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Adipose Tissue and Metabolic Factors Associated with Steatosis in HIV/HCV coinfection: Histology versus Magnetic Resonance Spectroscopy

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Abstract

Background—Hepatic steatosis is common in persons with HIV and hepatitis C virus (HCV); yet biopsy measurement of steatosis is prone to sampling error. We compared magnetic resonance spectroscopy (MRS) measurement of steatosis to histology in HIV/HCV-coinfected patients, and explored the associated adipose tissue and metabolic factors.

Methods—Cross-sectional analysis of 42 HIV/HCV-coinfected men and women. Logistic regression analysis identified factors [MRI-measured visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) and Homeostasis Model Assessment (HOMA) estimated insulin resistance] associated with histologic steatosis (≥5% of hepatocytes with fat) and MRS steatosis (≥5% of hepatic fat).

Results—MRS steatosis was strongly associated with histologic steatosis, when measured continuously [odds ratio: 10.2 per doubling of MRS-measured hepatic fat; 95% confidence interval (CI):2.9, 69.3] and dichotomously (Kappa coefficient=0.52; p=0.0007). Four of the 10 with MRS-measured steatosis did not have histologic steatosis; 3 of 9 with histologic steatosis did not have MRS-measured steatosis (67% sensitivity; 88% specificity). Associations of VAT and abdominal SAT were associated with both histologic and MRS-measured steatosis. Insulin resistance was also associated with both.

Conclusions—When compared to histology, MRS was similarly associated with adipose tissue and metabolic factors. MRS is a useful non-invasive alternative to biopsy in HIV/HCV coinfection.

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Keywords

Steatosis; Magnetic Resonance Spectroscopy; HIV; HCV; Adipose tissue; Insulin Resistance

Introduction

Hepatic steatosis (or fatty liver) is a frequent complication of HIV and hepatitis C virus (HCV) coinfection[1–3] and is associated with rapid fibrosis progression[4, 5]. Genotype 3 HCV infection is thought to be directly associated with steatosis; obesity may be a more common cause in those with genotype 1 HCV infection[6], which is highly prevalent in the U.S. Steatosis is also thought to be more prevalent in those with HIV/HCV coinfection compared with HCV monoinfection[1, 7]. Whether adipose tissue and HIV-related metabolic perturbations contribute to the higher prevalence is unclear.

Liver biopsy, which is the current clinical standard to measure steatosis, is prone to sampling error. Magnetic resonance spectroscopy (MRS) offers several advantages: 1) it is non-invasive, which is important in patients with HIV and/or HCV who may have contraindications to biopsy, including low platelet count and increased prothrombin time; 2) it estimates hepatic fat content from a larger region than a random core biopsy of the liver; and 3) it provides a continuous measure of hepatic fat that allows for monitoring of steatosis (as opposed to a categorical histologic grade of steatosis).

The few studies that have examined steatosis using MRS in HIV-infected persons have been small in sample size, did not compare with liver biopsy and did not include a comparison control group [8–10]. The aim of our study was to compare MRS measurement of steatosis to histology in HIV/HCV-coinfected patients, and explore the associated adipose tissue and metabolic factors.

Methods

Between December 2003 and June 2007, 43 HIV/HCV-coinfected (24 women, 19 men) without hepatitis B infection, prior HCV treatment, or decompensated cirrhosis underwent MRS and liver biopsy (within 3 months of MRS). All HIV/HCV-coinfected women from the Northern California site of the Women's Interagency HIV Study (WIHS) were approached for enrollment. WIHS recruitment, study design and characteristics have been described elsewhere[11]. Men were recruited via posted flyers from San Francisco Bay Area clinics. One woman was excluded from the final analysis, because MRS measurement of hepatic fat could not be calculated. MRS data from 18 controls (without HIV or HCV infection, self report of recent alcohol use, diabetes and BMI >25) were included for comparison. Controls were of normal weight (median BMI: 23) and had a median age of 33. Written informed consent was obtained from all participants following a protocol approved by the University of California, San Francisco Committee on Human Research.

