy according to a physical examination and routine laboratory tests. The participants had no history of liver disease and did not consume more than two alcoholic drinks per day. Serum aminotransferase levels were <2.5 times the upper limit of normal. In a first approach, out of 330 subjects who met the aforementioned requirements, i.e., had measurements of body fat distribution and liver fat content, using magnetic resonance techniques at baseline (12), we randomly selected 40 subjects who had NAFL for metabolomics analysis. We then measured the identified metabolomic parameters in 17 subjects who underwent the same phenotyping strategies and who did not have NAFL. Finally, we measured the relevant metabolites in a third group of 29 subjects from whom liver tissue samples were available. Informed written consent was obtained from all participants after the nature and possible consequences of the studies were explained and the local medical ethics committee had approved the protocol.

**Lifestyle intervention**
The 57 individuals who underwent precise phenotyping and the intervention also underwent a 75-g oral glucose tolerance test (OGTT) at baseline and after 9 months of lifestyle intervention. Whole-body insulin sensitivity was calculated from glucose and insulin values during the OGTT as previously described (14). NAFL was defined as liver fat content >5.56% (15). Intramyocellular lipid content of the tibialis anterior muscle was determined as previously described (14).

**Noninvasive measurement of liver fat content and intramyocellular lipids**
Liver fat content was noninvasively measured by localized proton magnetic resonance (1HMR) spectroscopy as previously described (14). NAFL was defined as liver fat content >5.56% (15). Intramyocellular lipid content of the tibialis anterior muscle was determined as previously described (14).

**Oral glucose tolerance test and clinical chemical analyses**
The 57 individuals who underwent precise phenotyping and the intervention also underwent a 75-g oral glucose tolerance test (OGTT) at baseline and after 9 months of lifestyle intervention. Whole-body insulin sensitivity was calculated from glucose and insulin values during the OGTT as proposed by Matsuda and DeFronzo (16). In the 29 subjects from whom liver samples were available, the homeostatic model assessment of insulin resistance (HOMA-IR) was calculated from fasting blood samples (glucose [mmol/L] \* insulin [mU/L]/22.5). Blood glucose was determined using a bedside glucose analyzer (YSI, Yellow Springs, CO). Plasma insulin was determined on an ADVIA Centaur XP and all other routine parameters on an ADVIA 1800 clinical chemistry system (Siemens Healthcare systems, Erlangen, Germany). Serum plasmaminogen activator inhibitor 1 was measured by ELISA (Bender MedSystems), and fetuin-A was measured by an immunoturbidimetic method (BioVendor Laboratory Medicine, Modreć, Czech Republic) (17). The metabolic syndrome was estimated based on the recommendation of the Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) (18).

**Liver samples**
The 29 Caucasians (62.8 ± 2.2 years of age; sex, 22 male/7 female, BMI, 25.5 ± 0.85 kg/m²) who underwent liver surgery were fasted overnight prior to the collection of blood samples and liver biopsies. Subjects tested negative for viral hepatitis and had no liver cirrhosis. Liver samples were taken from normal, nondegenerated tissue during surgery, immediately frozen in liquid nitrogen, and stored at −80°C.

**Metabolite profiling**
At baseline and after 9 months of lifestyle intervention, 265 blood metabolites were analyzed by the targeted IDQ metabolomics platform from Biocrates (Innsbruck, Austria). This platform combines flow injection (acylcarnitines and glycerophospholipids), liquid chromatographic (amino acids and bile acids), gas chromatographic (free fatty acids), and mass spectrometric approaches. For the replication of the findings on palmitoyl lysophosphatidylcholine (lyso-PC C16:0) in the lifestyle intervention study, lyso-PC C16:0 levels were measured with another method, targeted ultraperformance liquid chromatography mass spectrometry, in the fasting blood samples of the subjects from whom liver samples were available.

**Statistical analyses**
First, 40 subjects with NAFL were randomly selected from a larger group solely based on their liver fat content at baseline, when they had NAFL (liver fat content >5.56%). Subjects were then divided into two groups based on the median insulin sensitivity, which was measured from the OGTT as proposed by Matsuda and DeFronzo (16) (insulin-sensitive and insulin-resistant NAFL groups). This simple two-step approach was specifically chosen to not introduce a bias that may arise by an artificial matching process, e.g., when matching for age, sex, and or adiposity, because today it is not known how these parameters influence the relationship of liver fat content with insulin sensitivity. The same approach was chosen for the 17 subjects without NAFL (insulin-sensitive and insulin-resistant non-NAFL groups). Pearson correlations were used to test for relationships between continuous variables. Paired Student t tests were performed to compare data obtained from the same experimental group. When different groups were compared, two-sample Student t tests were applied. In addition, repeated-measures multivariate ANOVA (MANOVA) was used, which allowed us to test in a more general aspect for differences in the parameters between the insulin-sensitive and insulin-resistant groups, as well as for changes of the parameters within the subjects and to perform group \( X \) time interaction tests. For the evaluation of the mass spectrometric data, a signal-to-noise ratio of 3 was set as the limit of detection. If not otherwise stated, a significance threshold of \( \alpha = 0.01 \) was used as a reasonable compromise between type I (false-positive) and type II (false-negative) error, as previously suggested (19). Statistical analysis was performed using Matlab version.
Liver fat (MRS) (%) 8.6
Age (years) 52
Sex (male/female) 15/5, 7/13, 0.02
AST (units/L) 26.6
Hs-CRP (mg/dL) 0.20
2-h glucose (mmol/L) 7.5
Fasting glucose (mmol/L) 5.3

