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RESEARCH ARTICLE

Hepatic steatosis is associated with anthropometry, cardio-metabolic disease risk, sex, age and urbanisation, but not with ethnicity in adult Kenyans

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Abstract

Objective: We aimed to determine the associations of non-alcoholic fatty liver disease (NAFLD) with cardio-metabolic risk factors for diabetes in adult Kenyans.

Methods: A cross-sectional study was undertaken among rural and urban Kenyans of different ethnic origin. Ultrasonography scanning (USS) methods were used for the assessment of hepatic fat accumulation for NAFLD assessment and abdominal fat distribution, and simple anthropometry measurements were performed. All participants underwent a 2-h oral glucose tolerance test, and biochemical, haemodynamic and lifestyle data were obtained. Multivariate logistic regression analyses were used to assess sex, age, residency and ethnic differences in the association between NAFLD and various metabolic parameters.

Results: In total, 743 individuals (59.1% women) with a mean age of 38.0 (range 18–68) years participated in the study. Overall, 118 individuals (15.9%) had NAFLD, of whom 94.1% had mild steatosis. Age >40 years was significantly associated with having NAFLD compared with <30 years of age with no difference found in NAFLD between ethnic groups (Luo, Kamba, Maasai). All body composition and clinical measurements were associated with NAFLD ($p < 0.045$ for OR).

Conclusion: Finding lower odds for NAFLD in men was unexpected, as was the lack of differences in NAFLD among the ethnic groups, while higher odds for NAFLD with increasing age and in urban vs. rural populations was expected. Especially the sex-specific results warrant further studies in black African populations on biology of body composition for having NAFLD, and whether this translates into insulin resistance and higher risk of diabetes and consequently cardiovascular disease in black African women.

KEYWORDS
cardio-metabolic risk, fatty liver index, non-alcoholic fatty liver disease, sub-Saharan Africa

INTRODUCTION

Non-communicable diseases (NCDs) are becoming a significant burden in sub-Saharan Africa (SSA) [1]. Two decades ago, the 'Global Burden of Disease Study' showed that 20% of deaths in SSA were caused by NCDs [2]. More recently, in rural and urban populations in Kenya, we showed that obesity [3], diabetes [4], as well as hypertension and dyslipidaemia [5] are moderately prevalent in adults with a strong positive rural-urban and age gradient of obesity and diabetes prevalence independently of sex and ethnicity. However, central obesity as measured by visceral adipose tissue (VAT) was higher in men than women, and in Maasai compared with Luo and Kamba [3]. The diets of these ethnic groups are characterised by mainly milk and cereals, fish and cereals, and cereals, respectively, as staples in traditional, rural societies [6]. Furthermore, we have also shown that proprotein convertase substilisin/kexin type 9 – a key modulator of the degradation of the low-density lipoprotein cholesterol receptor – is associated with hepatic steatosis in adult Kenyans, and to some degree mediated by hepatic insulin resistance [7]. Thus, it is likely that hepatic steatosis will become more prevalent in SSA and contribute to the development of cardio-metabolic disease, especially type 2 diabetes in Kenyan as well as SSA populations in the light of increasing urbanisation.

Non-alcoholic fatty liver disease (NAFLD) covers a broad range of histologic manifestations ranging from simple deposition of adipose tissue to more progressive steatosis with associated hepatitis, fibrosis, cirrhosis and in some cases, hepatocellular carcinoma [8]. The term NAFLD is comprised of non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH) [9]. NAFL is characterised by steatosis of the liver that involves more than 5% of the parenchyma with no evidence of hepatocyte injury [10]. NASH is defined by a necro-inflammatory process where the liver cells become injured due to steatosis [10]. Even though simple steatosis follows a more benign course, NASH has the potential to progress into cirrhosis and thereby increase the risk of developing hepatocellular carcinoma [11,12]. To capture the hepatic steatosis, a liver biopsy is the gold standard [8]. A liver biopsy is an invasive technique, which may be associated with clinical complications. Ultrasonography is a cheaper, non-invasive and more practical solution and is validated and approved with regards to assessment of NAFLD [13].

