Non-alcoholic fatty liver disease in non-obese subjects of African origin has atypical metabolic characteristics

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Abstract

Background: Non-obese non-alcoholic fatty liver disease (NAFLD) is reported in several populations. However, as persons of African origin display unique fat accumulation, insulin resistance and lipid profiles, we investigated fatty liver in non-obese persons of African origin.

Method: We recruited 78 urban Jamaican volunteers. CT scan was used to estimate liver and abdominal fat; body composition by D-EXA. Fasting blood was collected for lipids, alanine aminotransferase (ALT), adiponectin and fetuin-A. Homeostatic model assessment of insulin resistance (HOMA), whole body insulin sensitivity index (WBISI), insulinogenic index (IGI) and oral disposition index (oDI) were calculated after a 75-g oral glucose tolerance test.

Results: 52% of the participants were male; mean age 28.5±7.8 years and BMI 22.4±3.0 kg/m^2 (±SDs). Mean liver attenuation (MLA) and liver: spleen (LS) ratio, both inversely correlated to liver fat, were 62.8±4.3 HU and 1.2±0.1 respectively, and 3.8% of the participants had liver fat >30% (LS ratio<1). In age, sex and BMI-adjusted correlations, MLA was negatively associated with weight (r=-0.30, p=0.009) and height (r=-0.28, p=0.017) and associated with fasting glucose (r=0.23, p=0.05), fasting insulin (r=0.42, $p \leq 0.001$) and HOMA-IR (r=0.35, p=0.004). Serum lipids, ALT, adiponectin, fetuin A, WBISI, IGI and oDI were not associated with liver fat.

Conclusions: In non-obese Afro-Caribbean participants, greater liver fat (lower MLA) was associated with weight and height and lower fasting insulin. Hyperinsulinaemia appears to be influential in the reduction of NAFLD in this group. These findings may be influenced by ethnicity, body size and the method of estimating liver fat.

Keywords: Non-obese non-alcoholic fatty liver disease, insulin resistance, adiponectin, fetuin A

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in Western countries[1] and is rapidly becoming the most common liver disease worldwide [2]. It is also a significant public health concern because of its association with cardiovascular risk factors [3]. NAFLD occurs when an imbalance between triglyceride accumulation and removal in hepatocytes results in fat accumulation greater that 5% of liver weight without significant alcohol intake. Although most commonly diagnosed in obese persons, NAFLD also occurs in lean/non-obese individuals. Non-obese NAFLD is defined as fatty liver in persons with a BMI < 25 kg/m^2 in Asians and <30 kg/m^2 in other races [4]. Its reported global prevalence rate ranges from 3% to almost 30% [5] and the prevalence in Western populations is 7% -21% [6-8].

Non-obese NAFLD is not well understood and the reports regarding its clinical and metabolic features are inconsistent. The third National Health and Nutrition Examination Survey (NHANES III) reports that in comparison to an overweight-obese NAFLD group, the lean NAFLD cohort was younger, more commonly female, with significantly lower prevalence of IR, DM, hypercholesteremia, and hypertension [8]. Similarly, in a meta-analysis of 16 studies including various ethnic groups, lean and obese patients with NAFLD share an altered metabolic and cardiovascular profile with the effects in lean patients being of a lesser magnitude [9]. In contrast, patients from Korea with non-obese NAFLD had significantly higher prevalence rates for blood pressure, impaired fasting glucose, low HDL-C and high TG than did obese NAFLD patients, especially among women [10].

The role of ethnicity in these conflicting findings is unknown, and very little is known about non-obese NAFLD in persons of African origin. Non-obese NAFLD (BMI < 30 kg/m^2) had a reported prevalence of 18% among Hispanic Americans, 9% among Caucasians and 6% among African

Americans [7]. Additionally, several metabolic variables commonly associated with fatty liver disease might not reliably predict liver fat in persons of African origin. LDL-cholesterol and triglycerides are associated with liver fat [11], but persons of African origin have normal triglyceride (TG) and low HDL-C as the characteristic lipid profile of insulin resistance, the so-called triglyceride paradox [12]. Notable also, is the fact that Blacks have a lower prevalence of fatty liver compared to Hispanics with similar levels of obesity and insulin resistance[6]. This distinct metabolic response to insulin resistance in African Americans (the insulin resistance paradox) [13] might also be a feature of non-obese NAFLD. Finally, visceral obesity is reported to play an important role in the pathogenesis of lean NAFLD [14]. However, African Americans may be less likely to accumulate visceral adipose tissue than Asians and Caucasians [15].

