Activation of the GP130-STAT3 axis and its potential implications in nonalcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) and obesity affect about a third of the US population (13, 26). Nonalcoholic steatohepatitis (NASH), the aggressive phenotype of NAFLD, leads to cirrhosis and is increasing as a cause of end-stage liver disease and an indication for liver transplantation (5, 18). The primary objective of this work was to determine the status of this pathway in humans with NAFLD and the potential consequences of its activation. The specific aims were to (1) define the status of the gp130-STAT3 axis in subjects with fatty liver or NASH and compare them with lean healthy controls and obese subjects without NAFLD, (2) determine whether the status of gp130 expression was linked to circulating IL-6 levels, (3) define the ability of palmitate to modulate the activity of the gp-130-STAT3 axis, and (4) define cross talk between IL-6 and palmitate in modulating gp130-STAT3-mediated effects on such pathways.

MATERIALS AND METHODS

Reagents. Antibodies for Western blotting, SP600125, and IL-6 protein were purchased from Cell Signaling (Beverly, MA). β-Actin, RIPA buffer, protease inhibitor mixture, STAT3 inhibitor (S3I-201), AR-42, IKK2 inhibitor, PD98059, and FFA-free BSA were purchased from Sigma Aldrich (St. Louis, MO). NF-κB antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). AR-42 was purchased from Thermo Fisher Scientific (Waltham, MA). Horseradish peroxidase (HRP)-conjugated secondary antibody and SuperSignal chemiluminescence kits were purchased from Pierce Biotechnology (Rockford, IL). Cell culture media, insulin, and Western blot supplies were obtained from Invitrogen (Carlsbad, CA).

Human subjects. Four groups of subjects were studied: 1) lean healthy normal controls, 2) obese controls without NAFLD, 3) nonalcoholic fatty liver, and 4) nonalcoholic steatohepatitis. Both effects are mediated via the direct cellular effects of fatty acids as well as by cytokine-mediated signaling (1).

The gp130 cell surface receptor mediates a multitude of cellular responses to external cues and is a key element of the cellular response to metabolic and other stresses (11, 25). It is engaged by numerous cytokines often referred to as the gp130 cytokine family (27). IL-6, a cytokine known to be increased in obesity and insulin-resistant states (20, 22), is a prototypic gp130 cytokine and activates Janus-activated kinase (JAK)-signal transduction and activator of transcription 3 (JAK-STAT3), Src-Ras, and the phosphatidylinositol 3-kinase-Akt pathways via gp130 (23, 28). Gp130-mediated signaling plays a role on hematopoiesis, immune regulation, inflammation, and carcinogenesis (3, 17). The status of gp130-STAT3 axis in NAFLD is unknown. It is also not known whether this pathway is affected by lipotoxic stress. The potential cross talk between palmitate and IL-6, which are both increased in NAFLD in modulating gp130 signaling, is also unknown.

The primary objective of this work was to determine the status of this pathway in humans with NAFLD and the potential consequences of its activation. The specific aims were to (1) define the status of the gp130-STAT3 axis in subjects with fatty liver or NASH and compare them with lean healthy controls and obese subjects without NAFLD, (2) determine whether the status of gp130 expression was linked to circulating IL-6 levels, (3) define the ability of palmitate to modulate the activity of the gp-130-STAT3 axis, and (4) define cross talk between IL-6 and palmitate in modulating gp130-STAT3-mediated effects on such pathways.

Materials and methods

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lean and obese controls had normal alanine aminotransferase (<19 IU/l for women and 31 IU/l for men) and normal liver histology. NAFL and NASH were confirmed by liver biopsy in all instances. Liver tissue from lean, normal subjects was obtained the Tissue and Data Acquisition and Analysis Core, Virginia Commonwealth University, whereas that from obese subjects was collected from liver biopsy obtained at the time of cholecystectomy or gastric bypass surgery. Liver histology was scored based on NAS Criteria (4). The nonalcoholic nature of the disease was established clinically by using a daily intake cutoff of <20 g/day of alcohol for female subjects and <30 g/day for male subjects within the past 5 years, as used in other studies of this disease (21). All subjects provided informed consent and the study was approved by the institutional review board (VCU IRB no. 1960). Blood samples were obtained from each patient at the time of liver biopsy, processed to plasma, and stored frozen at −80°C. The plasma was subsequently used for quantitative measurement of IL-6 by a commercially available ELISA kit (R&D Systems, Minneapolis, MN). All assays were performed in triplicate, and the absorbance was determined by a microplate reader (Infinite M200 PRO, San Jose, CA).

