Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies

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Hwang J-H, Stein DT, Barzilai N, Cui M-H, Tonelli J, Kishore P, Hawkins M. Increased intrahepatic triglyceride is associated with peripheral insulin resistance: in vivo MR imaging and spectroscopy studies. Am J Physiol Endocrinol Metab 293: E1663–E1669, 2007. First published October 2, 2007; doi:10.1152/ajpendo.00590.2006.—Recent studies have indicated that the mass/content of intramyocellular lipid (IMCL), intrahepatic triglyceride (IHTG), visceral fat (VF), and even deep abdominal subcutaneous fat (SF) may all be correlated with insulin resistance. Since simultaneous measurements of these parameters have not been reported, the relative strength of their associations with insulin action is not known. Therefore, the goals of this study were 1) to simultaneously measure IMCL, IHTG, VF, and abdominal SF in the same nondiabetic individuals using noninvasive 1H-magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) and 2) to examine how these fat stores are correlated with systemic insulin sensitivity as measured by whole body glucose disposal (Ra) during euglycemic-hyperinsulinemic clamp studies. Positive correlations were observed among IMCL, IHTG, and VF. There were significant inverse correlations between whole body Ra and both IMCL and VF. Notably, there was a particularly tight inverse correlation between IHTG and whole body Ra (r = −0.86, P < 0.001), consistent with an association between liver fat and peripheral insulin sensitivity. This novel finding suggests that hepatic triglyceride accumulation has important systemic consequences that may adversely affect insulin sensitivity in other tissues.

Recent technical advances in in vivo magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) have made it possible to accurately and noninvasively quantify tissue fat content in humans. Quantitative data from these methods have been validated against both biochemical analysis and morphometry (13, 41), and their noninvasive nature makes them very practical for human research. Sophisticated 1H-MRS of muscle lipid accumulation allows researchers to distinguish intramyocellular lipid (IMCL) deposition from intercellular triastring (17, 20, 24, 31, 41). This is a pragmatic advantage over muscle biopsy, in which manual separation of extra-myocellular lipids often results in contamination. With the use of this 1H-MRS method, it has recently been shown that IMCL is associated with insulin resistance in both nondiabetic and T2DM humans (17, 20, 24, 31, 41). However, it remains to be determined whether muscle triglyceride accumulation is merely a marker of other metabolic abnormalities or whether it plays a causal role in the development of insulin resistance.

Although liver biopsy is considered the “gold standard” for measuring intrahepatic triglycerides (IHTG), X-ray computerized tomography (CT) scanning and localized 1H-MRS offer noninvasive alternatives. MRS appears to provide a more sensitive measure of hepatic fat content than X-ray CT (5, 6, 11), facilitating accurate measurements even in nonobese subjects with very low liver fat content. Given the low sensitivity of X-ray CT, previous correlative studies between liver fat content and insulin sensitivity were often limited to subjects with T2DM or subjects with high levels of IHTG (5, 6, 11). Since insulin action can be impacted by hepatic dysfunction secondary to triglyceride accumulation (i.e., steatosis) (23), it would be helpful to determine whether less marked accumulation of IHTG is also linked with insulin resistance.

Questions of interest include the relative strength of associations between various tissue fat deposition patterns and insulin resistance (9) and why an individual might preferentially deposit more triglyceride in a given tissue. Since visceral (VF) and deep subcutaneous fat (SF) depots may contribute to IHTG via hepatic delivery of free fatty acids (FFA) and adipose-derived peptides (“adipokines”), it would be helpful to independently measure VF and SF and determine their association with insulin resistance. Therefore, the aims of the study were 1) to simultaneously measure IMCL, IHTG, VF, and SF in the same nondiabetic individuals using state-of-the-art 1H-MRS and MRI technology and 2) to comprehensively examine how each of these fat stores are correlated with insulin sensi-

