Methods and Reproducibility of Liver Fat Measurement Using 3-Dimensional Liver Segmentation From Noncontrast Computed Tomography in EVAPORATE Cohort

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Objective: Nonalcoholic fatty liver disease not only shares multiple risk factors with cardiovascular disease but also independently predicts its increased risk and related outcomes. Here, we evaluate reproducibility of 3-dimensional (3D) liver volume segmentation method to identify fatty liver on noncontrast cardiac computed tomography (CT) and compare measures with previously validated 2-dimensional (2D) segmentation CT criteria for the measurement of liver fat.

Methods: The study included 68 participants enrolled in the EVAPORATE trial and underwent serial noncontrast cardiac CT. Liver attenuation < 40 Hounsfield units (HU) was used for diagnosing fatty liver, as done in the MESA study. Two-dimensional and 3D segmentation of the liver were performed by Philips software. Bland-Altman plot analysis was used to assess reproducibility.

Results: Interreader reproducibility of 3D liver mean HU measurements was 96% in a sample of 111 scans. Reproducibility of 2D and 3D liver mean HU measurements was 93% in a sample of 111 scans. Reproducibility of change in 2D and 3D liver mean HU was 94% in 68 scans. Kappa, a measure of agreement in which the 2D and 3D measures both identified fatty liver, was excellent at 96.4% in 111 scans.

Conclusions: Fatty liver can be reliably diagnosed and measured serially in a stable and reproducible way by 3D liver segmentation of noncontrast cardiac CT scans. Future studies need to explore the sensitivity and stability of measures for low liver fat content by 3D segmentation, over the current 2D methodology. This measure can serve as an imaging biomarker to understand mechanistic correlations between atherosclerosis, fatty liver, and cardiovascular disease risk.

Key Words: liver fat, cardiovascular disease, cardiac computed tomography, volume segmentation

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Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of disease processes characterized by abnormal liver fat accumulation not from alcohol consumption. Simple fatty liver progresses to steatohepatitis (nonalcoholic steatohepatitis), characterized by hepatocellular inflammation and fibrosis in approximately 20% to 30% of cases, and approximately another 20% of cases advance to cirrhosis.[1,2] Mortality rates are significantly higher in patients with NAFLD as cirrhosis carries a poor prognosis given increased chances of liver failure and hepatocellular carcinoma.[1]

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The clinical burden of NAFLD extends beyond liver-related morbidity and mortality. Diagnosis of NAFLD may be seen as a gateway, for which a bidirectional relationship exists between traditional risk factors (eg, diabetes, hypertension, hyperlipidemia, obesity) and liver disease,[3] as well as conferring risk for cardiovascular disease (CVD), independent of previously mentioned risk factors.[3–5] Such associations have prompted an international consensus to propose the term *metabolic-associated fatty liver disease*, which requires hepatic steatosis and evidence of metabolic dysfunction.[6]

Nonalcoholic fatty liver disease has a global prevalence of 25% and varies significantly by ethnicity with the highest prevalence in Hispanics and the least in African Americans.[7–9] Naturally, there is continued interest in expanding methodologies to accurately screen, diagnose, and monitor fatty liver specifically using noncontrast computed tomography (CT).[8] Current techniques for evaluating hepatic steatosis, or fatty liver, include using cutoff values of attenuation expressed as Hounsfield units (HU) less than 40 as threshold for >30% histological fat.[9] More recently, studies have incorporated using spleen HU values for internal control: a liver-spleen difference less than −10 HU reports robust sensitivity and specificity as well as liver-to-spleen ratio < 1.0,[10–12] where quantifying liver fat relies on 2-dimensional (2D) segmentation from acquired CT imaging. This article will focus on 3-dimensional (3D) liver volume segmentation method in cardiac scans to evaluate the degree of hepatic steatosis in comparison with previously validated 2D segmentation methods.

MATERIALS AND METHODS

Study Population

EVAPORATE (Effect of Icosapent Ethyl on Progression of Coronary Atherosclerosis in Patients with Elevated Triglycerides on Statin Therapy) study was a multicenter (Harbor-UCLA, Lundquist Institute, and Intermountain Health Care), randomized, double-blind, placebo-controlled trial that evaluated the effect of icosapent ethyl on coronary plaque progression. We used the EVAPORATE cohort for our study because these participants had high levels of low-density lipoprotein and triglycerides, making them susceptible to have fatty liver.

