



Published in final edited form as:

*Gastroenterology*. 2014 March ; 146(3): 726–735. doi:10.1053/j.gastro.2013.11.049.

## Increased de novo Lipogenesis is a Distinct Characteristic of Individuals with Nonalcoholic Fatty Liver Disease

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### Abstract

**BACKGROUND & AIMS:** There have been few studies on 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 individuals 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 of intrahepatic triacylglycerol (LowLF,  $n=11$ ). 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 (non-esterified) fatty acid (FFA) and triacylglycerol flux and lipogenesis.

**RESULTS:** Individuals with HighLF ( $18.4\% \pm 3.6\%$ ) had higher plasma levels of FFA during the nighttime and concentrations of insulin than subjects with LowLF ( $3.1\% \pm 2.7\%$ ;  $P=.04$  and  $P<.001$ , respectively). No differences were observed between groups in adipose flux of FFA ( $414 \pm 195$   $\mu\text{mol}/\text{min}$  for HighLF vs  $358 \pm 105$   $\mu\text{mol}/\text{min}$  for LowLF;  $P=.41$ ) or production of very low-density lipoprotein triacylglycerols from FFA ( $4.06 \pm 2.57$   $\mu\text{mol}/\text{min}$  vs  $4.34 \pm 1.82$   $\mu\text{mol}/\text{min}$ ;  $P=.77$ ). By contrast, subjects with HighLF had more than 3-fold higher rates of de novo fatty acid synthesis than subjects with LowLF ( $2.57 \pm 1.53$   $\mu\text{mol}/\text{min}$  vs  $0.78 \pm 0.42$   $\mu\text{mol}/\text{min}$ ;  $P=.001$ ). As a percentage of triacylglycerol palmitate, de novo lipogenesis was 2-fold higher in subjects with HighLF ( $23.2\% \pm 7.9\%$  vs  $10.1\% \pm 6.7\%$ ;  $P<.001$ ); this level was independently associated with the level of intrahepatic triacylglycerol ( $r=0.53$ ;  $P=.007$ ).

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Author contributions

JEL, conducted the clinical trial, processed and analyzed samples, performed data and statistical analysis, wrote manuscript; MARR, collected data, analyzed samples, performed data analysis; JDB, provided medical and safety management, collected samples, edited and wrote manuscript; EJP, designed and directed entire study, collected and analyzed samples, performed data analysis, wrote manuscript.

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Author names in bold designate shared co-first authors.

None of the authors had any conflicts of interest.

**CONCLUSIONS:** By administering isotopes to individuals with NAFLD and control subjects, we confirmed that those with NAFLD increase synthesis of fatty acids. Subjects with NAFLD also had higher nocturnal plasma levels of FFA and did not suppress the contribution from de novo lipogenesis upon fasting. These findings indicate that lipogenesis might be a therapeutic target for NAFLD.

### Keywords

lipid metabolism; fatty acid kinetics; obesity; diabetes

## INTRODUCTION

The prevalence of NAFLD is increasing<sup>1</sup> and over the next 10 years, this condition will become the most common liver disease in the United States<sup>2, 3</sup>. A comprehensive understanding of metabolic mechanisms leading to accumulation of intrahepatic triglycerides (TG) is critically needed to support the development of treatments for NAFLD. With regard to the flux of fatty acids (FA) through the blood, the liver exists as somewhat of an ‘innocent bystander’ - taking up a constant proportion (~25%) of the FA that flow to the organ<sup>4</sup> and numerous studies have identified adipose insulin resistance as a key contributor to excess intrahepatic-TG<sup>5-8</sup>. Of note, most previous research focusing on FA flux in NAFLD has been conducted in the fasting state and thus, although postprandial insulin has been shown to be increased<sup>9, 10</sup>, little is known in NAFLD about how FA flux transitions between the fasted and fed states. For instance, if dietary FA are not cleared efficiently to the periphery, they can contribute to intrahepatic-TG<sup>11, 12</sup> and postprandial hyperlipidemia has been demonstrated in this condition<sup>13</sup>. Further, dietary carbohydrates could provide a potentially important source of excess liver FA<sup>14</sup> 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. The first is that early published data were derived from fasting subjects<sup>15, 16</sup> and the de novo lipogenesis pathway is suppressed by fasting<sup>17</sup>. Second, results from lean subjects, in which lipogenesis rates were found to be very low (e.g., <5% of liver-derived VLDL-TG palmitate) may not reflect values in obesity and insulin resistance<sup>18</sup>. The third reason for underestimation of the role of de novo lipogenesis in liver physiology is a technical one, in that the accurate assessment is dependent on a minimum length of isotope administration. Our lab<sup>14, 19-21</sup> and others<sup>22, 23</sup> have shown that a longer duration of labeling (>2d) is necessary to assess lipogenesis, possibly due to a delay in the movement of these FA through intrahepatocyte storage pools. Indeed, higher lipogenesis levels have been observed in lean, fasting subjects, if IV or oral administration of isotopes occurs concurrently with meal consumption, before the fasting measurement is made<sup>19-21</sup>. Such methodology, when used in insulin-resistant subjects, has shown that fasting lipogenesis is significantly greater than previously appreciated (>20%) and up to 5-fold higher in insulin-resistant, compared to insulin-sensitive individuals<sup>24</sup>. One last aspect of lipogenesis that contributes to its relevance in the pathology of intrahepatic-TG is that when the pathway is stimulated, the presence of a key intermediate of FA synthesis (malonyl-CoA) is thought to reduce the oxidation of fatty acids from any source<sup>25</sup>. This effect has been demonstrated in humans with fructose feeding, which increases lipogenesis

and is associated with higher postprandial TG concentrations<sup>20, 26</sup> and greater reesterification of dietary FA<sup>27, 28</sup>