In vitro experiments. The Huh-7 cell line was grown in DMEM containing 10% FBS, 100 IU/ml penicillin, and 100 mg/ml streptomycin as previously described (16). Huh-7 cells were grown in 2 ml of DMEM medium in a six-well plate at 37°C for overnight in a 0.5% CO2 incubator. On the next day, cells were treated with the indicated concentrations of 0.5 mM palmitate in the presence of 1% FFA-free BSA. For the gp130 inhibition experiments, Huh-7 cells were plated in a six-well plate in a 2.0-mL total volume at a density of 6 × 106 cells per well. Cells were treated with DMEM media containing 0, 0.01, and 1.0 μmol/l AR-42 for 1 h and then treated with 0.5 mM palmitate for 12 h. The plate was incubated with a 20 μg/ml of insulin at 37°C in a 5% CO2 incubator for 15 min. For the STAT3 inhibition experiments, cells were preincubated with STAT3 inhibitor (S31-201) (0, 10, 50, and 100 μM) for 4 h and then exposed with or without 0.5 mM palmitate in DMEM medium containing 1% FFA-free BSA for 12 h. For studies on cross talk between palmitate and IL-6 on insulin signaling pathways, Huh-7 cells were stimulated with palmitate (0.5 mM) for 12 h and then incubated with recombinant human IL-6 at 20 ng/ml for 15 min, and the cells were further exposed with insulin at 100 nM for 30 min.

Adenovirus-mediated gene transduction. Adenoviral vectors expressing STAT3 (Ad-STAT3) and control green fluorescent protein (GFP; Ad-GFP) were purchased from Vector BioLabs. Briefly, for infection of Ad-STAT3 or Ad-GFP in Huh-7 cells, 6 × 105 cells/well were seeded to six-well plates containing DMEM medium and allowed to adhere for 1 days and then cells were infected with multiplicity of infection (MOI) (0.001, 0.01, 0.1, or 1) of Ad-STAT3 or Ad-GFP for 12 h. After infection with adenovirus, the cells were treated with 0.5 mM palmitate for 8 or 12 h.

Protein extraction and Western blot analysis. All liver tissues were homogenized by using RIPA lysis buffer and then sonicated on ice with a sonicator cell disrupter, model 100 Sonic Dismembrator (Thermo Fisher Scientific, Waltham, MA; power 2, 6 pulses ×2). Huh-7 cells were washed with phosphate-buffered saline and lysed in RIPA buffer, containing a protease inhibitor mixture. All experiments for Western blot were performed as previously described (16). Briefly, the sample proteins were electrophoretically separated by using 4–12% NuPAGE Novex Bis-Tris Mini Gels (Invitrogen) and were transferred to a nitrocellulose membrane for 1 h at 40 V by use of a Western blot apparatus (Invitrogen). The membrane was blocked for 2 h in 5% nonfat dry milk in TBST buffer (0.1 M Tris (pH 7.5), 0.15 M NaCl, 0.1% (vol/vol) Tween-20) at room temperature. The primary antibodies were incubated overnight at 4°C or 2 h at room temperature and then removed. The membrane was washed three (5 min) each with TBST. The membranes were then incubated with HRP-conjugated secondary and were detected by use of the SuperSignal chemiluminescence kit (Pierce). All immunoblots were scanned by using a model Fluorchem M imaging system (ProteinSimple, San Jose, CA). Densitometry analysis for the expression of proteins was performed with Imaged software. The protein levels were normalized for β-actin or total protein as appropriate.