In a recent meta-analysis of studies either using imaging or liver biopsy, the regional prevalence of NAFLD was estimated to be 31.8% in the Middle-East, 30.5% in South America, 24.1% in North America, 23.7% in Europe, 23.4% in Asia and 13.5% in Africa [14]. It is noteworthy that the low prevalence in Africa is based on two studies with small sample sizes [15,16] hardly representative of SSA populations. Globally, the estimated prevalence of NAFLD is 25% and 23% of the affected individuals who also have type 2 diabetes, hyperlipidaemia (69%), hypertension (39%) or metabolic syndrome (43%) [14].

A prospective study of 1,051 middle-aged participants from the Framingham Heart Study found a bidirectional relationship between NAFLD and cardiovascular disease risk factors even if the underlying mechanisms of NAFLD and cardiovascular risk factors are not fully understood [17].

We aimed to identify anthropometric and cardio-metabolic correlates of NAFLD by sex, age, ethnicity and urban vs. rural residency in adult Kenyans. Based on current evidence, we hypothesised that NAFLD is more prevalent in urban populations, with increasing age, in men vs. women and in Maasai vs. Luo and Kamba ethnic populations.

METHODS

Study area and population

A community-based cross-sectional study was conducted in 2005–2006 in rural and urban Kenya. Inclusion criteria for the study were ≥18 years of age and Luo, Kamba or Maasai ethnicity or culturally related ethnic groups. Exclusion criteria were pregnancy, serious illnesses such as malaria, inability to walk unassisted and severe mental disease [3]. Liver scans were performed in 799 individuals. All participants gave written or thumb print informed consent following detailed information of the study in layman's terms given at local community meetings. Ethical permission was obtained from the National Ethical Review Committee in Kenya (SSC Protocol No. 936), and consultative approval was given by the Danish National Committee on Biomedical Research Ethics in Denmark. For more details concerning the selection procedure and the study participants, see Christensen et al. [3].

Liver scan, scoring methods and fatty liver index

Ultrasonography scan (USS) was used for the assessment of hepatic tissue with regards to steatosis using a portable scanner (Aquila Basic Unit, Pie Medical Equipment; Esaote, Masstricht, the Netherlands) with a 3C.RS 3.5/5.0 MHz curved transducer (Probe Article no. 410638 Curved Array HiD probe R40; Pie Medical Equipment, Maastricht, the Netherlands). The abdominal ultrasound investigation was performed with the participant holding his/her breath. Multiple images were acquired on both sides of the main liver lobes by two trained operators. A sub-costal approach was used, and four sweeps were performed using standard imaging protocol [13].

A semi-quantitative grading system was used to define normal, mild, moderate and severe steatosis. The liver scoring criteria were [13]:

1. Increased echogenicity of liver parenchyma (bright liver in comparison with the kidney)
2. Decreased visualisation of intra-hepatic vasculature

3. Attenuation of the ultrasound beam. The different scoring criteria were summed to derive a liver steatosis score. A score of ≤4 was classified as normal, score 5–7 for mild steatosis, score 8–10 for moderate steatosis and score ≥11 for severe steatosis. The usage of ultrasonography versus magnetic resonance spectroscopy was validated and approved with regards to assessment of NAFLD [13].

Fatty liver index (FLI) was calculated as previously described by Bedogni et al., and categorised as grade I (FLI < 30), grade II (FLI ≥ 30–59) and grade III (FLI ≥ 60) according to the formula: (e ^ 0.953*loge (triglycerides) +0.139*bmi +0.718*loge (ggt) +0.053*waist circumference - 15.745)/(1 + e ^ 0.953*ln(triglycerides) +0.139*bmi +0.718*loge (ggt) +0.053*waist circumference - 15.745)*100 [18]. We used the ultrasound-based scores to define NAFLD, while FLI was used as Table S1.

Anthropometry and ultrasonography for abdominal fat distribution

Anthropometric measurements were taken with the participants standing barefoot and wearing light clothing. Body mass index (BMI, kg/m^2) was calculated and waist circumference (WC) was measured with a body tape (WM02 Body Tape; Chasmors, Haechstmass, Germany) midway between the iliac crest and the costal margin following a quiet expiration. For further details, see Christensen et al. [3].

