Research Article
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The degree of hepatic steatosis associates with impaired cardiac and autonomic function

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Background & Aims: Cardiovascular disease is the principle cause of death in patients with elevated liver fat unrelated to alcohol consumption, more so than liver-related morbidity and mortality. The aim of this study was to evaluate the relationship between liver fat and cardiac and autonomic function, as well as to assess how impairment in cardiac and autonomic function is influenced by metabolic risk factors.

Methods: Cardiovascular and autonomic function were assessed in 96 sedentary individuals: i) non-alcoholic fatty liver disease (NAFLD) (n = 46, hepatic steatosis >5% by magnetic resonance spectroscopy), ii) Hepatic steatosis and alcohol (dual aetiology fatty liver disease [DAFLD]) (n = 16, hepatic steatosis >5%, consuming >20 g/day of alcohol) and iii) CONTROL (n = 34, no cardiac, liver or metabolic disorders, <20 g/day of alcohol).

Results: Patients with NAFLD and DAFLD had significantly impaired cardiac and autonomic function when compared with controls. Diastolic variability and systolic variability (LF/HF-sBP [n/1]; 2.3 (1.7) and 2.3 (1.5) *vs.* 3.4 (1.5), p <0.01) were impaired in patients with NAFLD and DAFLD when compared to controls, with DAFLD individuals showing a decrease in diastolic variability relative to NAFLD patients. Hepatic steatosis and fasting glucose were negatively correlated with stroke volume index. Fibrosis stage was significantly negatively associated with mean blood pressure ($r = -0.47$, $p = 0.02$), diastolic variability ($r = -0.58$, $p \leq 0.01$) and systolic variability ($r = -0.42$, $p = 0.04$). Hepatic steatosis was independently associated with cardiac function ($p \leq 0.01$); TNF-α ($p \leq 0.05$) and CK-18 ($p \leq 0.05$) were independently associated with autonomic function.

Conclusion: Cardiac and autonomic impairments appear to be dependent on level of liver fat, metabolic dysfunction, inflammation and fibrosis staging, and to a lesser extent alcohol intake. Interventions should be sought to moderate the excess cardiovascular risk in patients with NAFLD or DAFLD.

Lay summary: Increased levels of fat in the liver impair the ability of the cardiovascular system to work properly. The amount of fat in the liver, metabolic control, inflammation and alcohol are all linked to the degree that the cardiovascular system is affected.

Introduction

Current clinical care in chronic liver disease divides fatty liver disease into non-alcoholic fatty liver disease (NAFLD) or alcoholic fatty liver disease (ALD), predominantly based on alcohol intake.[1] In line with the increase in obesity NAFLD has become the leading cause of liver disease in developed countries,[2,3] closely followed by ALD, which together account for the 2 most common liver diseases worldwide.[4]

NAFLD and ALD encompass a spectrum of clinical features, with similar pathophysiology and histological features ranging from steatosis, steatohepatitis, fibrosis and cirrhosis, with more advanced forms of the disease causing considerable liver mortality and morbidity.[5] The clinical and economic burden of NAFLD and ALD is not only dependent on liver-related mortality, but is also due to extrahepatic diseases (type 2 diabetes, kidney disease) and increased risk of cardiovascular disease (CVD).[6,7]

CVD rather than liver-related death, is a more common outcome in patients with NAFLD,[8] however, liver-related deaths are more strongly associated with ALD. The risk of CVD in NAFLD increases in a dose dependent manner in line with the severity of NAFLD (especially, fibrosis stage)[9,10] and alcohol consumption in ALD.[11] Over 20 retrospective and prospective studies have investigated the relationship between NAFLD and CVD,

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with the majority of NAFLD cases showing increased CVD morbidity and mortality, independent of metabolic phenotypes.[12] Furthermore, the relationship between liver disease and CVD, extends to fibrosis stage. In comparison with a matched reference population, patients with NAFLD and fibrosis were more likely to die from CVD and liver-related disease, specifically those with stage 3 or 4 fibrosis.[13]

Although the relationship between liver disease and CVD is well defined, the underpinning mechanisms are less well understood. The presence of subclinical atherosclerosis has been shown to be linked with disease severity in NAFLD.[14] Furthermore, chronic low grade inflammation is a characteristic of metabolic disorders, and may contribute to extrahepatic complications.[15] In particular, higher levels of circulating inflammatory mediators associated with non-alcoholic steatohepatitis (NASH) may also play a pathogenic role in CVD,[16] activate sites in the brain[17] and increase sympathetic outflow.[18] NASH and fibrosis severity have also been linked with epicardial fat, morphological and functional cardiac alterations and inflammation.[19–23] Heavy alcohol consumption also increases the risk of cardiomyopathy, hypertension, arrhythmias, stroke and cardiac ischemia and altered autonomic function.[11,24] Furthermore, increased alcohol intake has shown to lead to weight gain and obesity, further exacerbating the risk factors and overlap between NAFLD and ALD.[25]

Changes in autonomic dysfunction predispose individuals to cardiac arrhythmias, coronary artery disease and increased mortality,[26–33] all of which increase in patients with NAFLD and ALD. Despite research supporting a link between NAFLD, CVD, alcohol intake and autonomic dysfunction, to date studies have not assessed both central haemodynamic and cardiac autonomic measures simultaneously in individuals with NAFLD and those with hepatic steatosis consuming >20 g/day of alcohol. Given that increased alcohol intake is linked to cardiac complications, and CVD dictates clinical outcomes in patients with NAFLD, a more detailed understanding of the potential mechanisms leading to CVD in patients with >5% liver fat, in those consuming greater than and less than 20 g/day of alcohol, would enable appropriate therapeutic interventions to be developed. The primary aim of this study was to evaluate the relationship between central haemodynamic and autonomic function in combination with liver fat, while also assessing how any impairment in cardiac and autonomic function is influenced by additional risk factors including alcohol, obesity, inflammation and diabetes.

Patients and methods

A total of 96 sedentary patients (defined as <60 min of moderate to vigorous activity per week) were recruited into the study. All participants underwent assessment of liver fat, abdominal adiposity, autonomic function, body composition, blood biochemistry, and metabolic testing (Table 1). The cohort consisted of 3 groups: **Group 1 – NAFLD**: 46 patients with NAFLD, defined as having a hepatic triglyceride content (HTGC) greater than 5% by magnetic resonance spectroscopy (MRS), no other causes of liver disease and consuming no alcohol or <20 g of alcohol per day. This group included 27 patients who had histologically proven NASH (liver histology and NAFLD fibrosis reported in Table 1); **Group 2 – DAFLD**. Hepatic steatosis and alcohol drinkers (dual aetiology fatty liver disease [DAFLD]): 16 patients with HTGC greater than 5% using MRS, no other causes of liver disease and consuming >20 g but <50 g of alcohol per day[1,34] and **Group 3 – CONTROL**: 34 individuals who were non-smokers, had no overt evidence of cardiac, liver or metabolic disorders and who consumed no alcohol or <20 g of alcohol per day. The study was approved by Sunderland Research Ethics Committee, UK. All patients provided informed written consent and were recruited from primary (GP surgeries in Newcastle upon Tyne, UK) and secondary care (Freeman Hospital, Newcastle upon Tyne, UK) and had previously taken part in the following studies: UK (REC 13NE/0041 and 12/NE/0411, ISRCTN90597099: http://www.isrctn.com/ISRCTN90597099 and ISRCTN16070927: http://www.isrctn.com/ISRCTN16070927).

Individuals with evidence of other liver disease (autoimmune hepatitis, viral hepatitis, drug-induced liver injury, haemochromatosis, cholestatic liver disease or Wilson's disease) were excluded. Other exclusion criteria included: heart (alcoholic cardiomyopathy or otherwise related) or kidney disease, dietary change over the preceding 6 months and insulin sensitising treatment (for patients with type 2 diabetes, only diet and metformin-controlled individuals were accepted, and it was a requirement that they were stable for at least 6 months prior to assessment). Alcohol levels were assessed by a suitably trained member of the research team as an average over the last year and then confirmed by hepatologists (SM, SMc, CD and QA) (Table 1).

Screening visit

During the screening visit a full medical history and physical examination was completed. Individuals from Groups 1 and 2 then completed a progressive peak exercise test to screen for any undiagnosed cardiac disease. Supine resting 12-lead electrocardiogram (ECG) (Custo med GmbH, Ottobrunn, Germany) and blood pressure measures (Suntech Tango+, Suntech Medical Ltd, Oxford) were conducted. Patients were then seated on an electronically braked recumbent cycle ergometer (Corival Lode BV, Groningen, Netherlands) to assess cardiac function and VO_2 peak. Following a 5-min warm up at 25 W, resistance was increased by 1 W per 8 seconds until volitional exhaustion was reached or patients were unable to maintain a cadence of 60–70 rpm. The ECG was used to continuously monitor heart function and blood pressure was measured every 2 min during the exercise test. Expired gases were collected using a Hans Rudolf breathing mask and analysed online for oxygen, carbon dioxide and minute ventilation (CORTEX Biophysik, Leipzig, Germany).