A total of 106 subjects were enrolled in this randomized, double-blind, placebo-controlled trial. Inclusion criteria were age of 30 to 85 years with established coronary atherosclerosis (narrowing >20% in 1 coronary artery diagnosed by either invasive angiography or multidetector CT angiography), elevated fasting triglycerides (135–499 mg/dL), and elevated low-density lipoprotein cholesterol (40–115 mg/dL). Twenty-six subjects failed screening. The remaining 80 subjects were randomized to either icosapent ethyl (n = 40) or placebo (n = 40). A total of 68 patients successfully completed the 18-month visit (Fig. 1A). In subsequent fatty

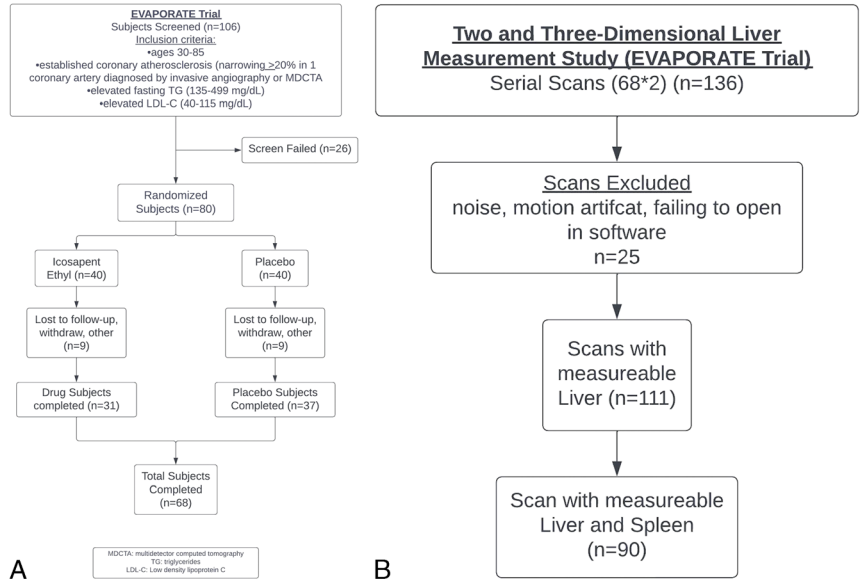

FIGURE 1. A–B, Flowcharts outlining derivation of the final population for the 3-dimensional liver measurement study. Images can be viewed in color online at www.jcat.org.

liver measurement studies, n = 136 serial scans (68 subjects × 2 scans/subject) were screened for exclusion criteria of noise, motion artifact, and failure to open in software (n = 25), resulting in 111 scans with measurable liver, and 90 scans with measurable liver and spleen. See flowchart in Figure 1B.

Population characteristics breakdown includes 57.4 ± 8.7 years, 37 or 54.4% being male, 82.4% White, and 54.4% Hispanic, with 100% statin use and a majority of study cohort having diabetes mellitus (69.1%) and hypertension (76.5%) (Table 1). All individuals gave informed consent, and the study protocol was approved by the review board at each institution in accordance with the Health Insurance Portability and Accountability Act. The original EVAPORATE study design and methods have been previously published.[13–15]

Noncontrast Cardiac CT Image Acquisition

Among the 3 EVAPORATE study sites, 1 site used a 64-slice coronary CT scanner (Aquilion 64; Toshiba America Medical Systems, Tustin, Calif) and 2 sites used a Revolution 256-detector scanner (GE Medical, Milwaukee, Wis), as previously reported.[14] Axial images of the thorax were acquired in supine position, and the current study will analyze images obtained during the inspiration phase. Scans have a slice thickness of 0.75 mm. Scans were performed from the carina to below the level of costophrenic angle on both sides of the chest. Scout scans were performed in all the subjects before acquiring noncontrast cardiac CT scans to identify these landmarks. Computed tomography scans with at least superior segments of the liver completely visible were included in the analysis. Superior segments of the liver were identified using transverse plane through the bifurcation of the main portal vein as landmark. All scans were read and analyzed in a blinded fashion by trained level II certified cardiac CT readers in accordance with Society of Cardiovascular Computed Tomography guidelines (more than 250 cardiac CT scans independently read and interpreted, with minimum of 40 hours of didactics). In addition, our readers had more than 1 year of experience in cardiac and chest imaging at the MESA cardiac CT core laboratory at Lundquist Institute of Harbor-UCLA Medical Center.