These findings suggest that there could be distinct mechanisms underlying the pathogenesis of NAFLD in persons of African origin. This study aimed to investigate the clinical and biochemical parameters associated with liver fat in non-obese Jamaican adults using an objective measure of both hepatic and visceral fat. A secondary aim was to identify predictors of liver fat in this study population. We hypothesized that fatty liver in non-obese persons of African origin is not associated with insulin resistance or lipids.

Methods

Subjects

We identified 84 individuals from urban Jamaican communities who were previously recruited by Community Health Aides as healthy community controls in a larger study involving Jamaican adults [16]. Each participant was recruited as follows: beginning at a specified address, visits were conducted house to house alternately on either side of the road. Failure to find a participant would result in adjacent streets being visited in a similar manner. Potential recruits were asked about their

general health status using a questionnaire and height and weight measurements were conducted in the field using a stadiometer and a digital scale that was calibrated daily [16]. Individuals with a BMI < 30 kg/m^2 were defined as non-obese. The exclusion criteria were a known history of liver disease, use of medications that cause liver abnormalities and self-reported alcohol intake of more than 14 drinks per week (men) and more than 7 drinks per week (women) [17]. The Faculty of Medical Sciences/ University Hospital of the West Indies Ethics Committee approved the study protocol (ECP 17, 14/15) and each participant gave written informed consent.

Measurements

After a 10 hour overnight fast, participants reported to the metabolic clinic at the Tropical Metabolism Research Unit and completed a staff-administered questionnaire. Body weight was measured to the nearest 0.1 kg and height and waist circumference to the nearest 0.1 cm using a standardized protocol [18]. A whole-body DEXA scan was performed on each participant to measure body composition (Lunar Prodigy, GE Health Care, USA). 10 mls of fasting blood was collected for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, alanine aminotransferase (ALT), adiponectin and fetuin-A assays. A 75-g oral glucose tolerance test was conducted with 5 ml samples taken at 0, 30, 60, 90 and 120 minutes into fluoridated and heparinized chilled tubes for plasma glucose and insulin measurements respectively.

Abdominal computerized tomography scans (Phillips Brilliance 64-slice scanner) were conducted to measure hepatic steatosis and visceral adiposity. A single cross-sectional 5mm-width CT scan (of 120 kVp, 100 mA) was taken at the mid-intervertebral disc space between T12 and L1 to include an image of both the liver and the spleen a second scan was located at the middle of the L4 / L5 disc space to measure total and subcutaneous adiposity. During the scans, the machine was operated in tissue optimization mode.

Assays

Glucose concentration was determined by the glucose oxidase method on an autoanalyzer. Insulin concentration was measured with an ELISA assay (ALPCO Diagnostics, Salem, NH, USA)[19], which had analytical sensitivity of 0·399 μIU/ml and an intra-assay coefficient of variation < 5% in our laboratory. Total cholesterol, HDL-cholesterol, triglycerides and ALT were measured by enzymatic techniques using a COBAS INTEGRA 400 Plus Analyzer (Roche Diagnostics, IN, USA). LDL-cholesterol was calculated by the Friedewald formula[20]. Adiponectin was measured using a commercial ELISA kit (EMD Millipore Corporation, MA, USA) [21]; the minimum detectable concentration was 0.78 ng/mL. The intra-assay CV was < 7.4% and inter-assay CV was <8.4 %. Fetuin-A was estimated by an ELISA method (ALPCO Diagnostics, Salem, NH, USA)[22]. The analytical sensitivity of the human fetuin-A ELISA was 5.0 ng/mL and the inter-assay and intra-assay coefficient of variation (CV) were ≤ 6.8%.

Data Analysis

Liver fat data was analyzed using eFilm software. Three (3) regions of interest (ROIs) were placed in the image of the liver and one in the image of the spleen, each measuring a minimum of 1 cm^2. Tissue attenuation was measured in Hounsfield Units (HU). The ratio of mean liver to spleen attenuation (LS ratio) was calculated and a ratio of ≤ 1 or a mean liver attenuation (MLA) of ≤ 40 HU used to indicate significant hepatic steatosis (> 30%) [17]. CT scans have a reported sensitivity of 82-93% and a specificity of 100% for steatosis >30% [23].

Total and intra-abdominal fat area and mass were measured using the commercial software package QCT Pro, Tissue Composition Module Beta 1.0 (Mindways, Austin, TX, USA). Images taken from the CT scanner were transferred to the Tissue Composition Module Beta 1.0 software package for analysis. The QCT Pro Tissue analysis report provided composition results for total

abdominal adiposity (TAA) and visceral adipose tissue (VAT) in terms of mass (g), area (cm^2) and volume (cm^3) [24]. Subcutaneous adipose tissue (SAT) was calculated by subtracting VAT from TAA.

