Insulin secretion is directly related to NASH, fibrogenesis and fibrosis in Non-diabetic patients with Non-Alcoholic Fatty Liver Disease

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INTRODUCTION

Liver damage in NAFLD has been related to the degree of insulin resistance (IR) and to the presence of metabolic syndrome. IR is invariably associated with compensatory hyperinsulinaemia until beta cell function is preserved. Insulin is secreted into the portal vein and mostly cleared by the liver. The effect of insulin per se on liver damage is still unknown although cellular studies suggest it can activate hepatic stellate cells and promote fibrosis.[1-3] Procollagen type III is up-regulated in the early development of liver fibrosis and its N-terminal neo-epitope (PRO-C3) is a non-invasive marker of fibrogenesis.[4-6]

AIM

We sought to investigate the association between increased pre-hepatic insulin by C-peptide (CP) levels, the degree of liver fibrosis in Non Diabetic (Non-T2DM) vs Type 2 Diabetic (T2DM) patients with NAFLD.

METHOD

- Study subjects: 205 subjects with biopsy proven NAFLD (N=50, 24%, T2DM)
- Fasting plasma PRO-C3 levels were measured by competitive ELISA → Nordic Bioscence
- Fasting CP levels were measured by chemiluminescence assay (LUMIPULSE G600, Fujirebio)
- *PNPLA3* rs738409 C>G was determined by RT-PCR
- Histology was scored according to SAF score
- NASH was defined by the joint presence of steatosis, lobular inflammation and ballooning degeneration

RESULTS

Clinical characteristics of the study cohort (N=205) are reported in **Table 1**.

Table 1

	Whole cohort (N=205)	Non T2DM (N=155)	T2DM (N=50)	p value
Age (y)	47 ± 12	45 ± 12	54 ± 10	< 0.001
BMI (kg/m^2)	28.6 ± 4.5	28.1 ± 4.4	30.3 ± 4.1	0.002
WC (cm)	99 ± 10	98 ± 10	104 ± 9	0.004
AST (IU/ml)	32 (30-34)	32 (29-34)	32 (29-36)	0.506
ALT (IU/ml)	52 (47-57)	53 (47-60)	47 (38-55)	0.253
GGT (IU/ml)	52 (45-63)	48 (41-57)	66 (38-55)	0.068
PLT (x10^9/l)	218 (213-225)	218 (207-225)	219 (207-243)	0.691
Hb (g/dl)	15.4 ± 9.6	14.8 ± 1.4	14.5 ± 1.4	0.216
Albumin (g/dl)	4.5 ± 0.4	4.5 ± 0.4	4.3 ± 0.4	0.008
TB (mg/dl)	0.84 ± 0.83	0.88 ± 0.92	0.72 ± 0.44	< 0.001
Fasting glucose (mg/dl)	93 (92-96)	99 (88-93)	126 (114-136)	< 0.001
Fasting insulin (mU/l)	13.2 (12.0-16.2)	12.2 (10.8-13.8)	23.2 (15.8-27.6)	0.009
HOMA	3.0 (2.7-3.7)	2.7 (2.4-3.1)	6.7 (3.7-8.2)	< 0.001
Tot-Chol (mg/dl)	190 (183-197)	193 (184-202)	187 (165-198)	0.103
HDL Chol (mg/dl)	47 (45-49)	48 (44-49)	47 (42-50)	0.865
TG (mg7dl)	123 (114-132)	116 (104-128)	144 (117-164)	0.044
*PNPLA3** CC/CG/GG, n (%)	55/79/37 (32/46/22)	39/64/30 (29/48/23)	16/15/7 (42/40/18)	0.205
CP (ng/ml)	2.73 ± 1.50	2.51 ± 1.40	3.37 ± 1.64	<0.001
PRO-C3 (ng/ml)	10.2 ± 5.2	9.78 ± 4.82	11.63 ± 6.28	0.016

* *PNPLA3* data avallale in 171 subjects (N=133 non T2DM, N=38 T2D subjects)

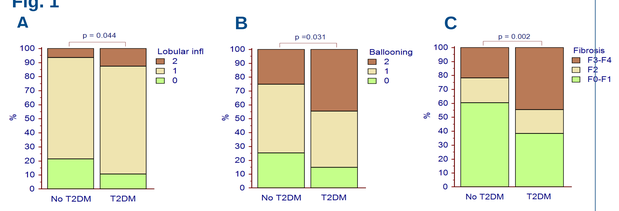
Histological characteristics of the study cohort (N=205) are reported in **Table 2**.