Two-Dimensional Liver Segmentation Measurement

Measurements were performed by 2 trained level II CT readers with more than 1 year of experience in cardiac and chest

TABLE 1. Patient Characteristics at Baseline of the EVAPORATE Cohort

	Subjects (N = 68)
Demographics	
Age, y	57.4 ± 8.7
Body mass index, kg/m^2	33.7 ± 6.7
Male, %	37 (54)
Hispanic/Latino	37 (54)
Race: White, %	56 (82)
Follow-up time, mo*	17.8 ± 3.8
Risk factors, %	
Hypertension	52 (77)
Hyperlipidemia	68 (100)
Family history of CAD	22 (32)
Past smoker	29 (43)
Laboratory values	
hsCRP, mg/L*	4.2 ± 4.2
Triglycerides, mg/dL	198.2 ± 93.1
HDL-C, mg/dL	37.2 ± 8.0
LDL-C, mg/dL	91.8 ± 35.2
Hemoglobin A_{1C}, %	7.7 ± 2.4
Medication, %	
Hypertension medication	52 (77)
Statin medication	68 (100)
Aspirin	37 (54)

Values are mean ± standard deviation or number (percentage).

*Median and interquartile range are reported.

CAD indicates coronary artery disease; HDL-C, high-density lipoprotein cholesterol; hsCRP, high sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.

imaging in a blinded fashion on noncontrast cardiac CT scans using Philips workstation (version 4.5; Philips Healthcare, Cleveland, Ohio). Measurements were taken via regions of interest (ROIs) greater than 100 mm^2 in area to measure hepatic and splenic Hounsfield attenuation (HU), specifically 2 ROIs placed in the right liver lobe (Fig. 2) along the anterior-posterior axis, taking care to avoid hepatic vascular and biliary structures, and 1 ROI on the spleen. Windowing parameters included a window level of 100 and a window width (1000) to capture and be inclusive of fatty liver variations. Nonalcoholic fatty liver disease was defined as a ratio of average liver HU values divided by spleen HU < 1.0. Subsequently, outcomes were stratified into mild (<1.0 to >0.7), moderate (<0.7 to >0.5), and severe (<0.5). Final measurements were obtained via the average of 2 readers' measurements (Table 2).[12]

Three-Dimensional Liver Segmentation and Liver Fat Measurements

Noncontrast CT cardiac scans were measured by the same readers as delineated previously for 2D measurements using Philips workstation (version 4.5; Philips Healthcare, Cleveland, Ohio). Tissue segmentation protocol was used to identify borders of the liver in axial, coronal, and sagittal planes (Figs. 3B–D). Extent of the liver visualized includes superior segments, as outlined previously. All tissue volume outside the liver was excluded. From the center plane of the 3D images, the border tracing of the liver was completed in all axes, and a 3D liver was derived (Fig. 3A). Next, Hounsfield threshold preset was changed to set appropriate parameters of window center at 30 and window width of 400. Using threshold-based segmentation technique, the average liver density (HU) and volume were obtained. Final measurements were obtained via the average of 2 measurements (Table 2).

Two-Dimensional and 3D Liver and Spleen HU Measurements

Two blinded level II readers (requirements are in line with Society of Cardiovascular Computed Tomography guidelines) measured 2D HU mean on subjects' liver and spleen (if visible on noncontrast CT). These readers also measured 3D liver and spleen using the new 3D segmentation model. Length of time for 3D measurements was approximately 1.5× compared with the 2D method. Final measurements were determined via the average of 2 readers' measurements. Bland-Altman plot analysis tested interreader reproducibility on each variable. Bland-Altman was also used to test reproducibility of 2D and 3D liver and spleen measures from 1 reader. Reproducibility of change in 2D and 3D liver mean HU (baseline visit to 18-month visit) was also examined. Using kappa, we examined the agreement of 2D and 3D measures in identifying fatty liver.