Abdominal fat distribution, that is VAT and subcutaneous adipose tissue (SAT), was measured in cm using USS (Aquila Basic Unit, Esaote, Pie Medical Equipment, Maastricht, the Netherlands) with a 3.5/5.0 MHz transducer (Probe Article no. 410638 Curved Array HiD probe R40 Pie Medical Equipment). We followed the protocol by Stolk et al. which has been validated in relation to computed tomography and magnetic resonance imaging [19].

Laboratory tests

Following an overnight fast (≥8-h), all participants had a blood test taken and subsequently underwent a standard 75-g oral glucose tolerance test (OGTT). Venous blood glucose was determined by the glucose dehydrogenase method using haemolysation and deproteinisation on a HemoCue B-Glucose 201+ device (HemoCue AB, Ängelholm, Sweden) with 5 mL of blood. Standard procedures were followed to centrifuge blood, which was stored as plasma and serum in cryotubes at −20°C at the nearest health facility while in the field, and later at −80°C at KEMRI, Nairobi, Kenya, before being shipped to Steno Diabetes Center Copenhagen in Denmark for insulin and standard lipid profile analyses; for further details, see [5]. Later, plasma samples were shipped to Canada for analysis of a standard liver enzyme profile at Montreal Clinical Research Institute; for further details, see [7]. Insulin resistance was calculated by the homoeostasis

model assessment of insulin resistance by computer model (HOMA-IR) [20]. Based on Matsuda et al., HOMA-IR was calculated according to the formula: (fasting insulin (pmol/L) × fasting glucose (mmol/L)/135 [21]. Insulin resistance was defined as follows: (1) BMI >28.9 kg/m^2, or (2) HOMA-IR >4.65 or (3) HOMA-IR >3.60 if BMI >27.5 kg/m^2 based on Stern et al. [22]. Dyslipidaemia was regarded as high triglyceride (>1.7 mmol/L) and/or low high-density lipoprotein cholesterol (HDL-C) levels (<1.03 for men, and <1.29 for women) [23].

Other clinical variables

Blood pressure was measured with each participant sitting upright and determined as the average of two measurements using a full-automatic device (Omron M6, HEM-7001-E, Kyoto, Japan). Hypertension was defined as ≥140 and/or 90 mmHg of systolic and diastolic blood pressure, respectively [24]. Blood haemoglobin was determined on site using a standard Coulter counter technique (model KX-21N, Sysmex Corporation, Kobe, Japan). Information concerning alcohol consumption and tobacco use was self-reported by the participants via an interactive Medical Health Assessment Form. Excessive alcohol consumption threshold was 20 g/day and 30 g/day for women and men, respectively [25].

Statistical analysis

Continuous variables were tested for normality using histogram and normal quantile plot. Skewed data were log-transformed prior to analysis. Differences between non-NAFLD and NAFLD groups were determined by using Student's t-test, while the chi^2 test was used to test for differences in proportions. Regression diagnostics showed that the associations between the outcome and exposure variables were linear, the residuals were normally distributed and the requirement for homoscedasticity was fulfilled. Logistic regression analyses adjusted for age and sex were used to assess the association between the binary outcome for not having or having hepatic steatosis in relation to age categories, sex, residence, ethnicity, waist circumference (per cm increase), VAT (per cm increase), SAT (per cm increase), HOMA-IR (per unit increase), glucose metabolism categories, HDL-C (per mmol/L increase), dyslipidaemia and hypertension expressed as odds ratio (OR) (95% CI). Parameters including sex, NAFLD, HOMA-IR, DM, IGT and IFG, dyslipidaemia and hypertension were categorised by FLI categories. In order to establish to what extent the differences in proportions of IFG, IGT and DM were driven by NAFLD, analyses of associations between IFG, IGT and DM without having and with NAFLD were carried out. Data that showed normal distribution are presented as means (SD) for continuous variables, and n (%) for categorical variables. Data with

skewed distribution are presented as geometric means (SD). Statistical analyses were performed using Stata/MP 14.0 (Stata Corp, College Station, USA).