Table 2

	Whole cohort (N=205)	Non T2DM (N=155)	T2DM (N=50)	p value
Hepatic fat (%)		34 ± 24	36 ± 22	0.770
Steatosis grade ≥ 33%	91 (44%)	67 (43%)	24 (48%)	0.397
Ballooning ≥ 1	159 (78%)	116 (75%)	43 (86%)	**0.031**
Lobular Inflammation ≥ 1	167 (81%)	122 (79%)	45 (90%)	**0.044**
Fibrosis				**0.002**
F0-F1	112 (55%)	94 (61%)	18 (36%)	
F2	38 (18%)	27 (17%)	11 (22%)	
F3-F4	55 (27%)	34 (22%)	21 (42%)	
NASH		109 (70%)	39 (78%)	0.111

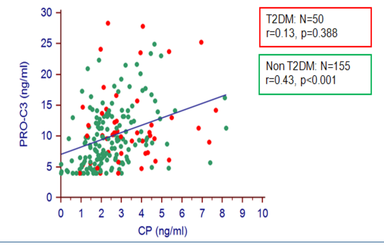
Concerning histology, the degree of lobular inflammation, the degree of ballooning and the stage of fibrosis progressively increased in Non T2DM but it was unchanged in T2DM subjects (**Fig. 1A-C**).

Fig. 1



Overall, there was a correlation between CP and PRO-C3 but when we grouped the cohort according to the presence of T2DM, we found that this correlation was significant only in the subgroup of Non-T2DM patients (**Fig. 2**).

Fig. 2



When grouped according to CP tertiles for specific group, in Non-T2DM subjects PRO-C3 levels had a stepwise increase (from 7.7 to 9.6 to 12.1 ng/ml, p<0.001) while no significance was found in T2DM patients (PRO-C3 range: 11.6 to 12.1 ng/ml) (**Fig. 3 A-B**).

Fig. 3

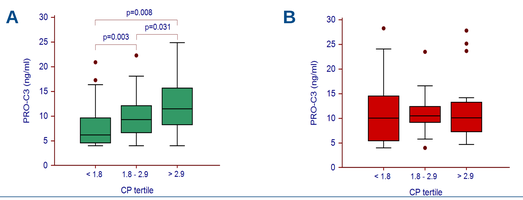
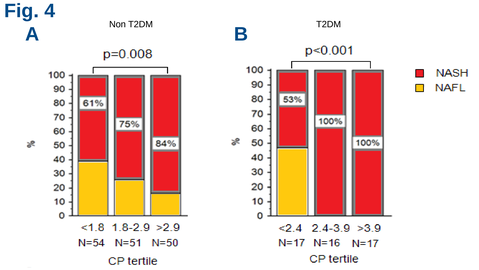
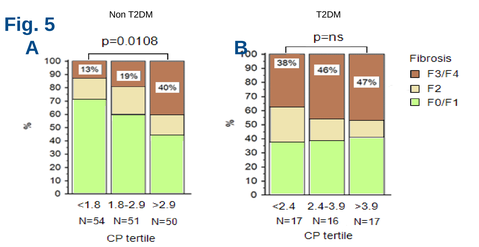


Fig. 4



The presence of NASH was directly related to CP tertiles only in the sub-group of non T2DM subjects (**Fig. 4 A-B**).

Fig. 5



The degree of hepatic fibrosis progressively increased according to CP tertiles only in non T2DM subjects (**Fig. 5 A-B**).

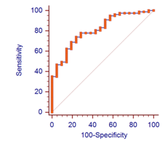
At multivariable logistic regression analysis adjusted for age, sex, BMI, waist, and PNPLA3 polymorphism, PRO-C3 levels were significantly associated to NASH while CP levels were significantly associated to severe fibrosis in Non-T2DM subjects (**Table 3 A-B**).

Table 3

A

NASH

Variable	OR	95% CI	P
Age	0.98	0.93 - 1.04	0.592
Male sex	1.19	0.22 - 6.32	0.836
BMI	1.27	0.98 - 1.64	0.067
Waist (cm)	0.99	0.89 - 1.09	0.879
PRO-C3 (ng/ml)	1.23	1.01 - 1.50	0.036
CP (ng/ml)	0.88	0.55 - 1.42	0.609
PNPLA3 (CC)	0.64	0.28 - 1.48	0.299

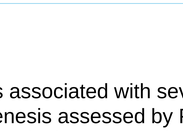


AUC = 0.81
95% CI = 0.719 - 0.883
PPV = 84.3 %
NPV = 77.8 %
% corr cases = 84 %

B

Severe fibrosis (F3-F4)

Variable	OR	95% CI	P
Age	1.01	0.97 – 1.06	0.625
Male sex	1.34	0.36 - 4.94	0.661
BMI	0.99	0.82 – 1.21	0.983
Waist (cm)	0.98	0.90 – 1.07	0.672
PRO-C3 (ng/ml)	1.10	0.97 – 1.24	0.134
CP (ng/ml)	1.75	1.12 – 2.75	0.015
PNPLA3 (CC)	0.93	0.43 – 2.01	0.853



AUC = 0.77
95% CI = 0.674 - 0.848
PPV = 85.7 %
NPV = 80.4 %
% corr cases = 78 %

CONCLUSIONS

Increased pre-hepatic insulin is associated with severe fibrosis and with increased liver fibrogenesis assessed by PRO-C3 in ND subjects with NAFLD, suggesting its important role in the onset and progression of NASH independently of T2D.

ACKNOWLEDGEMENTS

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