Despite the comorbidity of insulin resistance in NAFLD, lipogenesis has been measured in only two small cohorts of patients by our group (n=9)<sup>14</sup> and one other (n=5)<sup>29</sup>. Further, of the numerous investigations showing elevated adipose FFA flux as a prominent feature in NAFLD, a role for lipogenesis has been implicated indirectly<sup>30</sup>. In an elegant study by Fabbrini et al., it was noted that after labeling the plasma FFA pool into VLDL-TG, a high percentage of TG-FA remaining unlabeled<sup>7</sup> (60%), which contributed significantly to increased VLDL-TG secretion. The sources of these nonsystemic FA were hypothesized to include those derived from visceral fat or intrahepatic depots, or from hepatic lipogenesis. However, direct confirmation has been challenging due to the complexity of measuring the different FA sources as they contribute to TG production rates. Accordingly, we have systematically quantified the sources of FA used for TG synthesis in subjects with either high levels of intrahepatic-TG or low liver fat, who were matched for similar adiposity and blood lipids. 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 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 this condition.

## EXPERIMENTAL PROCEDURES

The methods are briefly described here and have been published previously<sup>14, 31,32</sup>; the reader is referred to the Appendix for a detailed description of the study design, laboratory procedures, and calculations. Research subjects were recruited from local community health fairs and physician referrals to determine the role of metabolic syndrome in the development of NAFLD<sup>1</sup>. The initial screening criteria included characteristics of metabolic syndrome<sup>33</sup> and liver enzymes were also 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 (with metabolic syndrome and/or ALT >30, AST >30) were invited to attend a more comprehensive screening visit to rule out diabetes and liver disease from other known causes, to obtain a medical and weight history, and measure intrahepatic-TG by 3.0 Tesla <sup>1</sup>H-MRS<sup>1</sup>. Subjects were excluded if they smoked, had known metabolic abnormalities including elevated thyroid hormone levels, or elevated alcohol consumption (>140 g/wk for men and >70 g/wk for women). Eligible and interested subjects were recruited sequentially over a 4-yr period; 3 wks were needed for each subject's in- and out-patient sample collection, followed by 5 wks of analytical analysis per subject studied. During the 3 wks of testing, each subject participated in two in-patient assessments; the first (admission #1) was designed to measure insulin sensitivity using a frequently-sampled, insulin-modified intravenous glucose tolerance test (IVGTT)<sup>34</sup> and the second (admission #2) designed to measure FA metabolism (Appendix fig. 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 Appendix). The

goal sample size was 22 metabolic syndrome subjects with (n=11) and without (n=11) high liver fat and this number was powered to test an absolute difference in fasting de novo lipogenesis of 5% (SD of that difference of 3.6%), while allowing for a 10% drop-out rate during the 3 wks of research data acquisition. This study was approved by the UT Southwestern Medical Center IRB (#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 to measure glucose and insulin responses during an IVGTT<sup>34</sup>, body composition by DEXA, and intrahepatic-TG by MRS. Between admissions #1 and #2, subjects consumed deuterated water to measure hepatic lipogenesis<sup>31,32</sup>. For admission #2, each subject consumed a standardized evening meal containing <sup>13</sup>C<sub>16</sub>-palmitate to trace meal fat incorporation into lipoprotein-TG and to trace spillover of dietary FA into the plasma FFA pool<sup>19</sup>. Subjects then fasted overnight and through the next morning (18h after the last meal) to allow for complete turnover of the plasma VLDL-TG pool. At midnight an IV infusion of <sup>13</sup>C<sub>4</sub>-palmitate was initiated to measure the contribution of plasma FFA to hepatic-TG synthesis<sup>19</sup> and the rate of adipose- FA and dietary spillover- FA flux (see below). The TG-FA composition and sources contributing to plasma VLDL- and liver-TG have been shown to be identical<sup>14, 35</sup>, and therefore the characteristics of VLDL-TG 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 (TRL) were isolated from plasma by fixed-angle ultracentrifugation<sup>19</sup>. At midnight, TRL contain a mixture of chylomicron (from the previous evening meal) and hepatically-derived VLDL particles<sup>36</sup>. With progressive fasting, chylomicrons are cleared from the plasma such that TRL isolated 18h after the last meal contains very small concentrations of chylomicrons. In the present study, 18h fasting chylomicron apoB48 was 1/60<sup>th</sup> of post-meal TRL values (data not shown). Accordingly, this fraction isolated at and after midnight is referred to herein as TRL (containing both chylomicrons and VLDL), whereas after an extended fast (18h after the last meal), this fraction is referred to as VLDL, as is commonly done<sup>7, 16</sup>. Lipoprotein-TG and FFA were isolated from plasma and prepared for GC/MS and GC analysis<sup>12, 19</sup>. Fractional lipoprotein-TG turnover rates were calculated by modeling the rise to plateau enrichment of <sup>13</sup>C<sub>4</sub>-palmitate into VLDL-TG<sup>19</sup> and the assumptions for this model<sup>37</sup> (e.g., the label used exclusively for hepatic-TG production) are presented in the Appendix.

### Calculations and statistical analysis

TG- fatty acid sources – Using an established multiple stable-isotope procedure<sup>12, 14, 16, 19</sup>, the potential FA sources that contribute to lipoprotein-TG palmitate production (dietary fat, adipose FA, and hepatic lipogenesis) are each labeled with a different palmitate isotope, and expressed as both absolute concentration and a proportion to reflect intracellular-TG synthesis<sup>14</sup>. Note that even with an extended period of labeling, a proportion of VLDL-TG will remain unlabeled<sup>14</sup> due to the use of visceral or intrahepatic stores. Plasma FFA sources include 1) adipose tissue, which supplies the majority of FFA in the fasting state, and 2)



During the morning fast, total FFA concentrations were significantly higher in the HighLF group (27%,  $P=0.001$ ) due to a greater adipose-derived FFA ( $P=0.002$ ), and a tendency for greater circulating residual meal-derived FFA at this time (0600-1200,  $P=0.059$ ). Similarly, the calculated *rates of appearance of FFA* during the night revealed that the peak RaSpillover between midnight and 0300 was two-fold greater in the HighLF group compared to the LowLF group ( $3.27\pm 2.17$  vs.  $1.82\pm 1.06$   $\mu\text{mol/kg FM/min}$ , respectively,  $P=0.056$ , data not shown). At the end of the extended fast, neither total RaFFA, adipose RaFFA, nor RaSpillover rates were different between the groups (table 2).