DECREASED INSULIN SENSITIVITY is a strong predictor for type 2 diabetes mellitus (T2DM) (28). Indeed, T2DM is associated with defects in the ability of insulin both to stimulate skeletal muscle glucose uptake (“peripheral” insulin resistance) and to suppress endogenous glucose production (“hepatic” insulin resistance) (12). Previous studies have indicated that abnormal accumulation of lipid in skeletal muscle, liver, and abdominal fat depots is associated with insulin resistance (5, 10, 17, 19, 20, 25, 29, 31, 34, 41, 41a). In addition to increased circulating levels of free fatty acids, intracellular accumulation of fatty acids and their metabolites may contribute to the pathogenesis of insulin resistance (31). Of note, abnormal lipid accumulation in skeletal muscle and liver was shown to be associated with insulin resistance in studies involving a wide variety of subjects (5, 10, 17, 19, 20, 25, 29, 31, 34, 41, 41a). However, given the lack of parallel measurements in the same individuals, it is not known how strongly each pattern of lipid accumulation is associated with insulin resistance.

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Fat content quantified using MRI and MRS, insulin sensitivity (Rd), and basal circulating plasma leptin levels during hyperinsulinemic-euglycemic clamps

Table 1. Fat content quantified using MRI and MRS, insulin sensitivity (Rd), and basal circulating plasma leptin levels during hyperinsulinemic-euglycemic clamps

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Race</th>
<th>Sex</th>
<th>BMI, kg/m²</th>
<th>Basal Insulin, mU/I</th>
<th>Basal Glucose, mg/dl</th>
<th>IMCL-Sol, mmol/kg</th>
<th>IMCL-TA, mmol/kg</th>
<th>IHTG, mmol/kg</th>
<th>Rd, mg/kg·min</th>
<th>SF L3-L4, g*</th>
<th>VF L3-L4, g*</th>
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<tr>
<td>1</td>
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<td>6</td>
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<td>3.9</td>
<td>3.8</td>
<td>3.2</td>
<td>12.1</td>
<td>403</td>
<td>122</td>
</tr>
<tr>
<td>2</td>
<td>Caucasian</td>
<td>F</td>
<td>24.0</td>
<td>4</td>
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</table>

MRI, magnetic resonance imaging; MRS, MR spectroscopy; Rd, glucose disposal; M, male; F, female; BMI, body mass index; IMCL, intramyocellular lipid; IHTG, intrahepatic triglyceride; Sol, soleus; TA, tibialis anterior; SF, subcutaneous fat; VF, visceral fat. *Fat content in a 6-cm slab located in L3–L4.

RESEARCH DESIGN AND METHODS

Subjects

All subjects were nondiabetic by standard 75-g oral glucose tolerance tests and had normal liver enzymes and function as reflected by plasma levels of alanine aminotransferase and aspartate aminotransferase and prothrombin time/partial thromboplastin time (PT/PTT). Twelve nondiabetic subjects (age, 34.0 ± 15.7 yr old; 10 males, 2 females; body mass index = 25.6 ± 3.2 kg/m²) were studied (Table 1). Reported physical activity levels varied from sedentary to less than two 12-oz glasses of beer per week. All subjects were nondiabetic by standard 75-g oral glucose tolerance tests and had normal liver enzymes and function as reflected by plasma levels of alanine aminotransferase and aspartate aminotransferase and prothrombin time/partial thromboplastin time (PT/PTT).

MR Methods

All MR measurements were performed using a 1.5 T GE Signa MR scanner (GE Medical Systems). Before MR spectroscopic measurements, fast spin-echo MR images [relaxation time (TR)/echo time (TE) = 400/8 ms] were obtained of the calf muscles and the abdomen (including the liver). Abdominal imaging is thoroughly described in MRI for abdominal fat, below. Three 1H-MRS measurements [IHTG, IMCL (soleus; Sol), and IMCL (tibialis anterior; TA)] were obtained from all 12 subjects. The quantification was achieved using an internal water reference after correcting the T1 and T2 relaxation times as previously described (41).