Statistical analysis. For levels of gp130, Tyk2, and STAT3 expression in humans, the data were compared across groups by using a nonparametric Kruskal-Wallis analysis of variance with a Dunn’s posttest for multiple comparisons. Each cell culture-based study was performed in triplicate. For comparisons of protein expression and phosphorylation in these studies, an unpaired two-tailed t-test was used to compare groups. Significance was set at P < 0.05. There were no prior data to base power calculations before the study was started. After seven subjects were enrolled in each arm, the sample size needed to have a power of 80% for the observed differences was estimated. The decision to terminate the study was based on having achieved 80% or higher power to demonstrate the key differences in gp130.

Table 1. Demographic, clinical, and laboratory profile

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lean Normal (N = 8)</th>
<th>Obese Normal (N = 12)</th>
<th>NAFL (N = 12)</th>
<th>NASH (N = 12)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>51.9 ± 6.0</td>
<td>49.8 ± 11.0</td>
<td>49.9 ± 14.1</td>
<td>57.4 ± 10.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Men:women, n</td>
<td>4:4</td>
<td>2:10</td>
<td>4:8</td>
<td>5:7</td>
<td>n.s.</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>60</td>
<td>60</td>
<td>75</td>
<td>100</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1 ± 3.8</td>
<td>41.6 ± 6.3</td>
<td>37.1 ± 7.0</td>
<td>41.8 ± 5.4</td>
<td>&lt; 0.0001*</td>
</tr>
<tr>
<td>Type 2 diabetes mellitus, n</td>
<td>0</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0.02‡</td>
</tr>
<tr>
<td>AST, IU/l</td>
<td>24.0 ± 7.3</td>
<td>28.2 ± 9.5</td>
<td>30.8 ± 14.7</td>
<td>41.7 ± 17.8</td>
<td>&lt;0.005†</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>25.1 ± 16.6</td>
<td>49.4 ± 31.5</td>
<td>70.1 ± 44.4</td>
<td>80.1 ± 16.8</td>
<td>&lt;0.008†</td>
</tr>
<tr>
<td>Alk phos, IU/l</td>
<td>86.2 ± 21</td>
<td>90.9 ± 9</td>
<td>97.8 ± 18</td>
<td>101 ± 21</td>
<td>n.s.</td>
</tr>
<tr>
<td>Bilirubin, μg/dl</td>
<td>0.2 ± 0.08</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Albumin, mg/dl</td>
<td>4.2 ± 0.2</td>
<td>4.0 ± 0.4</td>
<td>3.9 ± 0.3</td>
<td>4.3 ± 0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fasting blood sugar, mg/dl</td>
<td>88 ± 8</td>
<td>95 ± 8</td>
<td>98 ± 11</td>
<td>99 ± 12</td>
<td>n.s.</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>6.0 ± 2</td>
<td>16.2 ± 9</td>
<td>20.1 ± 11</td>
<td>22 ± 7</td>
<td>&lt;0.005*</td>
</tr>
<tr>
<td>Hemoglobin A1C, %</td>
<td>5.0 ± 0.2</td>
<td>6.1 ± 1.3</td>
<td>6.2 ± 2.1</td>
<td>6.5 ± 1.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>194.9 ± 20.4</td>
<td>190.0 ± 51.1</td>
<td>206.6 ± 70.7</td>
<td>216.0 ± 28.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>LDL-cholesterol, mg/dl</td>
<td>108.1 ± 21.4</td>
<td>116.6 ± 38.5</td>
<td>109.8 ± 57.3</td>
<td>139.8 ± 78.7</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>HDL-cholesterol, mg/dl</td>
<td>55.6 ± 15.4</td>
<td>50.0 ± 7.6</td>
<td>43.6 ± 13.7</td>
<td>43.6 ± 8.8</td>
<td>&lt;0.05†</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>99.1 ± 40.9</td>
<td>102.8 ± 48.2</td>
<td>171.8 ± 81.6</td>
<td>211.3 ± 44.7</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Values are means ± SD. BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alk phos, alkaline phosphatase. *Lean vs. other groups; †NAFL or NASH vs. either control group; ‡χ² for trend; n.s., not significant.
RESULTS