Anthropometry

For individuals from Group 1 and 2 body weight was measured using an electronic scale and air displacement plethysmography (BodPod, Life Measurement Inc., CA, USA).[35] Height was measured with a stadiometer (SECA 799, SECA UK).

Liver steatosis and abdominal fat measurement

For individuals from Groups 1 and 2, magnetic resonance studies were performed using a 3.0 Tesla Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands). HTGC was measured by localised T2-corrected 1H-magnetic resonance spectroscopy (PRESS, TR/TEs = 3,000 ms/36, 50, 75, 100, 125, 150, voxel size 3 × 3 × 3 cm). Lipid volume fractions were calculated according to previous research[36] by integration of the lipid resonances between 0.5–3.0 ppm and the water and lipid

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Table 1. Mean (SD) baseline characteristics.

	Control (n = 34)	NAFLD (n = 46)	DAFLD (n = 16)	Between group *p* value
Anthropometry				
Age (years)	52 (9)	54 (13)	55 (10)	0.66
BMI (kg/m^2)	27 (3)	32 (5)	32 (5)	0.01*
Gender (M/F)	23/11	28/18	12/4	0.55
VO_{2PEAK} (ml/kg/min)	–	22 (6)	22 (5)	0.87
LT (ml/kg/min)		15 (4)	14 (5)	0.53
Peak exercise HR (b/min)		152 (21)	153 (18)	0.70
Resting RQ		0.9 (0.2)	0.9 (0.1)	0.45
Alcohol intake (g/wk)				
Women	–	–	140–168	–
Men	–	–	231–273	–
Smoking status (%)	0	4	13	0.30
HTGC (%)	–	13 (7)	13 (6)	0.99
Visceral fat (cm^2)	–	202 (74)	173 (91)	0.25
Subcutaneous fat (cm^2)	–	351 (133)	341 (104)	0.78
Metabolic				
ALT (U/L)	–	64 (43)	52 (22)	0.27
AST (U/L)	–	46 (23)	44 (23)	0.74
GGT (U/L)	–	102 (144)	110 (103)	0.96
Cholesterol (mmol/L)	–	4.8 (1.4)	5.6 (1.2)	0.06
Triglyceride (mmol/L)	–	1.9 (0.9)	1.9 (0.9)	0.76
F–Glucose (mmol/L)	–	6.1 (1.8)	5.5 (0.5)	0.23
2 h fsOGTT (mmol/L)	–	9.3 (3.9)	8.5 (3.1)	0.45
Insulin (pmol/L)	–	19 (11)	18 (9)	0.80
HbA_{1c} (%)	–	47 (12)	39 (4)	0.01#
HOMA–IR	–	1.6 (1.1)	1.8 (0.9)	0.64
IL–6	–	1.2 (0.6)	1.7 (1.1)	0.08
TNFα	–	3.6 (1.8)	4.3 (1.6)	0.14
CK–18	–	633 (876)	247 (153)	0.04#
Type 2 diabetes (%)	–	48	56	0.07
Metformin	–	42	25	–
Sulfonylureas	–	13	1	–
Thiazolidinediones	–	8	–	–
GLP–1 Receptor Agonist	–	2	–	–
DPP–4 Inhibitors	–	4	–	–
Insulin	–	4	6	–
Hypertension (%)	–	38	62	0.05
ARB	–	6	6	–
ACE–1	–	19	63	–
BB	–	6	6	–
CCB	–	13	19	–
Liver histology and NAFLD fibrosis				
NAS	–	5 (2–7)	–	–
Steatosis	–	2 (1–3)	–	–
Inflammation	–	2 (1–3)	–	–
Ballooning	–	1 (1–2)	–	–
Fibrosis Stage	–	3 (0–3)	–	–
0	–	1 (4%)	–	–
1	–	2 (8%)	–	–
2	–	8 (33%)	–	–
3	–	13 (54%)	–	–
4	–	0 (0%)	–	–

Alcohol consumption presented as range. LT, lactate threshold; HR, heart rate; RQ, respiratory quotient; HTGC, hepatic triglyceride content; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; F-Glucose, fasting glucose, 2 h fsOGTT, 2 h frequently sampled oral glucose tolerance test; HbA1c, glycated haemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; TNF-α, tumour necrosis factor-alpha; IL-6, interleukin 6, CK-18, cytoketatin-18, BP, blood pressure. GLP-1, glucagon-like peptide-1; DPP-4, dipeptidyl peptidase 4: ARB, Angiotensin II receptor blockers; ACE, angiotensin-converting enzyme; BB, beta blockers; CCB, calcium channel blockers; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis. Medication – percentage of patients from total number of patients on medication. CK-18 (mean and SD), liver histology and fibrosis stage are median (range) for patients with NASH. Kruskal-Wallis test and within group differences using a Wilcoxon signed-rank (2 way) test both controlling for BMI. Values are means (SD). # and * denotes a between group difference at p <0.05 and <0.01, respectively.

resonances between 3.0–5.5 ppm, using the 3DiCSI software (Columbia University, USA). Subcutaneous and visceral fat were measured at the L4/L5 junction using a three-point Dixon sequence as previously described.[37] All measurements for HTGC, visceral and subcutaneous fat were blinded for all liver patients only (*i.e.* Groups 1 and 2).

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Liver histology

A subset of Group 1 patients (NAFLD, n = 27) had a histological diagnosis of NASH. These biopsies were conducted as part of their routine clinical care. The liver biopsies were scored by expert hepatopathologists (ADB and DT) according to the NASH Clinical Research Network criteria.[38] NAFLD activity score (NAS) ranges between 0–8, which included scoring for steatosis (0–3), lobular inflammation (0–3) and hepatocyte ballooning (0–2) and fibrosis is staged from 0 to 4. NASH was defined as steatosis with hepatocyte ballooning degeneration, hepatic inflammation ± fibrosis.

Cardiac haemodynamic and autonomic regulation

Patients were fasted overnight for a minimum of 8 h, instructed to consume only water prior to their visit and to avoid exercise for 24 h prior to this visit. All testing was performed at the same time of the day to exclude any diurnal variation in parameters. All cardiac haemodynamic and autonomic regulation data were recorded using a Task Force Monitor (TFM, CNSystem, Medizintechnik, Graz, Austria) where patients lay supine for 10 min acclimatisation, followed by 20 min of recording, as previously described.[39] Briefly, heart rate was assessed by continuous ECG, and beat-to-beat stroke volume, cardiac output and cardiac output index (CI) were assessed using impedance cardiography.[40] Beat-to-beat blood pressure was measured by a vascular unloading technique[41] which was corrected automatically to the oscillometric blood pressure measured on the contralateral arm. Beat variables measured included heart rate, diastolic blood pressure (DBP), systolic blood pressure (SBP) and mean blood pressure (MBP). Cardiac variables were normalised for total body surface area and included stroke volume index (SVI), CI, total peripheral resistance index (TPRI), end diastolic index (EDI), index of contractility (IC), acceleration index (ACI), left ventricular ejection time (LVET), thoracic fluid content (TFC), heather index (HI) and total arterial compliance (TAC).

Heart rate variability, blood pressure variability and baroreceptor variability were all assessed using power spectral analysis, which has demonstrated to be a simple and non-invasive method for analysing autonomic mechanisms.[42] Briefly, heart rate interval variability was calculated by comparing low frequency (LF – predominantly sympathetic activity) (0.05–0.17 Hz) with high frequency (HF – parasympathetic activity) (0.17–0.40 Hz) whilst correcting for the R-R interval of the ECG complex. Using power spectral analysis and applying an autoregressive methodology, SBP and DBP variability (0.05–0.17 Hz) were measured using absolute and normalised values.[41] LF/HF ratio for heart rate and blood pressure variability were measured as the ratio between LF (sympathetic activity) and HF (parasympathetic activity). Baroreceptor variability was measured using the sequence technique[43] and baroreceptor effectiveness index was calculated as the ratio of baroreceptor sequences (or events) as related to the number of blood pressure ramps.