### TG-rich lipoprotein TG concentrations and fatty acid sources used for TG synthesis

Fasting VLDL-TG concentrations were approximately 50% higher in the HighLF group (table 2). Findings on the contribution of each identified source of FA present in lipoprotein-TG are presented as the relative input into the TG pool over time in figure 2. As shown in figure 2A, previous evening-meal fat represented ca. 22% of FA in TG at midnight for both groups. This label would be carried in chylomicron remnants made following the evening meal and/or in VLDL made from the recycling of chylomicron-TG through the liver<sup>11, 12, 19</sup>. The proportion of meal-derived FA decayed throughout the night (fig. 2A) and at the end of the experiment the percentages measured by GC/MS were similar between the HighLF and LowLF groups ( $5.3\pm 0.6\%$  and  $5.4\pm 0.7\%$ , respectively,  $P=0.88$ ). The concentration of TRL apoB48 was measured during fasting and some residual apoB48 was found after the morning fast, without appreciable differences between the groups ( $2.3\pm 1.6$  vs.  $1.9\pm 1.2$  nmol/L,  $P=0.51$ ). Based on these apoB48 concentrations, we estimated the fasting contribution of chylomicron-TG to the TRL fraction, which was calculated to be  $5.9\pm 3.9\%$  and  $4.0\pm 4.0\%$  for the HighLF and LowLF respectively ( $P=0.32$ ). These values agreed with the proportion measured by GC/MS described above (e.g., 5.3% and 5.4%).

The proportion of lipoprotein-TG arising from the plasma FFA pool was significantly lower in the HighLF compared to the LowLF group from 0130 onward (fig. 2B,  $P<0.01$ ). With regard to lipogenesis (fig. 2C), at midnight the fractional contribution was 72% higher in subjects with HighLF ( $29.5\pm 3.2\%$  and  $17.1\pm 4.2\%$ , respectively,  $P=0.037$ ) and these group differences were significant at every time point thereafter ( $P<0.05$ ). Fractional contribution from lipogenesis decayed throughout the night for both groups, in agreement with other studies<sup>22, 23</sup>. However, within the HighLF group, every value from midnight until 1030 was higher than fasting values at 1030 and 1145 ( $P<0.05$ ). In other words, it took at least 16.5h without food for the HighLF subjects to significantly suppress lipogenesis down to basal levels. By contrast, in the LowLF group the fasting level of lipogenesis was reached by at least 0800. The final fasting lipogenesis values at 12 noon were more than doubled in the HighLF group compared to the LowLF group (fig. 2C,  $P<0.001$ ). Finally, for the sum of all the isotopically-identified FA sources found at 1200 (fig. 2D), an identical total proportion of VLDL-TG was identified in the HighLF ( $66\pm 4\%$ ) and LowLF ( $67\pm 4\%$ ) subjects ( $P=0.84$ ). Fatty acid sources remaining unlabeled are anticipated<sup>7, 14, 16</sup> and could be derived from visceral stores or previously stored dietary and plasma-derived FA used to make intrahepatic-TG droplets prior to the hours of labeling<sup>7</sup>.

### Absolute contributions of fatty acid sources to TRL-TG

While the proportional contribution of FA sources to VLDL-TG reflects intracellular lipid synthesis<sup>14, 35</sup>, the absolute quantity of VLDL-TG derived from each source (mmol/L) reflects a balance of the processes of TG secretion, lipoprotein turnover, and clearance from the plasma. As shown in figure 3A, at the end of fasting, the absolute quantities of VLDL-TG made from adipose FFA flux were not different between the HighLF and LowLF groups ( $0.23 \pm 0.05$  vs.  $0.19 \pm 0.03$  mmol/L, respectively;  $P = .44$ ), in agreement with their similar RaFFA rates. This was also true for the residual TG derived from the evening meal ( $0.03 \pm 0.01$  and  $0.02 \pm 0.01$  mmol/L,  $P = .38$ ). The significant difference between the groups was found in the absolute quantity of FA made via de novo lipogenesis, which was 3.5-fold higher in the HighLF group ( $0.14 \pm 0.03$  mmol/L) compared to the LowLF group ( $0.04 \pm 0.01$  mmol/L,  $P = .001$ ). The quantity of unidentified VLDL-FA was not different between the groups ( $P = .22$ ). When the kinetic measurements of VLDL-TG production were applied to these FA sources, the contribution of the de novo lipogenesis pathway was again evident as a 3-fold increase in TG production from this source (table 2). No significant differences were found between the groups in production rate of total-TG or from adipose and meal FA, while the production of TG from lipogenesis was more than three-fold higher in the HighLF group ( $P = .004$ ). Fractional TG catabolic, TG half-life, and clearance rates were similar between the groups (table 2).

### Relationships between fatty acid flux, lipoprotein-TG sources and intrahepatic-TG

Among the factors tested for prediction of liver-TG accrual in these subjects, no associations were found between intrahepatic-TG and BMI, trunk fat, fasting glucose and insulin (data not shown). By contrast, intrahepatic-TG was positively associated with de novo lipogenesis, when lipogenesis was expressed as an absolute quantity of lipogenic FA within VLDL-TG ( $r = 0.60$ ,  $P = .003$ ) and as a proportion of FA VLDL-TG (fig. 3B). Similarly, intrahepatic-TG was positively correlated with fasting TG secretion arising from lipogenic FA ( $\mu\text{mol}/\text{min}$ ) (fig. 3C).