The gp130-Tyk2-STAT3 pathway is suppressed in obese controls without steatosis but enhanced in subjects with NAFL or NASH. A total of 12 subjects each with nonalcoholic fatty liver (NAFL) and NASH without bridging fibrosis or cirrhosis were studied and compared with 8 lean normal control subjects and 12 obese weight-, age-, gender-, and race-matched controls without NAFLD. The summaries of demographic, clinical, and laboratory data are shown in Table 1. The gp130 and Tyk2 expression were significantly lower in obese controls compared with lean normal subjects (Fig. 1A). Tyk2 and STAT3 phosphorylation (p-Tyk2 and p-STAT3, respectively) was virtually undetectable in obese control subjects. In terms of gp130 expression, two distinct patterns were noted in subjects both with NAFL and with NASH. The expression was high in 7/12 subjects with NAFL and 7/12 subjects with NASH whereas it was undetectable in the rest (Fig. 1B). Overall, both NAFL and NASH still had significantly higher gp130 expression compared with obese controls as well as lean controls (Fig. 1B, *P* < 0.01 for both NAFL and NASH vs. either control). Similarly, there was also greater Tyk2 and STAT3 phosphorylation in NAFL.

![Western blot data of representative samples are shown in A. B demonstrates data for individual groups with mean shown as horizontal line. Mean (± SD) IL-6 levels in subjects with NAFL and NASH were significantly higher than in lean and obese controls combined (C, *P* < 0.01). Gp130 expression was closely related to IL-6 levels (r = 0.44, *P* < 0.01) (D). **P* < 0.01. OD, optical density.](http://ajpgi.physiology.org/)

Fig. 1. The expression of gp130 and its downstream signaling elements Tyk2 and STAT3 was examined in subjects with nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH) (*n* = 12 each) and compared with lean (*n* = 8) and weight-matched controls (*n* = 12) with normal liver histology. Obese controls had decreased gp130, p-Tyk2, and p-STAT3 expression whereas these were increased in both NAFL and NASH compared with lean controls (A and B; *P* < 0.01). Western blot data of representative samples are shown in A. B demonstrates data for individual groups with mean shown as horizontal line. Mean (± SD) IL-6 levels in subjects with NAFL and NASH were significantly higher than in lean and obese controls combined (C, *P* < 0.01). Gp130 expression was closely related to IL-6 levels (r = 0.44, *P* < 0.01) (D). **P* < 0.01. OD, optical density.
and NASH compared with controls ($P < 0.01$ for both NAFL and NASH vs. either control, Fig. 1B).

*gp130 expression is directly related to circulating IL-6 in NAFLD.* To further determine the potential factors driving the expression and activation of the gp130-STAT3 axis in NAFLD, the levels of circulating IL-6 was compared in controls vs. those with NAFL or NASH. Both those with NAFL and those with NASH had significantly increased levels of IL-6 compared with controls (Fig. 1C, $P < 0.01$). IL-6 levels were directly related to gp130 expression in these subjects (Fig. 1D, $r^2 = 0.44, P < 0.01$).

Palmitate inhibits gp130 protein and STAT3 signaling pathway in hepatocytes. Human hepatoma cells (Huh-7) were cultured in the presence of normal (5.5 mM) or high glucose (30.0 mM) concentration with or without 0.5 mM palmitate up to 12 h. Palmitate produced a modest and nonsignificant downregulation of both gp130 and phosphorylated STAT3 (Y705) proteins at 4 h (Fig. 2, A and B) and completely suppressed both gp130 expression and STAT3 (Y705) phosphorylation at 12 h (Fig. 2, A and B; $P < 0.01$). Of note, serine phosphorylation of STAT3 (S727) remained unchanged at both 4 and 12 h. Total STAT3 and Tyk2 also decreased after 12 h of exposure to palmitate (Fig. 2, A and B; $P < 0.01$). These data demonstrate that palmitate inhibits the STAT3 signaling pathway in a time-dependent manner.