MRS was performed on a 1.5 Tesla whole body clinical scanner (General Electric Medical Systems, Milwaukee, WI). Spectra were obtained from an 8 cubic centimeter, single voxel region of tissue and were analyzed with motion correction algorithms using a standardized MRS protocol [12]. The peak areas under the resonance frequencies of lipids and unsuppressed water were calculated for each participant and the percent (%) hepatic fat derived as the ratio of the total lipids measure to the total lipids plus unsuppressed water measures. A mean inter-examination coefficient of variation for MRS-measured % hepatic fat of 11.9% (range 2.8 – 20.3%) in a sample of 9 controls confirmed its reproducibility.

Liver tissue was obtained in the HIV/HCV-coinfected participants via percutaneous core biopsy and reviewed by a single pathologist unaware of imaging findings. The median length of liver tissue was 1.7cm [interquartile range (IQR): 1.4 – 2cm]. Steatosis grade was determined by estimating the percentage of fat-containing hepatocytes on hematoxylin-eosin-stained specimens using the Non-Alcoholic Steatohepatitis Clinical Research Network grading system: grade 0 representing < 5% steatosis; grade 1: 5%–33%; grade 2: 34%–66%; and grade 3: > 66% steatosis.

Visceral adipose tissue (VAT) and abdominal subcutaneous adipose tissue (SAT) were calculated as the mean area [centimeter squared (cm^2)] of the visceral and subcutaneous compartments, respectively by MRI of the L2/3, L3/4, and L4/5 intervertebral disk levels. Body mass index (BMI) was calculated from height and weight in kilogram per meter squared (kg/m^2). Leg fat mass (kg) was determined from dual energy X-ray absorptiometry scan (Lunar Prodigy, Madison, Wisconsin). The Homeostasis Model Assessment estimated insulin resistance (HOMA-IR) [fasting insulin (μU/mL) × glucose (mg/dL)/405].

Statistical Analysis

Characteristics of the 42 HIV/HCV-coinfected men and women included in the analysis were compared and tested for statistical significance using the Mann-Whitney U test for continuous variables, and Fisher's exact test for categorical variables. Logistic regression models examined the association of adipose tissue, metabolic, viral and liver-related factors with histologic steatosis (grade 0 versus grade 1 or more) and with MRS-measured steatosis (<5% versus 5% or more) in the HIV/HCV-coinfected participants. The 5% cutoff was validated in a large clinical study as being diagnostic of clinically significant steatosis[13] and was used in a recent study in HIV-infected individuals [8]. Factors assessed in unadjusted and gender-adjusted models included demographics (age, race, and gender), body composition (BMI, leg fat, VAT and abdominal SAT), HOMA-IR, alcohol use, menopausal status, HIV/HCV-related factors (HCV and HIV viral load, HCV genotype, current CD4 count, and current antiretroviral drug use), and liver related factors [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)]. The linearity assumption for continuous predictors was tested. Interactions between gender and other factors in the model were assessed.

Factors with right-skewed distributions were log-transformed in all analyses. Exact logistic regression analysis was used because of the small sample size. All analyses were conducted using the SAS system, version 9.1 (SAS Institute, Inc., Cary, North Carolina).

Results

Table 1 shows the demographic and clinical characteristics of HIV/HCV-coinfected men compared to women. Median age, BMI, CD4 count, as well as HCV genotype distribution and percentage on antiretroviral therapy were similar. Men were more often Caucasian; women more often African-American, which is reflective of the race/ethnic distribution of HIV infection in the US. Men had less leg fat and abdominal SAT, but more VAT than women. None were on metformin or pioglitazone.

Figure 1 shows levels of MRS % hepatic fat for three groups: controls, HIV/HCV-coinfected with grade 0 steatosis, and HIV/HCV-coinfected with grade 1 steatosis. Those with grade 1 steatosis had a higher MRS % hepatic fat than those with grade 0 steatosis (median 5.8% vs. 1.9%; p=0.001); those with grade 0 steatosis had a higher MRS % hepatic fat than controls (1.9% vs. 1.0%; p=0.036). Among the HIV/HCV-coinfected, greater amounts of MRS % hepatic fat were strongly associated with histologic steatosis (OR 10.2 per doubling; 95% confidence interval: 2.9, 69.3; p=0.003).