RESULTS

The study population included 799 Kenyans. Of these, potential participants were excluded due to not reaching the 18-years-old age-limit ($n = 2$), missing values for age, height, weight, sex, BMI and steatosis score ($n = 28$), or if they exceeded the criteria for AFLD [25], that is excessive alcohol consumption ($n = 26$). Thus, 743 participants with a mean age (SD) of 38.0 (11.0) (range 18–68) years participated in the study whereof 439 (59.1%) were women. In total, 587 (79%) were rural residents, and ethnic distribution was 68 (9.2%) Luo, 353 (47.5%) Kamba and 307 (41.3%) Maasai, respectively, while 15 (2%) belonged to other ethnic groups. Eight (1.1%) participants were diagnosed with DM during the study, while 16 (2.2%) had known DM, of whom 11 took medication. Background characteristics of participants without and with NAFLD are presented in Table 1. Based on ultrasound scanning, 118 (15.9%) had NAFLD, with 111 (94.1%) and 7 (5.9%) having mild and moderate steatosis, respectively. None had severe steatosis. The proportion of NAFLD was 13.3% ($n = 78$) and 25.6% ($n = 40$) of the rural and urban participants, respectively ($p < 0.001$), respectively. Among those with NAFLD, 21 (17.8%) and 66 (55.9%) were women <50 and ≥50 years of age, and 31 (26.3%) were men, respectively. Men were less likely to have NAFLD than women (OR = 0.48 (95% CI: 0.31, 0.75), $p = 0.001$). When further adjusted for WC or VAT, the results for sex difference remained statistically significant and more pronounced (0.39, 95% CI: 0.23, 0.65 and 0.29, 95% CI: 0.16;0.53, respectively, both $p < 0.001$). After further adjustment for BMI or SAT, there was no longer a difference in OR between women and men for having NAFLD (OR = 0.78 95% CI: 0.47, 1.30, $p = 0.34$)) and (OR = 1.35 95% CI: 0.79, 2.30, $p = 0.28$), respectively. For individuals having NAFLD, mean BMI in women <50 and ≥50 years was 29.7 (5.8) and 26.9 (5.5) kg/m^2, respectively, and for men it was 27.0 (6.4) kg/m^2. Mean SAT was 3.56 (1.00), 2.84 (1.50) and 2.40 (1.19) cm, for women <50 years, women ≥50 years and men, respectively. Among those with NAFLD, WC and VAT were higher in men, while BMI and SAT were higher in women (results not shown). All body composition, biochemical and haemodynamics data were positively associated with NAFLD ($p < 0.045$) (see Table 2).

Except for sex distribution and the distribution of IFG between the three FLI grade groups, all cardio-metabolic diseases showed significant associations across FLI grade groups going from grade I to grade III ($p < 0.001$) (see Table S1).

The proportion of IFG, IGT and DM in the participants with NAFLD was 4.2, 2.0 and 3.8 times higher, respectively, compared with those without NAFLD (Figure 1). Blood glucose levels at 0, 30 and 120 min. were significantly higher in the participants with NAFLD ($p < 0.002$) (Figure 2).

TABLE 1 General characteristics of participants without and with non-alcoholic fatty liver disease ($n = 743$) presented as mean (SD) and proportion (%)

Variable	Non-NAFLD $n = 625$	NAFLD $n = 118$	p-value
Age (years)	36.7 (10.8)	41.6 (11.4)	0.001
Sex (F:M %)	352:273 (56:44)	87:31 (74:26)	0.001
Urban residence, n (%)	116 (19)	40 (34)	0.001
BMI (kg/m^2)	21.3 (3.8)	27.4 (5.8)	0.001
Waist circumference (cm)	76.8 (9.3)	91.5 (14.2)	0.001
VAT (cm)	6.3 (1.31)	7.7 (2.0)	0.001
SAT^a (cm)	1.4 (1.0)	2.9 (1.4)	0.001
Fasting plasma glucose (mmol/L)^a	4.5 (1.4)	4.9 (1.8)	0.012
Fasting serum insulin (pmol/L)	27.6 (20.5)	48.6 (41.5)	0.001
HOMA-IR	0.82 (0.76)	1.65 (2.7)	0.001
DM, n (%)	14 (2.2)	10 (8.5)	0.001
IGT, n (%)	45 (7.2)	17 (14.4)	0.010
IFG, n (%)	6 (1.0)	5 (4.2)	0.007
Total cholesterol (mmol/L)	3.9 (0.9)	4.4 (1.1)	0.001
LDL-C (mmol/L)	2.2 (0.9)	2.7 (1.0)	0.001
HDL-C (mmol/L)	1.15 (0.29)	1.08 (0.28)	0.015
Triglycerides (mmol/L)	0.9 (0.5)	1.2 (0.65)	0.001
Dyslipidaemia, n (%)	167 (26.7)	45 (38.1)	0.017
ALP (U/L)	80.4 (31.6)	84.1 (26.5)	0.264
ALT (U/L)	8.7 (5.1)	8.6 (4.4)	0.750
AST (U/L)	21.4 (10.2)	19.6 (7.79)	0.107
Total bilirubin (μmol/L)	4.7 (3.8)	6.5 (5.93)	0.003
GGT (U/L)	22.5 (19.6)	27.4 (31.8)	0.040
Systolic BP (mmHg)	119 (16)	127 (17)	0.001
Diastolic BP (mmHg)	74 (10)	78 (11)	0.001
Hypertension, n (%)	60 (9.6)	30 (25.4)	0.001
Haemoglobin (g/dL)	13.7 (2.1)	13.4 (2.2)	0.145
Tobacco use, n (%)^b	76 (12.2)	2 (1.7)	0.001
Alcohol, n (%)^c	46 (7.4)	7 (5.9)	0.543