### Table 1—Selected characteristics of the 40 subjects with NAFL at baseline and after 9 months of lifestyle intervention

| Characteristics                | Insulin-sensitive subjects | Insulin-resistant subjects | P value at baseline | P value at follow-up | P-MANOVA group × time |
|--------------------------------|----------------------------|----------------------------|---------------------|----------------------|-----------------------|
|                                | Baseline (n = 20)          | Follow-up                  |                     |                      |                       |
|                                |                            |                            |                     |                      |                       |
| Insulin sensitivity (AU)       | 14.7 ± 1.10                | 18.4 ± 1.72                | 7.3 ± 0.52          | 7.9 ± 0.75           | <0.0001               |
| Liver fat (MRS) (%)            | 8.6 ± 0.38                 | 5.8 ± 0.85*                | 8.5 ± 0.51          | 5.6 ± 0.65*          | 0.72                  |
| Sex (male/female)              | 15/5                       |                            | 7/13                | 0.02                 |                       |
| Age (years)                    | 52 ± 2                     | 44 ± 2                     | 0.016               |                      |                       |
| BMI (kg · m⁻²)                 | 30.1 ± 0.76                | 28.9 ± 0.93                | 32.4 ± 0.81         | 31.8 ± 0.81          | 0.05                  |
| Waist circumference (cm)       | 103.5 ± 1.99               | 98.1 ± 2.63                | 105.1 ± 2.12        | 101.9 ± 2.14*        | 0.61                  |
| Metabolic syndrome (%)         | 20                         | 22                         | 30                  | 50                   | 0.46                  |
| Total body fat (MRT) (kg)      | 24.4 ± 1.82                | 20.5 ± 2.27                | 32.0 ± 1.50         | 29.1 ± 2.06*         | 0.002                 |
| Visceral fat (MRT) (kg)        | 4.5 ± 0.41                 | 3.8 ± 0.54                 | 3.7 ± 0.35          | 3.6 ± 0.50*          | 0.14                  |
| LBMMRT (kg)                    | 61.3 ± 2.0                 | 59.3 ± 2.1                 | 58.6 ± 2.5          | 60.9 ± 3.1           | 0.40                  |
| IMCl (l/height)                | 4.2 ± 0.6                  | 3.1 ± 0.3                  | 3.9 ± 0.4           | 4.1 ± 0.4            | 0.99                  |
| AST (units/L)                  | 26.6 ± 1.46                | 26.6 ± 3.44                | 27.1 ± 2.58         | 22.0 ± 1.51*         | 0.89                  |
| ALT (units/L)                  | 27.3 ± 2.33                | 26.8 ± 4.58                | 29.4 ± 3.30         | 26.5 ± 2.99          | 0.78                  |
| Hs-CRP (mg/dL)                 | 0.20 ± 0.05                | 0.17 ± 0.04                | 0.36 ± 0.09         | 0.16 ± 0.04*         | 0.32                  |
| Fasting glucose (mmol/L)       | 5.3 ± 0.15                 | 5.1 ± 0.15                 | 5.3 ± 0.12          | 5.2 ± 0.12           | 0.96                  |
| 2-h glucose (mmol/L)           | 7.5 ± 0.37                 | 6.9 ± 0.50*                | 7.3 ± 0.43          | 6.4 ± 0.32*          | 0.58                  |
| PAI-1 (ng/mL)                  | 4.5 ± 0.51                 | 4.6 ± 0.43                 | 5.5 ± 0.53          | 6.5 ± 0.79           | 0.10                  |
| Fetuin-A (µg/mL)               | 237 ± 9                    | —                           | 293 ± 14            | —                    | 0.002                 |

Values are means ± SEM. ALT, alanine transaminase; AST, aspartate transaminase; AU, arbitrary units; IMCL, intramyocellular lipid; LBMMRT, lean body mass; MRS, magnetic resonance spectroscopy; MRT, magnetic resonance tomography; PAI-1, plasminogen activator inhibitor 1. At follow-up, magnetic resonance measurements were only available in 30 subjects. ※χ² test. *P < 0.05 for change within each group.
Figure 1—A plasma metabolite pattern predicts insulin sensitivity in NAFL. Plasma pattern of seven metabolites consistent with lyso-PC C16:0, ornithine, leucine, isoleucine, and C3:0-, C16:0-, and C18:0-carnitine for insulin-sensitive (blue) and insulin-resistant (red) subjects with NAFL at baseline (A) and after 9 months of lifestyle intervention (D). Each axis represents one metabolite, and all axes have their common origin in the center of the circle. The circle has radius 1 and intersects all axes at 1 (at the arrowhead) and −1 (in the lower half). The blue and red stars denote the individuals represented by the levels of the corresponding metabolites, projected to the axes after mean centering and scaling to unit variance. An individual’s metabolite level was combined by equally weighted linear combination. The connecting lines between the stars meet at the center (i.e., mean) of all individuals of each group to indicate the separation of the groups in this projection. The closer the center of one of the groups is to 1, the
The metabolic fingerprints of insulin-sensitive and -resistant subjects with NAFL

Next, we investigated which of the individual metabolites were different between the insulin-sensitive and the insulin-resistant subjects with NAFL. The plasma levels of total lyso-PCs reached the significance level $P < 0.01$ at baseline (Fig. 2A). Within the metabolite group of lyso-PCs, lyso-PC C16:0 levels were different, with 19.4% lower plasma levels in insulin-resistant subjects (mean, 70.42 vs. 86.34 µmol/L; $P = 0.0039$) (Fig. 2B). Because there was a sex difference between the NAFL groups, we also adjusted for sex using general linear regression models, and the differences between the groups for total lyso-PCs and lyso-PC C16:0 were not largely affected ($P = 0.008$ and 0.015, respectively). Interestingly, the relationships were also not affected by the lifestyle intervention (e.g., for lyso-PC C16:0: insulin resistant, 71.79 µmol/L vs. insulin sensitive, 88.64 µmol/L), and the same differences in the metabolite parameters were found between the insulin-resistant and the insulin-resistant NAFL groups after the intervention (Fig. 2C and D).

Then, we investigated which metabolites correlated with insulin sensitivity in all 40 subjects and in the 20 insulin-sensitive and in the 20 insulin-resistant NAFL groups analyzed separately. At baseline, the most robust correlations (significant relationships with insulin sensitivity were found in all subjects and in both NAFL groups analyzed separately) were present among the lyso-PCs (Supplementary Table 4). In all 40 subjects, for lyso-PC C16:0 correlated not only positively with insulin sensitivity ($r = 0.38, P = 0.016$) but also negatively with circulating hs-CRP ($r = -0.54, P = 0.0004$), a marker of subclinical inflammation. During the lifestyle intervention, insulin sensitivity increased ($P = 0.04$) and hs-CRP levels decreased ($P = 0.006$), but lyso-PC C16:0 levels did not change ($P = 0.37$). Interestingly, at follow-up, the relationship of lyso-PC C16:0 levels with insulin sensitivity was similarly strong ($r = 0.39, P = 0.014$) compared with baseline; however, the relationship of lyso-PC C16:0 levels with hs-CRP levels was no longer significant ($r = -0.23, P = 0.16$).