## DISCUSSION

The present study represents the first direct confirmation of the significant role of de novo lipogenesis in the etiology of fatty liver disease. Compared to subjects with LowLF, those with HighLF demonstrated a doubling of the fractional contribution from de novo lipogenesis to VLDL-FA ( $10.1 \pm 6.7\%$  vs  $23.2 \pm 7.9\%$ , respectively,  $P < .001$ ), a 3-fold higher rate of production of VLDL-TG from lipogenesis, accounted for through extended labeling of the lipogenic pathway and, among all subjects, a positive association between fasting lipogenesis and intrahepatic-TG.

It should be noted that the importance of the de novo lipogenesis pathway in human physiology has been the subject of debate<sup>40</sup>. Indeed, in lean individuals in energy balance, the contribution of lipogenesis to liver-TG synthesis in the fasting state is low,  $<5\%$  of VLDL-TG<sup>16,17</sup>. However, over the past 10 years, data from our lab<sup>14, 19</sup> and others<sup>17, 18, 23, 41</sup> have demonstrated a significant stimulation of this pathway in the fed state ( $>25\%$  of VLDL-TG), particularly when dietary carbohydrates are more simple in

structure<sup>42</sup>, consumed in liquid form<sup>42</sup>, and enter the body at a higher rate<sup>12</sup>. Furthermore, numerous studies have now documented significant elevations in both fasting and fed lipogenesis in obesity<sup>18</sup>, insulin resistance<sup>41,43, 44</sup>, and diabetes<sup>23, 24</sup>. Previously, indirect evidence of elevated lipogenesis in NAFLD has come from the findings of Fabbrini et al<sup>7</sup> and Sevastianova et al<sup>30</sup> and muscle insulin resistance has been hypothesized to contribute to NAFLD by shifting dietary carbohydrate away from muscle glycogen synthesis and into hepatic lipogenesis.<sup>43</sup> Direct evidence of the role of lipogenesis in NAFLD has come from our lab<sup>14</sup> and Diraison et al<sup>29</sup>, however these observations were made in a small group of subjects (n=5–9) and lacked an obese, non-NAFLD comparison group. By matching control and NAFLD subjects closely for body composition and blood chemistries, the present study significantly strengthens our previous observations in NAFLD<sup>14</sup> and underscores elevated lipogenesis as a prominent characteristic of this condition.

The role of insulin resistance appears central to NAFLD development, particularly when it occurs in multiple tissues<sup>6, 8</sup>. The present data join an established literature supporting adipose insulin resistance as the key contributor to the development of NAFLD<sup>5, 7</sup>. In the present study, a measure of adipose insulin resistance, the AdipoIR<sup>6</sup> provided evidence of excess FA release in the subjects with HighLF, yet the greater availability of plasma FFA did not contribute to significantly greater liver-TG secretion in the form of VLDL. This disconnect between plasma FA flux and VLDL-TG secretion rates has been recently highlighted by Koutsari et al.<sup>45</sup>. These findings raise a key unanswered question, “What is the fate of excess plasma FA in the liver?” We have recently shown in a subset of these same subjects<sup>46</sup>, that those with HighLF exhibited greater rates of liver TCA cycle activity, which is a major pathway of FA utilization. Increased TCA cycle activity would supply the energy needed to support the elevated gluconeogenesis and excess hepatic glucose production<sup>47</sup>, which we<sup>46</sup> and others<sup>48, 49</sup> have demonstrated in NAFLD. In the context of these observations, the higher lipogenesis observed here in the HighLF subjects, fits into an overall picture of hepatic insulin resistance in which excess glucose carbon flux provides a common metabolic mechanism for both elevated glucose production and de novo lipogenesis. Indeed, in rodent studies, hepatic insulin resistance is characterized by a bifurcation of insulin signaling in which glucose production is resistant to insulin’s inhibitory effects whereas lipogenesis is not, and is, in fact, stimulated<sup>50</sup>.

Mounting data have supported the concept that stimulation of lipogenesis can lead to an increased hepatic lipid burden by multiple distinct mechanisms. These include the fact that 1) elevated lipogenesis is the end result of a high flux of carbons down glycolysis (as occurs in fructose feeding, for example<sup>20, 26</sup>) supplying excess triose phosphates, which serve as the glycerol backbone for TG synthesis<sup>51</sup>; 2) stimulation of lipogenesis produces FA directly through the *fatty acid synthase* enzyme complex<sup>52</sup>, while at the same time 3) elevating intracellular malonyl-CoA concentrations which should inhibit FA oxidation<sup>25</sup>. If FA oxidation is actually increased in NAFLD<sup>46</sup> this would suggest that the production of malonyl-CoA is insufficient to have a suppressive effect. Lastly, the primary product of lipogenesis is palmitate, a saturated FA<sup>24</sup>, which may promote inflammation and endoplasmic reticulum stress<sup>53, 54</sup>.



The strengths of the present study included the comprehensive assessments of characteristics that impact intrahepatic-TG, and the similarity between groups for adiposity, food intake, and physical activity. The primary study limitation relates to the nature of tracer studies, as these intensive protocols typically limit the sample size. Furthermore, the fact that the lipogenesis pathway was labeled longer than the dietary or plasma NEFA pools leaves some question as to the true contribution of the previously unlabeled diet and adipose flux to the unlabeled TG pools remaining at the end of the study. However, within a given source (e.g., diet or lipogenesis), both groups of subjects underwent the same timing of label administration and yet only the fractional contribution from lipogenesis was different between the LowLF and HighLF groups. In addition to these findings, more research is needed to understand the relative lack of circadian control of lipogenesis in NAFLD and whether elevated nocturnal insulin concentrations play a role in the continued stimulation of this pathway during fasting.