IHTG content. IHTG content was obtained with water-suppressed 1H-MRS using single-voxel stimulated acquisition mode (STEAM) (TR/TE = 5,000/12 ms) with a GE body coil. Typical voxel size was ~35 ml.

IMCL content of Sol and TA. The right calf was positioned in a linear GE calf coil. Single-voxel STEAM sequence (TR/TE = 2,000/24 ms) was used. Typical voxel size was 1.5–2.5 ml.

MRI for abdominal fat. On the basis of quick coronal scout images of the abdomen, T1-weighted sagittal images were acquired using a fast spin-echo sequence (TR/TE = 400/8 ms). To obtain abdominal fat from an anatomically consistent area in all subjects, sagittal MRI of the vertebral column served as a marker (Fig. 1A). Transverse MRI (TR/TE = 400/8 ms, slice thickness = 0.4 cm, gap = 0.6 cm) images were also acquired by fast multislice spin echo, and a slice containing liver regions was employed as a localization for 1H-MRS study of liver. In all subjects, SF and VF content in the periumbilical region of L3-L4 (in a 6-cm slab) was quantified from the transverse MRI using imaging processing functions in Adobe Photoshop. To minimize spatial distortion induced by Z-gradient nonlinearity, a multiblock T1 spin-echo imaging method was used. Thus a block for MRI to locate liver and umbilicus area was separately performed by moving the MR table to keep the volume of interest centered in the magnet. To

Fig. 1. A: sagittal image of abdomen, which was used to set the landmark to select location for subcutaneous and visceral fat analysis. Lumbar spine L3–L4 denoted. B: transverse image of abdomen. Subcutaneous fat and visceral fat were separated (bottom), and the content was quantified after converting those images to black-and-white binary images.
minimize breathing artifacts, a respiratory gating mode was employed. Fat content from MRI was obtained using a threshold method. In brief, two compartments of fat were separated as shown in Fig. 1B. A threshold for each compartment was set to separate fat and nonfat regions, and then the gray-scaled image was converted to a binary image to obtain fat volumes. Conversion from fat volume to mass was calculated as previously described (1).

Spectral Processing and Quantification

After transfer of the MR data from a GE Signa MRI console onto a personal computer, offline processing of MR spectra was done using the NUTS program (Acorn NMR). All peak areas were obtained using a consistent fitting routine. For lipid quantity, the signal from middle chain methylene groups (-CH2-, 1.3 ppm) was evaluated relative to water signals in the same voxel. Assumptions of 80% water in muscle and 72% water in liver were made for quantification based on established methodology (41).

Euglycemic-Hyperinsulinemic Clamp Studies

All subjects were fasted overnight and underwent a 5-h constant insulin (40 mU/m2.min) infusion the next morning, using a variable infusion of 20% dextrose to maintain euglycemia.

Two intravenous cannulas were established in each subject, one for infusions and a second retrograde cannula in a dorsal vein of the opposite arm for blood sampling. To obtain arterialized venous blood samples, this arm was maintained at 65°C in a thermoregulated Plexiglas box. At time t = 0, primed continuous infusions of insulin were initiated with a bolus of 80 mU/m2.min for 10 min and then maintained at a rate of 40 mU/m2.min for 5 h (18). This insulin infusion rate was selected to optimally assess peripheral insulin sensitivity, as measured by whole body glucose disposal (Rd). Primed continuous infusions of HPLC-purified [3-3H]glucose (21.6-μCi bolus; 0.15 μCi/min) were maintained for the entire study to quantify glucose turnover, specifically rates of peripheral Rd and endogenous glucose production. Plasma glucose was measured every 5 min in duplicate by a Beckman glucose analyzer, and variable 20% dextrose infusions were used to maintain euglycemia (5 mM) throughout the studies.