Fasting biochemistry

Fasting samples were analysed for Groups 1 and 2 in a Clinical Pathology Accredited laboratory (*Newcastle Upon Tyne Hospital NHS Foundation Trust, Department of Clinical Biochemistry).* Glucose, insulin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), total cholesterol, triglycerides and glycated haemoglobin (HbA_{1c}) were measured. Plasma samples were collected in silica clot activator polymer gel containing vacutainers (BD Diagnostics, Plymouth, UK). Total cholesterol, triglycerides, ALT, AST, and GGT were measured using a Roche Modular P and test kits (Roche Diagnostics Ltd, Burgess Hill, UK). HbA_{1c} was measured using a TOSOH HLC-723G7 (Tosoh Corporation, Tokyo, Japan). Cytokines (tumour necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6)) were measured V-PLEX plate (K15049D plate, Meso-Scale) and non-invasive scores for disease activity measured cell death (fragments of CK-18 using M30-Apoptosense ELISA kit, PEVIVA, Bromma, Sweden).

Statistics

Between group differences were assessed using a Kruskal-Wallis test and within group differences using a Wilcoxon signed-rank (2-way) test both controlling for body mass index (BMI). Bivariate correlations using Spearman rank correlations were conducted to investigate any associations between baseline characteristics, resting central haemodynamic and cardiac autonomic measures for liver patients only. Multiple linear regression models were used to investigate the associations between HTGC, fibrosis score, CK-18 and TNF-α and cardiac variables (CI, SVI and EDI) and autonomic variables (baroreceptor sensitivity, LFnu-DBP and SBP and HF-DBP and SBP). Models were fit to estimate associations after the adjustment of age, gender, BMI, diagnosis of type 2 diabetes, smoking status and HbA_{1c}. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using SPSS statistical analysis software (Version 19, IBM, predictive Analysis Software, USA). All authors had access to the study data and reviewed and approved the final manuscript.

Results

Baseline characteristics of participants are presented (Table 1). The mean age and gender distribution were similar across the 3 groups. The 2 groups of patients with hepatic steatosis had a significantly higher BMI ($p < 0.01$) when compared to the control group. The patients with NAFLD had similar baseline characteristics to the DAFLD group. HbA_{1c} and CK-18 levels were significantly higher in the NAFLD group ($p < 0.05$).

Comparison of resting central haemodynamic and autonomic measures between individuals with hepatic steatosis (Groups 1 and 2) and controls

Table 2 presents a comparison of the central haemodynamics and cardiac autonomic measures between individuals with NAFLD, DAFLD and controls. Beat variables including heart rate, blood pressure (Fig. 1) and SVI were significantly different between the controls and patients with >5% HTGC ($p < 0.01$). Cardiac variables (EDI, IC, ACI, LVET, TFC, HI and TAC) were all significantly lower in the patients with hepatic steatosis compared to controls ($p < 0.01$), indicating that the hearts of individuals with >5% HTGC were less efficient in filling and contracting than those of the controls.

There were significant differences in heart rate variability and DBP and SBP variability between the control group and those with hepatic steatosis ($p < 0.05$). LFnu-RRI (sympathetic activity), LF/HF-RRI (sympathetic activity/parasympathetic activity ratio) for heart rate were both lower and HFnu-RRI (parasympathetic activity) variability was higher in the control group ($p < 0.01$) when compared to individuals with hepatic steatosis. HFnu-DBP and HFnu-SBP were higher and LF/HF-dBP

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Table 2. Mean (SD) resting central haemodynamic and cardiac autonomic measures for controls and patients with hepatic steatosis.

	Control (n = 34)	NAFLD (n = 46)	DAFLD (n = 16)	Between group *p* value
Beat				
HR (n/1)	63 (9)	68 (9)	70 (9)	0.01#
SBP (mmHg)	119 (9)	127 (15)	131 (11)	0.01#
DBP (mmHg)	79 (7)	84 (10)	85 (10)	0.01#
MBP (mmHg)	94 (8)	99 (12)	104 (9) ¥	0.01#
SV (ml)	81 (26)	74 (18)	68 (15)	0.09
SI (ml/m^2)	42 (12)	35 (9)	35 (9)	0.01#
CO (L/min)	5.0 (1.6)	4.9 (1.1)	4.6 (0.5)	0.60
CI (L/min/m^2)	2.6 (0.77)	2.4 (0.5)	2.4 (0.6)	0.50
TPRI (dyn*s*m^2/cm^5)	3,007 (854)	3,449 (959)	3,603 (1,044)	0.08
Cardiac				
EDI (ml/m^2)	68 (18)	57 (18)	53 (13)	0.01#
IC (1,000/s)	44 (18)	33 (12)	32 (13)	0.01#
ACI (100/s^2)	58 (23)	42 (19)	44 (19)	0.01*
LVET (ms)	322 (15)	309 (20)	308 (18)	0.01*
TFC (1/Ohm)	29 (5)	24 (5)	26 (6)	0.01*
HI (1/s^2)	0.20 (0.11)	0.19 (0.10)	0.18 (0.10)	0.01#
TAC (ml/mmHg)	2.0 (0.7)	1.7 (0.5)	1.6 (0.4)	0.02
Heart rate variability				
LFnu-RRI (%)	61 (14)	51 (16)	54 (20)	0.01*
HFnu-RRI (%)	38 (14)	49 (16)	46 (20)	0.01*
LF/HF-RRI (n/1)	2.2 (1.4)	1.5 (1.0)	1.6 (1.4)	0.03#
Diastolic blood pressure variability				
LFnu-DBP (%)	45 (13)	43 (11)	48 (14)	0.61
HFnu-DBP (%)	11 (5)	24 (12)	17 (10)¥	0.01*
LF/HF-DBP (n/1)	4.8 (2.7)	3.0 (2.0)	3.8 (2.2)¥	0.01*
Systolic blood pressure variability				
LFnu-SBP (%)	44 (13)	39 (11)	42 (13)	0.20
HFnu-SBP (%)	14 (6)	26 (14)	24 (14)	0.01*
LF/HF-SBP (n/1)	3.4 (1.5)	2.3 (1.7)	2.3 (1.5)	0.01*
Baroreceptors reflex sensitivity				
Total-events event count (n/1)	46 (37)	76 (69)	55 (34)	0.20

HR, heart rate, SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; SV, stroke volume; SVI, stroke volume index; CO, cardiac output; CI, cardiac output index; TPRI, total peripheral resistance index; EDI, end diastolic index; IC, index of contractility; ACI, acceleration index; LVET, left ventricular ejection time; TFC, thoracic fluid content; HI, heather index: TAC, total arterial compliance; HRV, heart rate variability; LF, low frequency; nu, normal units; RRI, R to R interval; HF, high frequency; BRS, baroreceptor sensitivity; DAFLD, dual aetiology fatty liver disease; NAFLD, non-alcoholic fatty liver disease. Kruskal-Wallis test and within group differences using a Wilcoxon signed-rank (2-way) test both controlling for BMI. Values are means (±SD). # and * denote a between group difference of $p \leq 0.05$ and <0.01, respectively. ¥denotes a significant difference between NAFLD and DAFLD group at $p \leq 0.05$. Full comparison between NAFLD and DAFLD group table is presented in Table S1.

and LF/HF-sBP were significantly lower in those with hepatic steatosis when compared with the controls ($p \leq 0.01$, Table 2, Fig. 1). CI, baroreceptor sensitivity, total peripheral resistance, LFnu-DBP (sympathetic activity) and LFnu-sBP (sympathetic activity) were not significantly different between the groups ($p > 0.05$). Overall, these findings indicate that individuals with hepatic steatosis have a greater degree of autonomic dysfunction compared to controls.

Comparison of resting central haemodynamic and cardiac autonomic measures between patients with NAFLD and DAFLD

The baseline demographic and clinical characteristics were very similar in the NAFLD and DAFLD groups (Table 1). However, MBP was 5% higher in the DAFLD group ($p \leq 0.02$). Moreover, the parasympathetic activity was 29% lower (% HFnu-DBP) and 26% higher (LF/HF-DBP ratio) in the DAFLD group than those with NAFLD ($p = 0.03$ and 0.04, respectively, Table S1).