As the understanding of NAFLD has evolved, scientists have questioned whether it is excess lipolysis or lipogenesis that causes fatty liver disease<sup>55</sup>. The present data join the large literature currently available demonstrating that elevated lipogenesis, in combination with excess adipose fatty acid release, is a significant contributor to this condition. Excess lipolysis is found in other conditions such as hypertriglyceridemia and obesity, but the distinctive elevation of lipogenesis in NAFLD suggests a causative role. As a result, these data provide strong support for the recent emergence of carbohydrate restriction as a goal of dietary therapy<sup>56, 57</sup>. Whether lipogenesis, as a target for pharmaceutical development, will benefit fatty liver has yet to be determined.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENTS

The authors would like to express their appreciation to the research subjects for their participation, to the staff of the Clinical Translational Research Center, and to Dora Bradford, RN-C, WHNP for recruitment and screening of the subjects and excellent patient care. The authors would also like to thank Maressa Valdez, RD, for subject recruitment, clinical coordination, and data collection, and to Joseph Lee, BS, Yelena Hovhannisyan, MS, and Kimberly Borke, MD, for data generation, analysis, and management. We thank Drs. Jay Horton and Scott Grundy for support, Dr. Elizabeth Murphy for advice in planning the D<sub>2</sub>O labeling procedures, and Dr. Robert Phair for insightful discussions of the data.

Financial Support:

National Institutes of Health grant RL1DK081187 (Elizabeth Parks, PI), the Task Force for Obesity Research at Southwestern (TORS) NIH UL1DE019584 (Jay Horton, PI), and the Clinical Translational Science Award, NIH/NCATS UL1-RR024982 (Robert Toto, PI).

## Abbreviations

<b>apoB48</b>	Apolipoprotein B48
<b>FA</b>	fatty acids
<b>FFA</b>	plasma free (non-esterified) fatty acids

<b>GC/MS</b>	gas chromatography/mass spectrometry
<b>IHTG</b>	intrahepatic triacylglycerols
<b>MIDA</b>	mass isotopomer distribution analysis
<b><sup>1</sup>H-MRS</b>	magnetic resonance spectroscopy
<b>NAFLD</b>	non-alcoholic fatty liver disease
<b>RaFFA</b>	rate of appearance of plasma free fatty acids
<b>RaSpillover</b>	rate of appearance of free fatty acids from lipoprotein-TG lipolysis
<b>SI</b>	Insulin sensitivity as calculated using the MINMOD program
<b>TG</b>	triacylglycerols
<b>TRL</b>	TG-rich lipoproteins
<b>VLDL</b>	very low-density lipoproteins