Relationships of lyso-PC C16:0 levels with insulin sensitivity in subjects without NAFL

We then tested whether the blood lyso-PC C16:0 levels were also different between the respective non-NAFL groups (Supplementary Table 5). No difference in the lyso-PC C16:0 levels was found (insulin sensitive, 85.5 ± 3.3 µmol/L; insulin resistant, 84.1 ± 6.7 µmol/L; $P = 0.86$). Power analyses revealed that we had a power of 0.97 to find the same difference in lyso-PC C16:0 that we observed in the NAFL groups.

Relationships of lyso-PC C16:0 levels with insulin sensitivity and hepatic inflammation in subjects with liver samples

Finally, we investigated whether similar relationships were apparent in blood from subjects who underwent liver tissue sampling. Subjects in the upper ($n = 7$; triglyceride content, 4.8 ± 0.6%) and lower ($n = 7$; triglyceride content, 0.7 ± 0.07%) quartiles of liver triglyceride content were divided by the medians in fasting HOMA-IR. In the upper quartile, liver triglyceride content was not different (Supplementary Fig. 1A); however, the HOMA-IR was lower (Supplementary Fig. 1B) and lyso-PC C16:0 levels were higher (Supplementary Fig. 1C) and a trend for less inflammation was found (Supplementary Fig. 1D–F) in the insulin-sensitive compared with the insulin-resistant group. No difference in lyso-PC C16:0 levels was seen in the respective groups of the lower quartile (74.5 ± 2.4 vs. 64.8 ± 2.4 µmol/L, $P = 0.56$).

CONCLUSIONS—Considering that among body fat and ectopic lipid compartments, liver fat content correlates most strongly with insulin sensitivity (9,10), the fact that some subjects can accumulate large amounts of fat within the liver yet remain insulin sensitive is an unexpected finding for many researchers. However, there is increasing data supporting the existence of such a phenotype (11,12,23), and the investigation of mechanisms determining this phenotype may help to advance our knowledge, specifically about signaling and detoxification pathways that are involved in the regulation of insulin sensitivity in NAFL.

In the current study, using a plasma metabolomic approach, we first found that a metabolite pattern including the BCAAs leucine and isoleucine, ornithine, the acylcarnitines C3:0-, C16:0-, and C18:0-carnitine, and lyso-PC C16:0 was able to separate the insulin-sensitive from the insulin-resistant NAFL groups with a relatively high discriminative power. Interestingly, the same metabolomic pattern separated the NAFL groups after a lifestyle intervention, after liver fat content and insulin sensitivity had changed, yet similar relationships for liver fat and insulin sensitivity were still apparent between the groups. When we then studied the individual metabolites comprising this metabolomic pattern, we found lyso-PCs to most strongly correlate with insulin sensitivity in subjects with NAFL. These findings support that a metabolomic pattern, particularly one including lyso-PC C16:0, may be a circulating marker of insulin sensitivity in NAFL.

The metabolomic pattern that we have identified to separate the NAFL groups includes BCAAs. They were shown by interesting studies from Newgard et al., Gerszten, and others (19,24–26) to be important for the regulation of glucose metabolism. In addition, Perseghin and colleagues (27) showed that in first-degree relatives of type 2 diabetic individuals, not only glucose and fatty acid metabolism but also leucine metabolism showed resistance to insulin. In our present study, we also detected correlations of leucine and isoleucine with insulin sensitivity at baseline in our 40 subjects with NAFL. However, and in contrast to what
we had expected, these relationships were positive. Interestingly, in the smaller group of 17 subjects without NAFL, these relationships were negative (both $r < -0.42$ and both $P < 0.09$). In addition, we detected a strong interaction effect of these circulating BCAAs with NAFL (both $P < 0.006$) but not with BMI, to determine insulin sensitivity. Although we have no explanation for this novel observation, and cannot rule out that the relatively small sample size may have brought about this finding, it may be of importance for further studies on the role of BCAAs in the regulation of insulin sensitivity, particularly in the aspect of NAFL.

What is known about the role of lysophosphatidylcholines (lyso-PCs) in metabolism? Lyso-PCs in plasma are primarily generated from PCs of lipoproteins or from membrane-derived PCs by the action of secretory or lipoprotein-bound phospholipase A2. Another source of plasma lyso-PC levels is the production from PCs by endothelial lipase as well as from HDL or oxidized LDL by lecithin-cholesterol acyltransferase, which is secreted from the liver. On the other hand, lyso-PCs can also be recycled to PC by the action of lyso-PC acyltransferase. Interestingly, most recently, a study in mice identified palmitoyl-, stearoyl-, and oleoyl-lyso-PCs to be lower in the serum of NASH animals compared with animals with simple steatosis and suggested that high lyso-PC acyltransferase activity is involved in this process (28). By the action of lysophospholipase D/autotoxin plasma, lyso-PC can also be transformed to lysophosphatid acid and sphingosine-1-phosphate, important extracellular signaling lipids with multiple biological functions (29). Finally, the bioavailability of choline, which is required for hepatic PC genesis, plays an important role in the pathophysiology of NAFL and its progression, and is regulated by the gut microbiome (30), may determine the plasma lyso-PC levels. Interestingly, recently lyso-PC C16:0 was found to enhance glucose uptake in an insulin-independent and protein kinase C-δ-dependent manner in adipocytes (31). This finding was confirmed in vivo by glucose-lowering effects of lyso-PC C16:0 in type 1 and type 2 diabetes mouse models (31). Whether these effects and putatively beneficial effects of lyso-PC C16:0 on glucose uptake in other tissues translate into a positive effect on whole-body glucose uptake needs to be investigated in future studies.