Calculations

The following formulae were used in the analyses:

1. Homeostatic model assessment-insulin resistance (HOMA-IR) = I_0 x G_0/22.5, where G_0 and I_0 reflect basal (fasting) glucose and insulin in SI units [25].
2. Whole-body Insulin Sensitivity Index (WBISI) = $10{,}000/(G_0 \times I_0 \times G_m \times I_m)^{0.5}$, where G_0 and I_0 reflect basal glucose and insulin, and G_m and I_m the mean concentrations of glucose and insulin during OGTT
3. Insulin secretion was estimated using the insulinogenic index (IGI) = $(I_{30} - I_0)/ (G_{30} - G_0)$ where I_{30} and I_0 are insulin concentrations at 30 and 0 minutes, and G_{30} and G_0 are glucose concentrations at 30 and 0 minutes
4. Oral disposition index (oDI); beta-cell function adjusted for insulin sensitivity = IGI x WBISI. oDI is a biomarker for predicting the development of type 2 diabetes.

Statistical Analysis

Sample size, based on the reported 11% prevalence of nonobese NAFLD in African Americans (by CT scan) [7], a precision of 7% and 80% power was 69. Continuous variables were expressed as means ± SDs where data were normally distributed and medians (quartiles) where data were not normally distributed. Characteristics of men and women were compared using the independent t-test. Variables that were not normally distributed were log transformed to a normal distribution. Using LS ratio and MLA as continuous outcome variables, partial correlations were conducted with age, sex, and BMI as control variables. An informal forwards variable selection approach was

used to identify predictors of fatty liver using a p-value < 0.05 as the criterion for inclusion. 13 independent variables were identified *a priori* for this analysis: age, sex, height, weight, BMI, waist circumference, total cholesterol, LDL-cholesterol, triglyceride, fasting glucose, fasting insulin, alanine transaminase and the presence of type 2 diabetes. These variables were selected based on their documented associations with fatty liver disease as well as their routine use in clinical practice. SPSS 19.0 for Windows was used for the statistical analyses. Two-sided p-values were reported and a p-value ≤ 0.05 considered statistically significant.

Results

84 participants were recruited of which 81 consented to undergo an abdominal CT scan. Three additional participants were excluded from the analysis due to insufficient CT data. Of the remaining 78 participants, 56% were male; age 28.5 ± 7.8 years and BMI 22.4 ± 3.0 kg/m^2 (mean ± SD). Liver attenuation was 62.8 ± 4.3 HU with a minimum of 53.4 HU and a maximum of 73.5 HU. Mean LS ratio was 1.2 ± 0.1 and the range was 0.95 -1.78. Liver fat > 30% was detected in 3.8% of the participants based on LS ratio ≤1. However, using the mean liver attenuation cutoff of ≤ 40 HU, no participant met the diagnostic criteria for moderate to severe fatty liver disease. Approximately 9% of the participants had impaired glucose tolerance (i.e. blood glucose ≥ 7.8 mmol/L but < 11.1 mmol/L after a 2 hour OGTT) [26].

Men weighed more, were taller and had greater lean mass and greater ratio of visceral to subcutaneous fatty tissue (VAT: SAT) while women had greater fat mass, VAT and SAT. Men had greater concentrations of fasting glucose, triglycerides, ALT and had a higher oral disposition index while women had higher concentrations of fasting insulin, total cholesterol, LDL-cholesterol and were more insulin resistant (HOMA-IR and WBISI) than men. Despite this, men and women had similar amounts of liver fat. (**Table 1**)

Anthropometry, Body Composition and Liver Fat

Adjusting for age, sex and BMI, MLA had a negative association with adult body weight (p = 0.009), height (p = 0.017) and lean mass (p = 0.003). The association between lean mass and MLA remained after further adjusting for fat mass (r = -0.27, p = 0.02) but was lost after adjusting for height (r = -0.11, p = 0.35) (data not shown). Lean mass was inversely correlated to fat mass (r = -0.51, $P < 0.001$) and BMI was not associated with either measure of liver fat, adjusting for age and sex (data not shown). LS ratio had a tendency towards an inverse association with weight (p = 0.06) and VAT (p = 0.06), adjusting for age, sex and BMI.