Palmitate attenuates IL-6 effects on the gp130-STAT3 axis. Next, the interactions between IL-6, a cytokine that activates gp130 and is produced in hepatocytes, and palmitate were studied in Huh-7 cells. Regardless of ambient glucose levels (N, normal: 5.5 mM glucose; H, high: 30 mM), palmitate (0.5 mM) produced a time-dependent inhibition of gp130, Tyk2, and tyrosine-phosphorylated p-STAT3 (Y705), whereas the serine-phosphorylated p-STAT3 (S727) did not change significantly (A and B; $P < 0.01$ baseline vs. 12 h). IL-6 increased gp130 (C and D; $P < 0.05$) and p-STAT3 compared with controls (C and D; $P < 0.01$) whereas palmitate inhibited their expression (C and D; $P < 0.01$). Palmitate inhibited IL-6-mediated increase in p-STAT3 (C and D; $P < 0.01$). AR-42, a gp130 antagonist, directly inhibited gp130 in a dose-dependent manner (C, top) and a dose-dependent blockade of IL-6 mediated STAT3 phosphorylation (C, bottom, and D; $P < 0.01$ for 1 $\mu$M). Means $\pm$ SD from 3 independent experiments are shown for all graphical data. *$P < 0.05$; **$P < 0.01$. 

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Fig. 3. Palmitate-induced inhibition of STAT3 is independent of JNK, NF-κB, and ERK activation. Prior treatment of cells with a JNK inhibitor SP600125 (A and B), IKK2 inhibitor (iIKK2, C and D), or ERK inhibitor PD98059 (E and F) did not affect palmitate-induced suppression of the gp130-STAT3 axis. Palmitate-induced inhibition of STAT3 phosphorylation is thus independent of these pathways. Means ± SD from 3 independent experiments are shown for all graphical data. PA, palmitate; SP, SP600125; PD, PD98059.
studied. Palmitate inhibited the gp130-STAT3 axis whereas IL-6 activated the axis as expected (Fig. 2, C and D; P < 0.01 for palmitate and P < 0.05 for IL-6). Palmitate also attenuated the IL-6-mediated activation of STAT3 (Fig. 2, bottom, lane 4, P < 0.01). The specificity of these effects for the gp130-STAT3 pathway was confirmed by abrogation of the IL-6 effects by the gp130 antagonist AR-42 (31) (P/H11021 0.01 IL-6 vs. IL-6 + AR42, Fig. 2, C, top, and D).

**Palmitate-induced inhibition of STAT3 is independent of JNK, NF-κB, and ERK activation.** Serum-starved cells in DMEM medium were incubated with specific MAPK- and IKK2 inhibitors 1 h before treatment with 0.5 mM palmitate for 8 h. Palmitate increased the phosphorylation of JNK, NF-κB, and ERK compared with controls (Fig. 3, A–F, P/H11021 0.01 for all three targets). As expected, pretreatment with JNK, IKK2, and ERK inhibitors before palmitate exposure significantly reduced phosphorylation of each of the corresponding proteins compared with palmitate-treated controls (Fig. 3, A–F). Neither palmitate-mediated changes in Tyk2 levels nor STAT3 phosphorylation were significantly affected by inhibition of NF-κB, JNK, or ERK. These data demonstrate that palmitate-induced inhibition of STAT3 phosphorylation is independent of these pathways.

**The potential role of STAT3 in IL-6 and palmitate-induced impairment in insulin signaling.** Hepatic insulin resistance is considered to be a key pathophysiological factor in the genesis of NAFLD (14, 24). IL-6 increased p-STAT3 (Y705) and also inhibited insulin-mediated p-Akt (Fig. 4, A and B; P/H11021 0.01 for both targets). These effects were abrogated by pretreatment with the STAT3 inhibitor S31-201 (Fig. 4, A, lanes 3 vs. 5, and B; P < 0.01 for both targets). On the other hand, palmitate reduced IL-6-mediated STAT3 phosphorylation yet still had an additive effect with IL-6 suppression of insulin-mediated Akt-phosphorylation (Fig. 4, A, lane 6, and B; P < 0.01 for both targets). Interestingly, STAT3 inhibition even in the absence of IL-6 increased insulin-mediated p-Akt levels to levels greater than those seen with insulin alone (Fig. 4, A, lane 4, and B; P < 0.01). Inhibition of STAT3 prior to palmitate exposure also

![Image](https://example.com/figure4.png)