In contrast to glucose metabolism, the role of lyso-PCs in the regulation of inflammation has been studied more extensively. It has been shown that lyso-PC C16:0 is able to inhibit reactive oxygen species production in stimulated polymorphonuclear leukocytes (32,33). As putative mechanisms of action, lyso-PCs activate G protein–coupled receptors (GPR4, G2A, and GPR119) that are involved, for example, in migration and apoptosis of immune cells, osteoclastogenesis and angiogenesis, and insulin secretion from B-cells (29). Importantly, lyso-PCs were recently found to enhance the suppressive function of human CD4+CD25+ regulatory T cells (Treg) (34). Treg secrete anti-inflammatory signals, inhibit macrophage migration, and induce noninflammatory M2-like macrophage differentiation (35). In addition, defects in Treg function are considered to play a role not only in autoimmune diseases, such as autoimmune hepatitis (36), but also in other causes of hepatic inflammation (37,38). In agreement with an anti-inflammatory property of
lyso-PCs, we detected strong negative relationships of total lyso-PCs and lyso-PC C16:0 with hs-CRP levels in our subjects with NAFL. We then asked whether lyso-PC C16:0 levels also differ between insulin-sensitive and insulin-resistant subjects without NAFL. No such relationship was found. Importantly, the insulin-sensitive and insulin-resistant subjects in the respective NAFL and non-NAFL groups had very similar insulin sensitivity (Fig. 3). This indicates that lyso-PC C16:0 levels may be a good marker of subclinical inflammation and insulin resistance and may putatively protect from inflammatory processes, specifically in NAFL. In support of this hypothesis, we found lyso-PC C16:0 to be negatively associated with insulin resistance in subjects with high, but not low, liver tissue lipid content. Certainly, because of the very small sample size of this group, we cannot draw definite conclusions from this observation. Importantly, the replication was not only done in a separate population than the initial screening population but another method of measurement of lyso-PC C16:0 levels was also applied. Interestingly, in the large Relationship Between Insulin Sensitivity and Cardiovascular Risk (RISC) study, lyso-PCs were also found to negatively correlate with estimated hepatic insulin resistance (39). Also in agreement with our present data, the correlations were stronger in the subjects with NAFL compared with the subjects without hepatic steatosis (A.G., personal communication). Moreover, and in agreement with our hypothesis, our liver histology data indicate that hepatic inflammation is present when plasma lyso-PC C16:0 levels are low.

In summary, from a panel of 180 metabolites, the lyso-PC concentrations differed most strongly between insulin-sensitive and insulin-resistant subjects with NAFL. These relationships, as well as the correlations with insulin sensitivity, were still apparent after 9 months of lifestyle intervention. Interestingly, lyso-PC levels did not change during this intervention, indicating that they are genetically determined. The fact that the correlations of lyso-PC levels with insulin sensitivity and with systemic and hepatic inflammation were less strong or absent when liver fat content was low, and the knowledge about the signaling properties of lyso-PCs, supports the hypothesis that a genetically determined high lyso-PC availability may protect specifically from fatty acid–induced insulin resistance and hepatic inflammation when NAFL is present. Alternatively, elevated circulating lyso-PCs may not be functionally active in this process but represent activated pathways in the synthesis or metabolism of phospholipids and phosphatidylcholine and, thus, in the generation of hepatic endoplasmic reticulum stress (40) (Supplementary Fig. 2).

In conclusion, in the future, a metabolomic fingerprint, most robust in the class of lyso-PCs, may be able to differentiate insulin-sensitive from insulin-resistant subjects with NAFL. Furthermore, as low lyso-PC levels appear to specifically be a marker of NAFL-associated insulin resistance, these findings may highlight novel and interesting pathways for the studies on the pathogenesis of insulin resistance in NAFL.

| Insulin sens. (arb. u.) | Lyso-PC 16:0 (μmol/l) |
|------------------------|----------------------|
| 17.1 (1.8)             | 85.5 (3.3)           |
| 14.7 (1.1)             | 86.4 (4.3)           |
| 8.5 (0.7)*             | 84.1 (6.7)           |
| 7.3 (0.5)*             | 70.5 (2.8)*          |

Figure 3—Relationships of liver fat content with insulin sensitivity at the baseline visit. The insert depicts the mean (SE) values for the respective groups and the level of statistical significance. *P < 0.05 for differences between the insulin-resistant and insulin-sensitive groups. \(\chi^2 = 0.20\) for differences between the insulin-sensitive and \(\chi^2 = 0.18\) for differences between the insulin-resistant groups. \#P < 0.05 for differences between the insulin-resistant NAFL group and all other groups. AU, arbitrary units.

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R.L., H.F., and N.S. researched data and wrote the manuscript. S.D., A.Z., M.H.deA., M.S., H.S., and H.-U.H. designed the study, contributed to the discussion, and reviewed the manuscript. L.R., K.K., P.A., J.L., G.X., A.K., J.M., F.S., E.S., A.G., and A.F. researched data and edited the manuscript. All authors provided substantial contributions to conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published. N.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Circulating Lyso-Phosphatidylcholines are Markers of a metabolically benign Nonalcoholic Fatty Liver

**Supplementary Table 1** Serum lipid levels of the 40 subjects with nonalcoholic fatty liver at baseline and after 9 months of lifestyle intervention

| Characteristics         | Insulin sensitive subjects | Insulin resistant subjects | p-value at baseline | p-value at follow-up | p-MANOVA group*time |
|-------------------------|----------------------------|----------------------------|---------------------|----------------------|---------------------|
|                         | Baseline (N=20)            | Follow-up                  | Baseline (N=20)     | Follow-up            |                     |
| Free fatty acids (µmol/l) | 650 ± 45                  | 555 ± 43*                  | 645 ± 54            | 591 ± 43             | 0.77                |
|                         |                            |                            |                     |                      | 0.51                | 0.60                |
| Triglycerides (mg/dl)   | 190 ± 42                   | 190 ± 50                   | 140 ± 16            | 138 ± 13             | 0.41                |
|                         |                            |                            |                     |                      | 0.67                | 0.98                |
| Total cholesterol (mg/dl)| 202 ± 2                    | 202 ± 11                   | 201 ± 7             | 195 ± 6              | 0.85                |
|                         |                            |                            |                     |                      | 0.72                | 0.72                |
| HDL cholesterol (mg/dl) | 49 ± 3                     | 50 ± 3                     | 50 ± 3              | 48 ± 3               | 0.76                |
|                         |                            |                            |                     |                      | 0.80                | 0.23                |
| LDL cholesterol (mg/dl) | 130 ± 6                    | 120 ± 8*                   | 131 ± 6             | 4127 ± 6             | 0.94                |
|                         |                            |                            |                     |                      | 0.38                | 0.65                |