Biochemical Variables and Liver Fat

Serum triglyceride, cholesterol and ALT were not associated with liver fat after adjusting for age, sex and BMI. Fasting glucose, fasting insulin and HOMA-IR were associated with MLA (**Figure 1**), however, other measures of glucose metabolism (WBISI, IGI and oDI) were not related to either measure of liver fat (**Table 2**). Adiponectin was not associated with liver fat ($P > 0.6$) but was associated with HDL-C (adjusting for age, sex and BMI (r = 0.36; p = 0.002). Adiponectin was associated with WBISI (r = 0.30; p = 0.05) but had no association with HOMA-IR (p = 0.5). However, the association between adiponectin and WBISI was nullified by adjusting for BMI (p = 0.08) (data not shown). Fetuin-A was not associated with either outcome measure of liver fat (p = 0.6), HOMA-IR, WBISI, oDI or adiponectin (p-values > 0.27) (data not shown).

Predictors of liver fat

In the informal forwards variable selection analysis, MLA was associated with fasting glucose (β = 0.28, p = 0.05) and fasting insulin (β = 0.5, p = 0.03) and negatively associated with weight in men (β = -0.5, $p < 0.001$), and, in women, MLA was associated with fasting insulin (β = 0.42, p =

212 0.01) (data not shown). When both sexes were included, MLA had a negative association with
213 weight and a positive association with fasting insulin (**Table 3**).
214 In men, LS ratio was associated with fasting glucose ($\beta = 0.39$, $p = 0.03$) and negatively associated
215 with BMI ($\beta = -0.71$, $p < 0.001$), while in women, LS ratio was associated with mean waist
216 circumference ($\beta = 0.5$, $p = 0.04$) (data not shown). However, none of the variables was associated
217 with LS ratio after adjusting for sex (**Table 3**).
218
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Discussion

To our knowledge, this is the first report that describes non-alcoholic fatty liver disease and its metabolic features in a non-obese population exclusively of African origin. As we hypothesized, some of our findings were distinct from those reported in other populations. These include associations between liver fat and reduced HOMA-IR and reduced fasting insulin concentrations. Additionally, serum triglyceride, and LDL-C did not show the characteristic associations with liver fat, nor was HDL-C related to liver fat.

Liver fat > 30% was found in less than 4% of the participants based on LS ratio ≤1 and we posit that there are several possible explanations for this low occurrence. Persons of African origin have the lowest burden of NAFLD compared with Hispanics and Caucasians; in a nationally representative sample of the U.S. population, age-adjusted prevalence of NAFLD was highest in Mexican-Americans (21.2%), followed by non-Hispanic whites (12.5%), and was lowest in non-Hispanic blacks (11.6%) [27]. Second, CT scans are less sensitive at detecting liver fat < 30%. These factors coupled with the young age of our participants are likely to have influenced the low prevalence of fatty liver in our study.

Our findings suggest that LS ratio is more sensitive in the detection of liver fat than MLA. Similarly, Rogier et al reported that LS ratio was more accurate than mean liver attenuation for detecting macro-vesicular steatosis > 30% (AUC = 0.94 vs. 0.89), with LS ratio having a higher positive predictive value. [28] Additionally, different CT scanners as well as different reconstruction algorithms can affect the absolute attenuation value of liver parenchyma [29], and these potential errors in measurement of attenuation can be avoided by using spleen attenuation as an internal control. Finally, the seemingly higher sensitivity of LS ratio may also result from the inclusion of milder cases of hepatic steatosis.

Both LS ratio <1 and MLA < 40 HU are reported to indicate moderate to severe hepatic steatosis [30,31] although other studies suggest that different thresholds might be more relevant. Zeb et al demonstrated that the prevalence of fatty liver as estimated by L/S ratio < 1.0 was higher than that provided by liver attenuation < 40 HU (17.2% vs 6.3%), and the MLA corresponding to the prevalence provided by L/S ratio <1.0 was <51 HU [17]. While several other authors suggest using a higher cutoff for MLA (i.e. 48 HU) to indicate moderate to severe liver fat accumulation [32 33], it is important to note that utilizing these threshold values did not affect our findings.

Body Composition and Liver Fat

Liver fat was associated with body weight and height and had a tendency toward a positive association with VAT but not fat mass. We posit that fat mass in this population may be influenced by higher SAT (reported to be the preferred fat storage depot in persons of African origin) [34]. Although women had twice as much SAT as men, they had comparable liver fat, similar to findings reported by Westerbacka et al [35]. It has been theorized that SAT acts as a metabolically neutral fat reservoir which protects against fat spilling over into ectopic depots such as visceral fat and hepatic fat that are associated with greater metabolic risk [36].

