Increased De Novo Lipogenesis Is a Distinct Characteristic of Individuals With Nonalcoholic Fatty Liver Disease

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BACKGROUND & AIMS: There have been few studies of the role of de novo lipogenesis in the development of nonalcoholic fatty liver disease (NAFLD). We used isotope analyses to compare de novo lipogenesis and fatty acid flux between subjects with NAFLD and those without, matched for metabolic factors (controls). **METHODS:** We studied subjects with metabolic syndrome and/or levels of alanine aminotransferase and aspartate aminotransferase >30 mU/L, using magnetic resonance spectroscopy to identify those with high levels (HighLF, n = 13) or low levels (LowLF, n = 11) of intrahepatic triacylglycerol. Clinical and demographic information was collected from all participants, and insulin sensitivity was measured using the insulin-modified intravenous glucose tolerance test. Stable isotopes were administered and gas chromatography with mass spectrometry was used to analyze free (nonesterified) fatty acid (FFA) and triacylglycerol flux and lipogenesis. **RESULTS:** Subjects with HighLF (18.4% ± 3.6%) had higher plasma levels of FFAs during the nighttime and higher concentrations of insulin than subjects with LowLF (3.1% ± 2.7%; $P = .04$ and $P < .001$, respectively). No differences were observed between groups in adipose flux of FFAs (414 ± 195 μmol/min for HighLF vs 358 ± 105 μmol/min for LowLF; $P = .41$) or production of very-low-density lipoprotein triacylglycerol from FFAs (4.06 ± 2.57 μmol/min vs 4.34 ± 1.82 μmol/min; $P = .77$). In contrast, subjects with HighLF had more than 3-fold higher rates of de novo fatty acid synthesis than subjects with LowLF (2.57 ± 1.53 μmol/min vs 0.78 ± 0.42 μmol/min; $P = .001$). As a percentage of triacylglycerol palmitate, de novo lipogenesis was 2-fold higher in subjects with HighLF (23.2% ± 7.9% vs 10.1% ± 6.7%; $P < .001$); this level was independently associated with the level of intrahepatic triacylglycerol ($r = 0.53$; $P = .007$). **CONCLUSIONS:** By administering isotopes to subjects with NAFLD and control subjects, we confirmed that those with NAFLD have increased synthesis of fatty acids. Subjects with NAFLD also had higher nocturnal plasma levels of FFAs and did not suppress the contribution from de novo lipogenesis on fasting. These findings indicate that lipogenesis might be a therapeutic target for NAFLD.

Keywords: Lipid Metabolism; Fatty Acid Kinetics; Obesity; Diabetes.

The prevalence of nonalcoholic fatty liver disease (NAFLD) is increasing,[1] and this condition will become the most common liver disease in the United States over the next 10 years.[2,3] A comprehensive understanding of the metabolic mechanisms leading to accumulation of intrahepatic triglycerides (TGs) is critically needed to support the development of treatments for NAFLD. With regard to the flux of fatty acids (FAs) through the blood, the liver is somewhat of an "innocent bystander," taking up a constant proportion (~25%) of the FAs that flow to the organ,[4] and numerous studies have identified adipose insulin resistance as a key contributor to excess levels of intrahepatic TG.[5–8] Most previous research focusing on FA flux in patients with NAFLD has been conducted in the fasting state; thus, although postprandial insulin levels have been shown to be increased,[9,10] little is known about how FA flux transitions between the fasted and fed states in NAFLD. For instance, if dietary FAs are not cleared efficiently to the periphery, they can contribute to intrahepatic TG levels,[11,12] and postprandial hyperlipidemia has been shown in this condition.[13] Further, dietary carbohydrates could provide a potentially important source of excess levels of liver FAs[14] through the process of de novo lipogenesis.

Theoretically, de novo lipogenesis has been considered a minor contributor to TG synthesis in humans for a number of reasons. First, early published data were derived from fasting subjects,[15,16] and the de novo lipogenesis pathway is suppressed by fasting.[17] Second, results from lean subjects, who were found to have very low rates of lipogenesis (eg, <5% of liver-derived very-low-density lipoprotein [VLDL]-TG palmitate), may not reflect values in obesity and insulin resistance.[18] Third, accurate assessment is dependent on a minimum length of isotope administration. Our laboratory[14,19–21] and others[22,23] have shown that a longer duration of labeling (>2 days) is necessary to assess lipogenesis, possibly due to a delay in the movement of these FAs through intrahepatocyte storage pools. Indeed, higher levels of lipogenesis have been observed in lean, fasting subjects if intravenous or oral administration of isotopes occurs concurrently with meal consumption, before the fasting measurement.[19–21] Such methodology, when used in insulin-resistant subjects, has shown that the incidence of

Abbreviations used in this paper: **FA, fatty acid; FFA, free (nonesterified) fatty acid; HighLF, high levels of liver fat; LowLF, low levels of liver fat; NAFLD, nonalcoholic fatty liver disease; RaFFA, rate of appearance of adipose fatty acids; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VLDL, very-low-density lipoprotein.**

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fasting lipogenesis is significantly greater than previously appreciated (>20%) and up to 5-fold higher in insulin-resistant compared with insulin-sensitive subjects.[24] One last aspect of lipogenesis that contributes to its relevance in the pathology of intrahepatic TGs is that when the pathway is stimulated, the presence of a key intermediate of FA synthesis (malonyl-CoA) is believed to reduce the oxidation of FAs from any source.[25] This effect has been shown in humans with fructose feeding, which increases lipogenesis and is associated with higher postprandial TG concentrations[20,26] and greater re-esterification of dietary FAs.[27,28]