Values are means ± SEM. *p < 0.05 for change within each group; MANOVA: multivariate analysis of variance.
**Supplementary Table 2** List of all measured metabolites and their biochemical classification.

| Metabolite | Metabolite class |
|------------|------------------|
| Ala        | amino acids      |
| Arg        |                  |
| Asn        |                  |
| Asp        |                  |
| Cit        |                  |
| Gln        |                  |
| Glu        |                  |
| Gly        |                  |
| His        |                  |
| Ile        |                  |
| Leu        |                  |
| Lys        |                  |
| Met        |                  |
| Orn        |                  |
| Phe        |                  |
| Pro        |                  |
| Ser        |                  |
| Thr        |                  |
| Trp        |                  |
| Tyr        |                  |
| Val        |                  |
| C0         | acylcarnitines   |
| C2         |                  |
| C3         |                  |
| C3-DC (C4-OH) |        |
| C3-OH      |                  |
| C3:1       |                  |
| C4         |                  |
| C4:1       |                  |
| C5-M-DC    |                  |
| C5-OH (C3-DC-M) |    |
| C5:1       |                  |
| C5:1-DC    |                  |
| C5         |                  |
| C5-DC (C6-OH) |              |
| C6 (C4:1-DC) |             |
| C6:1       |                  |
| C7-DC      |                  |
| C8         |                  |
| C8:1       |                  |
| C9         |                  |
| C10        |                  |
| C10:2      |                  |
| C10:1      |                  |
|        | C12-DC | C12:1 | C12     | C14:1 | C14:2 | C14     | C14:1-OH | C14:2-OH | C16    | C16:2 | C16-OH | C16:1 | C16:1-OH | C16:2-OH | C18:1-OH | C18     | C18:1 | C18:2 |
|--------|--------|-------|---------|-------|-------|---------|----------|----------|--------|-------|--------|-------|----------|----------|----------|---------|-------|-------|
| **biogenic amines** |        |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Dopamin |        |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Histamine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Kynurenine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Methionine-Sulfoxide |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Nitro-Tyrosine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Hydroxykynurenine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Hydroxyproline |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Phenylethylamine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Putrescine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Symmetric dimethylarginine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Asymmetric dimethylarginine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Sarcosine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Serotonin |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Spermidine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Spermine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| Taurine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| total dimethylarginine |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| **lyso-PCs** |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C14:0 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C16:0 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C16:1 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C17:0 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C18:0 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C18:1 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C18:2 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C20:3 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C20:4 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C24:0 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C26:0 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
| lysoPC C26:1 |       |       |         |       |       |         |          |          |        |       |        |       |          |          |          |         |       |       |
|   | lysoPC C28:0 | lysoPC C28:1 |
|---|-------------|-------------|
| 94 | PC aa C24:0  |             |
| 95 | PC aa C26:0  |             |
| 96 | PC aa C28:1  |             |
| 97 | PC aa C30:0  |             |
| 98 | PC aa C30:2  |             |
| 99 | PC aa C32:0  |             |
|100 | PC aa C32:1  |             |
|101 | PC aa C32:2  |             |
|102 | PC aa C32:3  |             |
|103 | PC aa C34:1  |             |
|104 | PC aa C34:2  |             |
|105 | PC aa C34:3  |             |
|106 | PC aa C34:4  |             |
|107 | PC aa C36:0  |             |
|108 | PC aa C36:1  |             |
|109 | PC aa C36:2  |             |
|110 | PC aa C36:3  |             |
|111 | PC aa C36:4  |             |
|112 | PC aa C36:5  |             |
|113 | PC aa C36:6  |             |
|114 | PC aa C38:0  |             |
|115 | PC aa C38:1  |             |
|116 | PC aa C38:3  |             |
|117 | PC aa C38:4  |             |
|118 | PC aa C38:5  |             |
|119 | PC aa C38:6  |             |
|120 | PC aa C40:1  |             |
|121 | PC aa C40:2  |             |
|122 | PC aa C40:3  |             |
|123 | PC aa C40:4  |             |
|124 | PC aa C40:5  |             |
|125 | PC aa C40:6  |             |
|126 | PC aa C42:0  |             |
|127 | PC aa C42:1  |             |
|128 | PC aa C42:2  |             |
|129 | PC aa C42:4  |             |
|130 | PC aa C42:5  |             |
|131 | PC aa C42:6  |             |

**Phosphatidylcholines (diacyl)**

|   | PC ae C30:0  |             |
|---|-------------|-------------|
|132 | PC ae C30:1  |             |
|133 | PC ae C30:2  |             |
|134 | PC ae C32:1  |             |
|135 | PC ae C32:2  |             |
|136 | PC ae C34:0  |             |
|137 | PC ae C34:1  |             |

