Hepatic macrophage activation is associated with adipose tissue insulin resistance in non-diabetic patients with Non-Alcoholic Fatty Liver Disease

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BACKGROUND

The onset and progression of liver damage in Non-Alcoholic Fatty Liver Disease (NAFLD) is tightly associated with insulin resistance (IR) in a dysfunctional adipose tissue (AT).
Macrophages activation is a key step for both the chronic low inflammatory state of IR and for hepatic damage. To date, a direct pathway linking AT-IR to the liver damage has not yet been described.

AIM

To elucidate the pathways linking IR in the AT, circulating/hepatic macrophages activation markers and liver damage in 40 non-diabetic patients with biopsy-proven NAFLD.

METHODS

Soluble CD163 (sCD163), a marker of macrophages activation, was measured by an in-house sandwich ELISA

AT-IR was calculated in two ways:

AT-IR1 = Ra Glycerol x Fasting Plasma Insulin
AT-IR2 = FFAs x Fasting Plasma Insulin

The infusion of [$^2H_5$]glycerol was used to evaluate glycerol Rate of Appearance (Ra) and lipolysis.

The hepatic expression of CD163, ADAM-17 and TNF-a were assessed by qPCR using a CFX96 real-time instrument (Bio-Rad).

Liver histology was scored according to Kleiner classification.

RESULTS

Clinical, biochemical and histological characteristics of NAFLD patients are reported in **Table 1**.

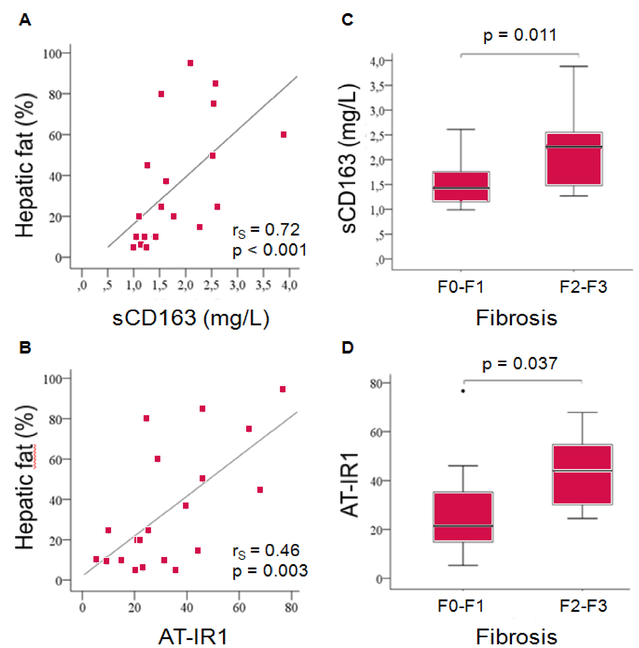
RESULTS

Variables	Study cohort (n = 40)
Age, years	41.9 11.2
M/F, n (%)	31/9 (77.5/22.5)
BMI, kg/m^2	26.9 4.4
Waist circumference, cm	93.5 10.9
AST, IU/ml	33 (18-77)
ALT, IU/ml	62 (26-154)
gGT, IU/ml	54 (18-317)
Platelets, x 10^9/l	216 (111-319)
Total cholesterol, mg/dl	195 (115-256)
HDL cholesterol, mg/dl	42 (27-81)
LDL cholesterol, mg/dl	121 (55-198)
Triglycerides, mg/dl	78 (50-281)
SC, kg	4.0 1.8
VF, kg	2.6 1.1
sCD163 (mg/l)	1.62 (1.07-4.42)

Histological features	
Steatosis, %	25 (5-85)
NAS score, n (%)	
1-2	6 (15)
3-4	21 (52.5)
5-6	13 (32.5)
Ballooning, n (%)	
0	5 (12.5)
1	16 (40)
2	19 (47.5)
Lobular Inflammation, n (%)	
0	10 (25)
1	30 (75)
Fibrosis Score, n (%)	
F0/F1	18 (45)
F2	11 (27.5)
F3/F4	11 (27.5)