Fig. 4. The effects of IL-6 and palmitate on hepatic insulin signaling were evaluated in Huh-7 cells. IL-6 increased p-STAT3 and inhibited insulin-stimulated Akt phosphorylation (A and B). Administration of a STAT3 inhibitor S31-201 prior to insulin (INS) exposure significantly increased p-Akt to levels greater than those seen with insulin alone (A and B; P < 0.01). It also reduced IL-6-mediated suppression of insulin-stimulated Akt phosphorylation (A, lane 5, and data shown graphically in B). Palmitate and IL-6 had an additive suppressive effect on insulin-stimulated Akt phosphorylation (A, lane 6, and graphical representation in B). Next, the role of STAT3 signaling in palmitate-induced decrease in insulin signaling was studied (C and D). Suppression of STAT3 abrogated palmitate-induced suppression of insulin-mediated Akt phosphorylation (C, lane 4 and D; P < 0.01). Means ± SD from 3 independent experiments are shown for all graphical data. *P < 0.05; **P < 0.01.
restored p-Akt levels to those seen with insulin alone (Fig. 4, C and D; P < 0.01).

**IL-6 increases autophagy and improves ER stress via STAT3.** Impaired autophagy and induction of endoplasmic reticulum (ER) stress are important pathophysiological factors in the genesis of NAFLD (2, 19). Palmitate induced ER stress as assessed by phosphorylation of the eukaryotic inhibitory factor-2 (p-eIF-2α) and modestly suppressed ATG7 a marker of autophagy (Fig. 5, A and B; P < 0.01). It also activated poly-(ADP-ribose) polymerase (PARP) cleavage, indicating activation of apoptosis. IL-6, in concentrations seen in insulin-resistant states (20 ng/ml) (20), had no significant effect on PARP cleavage or p-eIF-2α levels while increasing ATG7 (Fig. 5, C and D; P < 0.01).

To directly assess whether STAT3 affected these pathways known to be relevant for NASH, the effects of both gain and loss of function were studied in Huh-7 cells. STAT3 overexpression had no significant effects on palmitate-induced PARP cleavage (Fig. 6, A and B). It, however, inhibited palmitate-induced increase in p-eIF-2α (Fig. 6B, P < 0.01) except at very high MOI 1 where p-eIF-2α was increased. STAT3 overexpression also produced a dose-dependent increase in ATG7 in the presence of palmitate (Fig. 6, A and B; P < 0.01) and reversed palmitate-induced ATG7 suppression. Conversely, inhibition of STAT3 with pretreatment with S31-201 produced a dose-dependent significant decrease in ATG7 (Fig. 6, C and D; P < 0.01).

**DISCUSSION**

The present study demonstrates increased expression and activation of the gp130-STAT3 axis in the majority of subjects.
with either NAFL or NASH and links it to the levels of IL-6 in this population. Gp130-mediated signaling is a key component of the cellular response to metabolic stress and injury and affects cell survival and inflammation (12). These effects are largely mediated via STAT3, which has also been linked to inflammation and cancer (30). NAFLD, especially NASH, is associated with substantial metabolic stress, inflammation, and an increased risk of hepatocellular cancer (15, 29). It is therefore certainly within the realm of possibility that activation of the gp130-STAT3 axis plays a role in modulating development and progression of NASH.

It is interesting to note that weight-matched obese controls without NAFLD not only have less insulin resistance but also lower IL-6 levels and very low levels of gp130 expression. Gp130 levels may be decreased by increased turnover due to palmitoylation; however, existing literature suggests that palmitoylation in fact protects proteins from proteosomal degradation (6). The mechanism for decreased gp130 in obese subjects without NAFLD is thus unknown and is an area of future research.