The prevalence of MRS steatosis (defined as % hepatic fat ≥5%) was 24% in the HIV/HCV-coinfected participants. The prevalence of histologic steatosis was 21% (15% in men vs. 26% in women) and all were grade 1. Although MRS steatosis was strongly correlated with histologic steatosis (Kappa coefficient = 0.52, p=0.0007), there was some discordance: 4 of the 10 with MRS steatosis did not have histologic steatosis; 3 of 9 with histologic steatosis did not have MRS steatosis. MRS had a 67% sensitivity and an 88% specificity for detecting steatosis as measured with liver biopsy.

We next compared factors associated with MRS steatosis and histologic steatosis in HIV/HCV-coinfected participants (Table 2). In gender adjusted models, BMI, VAT, and abdominal SAT showed similar odds of being associated with MRS steatosis and histologic steatosis. Greater leg SAT was strongly associated with histologic steatosis, but the association with MRS steatosis did not reach statistical significance (OR=2.5, p=0.098). HOMA-IR showed a stronger association with MRS steatosis (OR=2.5, p=0.037) than with histologic steatosis (OR=2.0, p=0.070). HCV-related factors (HCV genotype 3, HCV viral load, ALT, and AST) were positively associated with both MRS and histologic steatosis (data not shown).

We also analyzed MRS % hepatic fat as a continuous outcome. We found a strong association of VAT with MRS % hepatic fat (+64% per doubling of VAT, 95% confidence interval (CI): 28, 105; p<0.001). Other associated factors included abdominal SAT (+45% per doubling, 95% CI: 10, 91; p=0.004), BMI (+5.5% per 1 kg/m2 increase, 95% CI: 0.69, 10; p=0.026), leg SAT (+42% per doubling, 95% CI: 6.5, 79; p=0.017), and HOMA-IR (+31% per doubling, 95% CI: 8.2, 53; p=0.010).

Discussion

Similar to studies in the general population[14], our study demonstrates that MRS is a useful non-invasive method to measure the amount of hepatic steatosis in HIV/HCV-coinfected persons. We found strong associations between the amount of MRS % hepatic fat and presence of histologic steatosis. Furthermore, we observed associations of BMI, VAT, and abdominal SAT with MRS steatosis that were similar to their associations with histologic steatosis. HOMA-IR was more strongly associated with MRS steatosis than with histologic steatosis. Adipose tissue and metabolic factors were strongly associated with continuously-measured MRS % hepatic fat. Taken together, our data support the use of MRS to quantify steatosis in the setting of HIV/HCV coinfection.

While we found a sensitivity of only 67% for MRS detection of steatosis, this was likely due to the use of liver biopsy as the gold standard. Biopsy is prone to sampling error. It is therefore noteworthy that in our study, a wide range of MRS % hepatic fat values was observed in participants without histologic steatosis, supporting the concept that a small random sample of liver tissue can miss heterogeneous or scattered steatosis. In addition, visual inspection of liver histology to determine steatosis grade can be subjective and prone to inter- and intra-reader variability, whereas MRS studies a larger area of the liver than biopsy, and estimates hepatic fat amount using an automated software-generated program.

MR imaging also allowed us to simultaneously acquire direct measurements of abdominal fat. Using direct measures of VAT, we confirmed prior reports that visceral obesity (measured using waist circumference) may be a better marker of steatosis than obesity [4]. Hadigan et al[8] also found VAT to be more strongly correlated with MRS % hepatic fat content than BMI in HIV-infected participants. However, in that study, BMI appeared slightly more correlated with MRS % hepatic fat than abdominal SAT, in contrast to our study, where abdominal SAT appeared to be more strongly correlated. We also found that abdominal SAT was associated

with categorically-measured MRS steatosis. The role of abdominal SAT in the pathogenesis of steatosis needs further study in a larger cohort of patients.