Note: Data are presented as means (SD) for continuous variables and n (%) for categorical variables.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; Diastolic BP, diastolic blood pressure; DM, diabetes mellitus; GGT, gamma-glutamyl transferase; HDL-C, high-density lipoprotein; HOMA-IR, homeostasis model assessment was calculated as (fasting plasma insulin X fasting plasma glucose)/22.5; IFG, impaired fasting glycaemia; IGT, impaired glucose tolerance; LDL-C, low-density lipoprotein; SAT, subcutaneous adipose tissue; Systolic BP, systolic blood pressure; VAT, visceral adipose tissue.

Waist circumference: $n = 741$; VAT: $n = 741$; SAT: $n = 741$; Fasting serum insulin: $n = 722$; HOMA-IR: $n = 722$; Total cholesterol: $n = 711$; LDL-C: $n = 734$; HDL-C: $n = 711$; Triglycerides: $n = 711$; Systolic BP: $n = 731$; Haemoglobin: $n = 699$; Smoking: $n = 706$; Alcohol: $n = 699$.

^{a}Presented as geometric mean (SD).

^{b}For most Maasai smoking is sniff and chewing tobacco.

^{c}Participants with an alcohol-consumption that does not exceed the criteria for NAFLD.

TABLE 2 Odds ratio for univariate and multivariate logistic regression for having non-alcoholic fatty liver disease

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95%CI)	p-value	Odds ratio (95%CI)	p-value
Age (years)[1]				
Age ≤30	1.00		1.00	
30 < Age ≤40	1.31 (0.72, 2.40)	0.383	1.22 (0.66, 2.22)	0.527
Age >40	2.67 (1.58, 4.50)	0.001	2.48 (1.46, 4.21)	0.001
Men[2]	0.47 (0.29, 0.71)	0.001	0.48 (0.31, 0.75)	0.001
Urban residence	2.25 (1.12, 3.46)	0.001	2.85 (1.81, 4.50)	<0.001
Ethnicity[a]				
Luo	1		1	
Kamba	0.68 (0.45, 1.03)	0.069	0.82 (0.53, 1.27)	0.317
Maasai	0.70 (0.15, 3.16)	0.641	1.13 (0.24, 5.38)	0.872
Body mass index (per kg/m^2 increase)	1.28 (1.22, 1.34)	<0.001	1.27 (1.21, 1.33)	<0.001
Waist circumference (pr cm increase)	1.11 (1.09, 1.13)	<0.001	1.11 (1.09, 1.14)	<0.001
VAT (pr cm increase)	1.70 (1.46, 1.98)	<0.001	1.81 (1.53, 2.14)	<0.001
SAT (pr cm increase)	2.57 (2.15, 3.10)	<0.001	2.65 (2.16, 3.27)	<0.001
HOMA-IR (per unit increase)	1.83 (1.50, 2.26)	<0.001	1.77 (1.43, 2.19)	<0.001
Glucose metabolism				
NGT	1		1	
Prediabetes (IFG/IGT)	2.64 (1.53, 4.55)	<0.001	2.43 (1.39, 4.24)	0.002
Diabetes mellitus	4.63 (1.99, 10.7)	<0.001	3.82 (1.59, 9.17)	0.003
Total cholesterol (per mmol/L increase)	1.64 (1.34, 2.00)	<0.001	1.57 (1.26, 1.95)	<0.001
HDL-C (per mmol/L increase)	0.42 (0.21, 0.85)	0.016	0.41 (0.20, 0.83)	0.014
LDL-C (per mmol/L increase)	1.18 (144, 2.29)	<0.001	1.73 (1.36, 2.03)	<0.001
Triglycerides (per mmol/L increase)	2.26 (1.61, 3.14)	<0.001	2.17 (1.51, 3.13)	<0.001
Dyslipidaemia	1.65 (1.01, 2.34)	0.018	1.51 (0.99, 2.31)	0.057
Hypertension	3.22 (1.97, 5.28)	<0.001	3.34 (1.96, 5.70)	<0.001