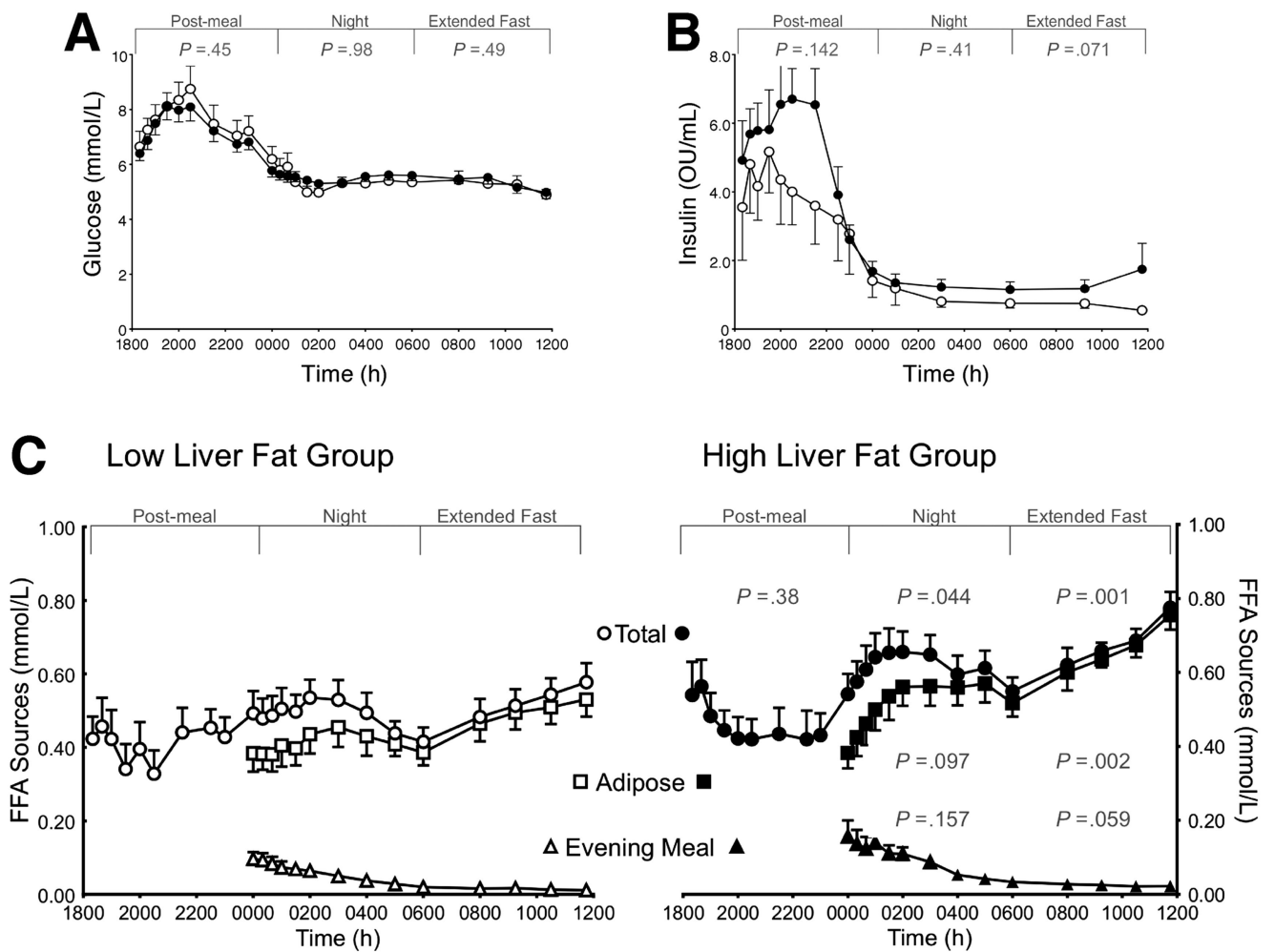
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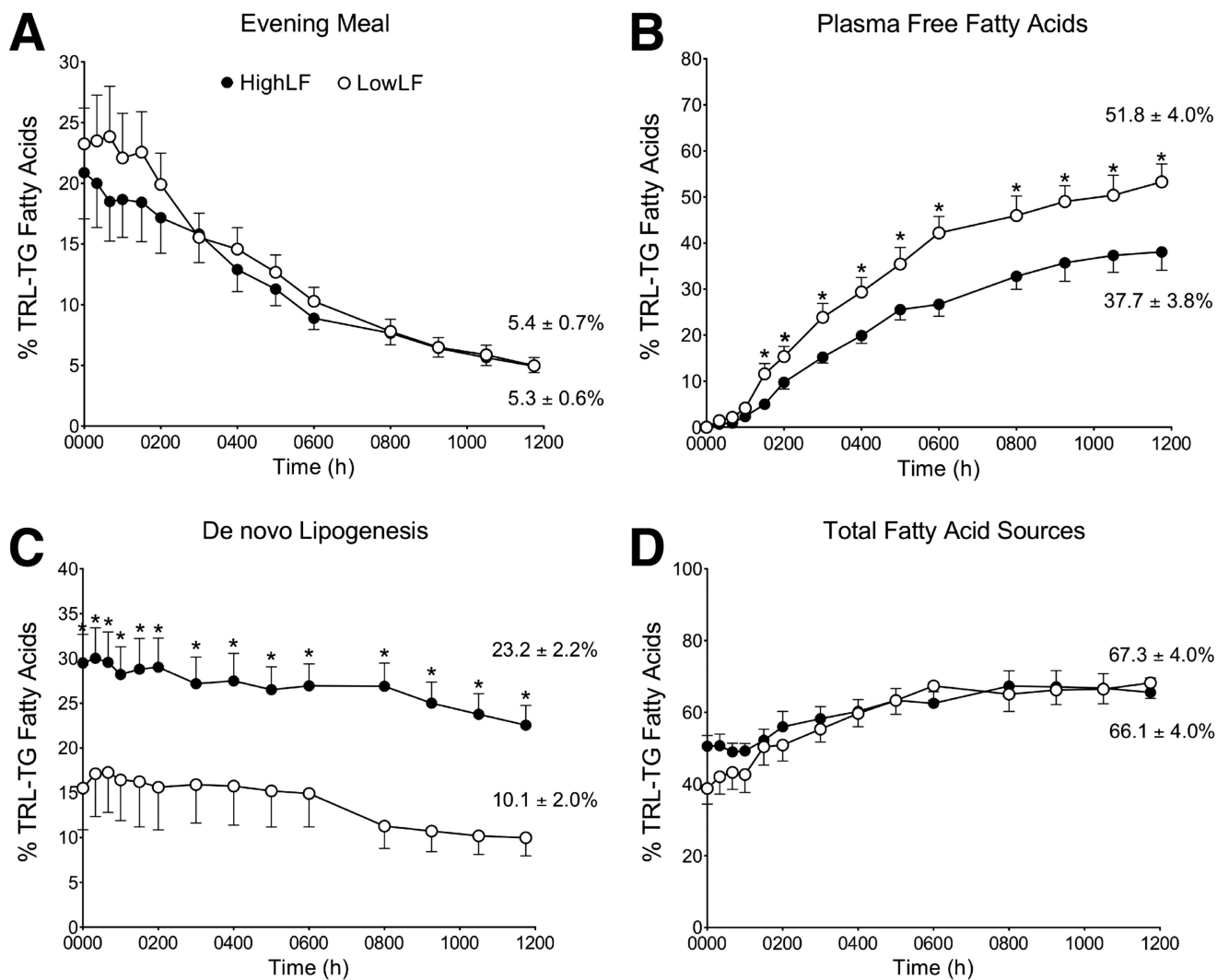
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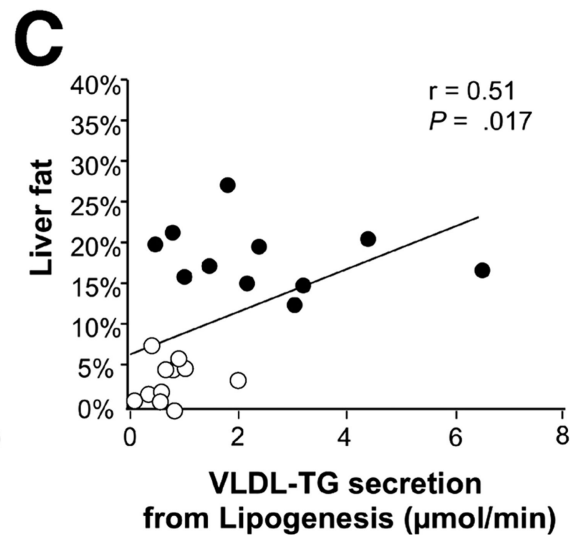
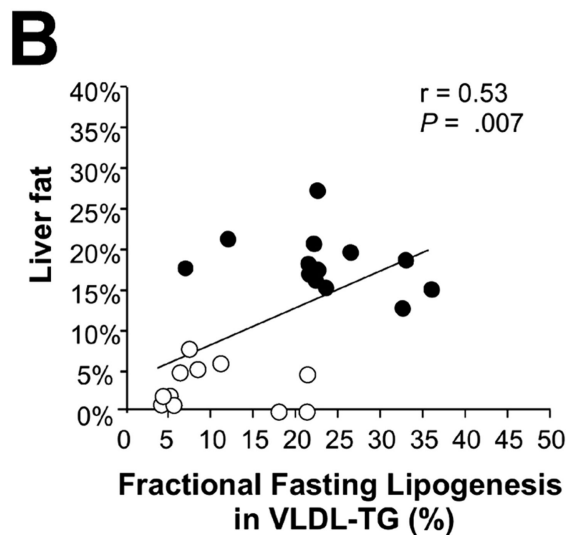
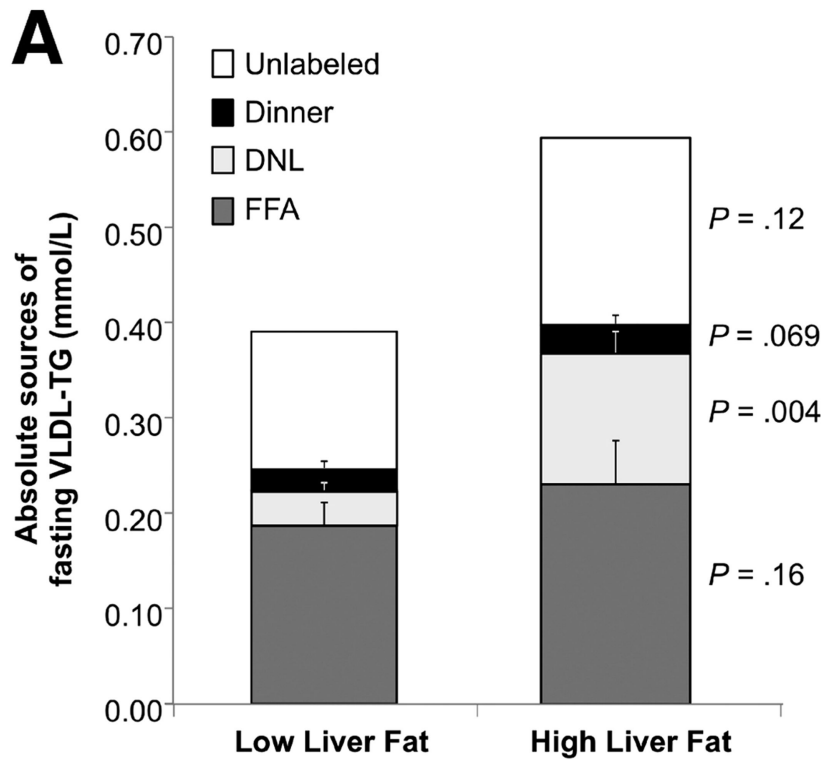
**Figure 1. Dynamics of plasma glucose, insulin, and fatty acids**