Unexpectedly, we found that leg fat was associated with steatosis, although the association with MRS steatosis was weak and did not reach statistical significance. Prior studies in patients with congenital and acquired lipodystrophy have shown an association of lipoatrophy with steatosis[15]. In our study, leg fat appeared to be a marker of obesity.

Limitations of our study include the small sample size, which precluded a fully adjusted analysis of associated factors. However, the aim of our study was to demonstrate that MRS is a sensitive technique to estimate steatosis in HIV/HCV-coinfected persons. We were limited in our comparison of MRS steatosis between controls and HIV/HCV-coinfected participants, because liver biopsy was not clinically indicated in controls. It is interesting that a wide range of hepatic fat values was observed in controls. We may have included controls with abdominal obesity or insulin resistance despite excluding those with factors potentially associated with steatosis [BMI>25 (a surrogate marker of obesity), self-report of diabetes, HIV or HCV infection]. A future study will examine the associated adipose tissue and metabolic factors in a large group of men and women with HIV and/or HCV infection, and those with neither infection.

In summary, we found that MRS is a useful technique to measure steatosis in HIV/HCV-coinfected persons. Furthermore, MRS similarly predicts the adipose tissue and metabolic factors associated with steatosis in HIV/HCV-coinfected persons when compared to histology. MRS provides a useful non-invasive tool for future comparative investigation of steatosis and its progression in those with HIV/HCV coinfection.

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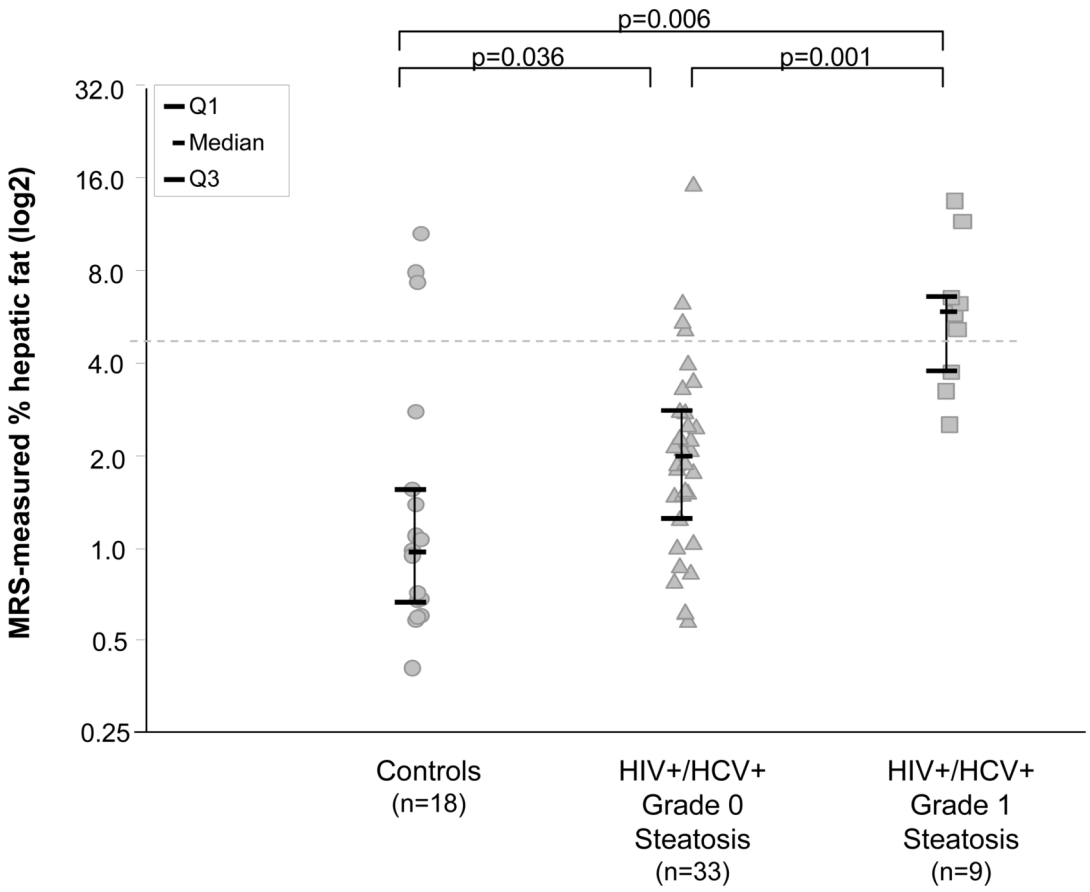