Note: All multivariable analyses adjusted for age and sex except for [1]only adjusted for sex and [2]only adjusted for age.

Abbreviations: HDL-C, high-density lipoprotein; HOMA-IR, homeostasis model assessment was calculated as (fasting plasma insulin × fasting plasma glucose)/135; IFG, impaired fasting glycaemia; IGT, impaired glucose tolerance; LDL-C, low-density lipoprotein; NGT, Normoglycaemia; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

[a]Excludes 15 participants of other ethnicities than Luo, Kamba, Maasai.

DISCUSSION

In Kenyan adults, we found lower OR (0.48) for having NAFLD in men than women with a female-to-male ratio of 2.8 to 1. Furthermore, being age >40 years showed an OR of ~2.5 for NAFLD versus being <30 years of age, and the urban population had a ~3-fold higher risk of NAFLD compared with the rural population. Cardio-metabolic risk parameters were all significantly associated with NAFLD (OR > 1.5), with hypertension and DM having the highest OR of 3.4 and 3.8, respectively.

The lower OR for having NAFLD in men than women that we found in this study is in contrast to previous findings [26,27]. Furthermore, this result is despite men in the current cohort having higher abdominal fat accumulation measured as WC and VAT, that is metabolically active fat tissue contributing to venous drainage of fat tissue going directly to the liver via the portal vein resulting in ectopic fat accumulation

and insulin resistance [28]. When adjusting for WC and VAT accumulation, the OR estimates became even more pronounced. This may be explained by women being more prone to NAFLD for a given amount of intra-abdominal fat accumulation, even if at this stage this suggestion remains pure speculation. Interestingly, when adjusting for BMI and SAT accumulation, both of which were higher in women, the sex-specific difference for having NAFLD disappeared. In a systematic review, Pang et al. showed that WC and VAT had a stronger association with NAFLD than BMI, even though all three obesity measures were independently associated with increased risk of NAFLD [29]. Furthermore, Fan et al. [30] found a dose-response relationship between BMI and liver steatosis risk in an approximate J-shaped fashion. In the current cohort, but including a higher number of participants (*n* = 1459), we previously showed that in women, BMI had the highest OR for glucose intolerance risk with increasing obesity as compared with WC, VAT and SAT, while for men,
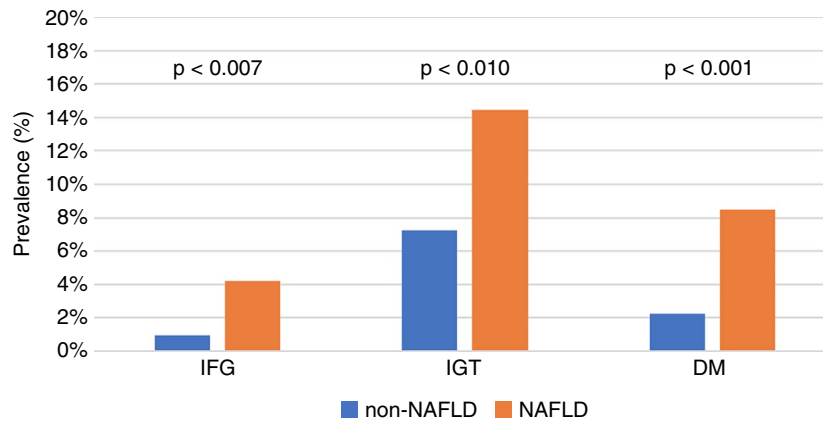
FIGURE 1 Prevalence of impaired fasting glucose (IFG*, 6.1–6.9 mmol/L** and <7.8 mmol/L***), impaired glucose tolerance (IGT*, <7.0 mmol/L** and ≥7.8 and <11.1 mmol/L***) and diabetes mellitus (DM*, ≥7.0 mmol/L** or ≥11.1 mmol/L***) among participants with non-NAFLD and NAFLD. *Plasma values; **fasting glucose test; ***2-h glucose tolerance test
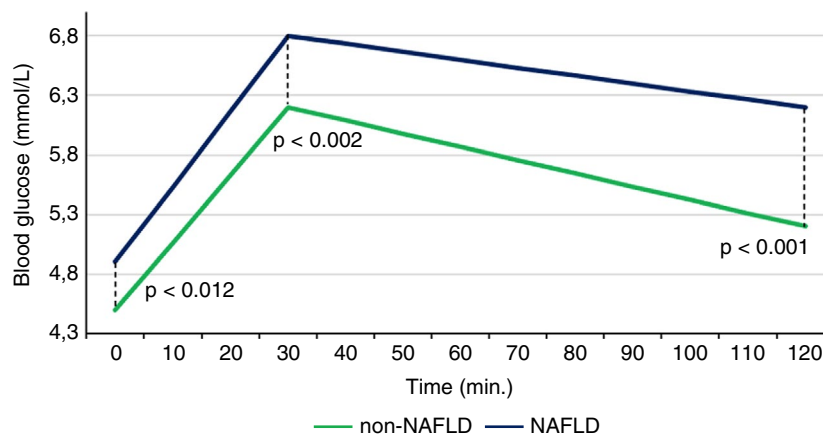