Relationship between cardiac and metabolic indices for patients with hepatic steatosis (NAFLD and DAFLD combined)

There was a significant positive association between HTGC and baroreceptors reflex sensitivity ($r = 0.27$, $p = 0.04$) and a negative association between HTGC and SVI ($r = -0.30$, $p = 0.02$) (Fig. 2) and EDI ($r = -0.28$, $p = 0.03$). HTGC was also negatively associated with CI ($r = -0.28$, $p = 0.03$) (Fig. 2), left ventricular work index ($r = -0.30$, $p = 0.02$), IC ($r = -0.29$, $p = 0.03$) and HI ($r = -0.28$, $p = 0.03$). Furthermore, there was a significant negative correlation between HTGC and VO_{2peak} ($r = -0.47$, $p = 0.01$), peak heart rate ($r = -0.23$, $p = 0.08$) and lactate threshold ($r = -0.27$, $p = 0.04$) (Fig. 3). The associations between HTGC, cardiac, autonomic and exercise variables suggest that greater HTGC may be associated with reduced cardiac function. BMI was significantly negatively associated with all cardiac variables ($p \leq 0.05$), beat variables (SVI and CI) ($p \geq 0.05$) and autonomic variables (LFnu-RRI (%), LFnu-DBP and LFnu-SBP) ($p = 0.05$). There were also significant positive associations between BMI and beat variables (heart rate, SBP, DBP, MBP and TPRI) ($p \leq 0.05$) and autonomic variables HFnu-RRI (%), HFnu-DBP and HFnu-SBP ($p = 0.05$). Subcutaneous fat (SAT) was also significantly positively correlated with TPRI ($r = 0.30$, $p = 0.01$), LFnu-DBP ($r = 0.30$, $p = 0.02$), LF/HF-DBP ($r = 0.23$, $p = 0.04$) and LF/HF-SBP ($r = 0.22$, $p = 0.05$), suggesting that body mass and body composition have a significant impact upon cardiac and autonomic function. There was also a significant negative association between fasting glucose and CI ($r = -0.27$, $p = 0.04$) (Fig. 2) and EDI ($r = -0.27$, $p = 0.03$). Cholesterol was negatively correlated with stroke volume ($r = -0.27$, $p = 0.03$), SVI ($r = -0.27$, $p = 0.04$) and TPRI ($r = -0.27$, $p = 0.04$). There was also a trend towards a negative association between triglyceride

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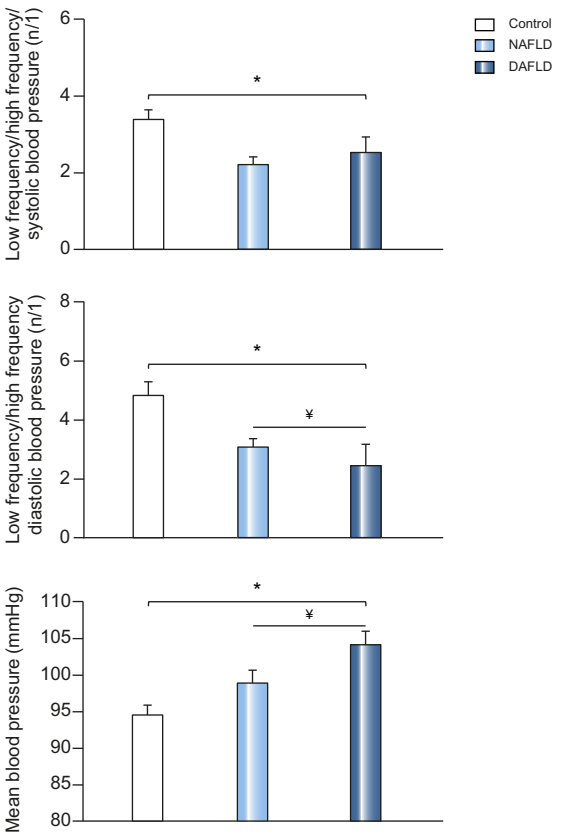

Fig. 1. Comparison of mean blood pressure, and low frequency/high frequency diastolic and systolic blood pressures between controls, and patients with NAFLD and DAFLD. Data are mean (SEM) for blood pressure, low frequency/high frequency ratio (sympathetic/parasympathetic, respectively) diastolic blood pressure and systolic blood pressure for controls (n = 34), patients with NAFLD (n = 46) and patients with DAFLD (n = 16) groups. * and ¥ denote a significant between group difference for all 3 groups (p <0.01) and a significant difference between NAFLD and DAFLD groups (p <0.05), respectively. Kruskal-Wallis test and within group differences using a Wilcoxon signed-rank (2-way) test both controlling for BMI. BMI, body mass index; DAFLD, dual aetiology fatty liver; NAFLD, non-alcoholic fatty liver disease and disease.

levels and stroke volume (r = −0.28, p = 0.08). Homeostasis model assessment-insulin resistance (HOMA-IR) was positively correlated with LF heart rate variability (r = 0.27, p = 0.03) and negatively correlated with HF heart rate variability (r = −0.27, p = 0.03). HbA_{1c} was significantly negatively associated with heart rate (r = −0.30, p = 0.01) (Fig. 2). There was a significant negative association between fasting glucose and SBP (r = −0.26, p = 0.04), SV (r = −0.26, p = 0.04) and CO (r = −0.31, p = 0.02) and a significant positive correlation between LF-nu DBP (r = 0.30, p = 0.02), SBP (r = 0.44, p <0.01), baroreceptor sensitivity (r = 0.33, p = 0.01) and TNF-α. Collectively, these findings suggest insulin resistance, dyslipidaemia and inflammation are associated with reduced cardiac and autonomic function in these individuals with hepatic steatosis.

Relationship between cardiac and metabolic indices for patients with histologically diagnosed NASH

In the 27 patients with NASH, fibrosis stage was significantly negatively associated with DBP (r = −0.47, p = 0.02), MBP (r = −0.47, p = 0.02), HFnu-dBP (r = −0.55, p ≤0.01), LF/HF-dBP (r = −0.58, p ≤0.01) and HFnu-sBP (r = −0.42, p = 0.04).

These findings suggest that there is an association between advanced liver fibrosis and reduced cardiac and autonomic function in these individuals with NASH.

Multiple linear regressions

The data presented in Table 3 generated from the multiple linear regressions show the associations of TNF-α, CK-18, HTGC and fibrosis scoring with cardiac and autonomic variables. There were strong inverse associations with cardiac variables CI, SVI and EDI and HTGC when controlling for age, gender, BMI, diagnosis of type 2 diabetes, HbA_{1c} and smoking. There were significant associations between TNF-α and autonomic variables (baroreceptor sensitivity, LFnu-DBP and SBP). CK-18 was also significantly associated with HFnu-DBP and SBP. These findings demonstrate that liver fat is independently associated with cardiac function, but not autonomic function. However, inflammation (TNF-α and CK-18) was independently associated with autonomic dysfunction but not cardiac function.

Discussion

This is the first study to explore cardiac *and* autonomic function in combination in a well characterised cohort of individuals with hepatic steatosis associated with NAFLD and alcohol intake. The main findings of this study were that patients with >5% HTGC had evidence of impaired cardiac and autonomic function when compared with controls. These differences appeared to be dependent on the degree of HTGC and metabolic dysfunction, inflammation and to a lesser extent the presence of increased alcohol consumption. Additional sub-analyses indicated that fibrosis staging may also be a key contributor towards the degree of cardiac and autonomic dysfunction, confirming previously published data.[13] These findings are of clinical importance as patients with NAFLD, especially those with advanced fibrosis have an increased risk of cardiac death. Defining the pathophysiology of cardiac and autonomic dysfunction in patients could help identify therapeutic interventions to reduce cardiac risk.

Using sensitive measures in a well-defined group, we have been able to demonstrate that liver disease is associated with impaired cardiac function, including reductions in CI and pumping capability, and increases in blood pressure and total peripheral resistance. It is important to acknowledge that body surface area indexed cardiac variables reported here, may not be as clinically accurate in obese liver patients as imaging techniques that account for left ventricular mass and height.[44] To account for this we controlled for BMI, and in addition to CI, we reported significant differences in additional cardiac variables, all of which contribute to cardiac function and pumping capability. These observations are supported by previous studies conducted by our group where MRI imaging techniques have been used.[19,45] These have shown that the hearts of patients with chronic liver disease, regardless of the aetiology, do not function as well as those of controls.[19,46–48] The cause of the underlying cardiac dysfunction associated with NAFLD is not known. Here we demonstrate that liver fat and fasting glucose were negatively correlated with cardiac output variables.