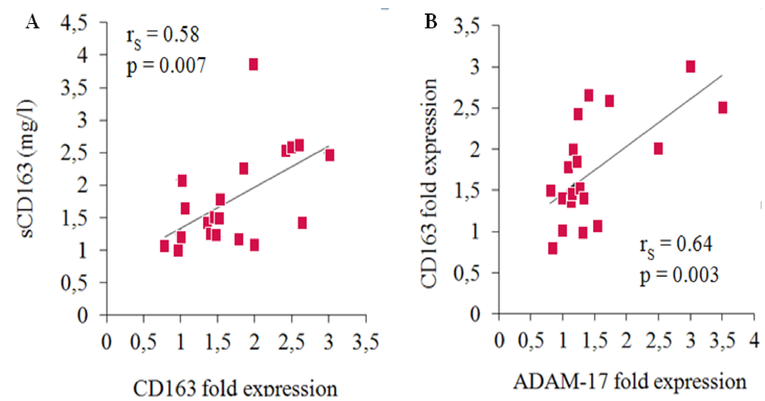
sCD163 plasma levels and AT-IR increased proportionally to the amount of hepatic fat (liver biopsy) (**Figure 1A-B**). Both circulating sCD163 and the degree of AT-IR were significantly higher in NAFLD subjects with F2/F3 fibrosis compared to F0-F1 patients (**Figure 1C & 1D**).

Figure 1



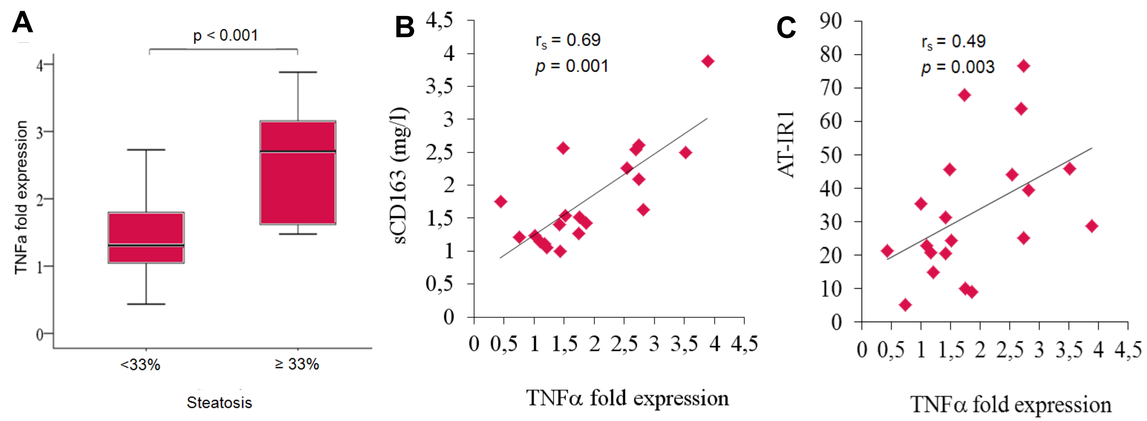
A liver tissue specimens was available for 20 NAFLD study subjects. We found a close correlation between circulating sCD163 and CD163 mRNA expression in the liver (**Figure 2A**). In addition we found a strong association between the hepatic expression of ADAM-17 metalloprotease and the CD163 (**Figure 2B**).

Figure 2



The hepatic expression of TNF-alpha was significantly higher in NAFLD subjects with steatosis ≥33% (**Figure 3A**) and associated with hepatic fat content (r_S=0.6, p=0.005). Both circulating sCD163 and the degree of AT-IR1 (**Figure 3B & 3C**) were related with TNF-alpha espression in the liver.

Figure 3



CONCLUSIONS

Our data support the hypothesis that in NAFLD patients hepatic macrophages activation may be directly stimulated by an increased flux of FFA due to AT-IR.
Despite the small size of the study cohort, these results highlight a direct pathway among IR, dysfunctional adipose tissue and liver damage.

Funded by FP7/2007-2013 under grant agreement n HEALTH-F2-2009-241762, project FLIP; PRIN 2009 ARYX4T; Horizon 2020 under grant agreement no. 634413, project EPoS