We demonstrated an association between liver fat (MLA) and height and weight. The unexpected association with lean mass was influenced by the height of the participants, suggesting that despite being highly co-linear, height (not lean mass) is influencing liver fat accumulation in this group. It appears that lean mass might act as a proxy for fat mass in obese healthy subjects (who tend to have greater lean mass). As evidence, lean body mass index was associated with liver fat measured by magnetic resonance spectroscopy ($r = 0.28$, $p = 0.002$) among 113 overweight and obese Canadian youth. [37]However, the same might not be true among our lean subjects where there was a negative association between lean and fat mass. In contrast, among 11,116 South Korean adults,

participants with the least liver fat (as estimated by fatty liver index) showed the highest skeletal muscle mass [38].

Biochemical Variables and Liver Fat

As we hypothesized, serum triglyceride, and LDL-C did not show the characteristic associations with liver fat, nor was HDL-C related to liver fat. Persons of African origins are known to have lower mean triglyceride and LDL-cholesterol concentrations compared to whites [39], and the associations between triglyceride concentration and insulin resistance, cardiovascular disease (CVD), and type 2 diabetes (T2D) are lower in Blacks than in other ethnic groups (the triglyceride paradox). [12]. Conversely, among non-obese Koreans, triglyceride levels were significantly associated with both the development and regression of NAFLD [40]. For this reason, our findings may reflect ethnic differences in lipid profiles, and so, indices such as fatty liver index, that utilize triglyceride concentrations may not be suitable to estimate liver fat in persons of African origin.

Alanine aminotransferase (ALT) was similarly unrelated to either outcome measure of liver fat; however, normal ALT values have been previously reported in patients across the entire histological spectrum of NAFLD [41,42] similar to the majority of our participants. This suggests that benign fat accumulation is occurring in the absence of liver injury (inflammation) with no attendant increase in ALT levels as most of our participants have ALT concentrations that fall well below the upper limit of normal.

Despite a documented inverse association with liver fat accumulation [43], adiponectin was not associated with liver fat in our participants. Since adiponectin is secreted by adipose tissue, our findings might reflect the lack of an association between fat mass and liver fat in our study population. It is also possible that the study may have been underpowered to detect an association between adiponectin and liver fat, although the expected associations between adiponectin and

289 HDL-C and insulin sensitivity (WBISI) were demonstrated. Fetuin-A, a hepatokine that suppresses
290 adiponectin production and has increased concentrations in persons with biopsy-proven NAFLD
291 [44], showed no correlation with adiponectin or liver fat in our group. Additionally, although fetuin-
292 A is also associated with impaired insulin sensitivity [45] and impaired glucose tolerance [46], we
293 demonstrated no association with HOMA-IR or WBISI. However, it is notable that rs738409, the
294 PNPLA3 variant associated with both fetuin-A concentration and NAFLD [47,48], is less common
295 in African Americans compared to Hispanic Americans (19% vs 40%)[49]. We are not aware of any
296 prior study reporting on fetuin-A levels in non-obese individuals with NAFLD, so it is unclear
297 whether our findings are specific to persons of African origin. Nevertheless, the study provided
298 reference data for fetuin-A in our population; the mean concentration of fetuin-A (0.5 ± 0.1 g/L)
299 being comparable to that reported by Jensen et al (0.43 ± 0.09 g/L) in 542 African Americans age
300 > 65 years [50].
301 Interestingly, in our study population, lower concentrations of fasting glucose and insulin were
302 associated with more liver fat as was decreased HOMA-IR. These findings are atypical, as
303 associations between non-obese NAFLD and insulin resistance are well documented [51,52] albeit
304 among middle aged Asians. Our findings are not without precedence, however, as Hakim et al
305 report a lack of an association between liver fat and hepatic insulin sensitivity in British men of
306 African origin [53]. Lipid intermediates which accumulate from excessive liver fat can cause
307 dysfunction of hepatic mitochondria, inflammation and increased VLDL-triglyceride production
308 with subsequent hepatic and systemic insulin resistance. However, the unique mechanisms of fat
309 distribution and metabolism that occurs in persons of African origin might render these lipid
310 intermediaries less damaging [53]. It is also important to note that our research group previously