Previous reports have demonstrated that liver fat predicts the presence of impaired myocardial metabolism and cardiac dysfunction.[49,50] Multiple linear regression analyses conducted for the present study have supported this by showing that liver fat was independently associated with cardiac function when

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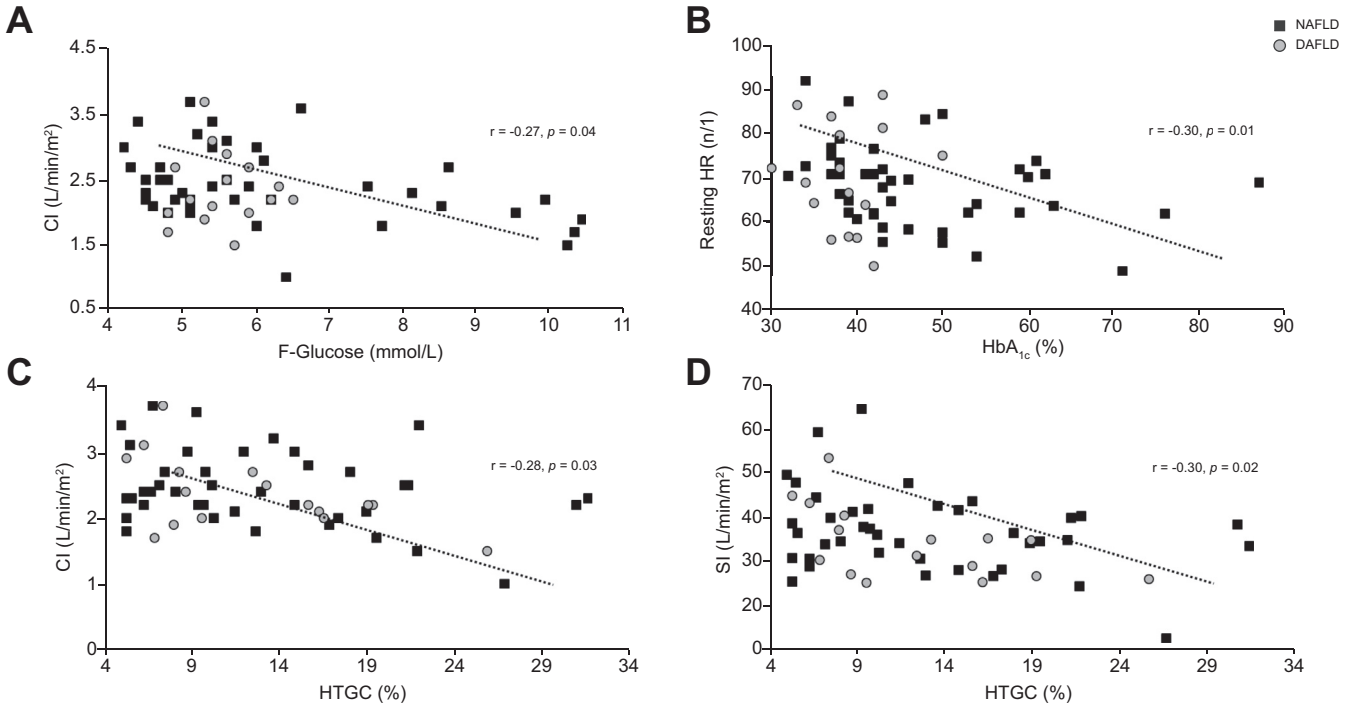

Fig. 2. Relationships between metabolic and cardiac parameters. (A) F-glucose and CI, (B) HbA1c and resting HR, (C) HTGC and CI, (D) HTGC and SVI. *Spearman rank correlations were conducted to investigate any associations between baseline characteristics, resting central haemodynamic and cardiac autonomic measures for liver patients only, however, here we separate NAFLD and DAFLD for contrasting purposes only. CI, cardiac index; DAFLD, dual aetiology fatty liver disease; F-glucose, fasting blood glucose; HbA1c, glycated haemoglobin; HR, heart rate; HTGC, hepatic triglyceride content; NAFLD, non-alcoholic fatty liver disease; SVI, stroke volume index.

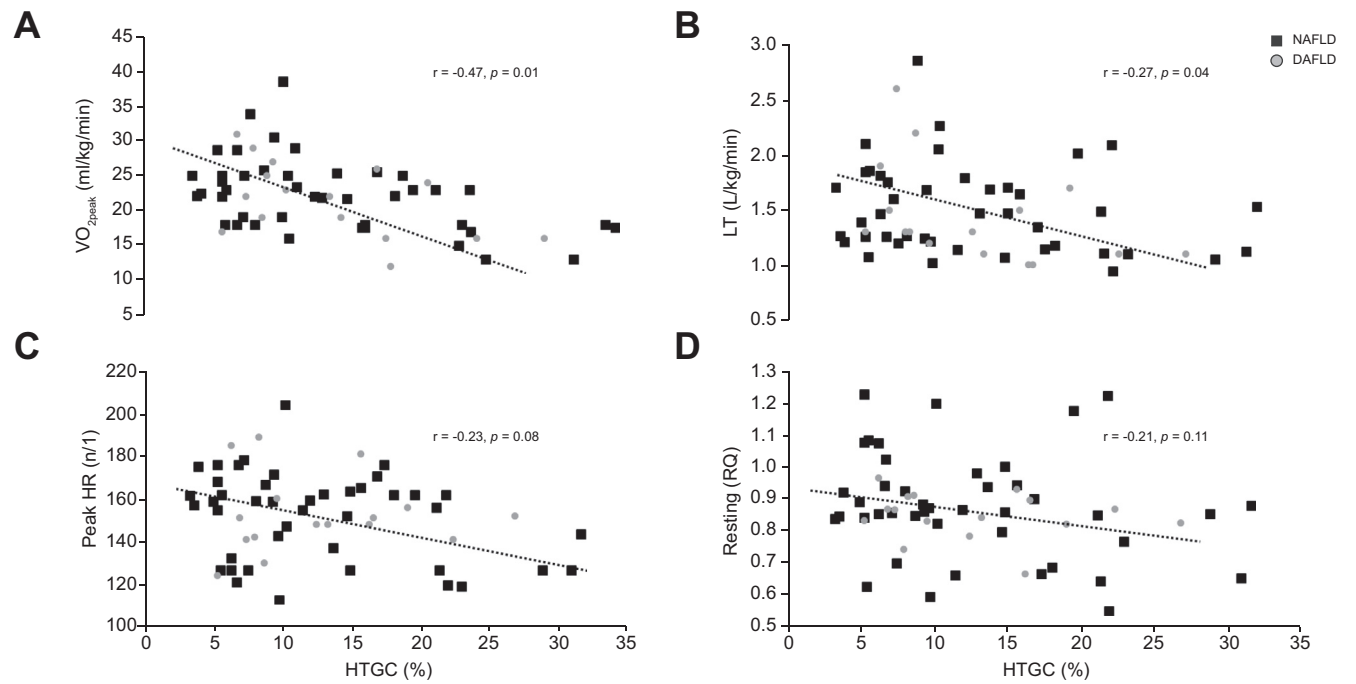

Fig. 3. Relationships between hepatic triglyceride content and cardiac autonomic measures. Relationship between HTGC and (A) peak oxygen uptake (VO_{2peak}), (B) peak HR, (C) LT, and (D) resting (RQ). *Spearman rank correlations were conducted to investigate any associations between baseline characteristics, resting central haemodynamic and cardiac autonomic measures for liver patients only, however, here we separate NAFLD and DAFLD for contrasting purposes only. DAFLD, dual aetiology fatty liver disease; HTGC, hepatic triglyceride content; HR, heart rate; LT, lactate threshold; NAFLD, non-alcoholic fatty liver disease; RQ, respiratory quotient.

controlling for type 2 diabetes diagnosis, HbA_{1c}, age, gender and BMI (Table 3). Hyperglycaemia, elevated levels of circulating triglycerides and cholesterol (surrogate biomarkers for non-esterified fatty acids (NEFA)) are commonly reported in metabolic disorders[51,52] and have been linked with altered cardiac metabolism and excessive epicardial fat accumulation.[12,53] High

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Table 3. Multivariate linear regression analyses.

	TNF-α (n = 62)		CK-18 (n = 62)		Fibrosis stage (n = 27)		HTGC (n = 62)	
	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value
Cardiac variables								
Cardiac index	0.04 (−0.80, 1.04)	0.80	0.29 (−13.43, 290.4)	0.08	−0.16 (−0.76, 0.29)	0.38	−0.45 (−8.90, −2.60,)	0.01#
Stroke index	0.03 (−0.05, 0.06)	0.82	0.14 (−5.02, 12.31)	0.42	−0.12 (−0.04, 0.02)	0.49	−0.42 (−0.54, −0.14)	0.01#
End diastolic index	0.06 (−0.03, 0.05)	0.67	0.13 (−3.50, 8.41)	0.42	−0.13 (−0.03, −0.02)	0.46	−0.40 (−0.35, −0.08)	0.01#
Autonomic variables								
Baroreceptor Reflex Sensitivity	0.35 (0.03, 0.12)	0.01#	−0.25 (−0.56, −4.03)	0.18	−0.25 (−0.02, −0.01)	0.18	0.21 (−0.1, 0.05)	0.22
LFnu-DBP (%)	0.42 (0.02, 0.12)	0.01#	0.04 (−7.10, 8.51)	0.51	−0.04 (−0.04, 0.03)	0.58	−0.28 (−0.59, −2.53)	0.60
HFnu-DBP (%)	0.14 (−0.03, 0.01)	0.92	0.57 (3.10, 19.32)	0.02*	−0.52 (−0.06, 0.05)	0.08	−0.12 (−0.40, 0.26)	0.48
LFnu-SBP (%)	0.55 (0.04, 0.13)	0.01#	0.04 (−0.7.45, −9.58)	0.41	0.13 (−0.03, 0.06)	0.31	0.18 (−0.34, 0.55)	0.73
HFnu-SBP (%)	0.15 (−0.02, 0.06)	0.59	0.63 (4.34, 17.88)	0.01#	−0.21 (−0.04, −0.02)	0.28	−0.19 (−0.39, 0.26)	0.25