Analytic Procedures

Details of analytic procedures were previously described (18). Plasma glucose was measured with a Beckman glucose analyzer (Fullerton, CA) by use of the glucose oxidase method. Glucose turnover was estimated using the steady-state equation of Steele (18). Plasma levels of leptin and other adipokines were measured by immunoassay (ELISA kits; Linco Research, St. Charles, MO). Given the technical obstacles related to conversion to a new methodology and poor quality of prior samples, only eight samples (7 male, 1 female) could be analyzed for leptin with consistent methods.

Statistical Analyses

Data presented are expressed as means ± SD unless otherwise stated. All statistical analyses were performed using SigmaSTAT (SPSS). To test the strength of correlations, the Pearson product moment was employed. A P value <0.05 was considered to be statistically significant.

RESULTS

Figures 1 and 2 show MR spectra acquired from liver and skeletal muscles (Sol and TA). The content of IHTG, IMCL.

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Fig. 2. A: transverse image showing the liver and the voxel where spectroscopic measurement was obtained. 1H-magnetic resonance (MR) spectrum from the liver clearly shows the methylene peak of triglycerides (TG). B: 1H-MR spectrum from soleus (Sol) muscle from a subject. C: 1H-spectrum from tibialis anterior (tibialis ant.; TA) muscle. Intramyocellular lipid (IMCL), total creatine (tCr), and extramyocellular lipid (EMCL) peaks are clearly shown.
VF, and SF was quantified in all 12 subjects. Intrahepatic and myocellular lipids and VF and SF content from the region L3 and L4 are summarized in Table 1, together with Rd. All correlations between various fat contents and Rd are summarized in Table 2.

Correlations Among Different Measures of Fat Content

The mean contents of IHTG, IMCL-Sol, and IMCL-TA were 12.5 ± 9.5, 8.8 ± 8.3, and 3.0 ± 1.3 mmol/kg, respectively. These values are consistent with previous reports showing that oxidative muscle fibers (Sol) contain higher IMCL than glycolytic muscle fibers (TA) by biopsy and 1H-MRS (15, 31). IHTG and IMCL-Sol showed a significant positive correlation (r = 0.86, P < 0.001; Fig. 3A), and the accumulation of triglyceride was in a similar range in both tissues (IHTG/IMCL-Sol ≈ 1.4). However, there were no correlations between IHTG and IMCL-TA measurements.

We consistently observed correlations among IMCL-Sol, IHTG, and VF. There were significant positive correlations between IMCL-Sol and VF (r = 0.86, P < 0.001), between IHTG and VF (r = 0.86, P < 0.001), and between IMCL-Sol and IHTG (r = 0.88, P < 0.001).

Relationships Between Fat Content and Insulin Sensitivity

As shown in Fig. 4, Rd (i.e., peripheral glucose uptake) measured at peak insulin stimulation during euglycemic-hyperinsulinemic clamp studies showed a strong and inverse correlation with IHTG (r = −0.86, P = 0.0003). Additionally, there were significant, although less striking, inverse correlations between IMCL and Rd (r = −0.60, P = 0.03) and between VF and Rd (r = −0.64, P = 0.03; Table 2). The latter findings are consistent with previous reports (5, 11, 20).

Relationships Between Plasma Levels of Cytokines and Tissue Fat Content

Table 3 shows correlations of plasma levels of several fat-derived proteins and cytokines with IHTG content and insulin sensitivity (Rd). Of interest, interleukin-6 (IL-6) and IHTG were positively correlated (r = 0.66, P = 0.038). Plasma resistin levels were inversely correlated with IHTG, consistent with previous observations (4). Given the technical challenges and the exclusion of female subjects because of the striking effect of gender on leptin levels, analysis of correlations with leptin was limited to a subset of seven male subjects. Within those subjects, a remarkably tight correlation was observed between IHTG and plasma leptin levels (r = 0.98, P < 0.0001).

