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

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Acknowledgements: CDB is supported in part by the Southampton NIHR Biomedical Research Centre.

Financial Support Statement: This work was supported by the New Zealand Health Research Council Grant 09/052, Developmental Adaptation to an Obesogenic Environment Program, the Caribbean Public Health Agency (CARPHA) and a UWI Graduate Studies Research and Publications Grant.

Conflict of Interest Statement: The authors have no conflicts to declare.

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Electronic word count: 4,268; Tables (3), Figures (1)
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/m² (±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 ≤ 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 than 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. 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, 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. 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. This distinct metabolic response to insulin resistance in African Americans (the insulin resistance paradox) 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. However, African Americans may be less likely to accumulate visceral adipose tissue than Asians and Caucasians.

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. 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. Individuals with a BMI < 30 kg/m² 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). 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. A whole-body DEXA scan was performed on each participant to measure body composition (Lunar Prodigy, GE Health Care, USA). 10 ml 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)\textsuperscript{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\textsuperscript{20}. Adiponectin was measured using a commercial ELISA kit (EMD Millipore Corporation, MA, USA)\textsuperscript{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)\textsuperscript{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%)\textsuperscript{17}. CT scans have a reported sensitivity of 82-93% and a specificity of 100% for steatosis >30%\textsuperscript{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 (cm²) and volume (cm³). 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) = I₀ x G₀/22.5, where G₀ and I₀ reflect basal (fasting) glucose and insulin in SI units.

2. Whole-body Insulin Sensitivity Index (WBISI) = 10,000/(G₀ x I₀ x Gₘ x Iₘ)⁰.⁵, where G₀ and I₀ reflect basal glucose and insulin, and Gₘ and Iₘ the mean concentrations of glucose and insulin during OGTT.

3. Insulin secretion was estimated using the insulinogenic index (IGI) = (I₃₀ - I₀)/(G₃₀ - G₀) where I₃₀ and I₀ are insulin concentrations at 30 and 0 minutes, and G₃₀ and G₀ 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), 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 $\leq 0.05$ 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 $28.5 \pm 7.8$ years and BMI $22.4 \pm 3.0$ kg/m$^2$ (mean ± SD). Liver attenuation was $62.8 \pm 4.3$ HU with a minimum of $53.4$ HU and a maximum of $73.5$ HU. Mean LS ratio was $1.2 \pm 0.1$ and the range was $0.95 -1.78$. Liver fat $> 30\%$ was detected in $3.8\%$ of the participants based on LS ratio $\leq 1$. However, using the mean liver attenuation cutoff of $\leq 40$ 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 $\geq 7.8$ 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)
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 ($\beta = 0.28, p = 0.05$) and fasting insulin ($\beta = 0.5, p = 0.03$) and negatively associated with weight in men ($\beta = -0.5, p < 0.001$), and, in women, MLA was associated with fasting insulin ($\beta = 0.42, p = 0.03$) and HOMA-IR ($\beta = 0.5, p = 0.02$).
0.01) (data not shown). When both sexes were included, MLA had a negative association with weight and a positive association with fasting insulin (Table 3).

In men, LS ratio was associated with fasting glucose ($\beta = 0.39, p = 0.03$) and negatively associated with BMI ($\beta = -0.71, p < 0.001$), while in women, LS ratio was associated with mean waist circumference ($\beta = 0.5, p = 0.04$) (data not shown). However, none of the variables was associated with LS ratio after adjusting for sex (Table 3).
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 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. While several other authors suggest using a higher cutoff for MLA (i.e. 48 HU) to indicate moderate to severe liver fat accumulation, 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). Although women had twice as much SAT as men, they had comparable liver fat, similar to findings reported by Westerbacka et al. 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.