**Phosphatidylcholines (acyl-alkyl)**
|   |   |
|---|---|
| 139 | PC ae C34:2 |
| 140 | PC ae C34:3 |
| 141 | PC ae C36:0 |
| 142 | PC ae C36:1 |
| 143 | PC ae C36:2 |
| 144 | PC ae C36:3 |
| 145 | PC ae C36:4 |
| 146 | PC ae C36:5 |
| 147 | PC ae C38:0 |
| 148 | PC ae C38:1 |
| 149 | PC ae C38:2 |
| 150 | PC ae C38:3 |
| 151 | PC ae C38:4 |
| 152 | PC ae C38:5 |
| 153 | PC ae C38:6 |
| 154 | PC ae C40:0 |
| 155 | PC ae C40:1 |
| 156 | PC ae C40:2 |
| 157 | PC ae C40:3 |
| 158 | PC ae C40:4 |
| 159 | PC ae C40:5 |
| 160 | PC ae C40:6 |
| 161 | PC ae C42:0 |
| 162 | PC ae C42:1 |
| 163 | PC ae C42:2 |
| 164 | PC ae C42:3 |
| 165 | PC ae C42:4 |
| 166 | PC ae C42:5 |
| 167 | PC ae C44:3 |
| 168 | PC ae C44:4 |
| 169 | PC ae C44:5 |
| 170 | PC ae C44:6 |
|   | sphingomyelins |
| 171 | SM (OH) C14:1 |
| 172 | SM (OH) C16:1 |
| 173 | SM (OH) C22:1 |
| 174 | SM (OH) C22:2 |
| 175 | SM (OH) C24:1 |
| 176 | SM C16:0 |
| 177 | SM C16:1 |
| 178 | SM C18:0 |
| 179 | SM C18:1 |
| 180 | SM C20:2 |
| 181 | SM C22:3 |
| 182 | SM C24:0 |
| 183 | SM C24:1 |
| 184 | SM C26:0 |
| 185 | SM C26:1 |
| 186 | Cholic Acid | bile acids |
|-----|-------------|------------|
| 187 | Chenodeoxycholic Acid | |
| 188 | Deoxycholic Acid | |
| 189 | Glycocholic Acid | |
| 190 | Glycochenodeoxycholic Acid | |
| 191 | Glycodeoxycholic Acid | |
| 192 | Glycolithocholic Acid | |
| 193 | Glycolithocholic Acid sulfate | |
| 194 | Glycoursodeoxycholic Acid | |
| 195 | Lithocholic Acid | |
| 196 | Taurocholic Acid | |
| 197 | Taurochenodeoxycholic Acid | |
| 198 | Taurodeoxycholic Acid | |
| 199 | Taurlithocholic Acid | |
| 200 | Taurlithocholic Acid sulfate | |
| 201 | Taursodeoxycholic Acid | |
| 202 | Ursodeoxycholic Acid | |
| 203 | Hexose | |
| 204 | C6:0 (caproic) | free fatty acids |
| 205 | C7:0 (heptanoic) | |
| 206 | C8:0 (caprylic) | |
| 207 | C10:0 (capric) | |
| 208 | C11:0 (undecanoic) | |
| 209 | C12:0 (lauric) | |
| 210 | C13:0 (tridecanoic) | |
| 211 | 12Me-C13:0 (iso-myristic) | |
| 212 | C14:0 (myristic) | |
| 213 | c-C14:1w5 (myristoleic) | |
| 214 | 13Me-C14:0 (iso) | |
| 215 | 13Me-C15:0 (anteiso) | |
| 216 | 14Me-C15:0 (iso) | |
| 217 | 4Me-C6:0 | |
| 218 | C12:2 | |
| 219 | C21:0 (heneicosanoic) | |
| 220 | C22:0 (behenic) | |
| 221 | C23:0 (tricosanoic) | |
| 222 | C24:0 (lignoceric) | |
| 223 | c-C15:1w5 (c-10-pentadecenoic) | |
| 224 | c-C16:1w5 | |
| 225 | c-C17:1w7 (c-10-heptadecenoic) | |
| 226 | c-C18:1w6 | |
| 227 | c-C18:4w3 (stearidonic) | |
| 228 | c-C19:5w3 | |
| 229 | c-C20:2w6 (c-11,14-eicosadinoic) | |
| 230 | c-C20:3w3 (c-11,14,17-C20:3) | |
|   |   |
|---|---|
| 231 | c-C20:3w6 (c-8,11,14-C20:3) |
| 232 | c-C20:3w9 (mead) |
| 233 | c-C20:4w3 |
| 234 | c-C20:5w6 |
| 235 | c-C22:1w9 (erucic) |
| 236 | c-C22:2w6 (c-13,16-C22:2) |
| 237 | c-C22:5w6 |
| 238 | c-C24:1w9 (nervonic) |
| 239 | t-C18:2w6 (linoleaidic) |
| 240 | 12Me-C14:0 (anteiso) |
| 241 | C15:0 (pentadecanoic) |
| 242 | C16:0 (palmitic) |
| 243 | c-C16:1w10 (sapienic) |
| 244 | c-C16:1w7 (palmitoleic) |
| 245 | 15Me-C16:0 (iso) |
| 246 | 14Me-C16:0 (anteiso) |
| 247 | C17:0 (heptadecanoic) |
| 248 | c-C17:1w8 |
| 249 | 16Me-C17:0 (iso) |
| 250 | C18:0 (stearic) |
| 251 | t-C18:1w9 (elaidic) |
| 252 | c-C18:1w9 (oleic) |
| 253 | c-C18:1w7 (vaccenic) |
| 254 | c-C18:2w6 (linoleic) |
| 255 | C19:0 (nonadecanoic) |
| 256 | c-C18:3w6 (gamma-linolenic) |
| 257 | c-C18:3w3 (linolenic) |
| 258 | C19:2 |
| 259 | C20:0 (eicosanoic) |
| 260 | c-C20:1w9 (c-11-eicosenoic) |
| 261 | c-C20:4w6 (arachidonic) |
| 262 | c-C20:5w3 (EPA) |
| 263 | c-C22:4w6 (adrenic) |
| 264 | c-C22:5w3 (DPA) |
| 265 | cis-C22:6w3 (DHA; cervonic) |
Supplementary Table 3 List of plasma metabolites that were excluded from data analysis. (Metabolites with >30% of the mass spectrometric signals below the limit of detection (LOD) were excluded from the data evaluation (LOD was defined as a signal-to-noise ratio of 3)).

| Lyso-PCs                          |          |
|----------------------------------|----------|
| lysoPC C14:0                     |          |
| lysoPC C24:0                     |          |
| lysoPC C26:0                     |          |
| lysoPC C26:1                     |          |
| lysoPC C28:0                     |          |

| Acylcarnitines                   |          |
|----------------------------------|----------|
| C3:1                             |          |
| C3-DC (C4-OH)                    |          |
| C3-OH                            |          |
| C4:1                             |          |
| C5-M-DC                          |          |
| C5-OH (C3-DC-M)                  |          |
| C5:1                             |          |
| C5:1-DC                          |          |
| C6:1                             |          |
| C10:1                            |          |
| C12-DC                           |          |
| C12:1                            |          |
| C14                              |          |
| C14:1-OH                         |          |
| C14:2-OH                         |          |
| C16-OH                           |          |
| C16:1                            |          |
| C16:1-OH                         |          |
| C16:2-OH                         |          |
| C18:1-OH                         |          |