Data are presented as mean  $\pm$  SEM. The 18h study period was divided into three segments for analysis: 1800 to midnight (post-evening meal), midnight to 0600 (at night), and 0600 to 1200 (during the extended fast). Panel A displays glucose and (B) insulin concentrations, which were not different between the groups during any of the segments. Panel C: postprandial changes in total plasma free fatty acid (FFA) concentrations (total; circles), those derived from adipose lipolysis (adipose; squares) or from evening-meal spillover (evening meal; triangles) in subjects with High liver fat (HighLF, filled symbols) and Low liver fat (LowLF, open symbols).  $P$ -values represent significant differences between the HighLF and LowLF groups for the FFA sources within the designated time period (determined by repeated measures ANOVA).



**Figure 2. Proportion of fatty acid sources becoming labeled in TRL-TG from midnight to noon in subjects with low or high liver fat**

Data represent the proportions of fatty acids arising from the (A) evening meal, (B) plasma FFA pool, (C) de novo lipogenesis, and (D) the proportion of total fatty acid sources accounted for in TRL particles at the end of the experiment. Values reported on the right edge of panels A-D represent the average of the last two measurements taken in the fasting state (e.g., 1030 and 1145). Data are presented as mean  $\pm$  SEM; asterisks denote significant differences between groups at individual time points ( $P < .05$ ); open circles = Low liver fat (LowLF) group; filled circles = High liver fat (HighLF) group.



**Figure 3. Absolute contributions of fatty acid sources to fasting VLDL-TG in subjects with low or high liver fat and the relationships between liver fat with de novo lipogenesis**

**A)** The absolute concentrations of fatty acids arising from the evening meal, plasma FFA pool, de novo lipogenesis, and the amount remaining unlabeled in fasting VLDL-TG particles. Values represent the mean group data from each subject in which the last two measurements were taken in the fasting state (e.g., 1030 and 1145) and data from these two time points were averaged for that subject. Relationships between liver fat content and de novo lipogenesis represented as **B)** newly-synthesized fatty acid in VLDL-TG in units of



mmol/L and C) as a fraction of VLDL-TG from lipogenesis (%). Open circles, Low liver fat (LF) group; filled circles, High liver fat (LF) group.

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**Table 1.**

Anthropometric characteristics, and biochemical and dietary intake data of subjects categorized by normal (LowLF) or elevated (HighLF) liver fat