TABLE 2. Two-Dimensional and 3D Liver and Spleen Mean HU Measures by Reader

	n	Reader 1	Reader 2
2D liver mean HU	111	46.7 ± 12.3	46.6 ± 12.8
2D spleen mean HU	90	37.6 ± 8.7	36.8 ± 8.7
3D liver mean HU	111	47.4 ± 9.5	47.6 ± 9.5
3D spleen mean HU	90	38.3 ± 6.3	38.7 ± 6.7

Values are mean ± standard deviation.

HU indicates Hounsfield units; 2D, 2-dimensional; 3D, 3-dimensional.

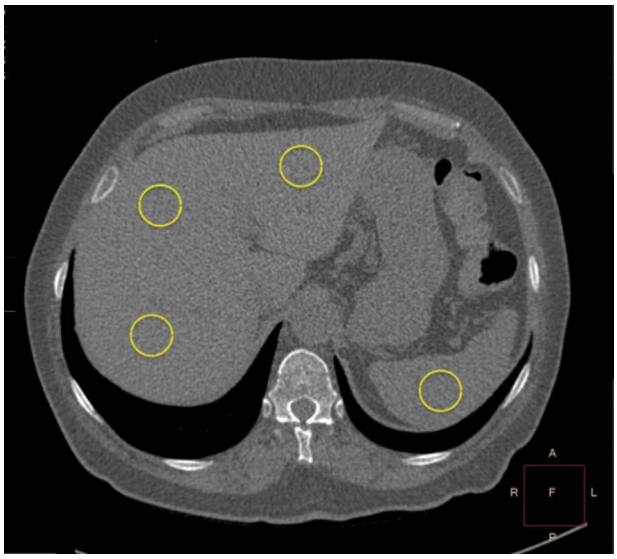

FIGURE 2. Noncontrast computed tomography scan in axial view demonstrating 2 regions of interest (ROIs) (>100 mm^2) in the right liver lobe, 1 ROI in the left liver lobe, and 1 in the spleen. Images can be viewed in color online at www.jcat.org.

Statistical Analysis

The EVAPORATE cohort had 68 subjects who completed at least 2 visits with coronary CT angiography and noncontrast cardiac CT scans. Baseline and 18-month scans were evaluated by 2 independent blinded readers. Noncontrast CT scans with motion artifact or with poor or fair image quality were excluded. Bland-Altman plot analysis was used to test reproducibility between interreader and intrareader variability.

RESULTS

Interreader reproducibility of 2D liver mean HU measurements was 93%, in a sample of 111 scans, and interreader reproducibility of 2D spleen mean HU measurements was 93% but was conducted on 90 scans because of visibility of the spleen (Bland-Altman; Figs. 4A, B)

Interreader reproducibility of 3D liver mean HU measurements was slightly better at 96% in 111 scans, and interreader reproducibility of 3D spleen mean HU measurements was also excellent at 97% in 90 scans with visibility of the spleen (Bland-Altman; Figs. 4C, D).

After establishing the method of both 2D and 3D measures to have minimal interreader bias, we examined the reproducibility of 2D and 3D liver and spleen measures from a single reader. Intrareader reproducibility of 2D and 3D liver mean HU measures was 93% in a sample of 111 scans, and spleen mean HU was 95% in a sample of 90 scans (Bland-Altman; Figs. 5E, F).

We also evaluated the reproducibility of change in 2D and 3D liver mean HU in serial scans at baseline and 18 months. Reproducibility was excellent at 94% in 68 scans (Fig. 5G).

Finally, 1 reader evaluated all available EVAPORATE noncontrast CT cohort (n = 111) scans in 2D and 3D mean HU liver measures. The 2D and 3D methods agreed in 80 scans positive for fatty liver and 27 negative for fatty liver. There were 3 scans where the 2D method evaluated as negative for fatty liver versus 3D evaluated as positive for fatty liver. There was 1 scan that the 3D