DISCUSSION

These studies compare for the first time IMCL, IHTG, and visceral fat in the same nondiabetic human subjects using state-of-the-art MR techniques to accurately measure fat con-

Table 2. Correlations between various components of fat content and insulin sensitivity (Rd)

<table>
<thead>
<tr>
<th></th>
<th>Leptin</th>
<th>IHTG</th>
<th>IMCL-Sol</th>
<th>IMCL-TA</th>
<th>VF</th>
<th>SF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rd</td>
<td>−0.84</td>
<td>−0.86</td>
<td>−0.60</td>
<td>NS</td>
<td>−0.64</td>
<td>NS</td>
</tr>
<tr>
<td>Leptin</td>
<td>0.983</td>
<td>0.84</td>
<td>NS</td>
<td>0.84</td>
<td>0.64</td>
<td>0.737</td>
</tr>
<tr>
<td>IHTG</td>
<td>0.84</td>
<td>−0.64</td>
<td>0.86</td>
<td>NS</td>
<td>0.61</td>
<td>NS</td>
</tr>
<tr>
<td>IMCL-Sol</td>
<td>0.84</td>
<td>0.86</td>
<td>−0.64</td>
<td>0.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VF</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>SF</td>
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</tbody>
</table>

Data are expressed as r, correlation coefficient (P value). NS, not significant.
tent along with a comprehensive analysis of in vivo insulin action. IHTG showed a very strong inverse correlation with peripheral insulin sensitivity, expressed as Rd. Although both IMCL and visceral fat were also significantly correlated with peripheral insulin resistance in this group of subjects, the relationship between IHTG and peripheral insulin resistance was particularly striking.

Increased hepatic fat content is receiving particular attention as a potential correlate with, and perhaps causative factor of, insulin resistance. In patients with stable T2DM, percent hepatic fat was the parameter most highly correlated with daily insulin dose (38). Nonobese nondiabetic subjects with biopsy-proven nonalcoholic fatty liver disease demonstrated ~50% reductions in both insulin-mediated suppression of hepatic glucose production and insulin-stimulated Rd (23). In fact, they were as insulin resistant as subjects with T2DM. Indeed, while associations between IHTG and hepatic insulin resistance are well established, the association with peripheral insulin resistance in this study is particularly novel. Since liver pathology can impact insulin action (33), we examined these associations in healthy nonobese individuals, and thus the associations with peripheral insulin resistance are particularly striking. Although the IHTG levels in these subjects were substantially lower than levels previously observed in T2DM, the inverse correlation of IHTG with insulin sensitivity was exceptionally tight. This was presumably aided by the sensitivity of measurements using in vivo 1H-MRS and insulin “clamp” methodologies. There was also a wide range of insulin action among these nonobese subjects, facilitating these correlations.

These observations in humans are consistent with intriguing studies in rodents in which decreasing IHTG levels improved muscle insulin action (2). In addition, liver-specific enhancement of fatty acid oxidation was associated with increased insulin-stimulated peripheral glucose uptake, even though IMCL actually increased in some muscle types. It has been proposed that the liver releases a humoral factor that either sensitizes skeletal muscle to insulin or has direct insulin-like effects (20a). Indeed, raw liver extracts increase glucose uptake into isolated rat hindquarter (32). Increased hepatic fat accumulation might interfere with the production/release of this factor, resulting in impaired peripheral insulin action. Another explanation might be that increased IHTG could impair hepatic insulin clearance (16), and secondary increases in insulin levels could downregulate insulin action. However, fasting plasma insulin levels did not correlate with IHTG in our subjects.

Additionally, chronic inflammation of the liver secondary to triglyceride infiltration could increase production of factors that cause systemic insulin resistance. Transgenic activation of the inflammatory mediators IKK-β and nuclear factor-κB (NF-κB) in the liver induced systemic insulin resistance as well as increased circulating levels of IL-6 and upregulated IL-6 target genes in muscle (8). Use of antibodies to neutralize circulating IL-6 normalized IL-6 target gene expression and reversed the insulin resistance. Importantly, similar activation of hepatic IKK-β and enhanced IL-6 production were seen in mouse models of obesity and fatty liver, indicating the potential relevance of this model to increased IHTG accumulation in humans. Of note, IL-6 levels were positively correlated with IHTG in our studies.