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. 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
HDL-C and insulin sensitivity (WBISI) were demonstrated. Fetuin-A, a hepatokine that suppresses adiponectin production and has increased concentrations in persons with biopsy-proven NAFLD, showed no correlation with adiponectin or liver fat in our group. Additionally, although fetuin-A is also associated with impaired insulin sensitivity and impaired glucose tolerance, we demonstrated no association with HOMA-IR or WBISI. However, it is notable that rs738409, the PNPLA3 variant associated with both fetuin-A concentration and NAFLD, is less common in African Americans compared to Hispanic Americans (19% vs 40%). We are not aware of any prior study reporting on fetuin-A levels in non-obese individuals with NAFLD, so it is unclear whether our findings are specific to persons of African origin. Nevertheless, the study provided reference data for fetuin-A in our population; the mean concentration of fetuin-A (0.5 ± 0.1 g/L) being comparable to that reported by Jensen et al (0.43 ± 0.09 g/L) in 542 African Americans age > 65 years.

Interestingly, in our study population, lower concentrations of fasting glucose and insulin were associated with more liver fat as was decreased HOMA-IR. These findings are atypical, as associations between non-obese NAFLD and insulin resistance are well documented albeit among middle aged Asians. Our findings are not without precedence, however, as Hakim et al report a lack of an association between liver fat and hepatic insulin sensitivity in British men of African origin. Lipid intermediates which accumulate from excessive liver fat can cause dysfunction of hepatic mitochondria, inflammation and increased VLDL-triglyceride production with subsequent hepatic and systemic insulin resistance. However, the unique mechanisms of fat distribution and metabolism that occurs in persons of African origin might render these lipid intermediaries less damaging. It is also important to note that our research group previously...
reported that HOMA-IR has limitations in our population and might be imprecise in lean individuals, and this is supported by the lack of an association between WBISI and liver fat. The inverse relationship between liver fat and insulin concentration might be a consequence of insulin’s inhibition of hormone sensitive lipase (HSL) which hydrolyzes fatty acids from triacylglycerols or diacylglycerols. Insulin resistant African-American women have a greater acute insulin response to glucose (AIRg) than insulin resistant Caucasian women after a frequently sampled intravenous glucose tolerance test. Further, this insulin response was out of proportion to their degree of insulin resistance. It was concluded that this hyperinsulinaemia in African American women accounted for the greater FFA clearance observed in African American women. The clear implication is that basal hyperinsulinaemia might be an important variable in our population and relative hyperinsulinaemia could thus reduce liver fat accumulation in persons of African origin. In addition, African-Americans appear to be more resistant to the accretion of fat in the liver associated with insulin resistance, the so-called insulin resistance paradox. Using forward variable selection, we again identified body weight and reduced fasting insulin as predictors of liver fat (as estimated by MLA). As discussed previously, our findings related to fasting insulin concentrations may be due to ethnic differences in the relative impact of insulin action and insulin sensitivity. Consequently, we cannot rule out our hypothesis that there might be a unique pathogenesis of liver fat accumulation independent of insulin resistance in our population. In the final analysis, it is likely that some of the observed differences in the prevalence and pathogenesis of NAFLD across ethnicities have genetic origins. The PNPLA3 gene variant, (rs738409), although occurring less frequently in persons of African origin, is neither associated with HOMA-IR nor concentrations of triglyceride, total cholesterol, HDL-C or LDL-C and has a reported significant association with ALT and AST only in Hispanics. 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.

Acknowledgements

We gratefully acknowledge the men and women who participated in this study. Additionally, we recognize Joan Patterson-McNamee, Kenesha Pencott-Brown, Hemoy Drummond, Lorraine Wilson, Diaham Knight and Stacey Chin for their work carried out at the Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica.

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.