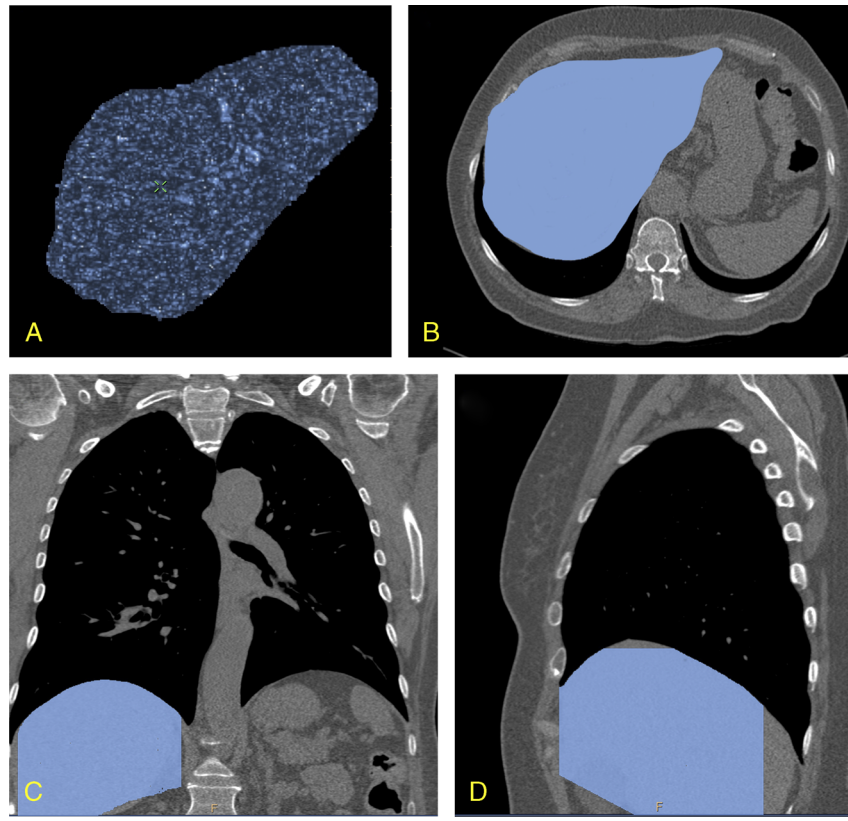

FIGURE 3. A, Semiautomated generation of the 3-dimensional model of the liver. B–D, From top to bottom, respectively, axial, coronal, and sagittal axis of tracings. Images can be viewed in color online at www.jcat.org.

method evaluated as negative fatty liver but the 2D method evaluated as positive. Kappa, the measure of agreement in which the 2D and 3D measures both identified fatty liver, was excellent at 96.4% in 111 scans.

DISCUSSION

In a recent consensus statement by Lazarus et al,[8] there is a dire need to advance global public health agenda on fatty liver disease, specifically NAFLD. In conjunction with proposed

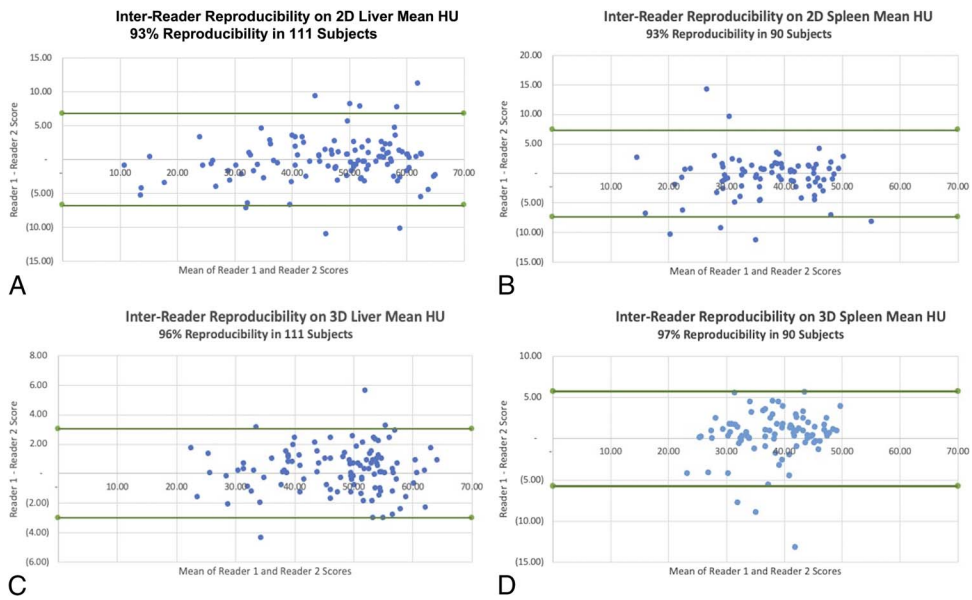

FIGURE 4. A–D, Bland-Altman plots' illustration of reproducibility. HU indicates Hounsfield units; 2D, 2-dimensional; 3D, 3-dimensional. Images can be viewed in color online at www.jcat.org.