311 reported that HOMA-IR has limitations in our population [54] and might be imprecise in lean
312 individuals, and this is supported by the lack of an association between WBISI and liver fat.
313 The inverse relationship between liver fat and insulin concentration might be a consequence of
314 insulin's inhibition of hormone sensitive lipase (HSL) which hydrolyzes fatty acids from
315 triacylglycerols or diacylglycerols. Insulin resistant African-American women have a greater acute
316 insulin response to glucose (AIRg) than insulin resistant Caucasian women after a frequently
317 sampled intravenous glucose tolerance test [55]. Further, this insulin response was out of proportion
318 to their degree of insulin resistance [55]. It was concluded that this hyperinsulinaemia in African
319 American women accounted for the greater FFA clearance observed in African American women
320 [55]. The clear implication is that basal hyperinsulinaemia might be an important variable in our
321 population and relative hyperinsulinaemia could thus reduce liver fat accumulation in persons of
322 African origin. In addition, African-Americans appear to be more resistant to the accretion of fat
323 in the liver associated with insulin resistance [13], the so-called insulin resistance paradox.
324 Using forward variable selection, we again identified body weight and reduced fasting insulin as
325 predictors of liver fat (as estimated by MLA). As discussed previously, our findings related to
326 fasting insulin concentrations may be due to ethnic differences in the relative impact of insulin
327 action and insulin sensitivity. Consequently, we cannot rule out our hypothesis that there might be
328 a unique pathogenesis of liver fat accumulation independent of insulin resistance in our population.
329 In the final analysis, it is likely that some of the observed differences in the prevalence and
330 pathogenesis of NAFLD across ethnicities have genetic origins. The PNPLA3 gene variant,
331 (rs738409), although occurring less frequently in persons of African origin, is neither associated
332 with HOMA-IR nor concentrations of triglyceride, total cholesterol, HDL-C or LDL-C [56] and has
333 a reported significant association with ALT and AST only in Hispanics [47]. Lean subjects with

NAFLD were shown to have an increased rate of rs738409 carriage compared to their obese counterparts (78.4% vs. 59.8%; $P = 0.001$)[57].

Our study has some limitations that should be discussed. The low sensitivity of CT in detecting milder degrees of liver fat might have led to the underestimation of the true prevalence of fatty liver disease. However, we are of the view that the cutoff values utilized in this study are justified as liver fat accumulation of about 30% is reported to correspond to a liver attenuation of 40 HU [30,31]. Unenhanced CT is less sensitive than magnetic resonance spectroscopy (MRS) and imaging (MRI), both of which were unavailable in our centre at the time of the study. The potential risk of radiation exposure attendant to CT was minimized by taking two single slices, using a reduced radiation dose and operating the machine in tissue optimization mode. Additionally, the aerobic fitness of our subjects, which could have the effect of reducing liver fat, was not accounted for in the study. A final limitation of this study was the modest sample size which may have resulted in the study being underpowered to detect some associations.

Despite the above limitations, our study had several strengths. The participants were well characterized using a range of clinical and biochemical variables and more than one outcome measure of insulin resistance was utilized. CT scans provided data that were objective, quantitative, and standardized by a phantom and provided the added benefit of objectively measuring visceral fat. Additionally, unenhanced CT scans were conducted to avoid the potential errors of contrast-enhanced CT scans and avoid the potential toxicity of iodinated contrast.

Conclusion

In summary, our data provides the first report of the characteristics of fatty liver in a non-obese population of African origin. The prevalence of NAFLD and features of metabolic syndrome were

low in normal weight Afro-Caribbean subjects. Liver fat had unexpected associations with lower fasting insulin concentration suggesting that hyperinsulinaemia may be influential in the reduction of liver fat in this population. The extent to which these findings are related to ethnicity, participant age, body size or the method of estimating liver fat is unknown and warrants clarification. It would therefore be instructive to investigate these in a larger group of individuals, using a more sensitive measure of liver fat.

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Author Contributions

DT, MB, TF and CB designed the research, IT-M, DS and DT participated in data collection and coordinating the clinical samples. CO and DT analyzed the data. DT conducted the literature review and wrote the first draft of the manuscript and MB had responsibility for final content. All authors read, contributed to and approved the final manuscript.

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523

524 Legends

525 **Table 1**: Age, anthropometry, body composition, biochemical characteristics, glucose

526 metabolism and liver fat of 78 non-obese urban Afro-Caribbean participants

527 **Table 2:** Correlations of liver fat measures with body composition and biochemical variables.