Analyses were controlled for age, gender, BMI, T2DM diagnosis, smoking Status and HbA_{1c} associations with TNF-α, CK-18, fibrosis stage and HTGC with cardiac and autonomic variables.
T2DM, type 2 diabetes mellitus, TNF-α, tumour necrosis factor-alpha, CK-18, Cytokeratin-18, HTGC, hepatic triglycerides content, LFnu, low frequency, DBP, diastolic blood pressure, HF, high frequency, SBP, systolic blood pressure. * and # denote a significant association at $p \leq 0.05$ and <0.01, respectively. Multiple linear regression models were used to investigate the associations between HTGC, fibrosis score, CK-18 and TNF-α and cardiac variables (CI, SVI and EDI) and autonomic variables (baroreceptor sensitivity, LFnu-DBP and SBP and HF-DBP and SBP). Models were fit to estimate associations after the adjustment of age, gender, BMI, diagnosis of type 2 diabetes, smoking status and HbA1c.

uptake of triglycerides and NEFA in cardiomyocytes prevents downstream insulin pathways, inducing cardiac insulin resistance.[54] Triglycerides and NEFA are then preferentially utilised by the heart over glucose, subsequently reducing cardiac contractile efficiency (reducing phosphocreatine/adenosine triphosphate ratio).[50] The findings presented here support this close relationship between metabolic and cardiac dysfunction, where liver fat, fasting glucose, triglycerides and cholesterol all negatively impact upon various cardiac variables. Understanding the pathophysiology of cardiac changes associated with hyperglycaemia and elevated lipids in patients is crucial to identifying potential therapeutic interventions that reduce the risk of CVD and liver disease.

The data presented extend beyond cardiac function to reveal significant changes in autonomic function recognised as a powerful predictor of morbidity and mortality in NAFLD.[30,55] This is unsurprising given the role of the sympathetic and parasympathetic systems, which are responsible for controlling cardiac function through information provided by the baroreceptors.[56] Our findings reveal increased baroreceptor sensitivity, both systolic and diastolic parasympathetic activity in patients with NAFLD, and impaired cardiac function. In contrast, Ziegler, *et al.*[31] reported that reduced parasympathetic activity was strongly associated with liver fat in newly diagnosed type 2 diabetes, but did not confirm any impairments in cardiac function. These differences may have been due to disease duration – *i.e.* the patients in the current study had a diagnosis of >5% hepatic steatosis of >3 years. Frith, *et al.*,[57] suggested that disease duration, alongside disease status may have a direct impact upon the degree of autonomic dysfunction. In patients with a confirmed diagnosis of >5% hepatic steatosis, among other metabolic risk factors shown here, increased parasympathetic activity may be a compensatory mechanism to maintain cardiac function, but eventually becomes part of the disease. Patients with >5% hepatic steatosis may therefore be suffering from pathological sympathetic activity,[57] leading to autonomic effects, such as heart and blood vessels becoming resistant to parasympathetic activity. Impaired response of the cardiovascular system to parasympathetic activity places additional stress on the cardiovascular system, evident here by increased heart rate, blood pressure, and impaired cardiac contractibility and output and attenuated heart rate variability, as previously reported in NAFLD and diabetes.[31,55]

Sustained sympathetic activity has been shown to be a stimulus for structural and functional changes in cardiomyocytes and interstitium, left ventricular re-modelling,[58] ventricular tachyarrhythmia and sudden cardiac death.[59] Although the precise mechanism linking autonomic dysfunction and chronic liver disease remains unclear, insulin resistance and inflammation have received interest for their potential contributions. Insulin resistance has been shown to increase circulating insulin, glucose and renal spill-over of noradrenaline.[60] Supporting the links with insulin resistance[56,60,61] the present data showed that HOMA-IR, a measure of insulin resistance, was significantly associated with heart rate variability, indicating increased sympathetic and decreased parasympathetic activity. Elevated levels of insulin (via glucose metabolism in the ventral medial hypothalamus) and noradrenaline increase sympathetic stimulation,[56] induce myocardial injury, impair β-adrenergic function[62] and damage nerve endings,[63] all of which could contribute to autonomic disturbance in individuals with excessive liver fat. Although the precise mechanism of autonomic

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function remains unknown, earlier detection of those at risk would allow hepatologists to co-ordinate with cardiologists to aggressively treat these patients with lifestyle interventions and/or pharmaceutical treatments.

Interestingly, increased alcohol consumption was associated with lower parasympathetic activity and increased blood pressure and sympathetic activity. The increase in blood pressure observed here appears to be driven by alterations in autonomic function during diastole (Fig. 1). Excessive alcohol is well established for its role in progressive liver disease[64] and increased risk of cardiovascular disease.[65] Elevated blood pressure is commonly reported when consuming excessive levels of alcohol,[66] although precise mechanisms are unknown. Amongst potential mechanisms, pre-clinical data have demonstrated that increased ethanol consumption was associated with increased sympathetic activity, SBP and attenuated baroreceptor sensitivity.[67] Therefore, patients consuming >20 g of alcohol per day and who have >5% liver fat may be exposed to a dual aetiology, and an increased risk of CVD,[11,24] when compared with NAFLD patients. Although patients with NAFLD are advised to drink <20 g of alcohol per day, recent reports have demonstrated that more patients are being recognised in the clinical setting who are obese with fatty liver, but are consuming >30 g/day of alcohol, and in some patients metabolic syndrome was more frequent in those consuming >30 g/day of alcohol.[68,69] The potential risk of consuming excessive amounts of alcohol, combined with metabolic dysfunction may further accentuate the clinical and economic burden of CVD and liver disease.

In addition to metabolic dysfunction, obesity and inflammation, have also been identified as potential contributing factors towards the progression of autonomic dysfunction.[18,70] In the current study, BMI and subcutaneous fat were associated with impaired cardiac and autonomic function, supporting previous observations.[71] Furthermore, elevated inflammation is commonly reported in obese and overweight individuals.[15] In the current study we were able to demonstrate that TNF-α was independently associated with sympathetic (low frequency) activity (DBP and SBP) and baroreceptor sensitivity, although it is difficult to ascertain whether TNF-α is specifically liver related or due to patients' body composition. However, we have also shown that CK-18, a blood indicator of hepatocellular injury was independently associated with parasympathetic activity (high frequency) for both DBP and SBP, further suggesting that increased parasympathetic activity may initially be a compensatory mechanism to maintain cardiac function in patients with excessive liver fat.[57] Furthermore, in a sub-analysis of patients with NASH, fibrosis staging was negatively correlated with cardiac and autonomic function, although this could not be confirmed in the multivariate linear regression analyses, likely due to the small sample size.

Combined, these data suggest that inflammation (TNF-α and CK-18) and NASH severity may have a direct impact upon cardiac and autonomic dysfunction, placing these patients at a greater risk of cardiac arrhythmias and increased mortality. This premise is supported by previous data linking severity of liver histopathology with atherogenesis, cardiomyopathy and arrhythmias[48,72] and increased mortality.[9] NASH is characterised by liver fat, liver inflammation and metabolic syndrome, all of which are associated with inflammation through various mechanisms.[73] Inflammation has also been identified in autonomic dysfunction, through its ability to activate a number of sites in the brain,[17] interacting with autonomic control and increasing sympathetic outflow,[18,70] and in doing so increasing the stress placed on the cardiovascular system. However, data linking inflammation and autonomic dysfunction in clinical practice is lacking.

Increased stress on the heart through altered sympathetic activity and a defective parasympathetic response could contribute to functional and structural cardiac changes. Hallsworth, *et al.*[19] and Cassidy, *et al.*[45] have previously reported cardiac structure and functional changes in similar cohorts of patients with NAFLD and type 2 diabetes mellitus in the presence of elevated liver fat and HOMA-IR. The present data, alongside these studies, further substantiate the suggestion that the degree of HTGC and metabolic dysfunction lead to autonomic dysfunction and cardiac impairments. Autonomic dysfunction has been previously reported in chronic liver diseases,[74] however, the link between alcohol, disease severity and the degree of autonomic dysfunction is equivocal due to conflicting results.[75–77] A potential explanation for the difference in observations may in part be the consequence of variations in methods and patient selection. The present study addresses this with the use of standardised methods and a well characterised patient cohort. In doing so the present autonomic data support the need to identify and treat patients with >5% HTGC, and those who continue to drink >20 g of alcohol per day and who have >5% HTGC as they may appear to have greater levels of autonomic dysfunction, placing them at a greater risk of CVD. Although longitudinal studies are required to assess the aetiology and time course of cardiac and autonomic changes associated with increased HTGC and metabolic dysfunction. One potential hypothesis may be that there is a 2-hit effect, with the first hit being hyperglycaemia and elevated circulating lipids inducing loss of contractile efficiency and cardiac hypertrophy, and the second, subsequent hit an impairment of the autonomic control due to cardiovascular remodelling. Alternatively, autonomic control may initially be impaired, followed by cardiac structural and functional changes. The direction of causality between impaired cardiac and autonomic function remains unknown, and prospective and longitudinal studies are warranted.