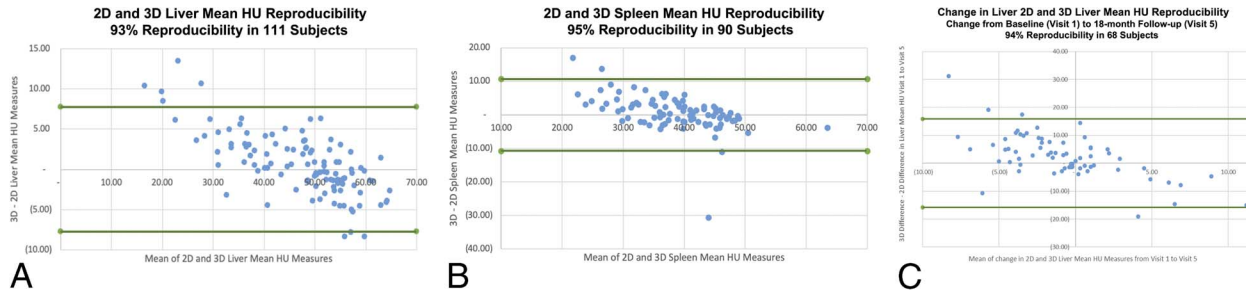

FIGURE 5. A–C, Bland-Altman plots' illustration of reproducibility. HU indicates Hounsfield units; 2D, 2-dimensional; 3D, 3-dimensional. Images can be viewed in color online at www.jcat.org.

advancements, there is a need for improved screening and diagnostic methodologies. A comprehensive review conducted by Zhang et al[9] revealed that previous studies diagnosing steatosis using absolute liver HU < 40 has sensitivity and specificity of 46% to 72% and 88% to 95%, respectively.[10] Diagnostic capabilities typically decrease as severity of steatosis decreases. On the basis of Bohte et al's[10] review, a subcategory of mild histological steatosis (10%–20%) results in lower sensitivity (52%–62%) with CT detection. Although the spleen may be used as internal control, disease processes such as hemochromatosis, albeit rare, may render comparisons unreliable.[9]

Our results mentioned previously affirm clear reliability in the proposed 3D liver segmentation method to measure liver fat, which is further bolstered by the kappa measurement. Evaluation of reproducibility both by readers and in stability for repeat serial scans 18 months apart emphasize potential utilization in clinical practice. Given that 64- and 256-slice multidetector CT angiography scanners were used, such protocol limits image processing artifacts compared with previous studies. Altogether, these findings suggest that fatty liver can be reliably measured and diagnosed through 3D liver segmentation using noncontrast cardiac CT scans.

The main benefit of 3D liver segmentation lies in the ability for a global survey of the liver. Multidimensional segmentation measurements also offer lower interslice variation and improved accuracy in comparison with previously validated 2D segmentation methods. Biopsy currently remains the criterion standard of diagnosing. However, it is a suboptimal tool for screening and monitoring because it is invasive and too focal. Furthermore, small volume samples and histological studies, which are often observer dependent, may hinder reproducibility.[9] In populations who already present with a need for coronary artery calcium scans, simultaneous 3D analysis of the liver provides reliable alternative screening modality with promising diagnostic potential, while reducing radiation exposure and risks associated with procedures.

This study differs from current literature regarding liver segmentation in that our methods highlighted utility of noncontrast cardiac CT scans versus traditional abdominal CT. The significance lies in the context of first establishing validity and reliability in quantifying fat using cardiac CT, eliminating need for additional testing. Such groundwork is necessary for further exploration in future studies to establish liver measurements as a potential biomarker for CVD.