<i>Characteristics</i>	<b>LowLF (n=11)</b>	<b>HighLF (n=13)</b>	<i>P-value</i>
Liver Fat (%)	3.1 ± 2.7	18.4 ± 3.6	< .001
Males : Females	4:7	6:7	.70
<i>Anthropometrics</i>			
BMI (kg/m <sup>2</sup> )	35.3 ± 7.7	34.9 ± 5.2	.89
Weight (kg)	103 ± 22	92 ± 18	.19
Body fat (%)	39.7 ± 10.5	39.2 ± 6.8	.89
Lean body mass (%)	57.6 ± 10.1	58.2 ± 6.6	.86
Trunk body fat (%)	20.1 ± 5.3	21.4 ± 4.5	.50
Waist:hip ratio	0.92 ± 0.07	0.96 ± 0.09	.20
<i>Fasting Biochemical Data</i> <sup>a</sup>			
ALT (U/L)	45 ± 35	66 ± 44	.20
AST (U/L)	36 ± 27	51 ± 39	.29
HbA1c (%)	5.8 ± 0.2	5.9 ± 0.3	.12
Glucose (mmol/L)	5.1 ± 0.5	5.5 ± 0.8	.20
Insulin (mU/L)	7 ± 4	11 ± 5	.05
AdipoIR <sup>b</sup>	4.1 ± 2.0	7.2 ± 3.4	.01
Plasma FFA (mmol/L)	0.57 ± 0.15	0.66 ± 0.12	.11
̂-hydroxybutyrate (̂mol/L)	114 ± 90	89 ± 61	.42
Plasma TG (mmol/L)	1.24 ± 0.57	1.51 ± 0.61	.28
Total cholesterol (mmol/L)	5.11 ± 0.35	5.16 ± 0.86	.87
LDL-cholesterol (mmol/L)	3.13 ± 0.29	3.27 ± 0.78	.59
HDL-cholesterol (mmol/L)	1.26 ± 0.40	1.21 ± 0.17	.70
<i>Dietary Intake</i>			
Total energy intake (kcal/kg BW/day)	25.7 ± 6.6	26.2 ± 6.2	.84
Fat (%E)	35 ± 8	36 ± 7	.80
Carbohydrate (%E)	48 ± 10	47 ± 10	.88
Protein (%E)	17 ± 4	17 ± 5	.96
Total sugars (%E)	24 ± 13	22 ± 8	.59
<i>Physical Activity</i>			
Steps/day <sup>c</sup>	6451 ± 2968	5254 ± 3452	.43
Daily activity <sup>c</sup>			
% of day Sedentary	79.8 ± 8.8	79.0 ± 10.5	.85
% of day in Light Activity	15.0 ± 6.0	16.8 ± 7.8	.56
% of day in Moderate Activity	5.2 ± 3.5	4.2 ± 3.1	.50

Data are mean ± SD. Abbreviations: %E, percent of total energy intake; BW, body weight.

<sup>a</sup>Biochemistry data represent the average concentrations of these analytes at 0600 and 0800.

<sup>b</sup> AdipoIR was calculated by multiplying the fasting plasma FFA concentration by the fasting insulin concentration <sup>6</sup>.

<sup>c</sup> METs were calculated based on counts per minute of activity. Sedentary activity corresponded to 0-1 MET, light activity 1-3 MET, and moderate activity 3-6 MET<sup>58</sup>. No detection of vigorous activity was found in any subjects' activity analyses.

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**Table 2.**

## Kinetic measures of metabolism

	LowLF (n=11)	HighLF (n=13)	P-value
<i>Energy Expenditure and Substrate Oxidation</i> <sup>a</sup>			
Energy expenditure (kcal/kg BW/day)	14.4 ± 2.9	15.5 ± 2.4	.32
Glucose oxidation (mg/kg BW/min)	1.07 ± 0.35	0.96 ± 0.36	.48
Fat oxidation (mg/kg BW/min)	0.55 ± 0.18	0.68 ± 0.19	.10
<i>Insulin Sensitivity Measures</i> <sup>b</sup>			
SI (10 <sup>-4</sup> * min <sup>-1</sup> per μU/mL)	3.1 ± 1.4	2.2 ± 1.4	.14
AIRg (μU/mL * min)	568 ± 407	489 ± 304	.60
Disposition index (unitless)	1431 ± 668	962 ± 637	.09
<i>Plasma FFA Kinetics</i> <sup>c</sup>			
Total RaFFA μmol/kg FM/min)	9.98 ± 2.80	12.73 ± 6.30	.20
Adipose RaFFA μmol/kg FM/min)	9.57 ± 2.65	12.28 ± 6.17	.19
RaSpillover μmol/kg FM/min)	0.37 ± 0.29	0.43 ± 0.23	.53
Mean FFA residence time (min) <sup>d</sup>	6.54 ± 1.66	6.75 ± 2.97	.84
T½ life (min)	4.5 ± 1.2	4.7 ± 2.1	.84
<i>VLDL-TG Concentrations and Kinetics</i> <sup>e</sup>			
VLDL-TG concentration (mmol/L)	0.39 ± 0.22	0.60 ± 0.29	.07
Total Production Rate (μmol/min)	8.6 ± 3.0	11.0 ± 4.5	.16
TG Production from Adipose (μmol/min)	4.34 ± 1.82	4.06 ± 2.57	.77
TG Production from Evening Meal (μmol/min)	0.46 ± 0.24	0.56 ± 0.25	.39
TG Production from Lipogenesis (μmol/min)	0.78 ± 0.42	2.57 ± 1.53	<b>.001</b>
Fractional Catabolic Rate (% / hr)	28.0 ± 11.1	24.8 ± 7.6	.44
T½ life (h)	2.9 ± 1.2	3.1 ± 1.3	.65
Clearance Rate (mL/min)	27.0 ± 12.2	20.1 ± 6.3	.11

Data are mean ± SD. Abbreviations: SI, insulin sensitivity index; AIRg, acute insulin response to glucose, RaFFA, rate of appearance of FFA.

<sup>a</sup>Fasting energy expenditure and substrate oxidation were determined at 0800.

<sup>b</sup>Data are calculated from the frequently-sampled insulin-modified IVGTT using MINMOD software.

<sup>c</sup>Plasma FFA kinetics were determined by the average values taken at the end of the study sampling period (1030, 1145).

<sup>d</sup>Mean residence time is the average time a FFA molecule spends in the volume of distribution.

<sup>e</sup>n=11/group; two subjects in the high liver fat (LF) group were excluded from this analysis because they had VLDL-TG concentrations that were more than 2 SD greater than the mean of all other subjects.