Funding Agencies

This work was supported by the New Zealand Health Research Council Grant 09/052, Developmental Adaptation to an Obesogenic Environment Program, the Caribbean Public Health Agency (CARPHA) and the UWI Graduate Studies Research and Publications Grant.
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**Legends**

**Table 1**: Age, anthropometry, body composition, biochemical characteristics, glucose metabolism and liver fat of 78 non-obese urban Afro-Caribbean participants

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

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

**Figure 1**: Correlation between Mean Liver Attenuation against HOMA-IR (adjusting for age, sex and BMI)
Table 1: Age, anthropometry, body composition, biochemical characteristics, glucose 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/m²)            | 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 (cm²)         | 31.4 (16.2, 51.4)       | 24.3 (15.2, 39.5) | 43.9 (25.7, 53.9) | 0.035 |
| SAT area (cm²)         | 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 |
Key: VAT- visceral adipose tissue, SAT-subcutaneous adipose tissue, HOMA-IR- homeostatic model assessment-insulin resistance, WBISI - whole body insulin sensitivity index, IGI - insulinogenic index, oDI - oral disposition index. Variables expressed as means ± SDs where data were normally distributed and medians (1st quartile, 3rd quartile) where data were not normally distributed.
Table 2: Correlations of liver fat measures with body composition and biochemical variables.

| Body Composition or Biochemical Variable | LS Ratio adjusted for Age and sex | Age, sex and BMI | Mean Liver Attenuation adjusted for Age and sex | Age, sex and BMI |
|-----------------------------------------|-----------------------------------|-----------------|-----------------------------------------------|-----------------|
| Weight                                  | -0.08 0.55                       | -0.25 0.06      | -0.16 0.16                                    | -0.30 0.009     |
| Height                                  | -0.21 0.10                       | -0.21 0.11      | -0.28 0.016                                   | -0.28 0.017     |
| Waist Circumference                     | 0.10 0.4                         | 0.07 0.6        | -0.01 1.0                                     | -0.05 0.7       |
| Fat Mass*                               | 0.04 0.8                         | -0.04 0.8       | -0.01 0.9                                     | -0.04 0.7       |
| Lean Mass*                              | -0.16 0.2                        | -0.20 0.1       | -0.32 0.005                                   | -0.35 0.003     |
| VAT Area*                               | -0.18 0.2                        | -0.24 0.06      | -0.01 1.0                                     | -0.02 0.9       |
| SAT Area*                               | -0.10 0.4                        | -0.18 0.2       | 0.11 0.3                                      | 0.12 0.3        |
| VAT: SAT*                               | -0.01 1.0                        | 0.02 0.9        | -0.15 0.2                                     | -0.15 0.2       |
| HDL-Cholesterol                         | 0.13 0.3                         | 0.15 0.3        | 0.03 0.8                                      | 0.03 0.8        |
| LDL-Cholesterol                         | -0.03 0.8                        | -0.03 0.8       | -0.02 0.9                                     | -0.02 0.9       |
| Triglyceride*                           | -0.10 0.4                        | -0.11 0.4       | 0.06 0.6                                      | 0.06 0.6        |
| ALT*                                    | -0.02 0.9                        | -0.02 0.9       | 0.10 0.4                                      | 0.10 0.4        |
| Adiponectin*                            | 0.02 0.9                         | 0.04 0.8        | -0.02 0.9                                     | -0.01 0.9       |
| Fetuin-A                                | -0.07 0.6                        | -0.06 0.6       | -0.06 0.6                                     | -0.06 0.6       |
| Fasting Glucose                         | 0.14 0.3                         | 0.14 0.3        | 0.23 0.04                                     | 0.23 0.05       |
| 2h Glucose*                             | 0.11 0.4                         | 0.11 0.4        | 0.09 0.5                                      | 0.08 0.5        |
| Fasting Insulin*                        | 0.18 0.2                         | 0.16 0.2        | 0.38 0.001                                    | 0.42 <0.001     |
| HOMA-IR*                                | 0.17 0.2                         | 0.15 0.3        | 0.33 0.007                                    | 0.35 0.004      |
| WBISI*                                  | -0.12 0.4                        | -0.10 0.5       | -0.04 0.7                                     | -0.04 0.8       |
| Insulinogenic Index                     | 0.05 0.7                         | 0.06 0.7        | 0.19 0.1                                      | 0.20 0.1        |
| Oral Disposition Index*                 | -0.08 0.6                        | -0.06 0.7       | 0.02 0.9                                      | 0.03 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)

$r = 0.35$
$p = 0.004$