Limitations of this study include a lack of liver biopsies to confirm diagnosis of steatosis. However, criteria discussed have been validated in comparison with histological studies for accuracy.[9] Despite an excellent kappa measurement, further studies are needed to explore whether having scans deemed as positive for fatty liver by the 3D method but as negative by 2D was a true increase in sensitivity versus random error. Possible overlap of normal and mild fatty liver using cutoff values of HU < 40, if present, would occur in both 2D and 3D methodologies, thereby increasing the probability of true detection. Reproducibility on repeat scans 18 months apart has potential changes in a patient's hepatic fat fraction. Finally, the authors of this article acknowledge fat in liver fissures was not excluded. Future methodologies should aim for automated liver fat measurement techniques that exclude fat in fissures to further optimize fat liver quantification.

CONCLUSION

When combined with other well-established advantages of noncontrast CT such as its noninvasive means for fast acquisition and quantitative results, this 3D live segmentation of noncontrast cardiac CT scan technique provides comprehensive, reliable, and stable liver fat measurements. As the liver is routinely imaged during cardiac CT imaging, liver fat calculations using segmentation may assist in improving risk classification of CVD seeing that NAFLD is associated with an increased cardiovascular risk.[3–5] Future studies are warranted to validate these 3D liver fat measurements in a larger population, and given the relationship between NAFLD and CVD as discussed, they may be a promising potential biomarker of CVD.

REFERENCES

1. Fernando DH, Forbes JM, Angus PW, et al. Development and progression of non-alcoholic fatty liver disease: the role of advanced glycation end products. *Int J Mol Sci*. 2019;20:5037.
2. Loomba R, Adams LA. The 20% rule of NASH progression: the natural history of advanced fibrosis and cirrhosis caused by NASH. *Hepatology*. 2019;70:1885–1888.
3. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. *J Gastroenterol Hepatol*. 2016;31:936–944.
4. Ma J, Hwang SJ, Pedley A, et al. Bidirectional analysis between fatty liver and cardiovascular disease risk factors. *J Hepatol*. 2017;66:390–397.
5. Zeb I, Katz R, Nasir K, et al. Relation of nonalcoholic fatty liver disease to the metabolic syndrome: the multi-ethnic study of atherosclerosis. *J Cardiovasc Comput Tomogr*. 2013;7:311–318.
6. Eslam M, Sanyal AJ, George J, et al. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. *Gastroenterology*. 2020;158:1999–2014.
7. Riazi K, Azhari H, Charette JH, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol*. 2022;7:851–861.

8. Lazarus JV, Mark HE, Anstee QM, et al. Advancing the global public health agenda for NAFLD: a consensus statement. *Nat Rev Gastroenterol Hepatol*. 2022;19:60–78.
9. Zhang YN, Fowler KJ, Hamilton G, et al. Liver fat imaging-a clinical overview of ultrasound, CT, and MR imaging. *Br J Radiol*. 2018; 91:20170959.
10. Bohte AE, van Werven JR, Bipat S, et al. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. *Eur Radiol*. 2011; 21:87–97.
11. Piekarski J, Goldberg HI, Royal SA, et al. Difference between liver and spleen CT numbers in the normal adult: its usefulness in predicting the presence of diffuse liver disease. *Radiology*. 1980;137: 727–729.
12. Zeb I, Li D, Nasir K, et al. Computed tomography scans in the evaluation of fatty liver disease in a population based study: the multi-ethnic study of atherosclerosis. *Acad Radiol*. 2012;19:811–818.
13. Defilippis AP, Blaha MJ, Martin SS, et al. Nonalcoholic fatty liver disease and serum lipoproteins: the multi-ethnic study of atherosclerosis. *Atherosclerosis*. 2013;227:429–436.
14. Budoff M, Brent Muhlestein J, Le VT, et al. Effect of Vascepa (icosapent ethyl) on progression of coronary atherosclerosis in patients with elevated triglycerides (200–499 mg/dL) on statin therapy: rationale and design of the EVAPORATE study. *Clin Cardiol*. 2018;41:13–19.
15. Budoff MJ, Bhatt DL, Kinninger A, et al. Effect of icosapent ethyl on progression of coronary atherosclerosis in patients with elevated triglycerides on statin therapy: final results of the EVAPORATE trial. *Eur Heart J*. 2020;41:3925–3932.