FIGURE 2 Whole blood glucose profile in participants with NAFLD and non-NAFLD during 2-h oral glucose tolerance test. Presented as geometric means

VAT showed the highest OR for glucose intolerance [4]. Thus, general obesity appears to be more detrimental to metabolic disorders in Kenyan women compared with men.

Nevertheless, SAT also explained the difference in sex-specific OR for having NAFLD. In the NAFLD individuals, women regardless of age group had higher SAT than men; furthermore, women <50 years (presumably premenopausal), had higher SAT accumulation than women ≥50 years (presumably postmenopausal). This difference could be explained by the high-energy demands of lactation in young women as SAT acts as an energy reservoir for women within reproductive age [31].

SAT in general is a depot for surplus triglycerides [32], and is regarded as protective in relation to cardio-metabolic disorders [33]. However, the USS technique does not distinguish between superficial and deep SAT divided by the Scarpa's fascia [34]. Deep SAT adipocytes have been shown to have higher lipolytic activity compared with superficial SAT adipocytes [35], and contain more saturated fat than superficial SAT [36]. Thus, deep SAT to some extent resembles VAT, and may therefore be responsible for ectopic fat accumulation,

including hepatic steatosis. It is important to note that more than half of the individuals with NAFLD in this study were postmenopausal women when using age ≥50 years as a proxy measure. Prevalence and incidence of NAFLD are higher in men and postmenopausal women compared with premenopausal women [37,38]. For the postmenopausal women, this is due to the loss of oestrogens, which among others regulate liver lipogenesis [39]. Nevertheless, Naran et al. have shown that SAT is a significant negative determinant of hepatic steatosis as measured by computed tomography in a small sample ($n = 29$) of black, South African women [40].

Cardio-metabolic disorders were significantly higher in the current study population with NAFLD whether measured as absolute numbers, by OR for each unit increase or by OR for standard cut-offs for disease. Increased HOMA-IR is regarded as the metabolic manifestation of hepatic steatosis, and as this feature progresses may result in DM [41]. We found significantly higher proportions of DM as well as prediabetes (IFG and IGT) in those with NAFLD. The results are based on a fasting blood test followed by an OGTT, and this indicates not only hepatic IR, but also peripheral IR as

demonstrated by higher 30 min and 2-h whole blood glucose levels in NAFLD individuals. Furthermore, DM showed the highest OR for having NAFLD at 3.80 compared with other cardio-metabolic and anthropometric correlates of hepatic steatosis. This is in line with previous studies showing that excess hepatic fat deposition is uniformly present before the onset of 'classical' type 2 DM [42,43], assuming that the DM sub-type in this study was predominantly if not entirely type 2 DM.