| Phosphatidylcholines (diacyl)    |          |
|----------------------------------|----------|
| PC aa C24:0                      |          |
| PC aa C26:0                      |          |
| PC aa C30:2                      |          |
| PC aa C38:1                      |          |
| PC aa C40:1                      |          |

| Phosphatidylcholines (acyl-alkyl)|          |
|----------------------------------|----------|
| PC ae C40:0                      |          |
| PC ae C42:0                      |          |

| Free fatty acids                 |          |
|----------------------------------|----------|
| 4Me-C6:0                         |          |
| C12:2                            |          |
| 13Me-C15:0 (anteiso)             |          |
| 14Me-C15:0 (iso)                 |          |
| c-C15:1w5 (c-10-pentadecenoic)   |          |
| c-C16:1w5                        |          |
c-C17:1w7 (c-10-heptadecenoic)
c-C18:1w6
t-C18:2w6 (linoleaidic)
c-C18:4w3 (stearidonic)
c-C19:5w3
c-C20:2w6 (c-11,14-eicosadinoic)
c-C20:3w3 (c-11,14,17-C20:3)
c-C20:3w6 (c-8,11,14-C20:3)
c-C20:3w9 (mead)
c-C20:4w3
c-C20:5w6
C21:0 (heneicosanoic)
C22:0 (behenic)
c-C22:1w9 (erucic)
c-C22:2w6 (c-13,16-C22:2)
c-C22:5w6
C23:0 (tricosanoic)
C24:0 (lignoceric)
c-C24:1w9 (nervonic)

**Biogenic amines**
Dopamin
Histamine
Kynurenine
Methionine-Sulfoxide
Nitro-Tyrosine
Hydroxykynurenine
Hydroxyproline
Phenylethylamine
Putrescine
Symmetric dimethylarginine
total dimethylarginine
Sarcosine
Serotonin
Spermidine
Spermine
Taurine

**Bile acids**
Chenodeoxycholic Acid
Deoxycholic Acid
Glycodeoxycholic Acid
Glycolithocholic Acid
Glycoursodeoxycholic Acid
Lithocholic Acid
Taurocholic Acid
Taurochenodeoxycholic Acid
Taurodeoxycholic Acid
Tauleolithocholic Acid
Supplementary Table 4 Significant correlations of metabolites with insulin sensitivity (ISI) in all 40 subjects with nonalcoholic fatty liver (NAFL) and the insulin sensitive (IS) and insulin resistant (IR) NAFL subgroups at baseline.

| Metabolite class | all | IS | IR |
|------------------|-----|----|----|
| **Lysophosphatidylcholines** |     |    |    |
| Total lyso-PC    | +   | +  | +  |
| Total lysoPC/PC  | -   | +  | +  |
| lysoPC C16:0     | +   | -  | +  |
| lysoPC C17:0     | +   | +  |  + |
| lysoPC C18:0     | +   | -  | +  |
| lysoPC C18:1     | +   | +  | +  |
| lysoPC C18:2     | +   | +  | +  |
| lysoPC C20:4     | +   | +  | +  |
| lysoPC C28:1     | +   | +  | +  |
| **Amino acids**  |     |    |    |
| Glu              | -   | -  | -  |
| Ile              | +   |    |    |
| Leu              | +   |    |    |
| **Sphingomyelins** |     |    |    |
| Total SM        | +   |    |    |
| Total SM(non-OH)| +   |    |    |
| Total SM(SM+PC) | +   | +  | +  |
| SM (OH) C16:1   | +   |    |    |
| SM (OH) C22:2   | +   | +  | +  |
| SM (OH) C24:1   | +   |    |    |
| SM C18:0        | +   | +  | +  |
| SM C16:1        | +   | +  | +  |
| SM C26:1        | +   | +  | +  |
| **Acylcarnitines** |     |    |    |
| C2 (acetyl)     | +   |    |    |
| C7-dicarboxylic (pimelyl) | + | + | + |
| C8 (octanoyl)   | +   |    |    |
| C10 (decanoyl)  | +   |    |    |
| C12 (dodecanoyl)| +   |    |    |
| **Phosphatidylycerolines (diacyl)** |     |    |    |
| Total PC        | -   |    |    |
| PC aa C32:2     | +   |    |    |
| PC aa C32:3     | +   |    |    |
| PC aa C34:3     | +   |    |    |
| PC aa C34:4     | +   |    |    |
| PC aa C36:0     | +   |    |    |
| PC aa C36:2     | +   |    |    |
| PC aa C36:3     | +   |    |    |

| Metabolite class | all | IS | IR |
|------------------|-----|----|----|
| **Phosphatidylcholines (acyl-alkyl)** |     |    |    |
| PC ae C30:1      | +   |    |    |
| PC ae C30:2      | +   |    |    |
| PC ae C34:0      | -   |    |    |
| PC ae C34:2      | -   |    |    |
| PC ae C36:2      | -   |    |    |
| PC ae C36:3      | -   |    |    |
| PC ae C38:2      | -   |    |    |
| PC ae C38:3      | -   |    |    |
| PC ae C40:1      | -   |    |    |
| PC ae C44:3      | -   |    |    |
| **Fatty acids**  |     |    |    |
| Total FFA        | -   |    |    |
| FFA (even)       | -   |    |    |
| FFA (u9)         | -   |    |    |
| FFA (u10)        | -   |    |    |
| total SFA        | -   |    |    |
| total MUFA       | -   |    |    |
| PUFA/SFA         | +   |    |    |
| C16:0 (palmitic) | -   |    |    |
| C16:1ω10 (sapienic) | - | - | - |
| C18:1ω9 (oleic)  | -   |    |    |
| C18:1ω7 (vaccenic) | - | - | - |
| C18:3ω6 (γ-linolenic) | - | - | - |
| C20:1ω9 (c-11-eicosenoic) | - | - | - |
| C22:4ω6 (adrenic) | - | - | - |