528 **Table 3**: Predictors of mean liver attenuation (MLA) and LS ratio in men and women

529 **Figure 1**: Correlation between Mean Liver Attenuation against HOMA-IR (adjusting for age,

530 sex and BMI)

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535 **Table 1**: Age, anthropometry, body composition, biochemical characteristics, glucose

536 metabolism and liver fat of 78 non-obese urban Afro-Caribbean participants

Clinical Variables	All Participants (N=78)	Men (N=44)	Women (N=34)	*P*-value M vs. W
Age (yrs)	28.5 ± 7.8	29.1 ± 8.2	27.8 ± 7.2	0.46
Weight (kg)	65.0 ± 10.7	69.1 ± 10.4	59.8 ± 8.7	<0.001
Height (cm)	170.0 ± 10.1	176.4 ± 7.7	161.7 ± 6.0	<0.001
BMI (kg/m^2)	22.4 ± 3.0	22.1 ± 2.5	22.9 ± 3.5	0.28
Fat Mass (kg)	9.9 (4.8, 20.0)	5.4 (3.8, 10.5)	20.6 (11.7, 25.4)	< 0.001
Lean mass (kg)	50.2 (38.1, 59.0)	58.7 (53.0, 63.4)	37.5 (35, 40.6)	< 0.001
VAT area (cm^2)	31.4 (16.2, 51.4)	24.3 (15.2, 39.5)	43.9 (25.7, 53.9)	0.035
SAT area (cm^2)	75.1 (21.63, 165.7)	37.6 (5.4, 90.2)	154.9 (81.3, 222.3)	< 0.001
VAT: SAT	0.5 (0.3, 0.8)	0.6 (0.5, 1.8)	0.3 (0.2, 0.4)	0.02
L: S Ratio	1.2 (1.1, 1.3)	1.2 (1.1,1.2)	1.2 (1.1,1.3)	0.28
Mean Liver Attenuation (HU)	62.8 ± 4.3	63.4 ± 4.6	62.2 ± 3.8	0.22
Total-C (mmol/L)	4.0 ± 0.8	3.8 ± 0.7	4.3 ± 0.9	0.008
HDL-C (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	0.54
LDL-C (mmol/L)	2.6 ± 0.9	2.3 ± 0.8	2.9 ± 0.8	0.007
Triglycerides (mmol/L)	0.7 (0.5, 0.8)	0.7 (0.6, 0.9)	0.6 (0.5, 0.8)	0.02
ALT (IU/L)	8.0 (6.0,10.0)	8.0 (7.0, 11.0)	7.0 (5.0, 8.0)	0.01
Adiponectin (µg/mL)	9.1 (6.9, 16.6)	8.1 (6.2, 12.3)	9.9 (8.9, 13.6)	0.08
Fetuin-A (g/L)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.51
Fasting Glucose (mmol/L)	4.5 ± 0.5	4.7 ± 0.4	4.4 ± 0.6	0.005
2-hour Glucose (mmol/L)	5.8 (4.9, 7.0)	5.8 (4.8, 6.5)	6.2 (4.9, 7.0)	0.2
Fasting Insulin (µIU/mL)	2.9 (1.5, 6.6)	2.0 (0.7, 3.5)	5.1 (2.7, 9.0)	0.006
HOMA-IR	0.6 (0.3, 1.3)	0.5 (0.2, 0.8)	1.0 (0.5, 1.8)	0.03
WBISI	161 (97, 245)	237 (156, 395)	109 (72, 160)	≤ 0.001
IGI	2.2 ± 0.9	2.4 ± 1.1	2.0 ± 0.4	0.08
oDI	304 (179, 685)	463 (229, 878)	221 (136, 337)	0.003

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538 **Key**: VAT- visceral adipose tissue. SAT-subcutaneous adipose tissue, HOMA-IR- homeostatic model
539 assessment-insulin resistance, WBISI - whole body insulin sensitivity index, IGI - insulinogenic index, oDI
540 - oral disposition index. Variables expressed as means ± SDs where data were normally distributed and
541 medians (1^{st} quartile, 3^{rd} quartile) where data were not normally distributed.
542

Table 2: Correlations of liver fat measures with body composition and biochemical variables.