We found no difference in OR for having hepatic steatosis between the three ethnic groups studied, that is the Luo, Kamba and Maasai. Based on results from the same but larger groups of participants, the Maasai had higher VAT accumulation (n = 1430), and higher hepatic insulin resistance based on HOMA-IR (n = 1087), respectively, compared with the other ethnic groups [3,44]. Therefore, a higher proportion of hepatic steatosis in the Maasai was expected. Thus, this adds to the evidence of a lack of linear relationship between VAT accumulation, insulin resistance and hepatic steatosis, but points to a more complex interaction between these interrelated parameters.

FLI is an algorithm used as a prediction for liver steatosis [16], which is often employed in clinical practice as the parameters (triglycerides, BMI, GGT and WC) included in the algorithm are routine measurements, albeit not in public clinics or hospitals in Kenya or SSA in general. According to Bedogni et al., FLI $\geq$ 60 is an indicator of hepatic steatosis when using USS as reference [18]. We found a significant association for having NAFLD across the three grades of FLI. Nevertheless, FLI may underestimate hepatic steatosis in black Africans due to low triglyceride levels in black African populations in general, which we also found in the current study population [5]. Importantly, HOMA-IR had an overlap of 83.8% when comparing FLI $\geq$ 60 with USS-derived diagnosis. Therefore, FLI seems to be relevant for cardio-metabolic disease risk in black African populations, and this algorithm may be easier to implement in clinical practice compared with analysing USS-captured images which is relatively time consuming.

The prevalence of NAFLD in this study was 15.9%, which is slightly higher than the prevalence reported by Younossi et al. based on a meta-analysis [14] including two very small studies in SSA. It is of note, that we only found mild and moderate steatosis, and that USS technique is limited by lower sensitivity for detecting milder degrees (<30%) of steatosis as compared with magnetic resonance imaging and magnetic resonance spectroscopy [45]. Thus, we may have underestimated the prevalence of NAFLD.

Our results show that NAFLD is not only an important factor for adverse cardio-metabolic traits, but also that hepatic steatosis becomes more prevalent with increasing age and urbanisation. The latter two factors are important to consider in Kenya and other SSA nations where life expectancy and urbanisation have been increasing over the past few decades – a trend which is expected to continue [46,47]. Thus, NAFLD prevalence is likely to increase, and along with this development cardio-metabolic disease risk is expected to increase as well. Special attention may be needed for women in this context, especially if NAFLD translates into higher incidence of diabetes and cardiovascular diseases in the female part of the population.

Strengths of this study were the large sample size in an African context, inclusion of rural and urban participants, and a comprehensive panel of USS, simple anthropometric, biochemical and haemodynamic measurements across a population with a wide age range (18–68 years).

We acknowledge several limitations to this study. Neither intra- nor inter-observer validity USS was carried out, and USS is not a gold standard technique, all of which could have compromised the quality of the data. However, NAFLD screening using USS at the population level in resource-poor settings may be the most cost-effective approach in a public health context. Furthermore, this is a cross-sectional study, and therefore the interpretation of the results from a cause-relationship perspective, must be done with caution. We also want to stress that using logistic regression analysis for association with frequent disease (>10% prevalence) such as hypertension or dyslipidaemia may inflate the estimates. It is of note that 11 and 10 study participants in the cohort were treated with hypoglycaemic and hypotensive drugs, respectively, and none of them were on lipid-lowering agents. Finally, we did not collect data on HIV including anti-retroviral drugs, hepatitis B or C or other biomarkers for infections, which could have affected the results.

In conclusion, NAFLD is more common in Kenyan women than men, which may be explained by general obesity (BMI) and SAT accumulation. Furthermore, increasing age and urbanisation are both associated with NAFLD, while ethnicity comparing Luo, Kamba and Maasai people showed no difference in NAFLD. Especially the sex-specific results warrant further studies in black African populations on body composition and NAFLD, and whether this translates into insulin resistance and higher risk of diabetes and consequently cardiovascular disease in black African women in particular.

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