The present study is not without limitation. Although the control group was matched for age and gender, BMI was lower than that of the hepatic steatosis groups. However, this was controlled for in the statistical analyses and accounted for when reporting beat and cardiac variables. Cardiac haemodynamic and autonomic variables were assessed using impedance cardiography, which is not the recognised gold standard. However, thermodilution methods were not considered appropriate due to ethical issues, *i.e.* it would have been ethically difficult to expose liver patients to this invasive procedure. There was no assessment of liver fat in the control group. MRS was used to determine HTGC; although it would have been beneficial to assess liver histology for all hepatic steatosis patients as it is likely that some may have had NASH and hepatic fibrosis, however, this was ethically difficult to justify exposing low risk patients to a liver biopsy. Medication is likely to have affected cardiac and autonomic functions, although, the levels across groups were similar in patients with hepatic steatosis. The data in the present study are observational in nature; it would have been beneficial to conduct longitudinal studies to assess causality of whether autonomic function leads to cardiac changes, whether cardiac changes lead to autonomic dysfunction, or whether they occur concurrently. However, this study represents an important first step.

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Conclusion

There is strong evidence linking liver disease and CVD, however, evidence linking autonomic dysfunction and liver disease in the pathology of CVD is lacking. Here we have shown that patients with hepatic steatosis greater than 5% had significant impairments in cardiac and autonomic function. These impairments appeared to be dependent on HTGC, metabolic dysfunction, inflammation and fibrosis staging. Alcohol intake enhanced the impact of liver fat on diastolic autonomic control. Combined, these data highlight cardiac and autonomic dysfunction as potential therapeutic targets in patients with hepatic steatosis. It is also important to understand the interaction between metabolic dysfunction, alcohol and liver fat on the development and progression of liver and cardiovascular disease. Clinical care teams should explore therapies to address these as a means to mitigate the excess CVD risk.

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Conflict of interest

None of the authors have any conflicts of interest.

Please refer to the accompanying ICMJE disclosure forms for further details.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhep.2019.01.035.

References

[1] EASL clinical practical guidelines. management of alcoholic liver disease. J Hepatol 2012;57:399–420.
[2] Harrison SA, Day CP. Benefits of lifestyle modification in NAFLD. Gut 2007;56:1760–1769.
[3] Miele L, Targher G. Understanding the association between developing a fatty liver and subsequent cardio-metabolic complications. Exp Rev Gastroenterol Hepatol 2015;9:1243–1245.
[4] Boyle M, Masson S, Anstee QM. The bidirectional impacts of alcohol consumption and the metabolic syndrome: cofactors for progressive fatty liver disease. J Hepatol 2018;68:251–267.
[5] Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat Rev Gastroenterol Hepatol 2013;10:330–344.
[6] Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia 2012;55:885–904.
[7] Musso G, Gambino R, Tabibian JH, et al. Association of non-alcoholic fatty liver disease with chronic kidney disease: a systematic review and meta-analysis. PLoS Med 2014;11 e1001680.
[8] Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010;363:1341–1350.
[9] Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology 2017;65:1557–1565.
[10] Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, et al. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease: a multi-national cohort study. Gastroenterology 2018;155:443–457, e17.
[11] Klatsky AL. Alcohol and cardiovascular diseases: where do we stand today? J Intern Med 2015;278:238–250.
[12] Byrne CD, Targher G. NAFLD: a multisystem disease. J Hepatol 2015;62: S47–S64.
[13] Ekstedt M, Hagstrom H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61:1547–1554.
[14] Adams LA, Anstee QM, Tilg H, Targher G. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut 2017;66:1138–1153.
[15] Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. Annu Rev Immunol 2011;29:415–445.
[16] Targher G, Marra F, Marchesini G. Increased risk of cardiovascular disease in non-alcoholic fatty liver disease: causal effect or epiphenomenon? Diabetologia 2008;51:1947–1953.
[17] Dunn AJ. Systemic interleukin-1 administration stimulates hypothalamic norepinephrine metabolism parallelling the increased plasma corticosterone. Life Sci 1988;43:429–435.
[18] Huang W, Hoffmann NV, West MJ. Spinal cord excitatory amino acid receptors and plasma catecholamine autonomic responses in the conscious rabbit. Aust N Z J Med 1997;27:479–484.
[19] Hallsworth K, Hollingsworth KG, Thoma C, et al. Cardiac structure and function are altered in adults with non-alcoholic fatty liver disease. J Hepatol 2013;58:757–762.
[20] Hung CS, Tseng PH, Tu CH, et al. Nonalcoholic fatty liver disease is associated with QT prolongation in the general population. J Am Heart Assoc 2015;4.
[21] Petta S, Argano C, Colomba D, et al. Epicardial fat, cardiac geometry and cardiac function in patients with non-alcoholic fatty liver disease: association with the severity of liver disease. J Hepatol 2015;62:928–933.
[22] Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391–397.
[23] Sung KC, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. Atherosclerosis 2009;203:581–586.
[24] Cohen EJ, Klatsky AL, Armstrong MA. Alcohol use and supraventricular arrhythmia. Am J Cardiol 1988;62:971–973.
[25] Fazzino TL, Fleming K, Sher KJ, Sullivan DK, Befort C. Heavy drinking in young adulthood increases risk of transitioning to obesity. Am J Prev Med 2017;53:169–175.
[26] Manco M, Giordano U, Turchetta A, et al. Insulin resistance and exercise capacity in male children and adolescents with non-alcoholic fatty liver disease. Acta Diabetol 2009;46:97–104.
[27] Newton JL, Jones DE, Henderson E, et al. Fatigue in non-alcoholic fatty liver disease (NAFLD) is significant and associates with inactivity and excessive daytime sleepiness but not with liver disease severity or insulin resistance. Gut 2008;57:807–813.
[28] Farrell TG, Bashir Y, Cripps T, et al. Risk stratification for arrhythmic events in postinfarction patients based on heart rate variability, ambulatory electrocardiographic variables and the signal-averaged electrocardiogram. J Am Coll Cardiol 1991;18:687–697.
[29] Zhang WSD, Sun J, Sun BXK, et al. Association between non-alcoholic fatty liver disease and autonomic dysfunction in a Chinese population. QJM 2015;108.
[30] Newton JL, Pairman J, Wilton K, Jones DE, Day C. Fatigue and autonomic dysfunction in non-alcoholic fatty liver disease. Clin Autonomic Res 2009;19:319–326.
[31] Ziegler D, Strom A, Kupriyanova Y, et al. Association of lower cardiovagal tone and baroreflex sensitivity with higher liver fat content early in type 2 diabetes. J Clin Endocrinol Metab 2018;103:1130–1138.
[32] Singh A, Kumar MS, Jaryal AK, et al. Diabetic status and grade of nonalcoholic fatty liver disease are associated with lower baroreceptor sensitivity in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2017;29:956–961.
[33] Kumar MS, Singh A, Jaryal AK, et al. Cardiovascular autonomic dysfunction in patients of nonalcoholic fatty liver disease. International J Hepatol 2016;2016:5160754.