Body Composition or Biochemical Variable	LS Ratio adjusted for				Mean Liver Attenuation adjusted for			
	Age and sex		Age, sex and BMI		Age and sex		Age, sex and BMI	
	r	*p*	r	*p*	r	*p*	r	*p*
Weight	-.08	0.55	-.25	0.06	-.16	0.16	-.30	0.009
Height	-.21	0.10	-.21	0.11	-.28	0.016	-.28	0.017
Waist Circumference	.10	0.4	.07	0.6	-.01	1.0	-.05	0.7
Fat Mass*	.04	0.8	-.04	0.8	-.01	0.9	-.04	0.7
Lean Mass*	-.16	0.2	-.20	0.1	-.32	0.005	-.35	0.003
VAT Area*	-.18	0.2	-.24	0.06	-.01	1.0	-.02	0.9
SAT Area*	-.10	0.4	-.18	0.2	.11	0.3	.12	0.3
VAT: SAT*	-.01	1.0	.02	0.9	-.15	0.2	-.15	0.2
HDL-Cholesterol	.13	0.3	.15	0.3	.03	0.8	.03	0.8
LDL-Cholesterol	-.03	0.8	-.03	0.8	-.02	0.9	-.02	0.9
Triglyceride*	-.10	0.4	-.11	0.4	.06	0.6	.06	0.6
ALT*	-.02	0.9	-.02	0.9	.10	0.4	.10	0.4
Adiponectin*	.02	0.9	.04	0.8	-.02	0.9	-.01	0.9
Fetuin-A	-.07	0.6	-.06	0.6	-.06	0.6	-.06	0.6
Fasting Glucose	.14	0.3	.14	0.3	.23	0.04	.23	0.05
2h Glucose*	.11	0.4	.11	0.4	.09	0.5	.08	0.5
Fasting Insulin*	.18	0.2	.16	0.2	.38	0.001	.42	<0.001
HOMA-IR*	.17	0.2	.15	0.3	.33	0.007	.35	0.004
WBISI*	-.12	0.4	-.10	0.5	-.04	0.7	-.04	0.8
Insulinogenic Index	.05	0.7	.06	0.7	.19	0.1	.20	0.1
Oral Disposition Index*	-.08	0.6	-.06	0.7	.02	0.9	.03	0.8

Note: * log transformed to a normal distribution. r = correlation coefficient; *p* = *p*-value

Table 3: Predictors of mean liver attenuation (MLA) and LS ratio in men and women

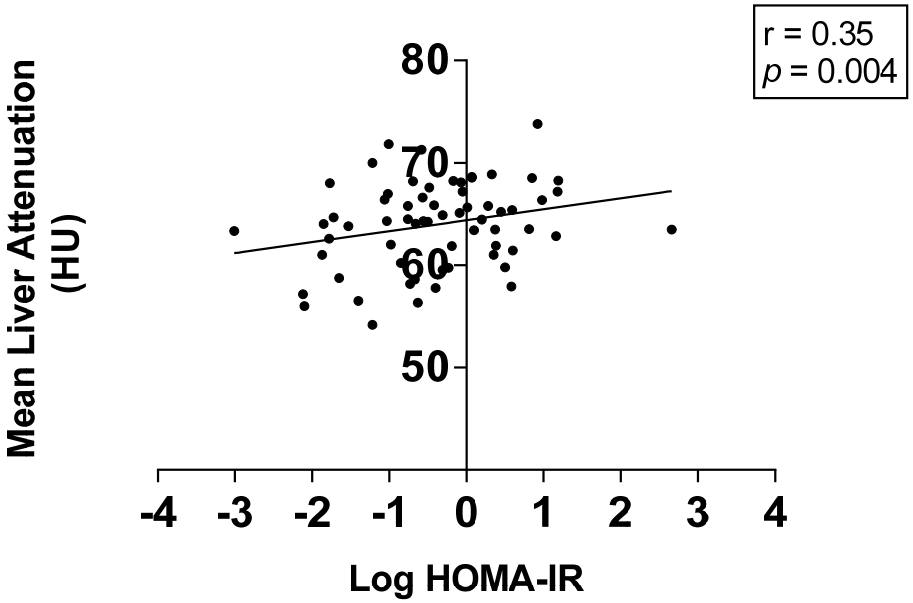
		Unstandardized Coefficient		Standardized Coefficient	*P*-value
		B*	Std. Error	Beta#	
MLA (HU)	(Constant)	69.92	3.83		.000
	Age (years)	.042	.069	.07	.544
	Sex	-5.09	1.27	-.58	.000
	Fasting Insulin (µIU/mL)	3.12	.72	.59	.000
	Adult weight (kg)	-.16	.06	-.35	.008
LS Ratio	(Constant)	1.218	.109		.000
	Age (years)	-.001	.002	-.048	.717
	Sex	.023	.036	.087	.537
	ALT (IU/L)	-.010	.037	-.038	.785

*The unstandardized coefficient (B) describes the number of units of the outcome associated with a one unit change in the predictor.

#The standardized (beta) coefficient describes the correlation when both the predictor and outcome are expressed in standardized units (i.e. mean = 0, standard deviation = 1).

Non-normally distributed variables were log transformed prior to inclusion in the regression.

Correlation between Mean Liver Attenuation and HOMA-IR (adjusting for age, sex and BMI)



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