Figure 1.
MRS-measured percent hepatic fat in controls and HIV/HCV-coinfected subjects (by steatosis grade)

Table 1

Demographic and Clinical Characteristics of HIV/HCV-coinfected men and women

(median (IQR))	Men (n=19)	Women (n=23)	P-value
Age (y)	46.0 (41.0–57.0)	50.0 (45.0–52.0)	0.60
Ethnicity, % (n)			
Caucasian	63% (12)	17% (4)	0.006
African-American	37% (7)	74% (17)	
Postmenopausal Status, % (n)	NA	74% (17)	NA
Drinks (last 90 days)	0 (0–0)	0 (0–5)	0.65
BMI (kg/m^2)	25.5 (22.9–27.3)	25.0 (21.9–31.0)	0.95
Leg SAT (kg)	5.3 (3.2–7.2)	10.1 (6.7–13.5)	0.002
VAT area (cm^2)	110.9 (68.3–155.7)	54.9 (35.2–76.9)	0.0003
Abdominal SAT area (cm^2)	125.4 (68.7–174.6)	196.3 (147.8–308.1)	0.008
Insulin (μU/ml)	12 (8–15)	12 (7–23)	0.74
Glucose (mg/dL)	97 (89–109)	83 (73–93)	0.001
HOMA	3 (2–4)	3 (1–5)	0.73
AST (U/L)	56 (45.84)	41 (37–58)	0.10
ALT (U/L)	30 (23–40)	33 (21–48)	0.83
HCV RNA × 10^4 (IU/mL)	15.9 (5.6–25.3)	8.9 (3.6–29.2)	0.89
HCV Genotype, % (n)			
1	79% (15)	87% (20)	0.89
2	5% (1)	0	
3	11% (2)	9% (2)	
4	5% (1)	4% (1)	
HIV RNA × 10^3 (copies/mL)	0.2 (0.1–4.3)	0.3 (0.1–2.2)	0.29
Current CD4 count (cells/μL)	422 (235–575)	392 (247–572)	0.81
Current ARV use, % (n)*	79% (15)	61% (14)	0.32

NA, not applicable; BMI, body mass index; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; HOMA, homeostasis model assessment;

*only 1 woman reported taking stavudine.

Table 2

Association of regional adipose tissue and insulin resistance with MRS-measured steatosis* and histologic steatosis**, after adjustment for gender

	MRS-measured Steatosis*		Histologic Steatosis**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
BMI (kg/m^2)	1.1 (0.98, 1.3)	0.11	1.1 (0.99, 1.3)	0.066
VAT (log_2)	2.3 (0.92, 7.1)	0.10	2.2 (0.87, 6.7)	0.13
Abdominal SAT (log_2)	3.3 (1.2, 12.1)	0.042	3.0 (1.1, 11.2)	0.056
Leg SAT (log_2)	2.5 (0.96, 9.2)	0.098	8.3 (1.9, 71.8)	0.019
HOMA-IR (log_2)	2.5 (1.2, 6.7)	0.037	2.0 (1.0, 4.9)	0.070

OR, odds ratio; CI, confidence interval; BMI, body mass index; VAT, visceral adipose tissue; HOMA-IR, homeostasis model assessment estimated insulin resistance

* outcome defined as presence (n=10) vs. absence (n=32) of MRS steatosis(≥5% hepatic fat by MRS)

** outcome defined as presence (n=9) vs. absence (n=33) of histologic steatosis (≥5% hepatocytes with fat)