Despite the comorbidity of insulin resistance in NAFLD, lipogenesis has been measured in only 2 small cohorts of patients by our group (n = 9)[14] and one other (n = 5).[29] Further, of the numerous investigations showing elevated adipose free fatty acid (FFA) flux as a prominent feature in NAFLD, a role for lipogenesis has been implicated indirectly.[30] In an elegant study by Fabbrini et al, after labeling the plasma FFA pool into VLDL-TG, a high percentage of TG-FA remained unlabeled[7] (60%) and contributed significantly to increased VLDL-TG secretion. The sources of these nonsystemic FAs were hypothesized to include those derived from visceral fat or intrahepatic depots or from hepatic lipogenesis. However, direct confirmation has been challenging because of the complexity of measuring the different FA sources as they contribute to TG production rates. Accordingly, we have systematically quantified the sources of FAs used for TG synthesis in subjects with either high levels of intrahepatic TGs or low levels of liver fat who were matched for similar adiposity and blood lipid levels. We used methodology optimized to accurately quantitate lipogenesis when liver lipid content was high, and the subjects underwent multiple clinical assessments to determine whether particular characteristics would predict elevated intrahepatic TG levels in NAFLD. The de novo lipogenesis pathway was found to be 3-fold higher in those with NAFLD, and lipogenesis was the key feature associated with fatty liver. These findings provide strong support for targeting lipogenesis in the future development of therapies for NAFLD.

Subjects and Methods

The methods are briefly described here and have been reported previously.[14,31,32] A detailed description of the study design, laboratory procedures, and calculations is provided in Supplementary Methods. Research subjects were recruited from local community health fairs and physician referrals to determine the role of metabolic syndrome in the development of NAFLD.[1] The initial screening criteria included characteristics of metabolic syndrome,[33] and liver enzyme levels were measured to increase the likelihood of finding subjects with NAFLD (as evidenced by elevated levels). Based on the results of this initial screen, eligible subjects (those with metabolic syndrome and/or levels of alanine aminotransferase and aspartate aminotransferase >30 mU/L) were invited to participate in a more comprehensive screening visit to rule out diabetes and liver disease from other known causes, obtain a medical and weight history, and measure intrahepatic TG levels by 3.0-T ^{1}H–magnetic resonance spectroscopy.[1] Subjects were excluded if they smoked, had known metabolic abnormalities (including elevated thyroid hormone levels), or an elevated level of alcohol consumption (>140 g/wk for men and >70 g/wk for women). Eligible and interested subjects were recruited sequentially over a 4-year period; 3 weeks were needed for each subject's inpatient and outpatient sample collection, followed by 5 weeks of analytical analysis per subject studied. During the 3 weeks of testing, each subject participated in 2 inpatient assessments; the first (admission 1) was designed to measure insulin sensitivity using a frequently sampled, insulin-modified intravenous glucose tolerance test,[34] and the second (admission 2) was designed to measure metabolism of FAs (Supplementary Figure 1). Subjects maintained habitual physical activity levels during the testing period, and all foods and beverages were provided to subjects before and during both admissions based on their habitual dietary patterns (see Supplementary Methods). The goal was to obtain a sample of 22 subjects who had metabolic syndrome with (n = 11) and without (n = 11) high levels of liver fat, and this number was powered to test an absolute difference in fasting de novo lipogenesis of 5% (SD of that difference, 3.6%) while allowing for a 10% dropout rate during the 3 weeks of research data acquisition. This study was approved by the University of Texas Southwestern Medical Center Institutional Review Board (062007-025), and subjects provided written informed consent.

Study Design and Laboratory Procedures

For admission 1, subjects reported to the Clinical and Translational Research Center for measurement of glucose and insulin responses during an intravenous glucose tolerance test,[34] body composition by dual-energy x-ray absorptiometry, and intrahepatic TG levels by magnetic resonance spectroscopy. Between admissions 1 and 2, subjects consumed deuterated water to measure hepatic lipogenesis.[31,32] For admission 2, each subject consumed a standardized evening meal containing $^{13}C_{16}$-palmitate to trace incorporation of meal fat into lipoprotein TG and to trace spillover of dietary FAs into the plasma FFA pool.[19] Subjects then fasted overnight and through the next morning (18 hours after the last meal) to allow for complete turnover of the plasma VLDL-TG pool. At midnight, an intravenous infusion of $^{13}C_4$-palmitate was initiated to measure the contribution of plasma FFAs to hepatic TG synthesis[19] and the rate of adipose FA and dietary spillover FA flux (see the following text). The TG-FA composition and sources contributing to plasma VLDL-TGs and liver TGs have been shown to be identical,[14,35] and therefore the characteristics of VLDL-TGs can be used to assess liver-TG fluxes. Energy expenditure and substrate oxidation were measured using indirect calorimetry. Immediately after blood collection, plasma was separated for measurement of FFA, TG, glucose, and insulin concentrations. Total TG-rich lipoproteins (TRLs) were isolated from plasma by fixed-angle ultracentrifugation.[19] At 12:00 AM, TRLs contained a mixture of chylomicrons (from the previous evening meal) and hepatically derived VLDL particles.[36] With progressive fasting, chylomicrons are cleared from the plasma such that TRLs isolated 18 hours after the last meal contain very small concentrations of chylomicrons. In the present study, the 18-hour fasting chylomicron apoB48 value was 1/60th the postmeal TRL value (data not shown). Accordingly, this fraction isolated