Several lines of evidence suggest that IL-6 is the principal driver of increased gp130 expression in NAFLD. These include the elevation of IL-6 in subjects with NAFL or NASH compared with either lean or obese controls (Fig. 1C), the direct relationship between IL-6 and gp130 expression (Fig. 1D), the IL-6-mediated increase in gp130 in vitro (Fig. 2C),

Fig. 6. The effects of STAT3 on palmitate lipotoxicity are shown. STAT3 was overexpressed by using an adenovirus vector (Ad-STAT3). Compared with controls (Ad-GFP), STAT3 overexpression did not affect palmitate-induced PARP cleavage (A and B). However, it produced a dose-dependent blockade of palmitate-induced activation of p-eIF2α (A and B; P < 0.01). It also produced a dose-dependent increase in ATG7 (A and B; P < 0.01). The effect of inhibition of STAT3 by the S31-201 was studied next (C and D). STAT3 inhibition was associated with decreased ATG7 (P < 0.01) without significant effects on cPARP or caspase 3 (C and D). Means ± SD from 3 independent experiments are shown for all graphical data; **P < 0.01. MOI, multiplicity of infection.
and the well-known effect of IL-6 as a gp130 cytokine (8). It is also interesting to note that all subjects with NAFLD did not have increased expression of gp130 and in fact had levels similar to obese controls. The IL-6 levels in these subjects were lower than in those with increased gp130.

The present study also provides insights on the effects of STAT3 on insulin signaling and the cross talk between lipotoxic stress and IL-6 in mediating impairment of hepatic insulin signaling. IL-6 activated p-STAT3 and impaired insulin-mediated Akt phosphorylation, and these effects could be blocked by pretreatment with a p-STAT3 inhibitor S31-201, indicating that this effect was mediated by p-STAT3. Palmitate inhibited p-STAT3 (the tyrosine-phosphorylated form Y705) but also impaired insulin-mediated Akt phosphorylation (Fig. 4, A–D). These data are compatible with the well-known palmitate-induced activation of other pathways such as JNK, etc., that also inhibit insulin signaling (9, 10). Thus IL-6 and palmitate contribute to impaired hepatic insulin signaling via STAT3-dependent and independent pathways, respectively.

Our data further suggest that STAT3 signaling provides an inhibitory control for Akt phosphorylation because, even in the absence of a gp130 agonist, inhibition of STAT3 raises insulin-mediated Akt phosphorylation to levels greater than those seen with insulin alone (Fig. 4C). Also, the abrogation of palmitate-induced suppression of insulin-mediated Akt phosphorylation by a STAT3 inhibitor suggests that this pathway is important for palmitate effects on Akt phosphorylation. STAT3 also had several effects that are expected to improve NASH. Autophagy has been demonstrated to be an important cellular mechanism to prevent steatosis, and decreased autophagy has been associated with increased steatosis. Similarly, continued activation of the unfolded protein response has been associated with inflammation, apoptosis, and disease progression in NASH (19). STAT3 increased ATG7 and inhibited palmitate-induced eIF-2α phosphorylation (Fig. 6, A and B); both of these effects would be expected to ameliorate NASH. These data provide a rationale to target STAT3 for the treatment of NASH. One must, however, remain cognizant of the potential for unrestrained STAT3 activation to promote inflammation and cancer (3, 30).

There are, however, some limitations of the present study and other open questions that can now be raised. The nature of the study precluded assessment of the specific hepatic cell type in which gp130 expression was altered in subjects with NAFLD; our studies in Huh-7 cells suggest hepatocytes are the likely cell type involved but these data are not definitive. Also, long-term prospective studies are now needed to evaluate whether STAT3 activation drives future development of hepatocellular cancer in humans with NAFLD.

In summary, the present study describes activation of the gp130-STAT3 axis in humans with NAFLD and demonstrates that, whereas palmitate and IL-6 have opposing effects on gp130 expression and signaling, they have additive effects on hepatic insulin signaling due to differing downstream signaling mechanisms that converge on Akt phosphorylation. It also demonstrates that palmitate inhibits autophagy by inhibiting the gp130-STAT3 axis. These data open the door for future investigations on the relevance of this pathway for disease progression in NASH, which may provide a rationale for targeting this pathway to prevent disease progression and development of hepatocellular cancer.

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DISCLOSURES

A. J. Sanay has stock options in Genfit. He has served as a consultant to AbbVie, Astra Zeneca, Nitto Denko, Nimbus, Salix, Tobira, Takeda, Fibrogen, Immuron, Exhalenz, and Genfit. He has been an unpaid consultant to Intercept and Echocons. His institution has received grant support from Gilead, Salix, Tobira, and Novartis. None of these are related to the present study.

AUTHOR CONTRIBUTIONS


REFERENCES