No metabolite out of the classes of bile acids and biogenic amines did show a significant correlation with ISI. Dark grey fields indicate p < 0.01, light grey fields indicate p < 0.05. A positive correlation is indicated by plus and a negative correlation by minus.
**Supplementary Table 5** Selected characteristics of the 17 subjects without nonalcoholic fatty liver at baseline and after 9 months of lifestyle intervention

| Characteristics                      | Insulin sensitive subjects |           | Insulin resistant subjects |           | p-value at baseline | p-value at follow-up | p-MANOVA time*group |
|--------------------------------------|-----------------------------|-----------|---------------------------|-----------|---------------------|----------------------|---------------------|
|                                      | Baseline (N=8)              | Follow-up | Baseline (N=9)            | Follow-up |                     |                      |                     |
|                                      |                             |           |                           |           |                     |                      |                     |
| Insulin sensitivity (arb. u)         | 17.1 ± 1.80                 | 19.3 ± 2.23 | 8.5 ± 0.66               | 10.36 ± 1.48 | <0.0001            | <0.0001             | 0.81               |
| Liver fat MRS (%)                    | 2.0 ± 0.51                  | 1.2 ± 0.30 | 3.0 ± 0.44               | 3.9 ± 0.92 | 0.16               | 0.005               | 0.22               |
| Gender (males / females)             | 1 / 7                       |           | 5 / 4                    |           | 0.13               |                      |                     |
| Age (years)                          | 51 ± 3                      |           | 43 ± 4                   |           | 0.13               |                      |                     |
| Body mass index (kg·m⁻²)             | 24.2 ± 1.57                 | 23.3 ± 1.28 | 28.7 ± 1.64              | 28.2 ± 1.38 | 0.06               | 0.02                | 0.46               |
| Waist circumference (cm)             | 81.3 ± 2.84                 | 77.5 ± 2.35 | 97.0 ± 4.92              | 94.4 ± 4.0 | 0.02               | 0.003               | 0.65               |
| Metabolic syndrome (%)               | 0                           | 22        | 0                         | 44        | 0.09               | 0.01                | .                  |
| Total body fat MRT (kg)              | 19.4 ± 3.70                 | 17.7 ± 3.43 | 22.9 ± 4.13              | 23.4 ± 4.24 | 0.55               | 0.28                | 0.48               |
| Visceral fat MRT (kg)                | 1.4 ± 0.23                  | 1.3 ± 0.26 | 3.2 ± 0.61               | 3.0 ± 0.60* | 0.06               | 0.08                | 0.60               |
| LBM MRT (kg)                         | 47.2 ± 2.4                  | 46.9 ± 2.7 | 58.7 ± 4.3               | 58.6 ± 5.0 | 0.04               | 0.07                | 0.28               |
| IMCL tibialis anterior (arb.units)   | 3.4 ± 0.8                   | 3.6 ± 0.4 | 3.5 ± 0.8               | 4.1 ± 0.5 | 0.80               | 0.51                | 0.92               |
| AST (U/L)                            | 22.8 ± 1.39                 | 23.4 ± 1.81 | 21.9 ± 1.66              | 23.1 ± 3.45 | 0.70               | 0.94                | 0.85               |
| ALT (U/L)                            | 18.1 ± 21.47                | 18.9 ± 2.27 | 26.6 ± 2.66              | 26.7 ± 6.44 | 0.02               | 0.30                | 0.91               |
| Hs-CRP (mg/dl)                       | 0.14 ± 0.04                 | 0.19 ± 0.11 | 0.14 ± 0.05              | 0.16 ± 0.07 | 0.56               | 0.70                | 0.95               |
| Free fatty acids (µmol/l)            | 686 ± 59                    | 614 ± 62 | 656 ± 79                | 568 ± 67 | 0.77               | 0.63                | 0.86               |
| Fasting glucose (mM)                 | 5.0 ± 0.15                  | 5.1 ± 0.20 | 5.6 ± 0.21               | 5.5 ± 0.23 | 0.07               | 0.29                | 0.38               |
| 2 h glucose (mM)                     | 7.8 ± 0.63                  | 8.1 ± 0.67 | 8.0 ± 0.56               | 8.8 ± 0.67 | 0.77               | 0.46                | 0.79               |
| PAI-1 (ng/ml) | 2.8 ± 0.57 | 3.0 ± 0.48 | 5.0 ± 0.76 | 4.5 ± 0.86 | 0.04 | 0.18 | 0.76 |
|---------------|------------|------------|------------|------------|------|------|------|
| Fetuin-A (µg/ml) | 251 ± 19 | . | 259 ± 19 | . | 0.79 | . |     |

Values are means ± SEM. *p < 0.05 for change within each group. MANOVA: multivariate analysis of variance; AT: adipose tissue; MRT: magnetic resonance tomography; MRS: magnetic resonance spectroscopy; LBM: lean body mass; IMCL: intramyocellular lipids; AST: aspartate transaminase; ALT: alanine transaminase; hs-CRP: high sensitivity C-reactive protein; PAI-1: plasminogen activator inhibitor 1
Supplementary Figure 1. Relationships of liver triglyceride (TG) content (A), insulin sensitivity (HOMA-IR) (B), palmitoyl lyso-PC (C) and liver histological parameters (D-F) in subjects in the upper quartile (N=7) of liver TG content (all N=29), who were divided by the median HOMA-IR. Panel D: p=0.08 ($\chi^2$ likelihood ratio) for having a cumulative value of mild, moderate, and advanced hepatic inflammation and ballooning. Mallory-Hyaline was not found in these subjects. Panel E: Liver histology of a patient representative for the insulin sensitive group (N=3). No inflammatory infiltrates are seen (original magnification 100x H&E). The hepatocytes show no ballooning and no Mallory-Hyaline (insert; original magnification 400x H&E). Panel F: Liver histology of a patient representative for the insulin resistant group (N=4). A mild lymphoid infiltrate, accentuated in the periportal tracts, but also in the lobules is shown (arrows; original magnification 100x H&E). Focally a cytoplasmic ballooning of hepatocytes is detected (insert; arrows; original magnification 400x H&E).
SUPPLEMENTARY DATA

E

Insulin sensitive

F

Insulin resistant
Supplementary Figure 2. Schematic working models of the potential role of lyso-PCs in NAFL-induced insulin resistance. Lyso-PCs may inhibit hepatic inflammation by regulating the function of naturally occurring CD4(+)CD25(+) regulatory T cells (Tregs) and, thereby, protect from insulin resistance (model 1). Alternatively, elevated circulating lyso-PCs may represent activated pathways in the synthesis or the metabolism of phospholipids and phosphatidylcholine and thus, in the generation of hepatic endoplasmatic reticulum stress (model 2).