Please cite this article in press as: Houghton D et al. The degree of hepatic steatosis associates with impaired cardiac and autonomic function. J Hepatol (2019), https://doi.org/10.1016/j.jhep.2019.01.035

[34] Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. J Hepatol 2010;53:372–384.
[35] Fields DA, Higgins PB, Radley D. Air-displacement plethysmography: here to stay. Curr Opin Clin Nutr Metabol Care 2005;8:624–629.
[36] Longo R, Pollesello P, Ricci C, et al. Proton MR spectroscopy in quantitative in vivo determination of fat content in human liver steatosis. J Magn Reson Imaging 1995;5:281–285.
[37] Hallsworth K, Fattakhova G, Hollingsworth KG, et al. Resistance exercise reduces liver fat and its mediators in non-alcoholic fatty liver disease independent of weight loss. Gut 2011;60:1278–1283.
[38] Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
[39] Jakovljevic DG, Hallsworth K, Zalewski P, et al. Resistance exercise improves autonomic regulation at rest and haemodynamic response to exercise in non-alcoholic fatty liver disease. Clin Sci 2013;125:143–149.
[40] Gratze G, Fortin J, Holler A, et al. A software package for non-invasive, real-time beat-to-beat monitoring of stroke volume, blood pressure, total peripheral resistance and for assessment of autonomic function. Comput Biol Med 1998;28:121–142.
[41] Fortin J, Habenbacher W, Gruellenberger R, Wach P, Skrabal F. Real-time monitor for hemodynamic beat-to-beat parameters and power spectra analysis of the biosignals. Engineering in Medicine and Biology Society 1998; Proceedings of the 20th Annual International Conference of the IEEE:360-363.
[42] Pagani M, Lombardi F, Guzzetti S, et al. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. Circ Res 1986;59:178–193.
[43] Parati G, Frattola A, Di Rienzo M, et al. Effects of aging on 24-h dynamic baroreceptor control of heart rate in ambulant subjects. Am J Physiol 1995;268:H1606–H1612.
[44] de Simone G, Daniels SR, Devereux RB, et al. Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. J Am Coll Cardiol 1992;20:1251–1260.
[45] Cassidy S, Thoma C, Hallsworth K, et al. High intensity intermittent exercise improves cardiac structure and function and reduces liver fat in patients with type 2 diabetes: a randomised controlled trial. Diabetologia 2016;59:56–66.
[46] Bonapace S, Perseghin G, Molon G, et al. Nonalcoholic fatty liver disease is associated with left ventricular diastolic dysfunction in patients with type 2 diabetes. Diabetes Care 2012;35:389–395.
[47] Kim NH, Park J, Kim SH, et al. Non-alcoholic fatty liver disease, metabolic syndrome and subclinical cardiovascular changes in the general population. Heart 2014;100:938–943.
[48] Targher G, Bertolini L, Padovani R, et al. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care 2006;29:1325–1330.
[49] Lautamaki R, Borra R, Iozzo P, et al. Liver steatosis coexists with myocardial insulin resistance and coronary dysfunction in patients with type 2 diabetes. Am J Physiol Endocrinol Metabol 2006;291:E282–E290.
[50] Rijzewijk LJ, Jonker JT, van der Meer RW, et al. Effects of hepatic triglyceride content on myocardial metabolism in type 2 diabetes. J Am Coll Cardiol 2010;56:225–233.
[51] Larsen TS, Aasum E. Metabolic (in)flexibility of the diabetic heart. Cardiovasc Drugs Ther 2008;22:91–95.
[52] Scheuermann-Freestone M, Madsen PL, Manners D, et al. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 2003;107:3040–3046.
[53] Rabkin SW. Epicardial fat: properties, function and relationship to obesity. Obes Rev 2007;8:253–261.
[54] Martins AR, Nachbar RT, Gorjao R, et al. Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function. Lipids Health Dis 2012;11:30.
[55] Liu YC, Hung CS, Wu YW, et al. Influence of non-alcoholic fatty liver disease on autonomic changes evaluated by the time domain, frequency domain, and symbolic dynamics of heart rate variability. PLoS ONE 2013;8 e61803.
[56] Landsberg L. Diet, obesity and hypertension: an hypothesis involving insulin, the sympathetic nervous system, and adaptive thermogenesis. Q J Med 1986;61:1081–1090.
[57] Frith J, Zalewski P, Klawe JJ, et al. Impaired blood pressure variability in chronic fatigue syndrome–a potential biomarker. QJM 2012;105:831–838.
[58] Karayannis G, Kitsios G, Kotidis H, Triposkiadis F. Left atrial remodelling contributes to the progression of asymptomatic left ventricular systolic dysfunction to chronic symptomatic heart failure. Heart Fail Rev 2008;13:91–98.
[59] Podrid PJ, Myerburg RJ. Epidemiology and stratification of risk for sudden cardiac death. Clin Cardiol 2005;28:I3–I11.
[60] Rumantir MS, Vaz M, Jennings GL, et al. Neural mechanisms in human obesity-related hypertension. J Hypertens 1999;17:1125–1133.
[61] Liao D, Cai J, Brancati FL, et al. Association of vagal tone with serum insulin, glucose, and diabetes mellitus–The ARIC Study. Diabetes Res Clin Pract 1995;30:211–221.
[62] Moller S, Henriksen JH. Cirrhotic cardiomyopathy: a pathophysiological review of circulatory dysfunction in liver disease. Heart 2002;87:9–15.
[63] Lehtinen JM, Uusitupa M, Siitonen O, Pyorala K. Prevalence of neuropathy in newly diagnosed NIDDM and nondiabetic control subjects. Diabetes 1989;38:1307–1313.
[64] Louvet A, Mathurin P. Alcoholic liver disease: mechanisms of injury and targeted treatment. Nat Rev Gastroenterol Hepatol 2015;12:231–242.
[65] Moller S, Bernardi M. Interactions of the heart and the liver. Eur Heart J 2013;34, 2804-+.
[66] Marchi KC, Muniz JJ, Tirapelli CR. Hypertension and chronic ethanol consumption: what do we know after a century of study? World J Cardiol 2014;6:283–294.
[67] Abdel-Rahman AA, Wooles WR. Ethanol-induced hypertension involves impairment of baroreceptors. Hypertension 1987;10:67–73.
[68] Baumeister SE, Alte D, Meyer C, John U. Health Risk drinking and problematic consumption of alcohol in Pomerania: comparative analysis of the Study of Health in Pomerania (SHIP) compared with the Federal German Health and Examination Survey in 1998. Gesundheitswesen 2005;67:39–47.
[69] Kotronen A, Yki-Jarvinen H, Mannisto S, et al. Non-alcoholic and alcoholic fatty liver disease – two diseases of affluence associated with the metabolic syndrome and type 2 diabetes: the FIN-D2D survey. BMC Public Health 2010;10:237.
[70] Herder C, Schamarek I, Nowotny B, et al. Inflammatory markers are associated with cardiac autonomic dysfunction in recent-onset type 2 diabetes. Heart 2017;103:63–70.
[71] Pimenta NM, Santa-Clara H, Cortez-Pinto H, et al. Body composition and body fat distribution are related to cardiac autonomic control in non-alcoholic fatty liver disease patients. Eur J Clin Nutr 2014;68:241–246.
[72] Lazzerini PE, Capecchi PL, Laghi-Pasini F. Systemic inflammation and arrhythmic risk: lessons from rheumatoid arthritis. Eur Heart J 2017;38:1717–1727.
[73] Tilg H, Moschen AR. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology 2010;52:1836–1846.
[74] Frith J, Newton JL. Autonomic dysfunction in chronic liver disease. Liver Int 2009;29:483–489.
[75] Fleckenstein JF, Frank S, Thuluvath PJ. Presence of autonomic neuropathy is a poor prognostic indicator in patients with advanced liver disease. Hepatology 1996;23:471–475.
[76] Hendrickse MT, Triger DR. Peripheral and cardiovascular autonomic impairment in chronic liver disease: prevalence and relation to hepatic function. J Hepatol 1992;16:177–183.
[77] Trevisani F, Sica G, Bernardi M. Autonomic neuropathy in advanced liver disease. Hepatology 1996;24:1549.

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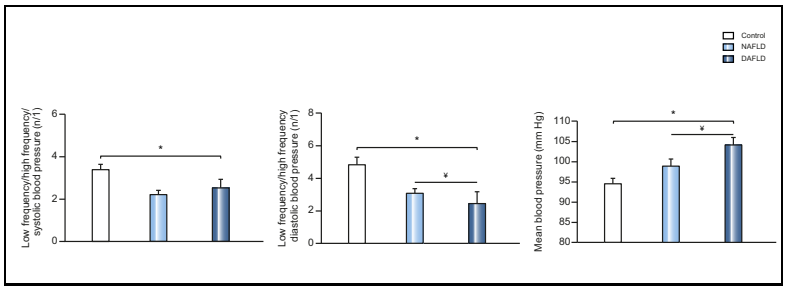
The degree of hepatic steatosis associates with impaired cardiac and autonomic function

Graphical abstract



Highlights

- Patients with elevated liver fat and poor metabolic control have impaired cardiac and autonomic function.
- Liver fat, metabolic dysfunction, inflammation and fibrosis staging correlate with cardiac and autonomic dysfunction.
- Elevated alcohol intake enhanced the impact of liver fat on diastolic autonomic dysfunction.

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Lay summary

Increased levels of fat in the liver impair the ability of the cardiovascular system to work properly. The amount of fat in the liver, metabolic control, inflammation and alcohol are all linked to the degree that the cardiovascular system is affected.

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