Table 3. Correlations between Cytokines and intrahepatic TG and Rd

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Average Value</th>
<th>Correlation With IHTG</th>
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<tr>
<td>IL-6</td>
<td>14.92±4.55</td>
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<tr>
<td>Resistin</td>
<td>7.89±1.25</td>
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<td>Adiponectin</td>
<td>9.155±1.734.3</td>
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<td>0.054</td>
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<tr>
<td>TNF-α</td>
<td>1.99±0.31</td>
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<tr>
<td>MCP</td>
<td>147.86±21.69</td>
<td>–0.345</td>
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</table>

TNF, tumor necrosis factor; MCP, macrophage chemoattractant protein. *Significance.
levels and peripheral insulin sensitivity approached significance \((P = 0.069)\). IL-6 has been shown to cause insulin resistance (39) as well as increase lipolysis, therefore contributing to the increased FFA levels in obesity (27). Raising circulating FFA for only 4 h doubled liver diacylglycerol levels and rapidly activated the proinflammatory NF-\(\kappa\)B pathway and cytokine expression in rat liver (7), suggesting that IHTG is not inert but impacts hepatic fat metabolism.

In contrast to the strong relationship between IHTG and insulin resistance, the inverse correlations of both IMCL and visceral fat with peripheral insulin action were significant but considerably less strong. The difference in strengths of association is more apparent when comparing the “explained variability” of the correlations, i.e., the \(r^2\) values. Thus, while the correlation with liver fat explains \(\approx 74\%\) of the variability in whole body \(R_a\) in this model, the correlation with visceral fat accounts for \(\approx 41\%\), and the correlation with IMCL accounts for only \(\approx 36\%\) of the variability. Indeed, studies examining the relationship between IMCL and insulin action have uncovered many interesting paradoxes, including increased hepatic triglycerides in muscle of highly trained athletes (43) and optimal insulin therapy (3). It has been suggested that triglyceride may not have adverse metabolic consequences in muscle provided that lipid utilization is efficient (21), and accumulation of intracellular triglyceride may protect against toxic effects of fatty acids (22). Of note, there may be a more consistent relationship between peripheral insulin resistance and skeletal muscle accumulation of long-chain fatty acyl-CoA rather than triglyceride (37).

The etiology of increased liver triglyceride accumulation in association with obesity and the metabolic syndrome has not been fully elucidated. A dietary excess of saturated fat is likely to be an important contributing factor (36), and moderately hypocaloric, fat-reduced diets have been shown to decrease IHTG levels (44). Furthermore, there may be considerable interindividual differences in the handling of a given oral fat load, such that postprandial uptake and storage of dietary fat may be a critical determinant of tissue triglyceride levels (35). Intriguingly, we found a very tight correlation between plasma leptin levels and IHTG content \((r = 0.98, P < 0.0001)\), although our analysis was limited to a subgroup of subjects. Since elevated leptin levels tend to be indicative of leptin resistance in humans (14), it is possible that resistance to the effects of leptin on fatty acid oxidation (25, 26) may contribute to increased hepatic triglyceride accumulation. However, clearly such speculations are beyond the scope of this observational study.

In conclusion, these studies compare for the first time IMCL, IHTG, visceral fat, and whole body \(R_a\) in the same nondiabetic subjects using state-of-the-art MR and insulin clamp techniques. While both IMCL and visceral fat were inversely correlated with whole body \(R_a\), the inverse correlation between IHTG and peripheral insulin sensitivity was both unexpected and striking. These novel findings in humans are consistent with in vivo animal studies suggesting that the liver impacts peripheral insulin action and lend further support to the concept of IHTG as an important therapeutic target in individuals with insulin resistance and type 2 diabetes. Further study may better elucidate the pathogenesis of systemic insulin resistance related to hepatic fat accumulation.

ACKNOWLEDGMENTS

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REFERENCES

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