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Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Circulating adiponectin levels in type 2 diabetes mellitus patients with or without non-alcoholic fatty liver disease: Results of a small, open-label, randomized controlled intervention trial in a subgroup receiving short-term exenatide

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ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form
19 October 2015
Accepted 27 December 2015
Available online 9 January 2016

Keywords:
Glucagon-like peptide-1
Exenatide
Adiponectin
Non-alcoholic fatty liver disease
Diabetes mellitus type 2

ABSTRACT

Aim: Diabetes mellitus type 2 (DMT2) and non-alcoholic fatty liver disease (NAFLD) are both characterized by decreased circulating adiponectin. Recently, glucagon-like peptide-1 receptor agonists have been shown to induce adiponectin's expression. However, their interaction on clinical grounds needs to be further elucidated.

Methods: DMT2 patients with abnormal aminotransferases were screened for NAFLD and subjected to liver biopsy (group A, $n = 17$). A subgroup of patients ($n = 110$), after assessed for eligibility criteria, was blindly randomized to receive either 6-month exenatide supplementation on glargine insulin (group B) or intense, self-regulated, insulin therapy alone (group C).

Results: Baseline patient characteristics: 49(38.6%) males, aged 63.1 ± 7.5 years-old, BMI 32.9 ± 4.9 kg/m^2, HbA1c 8.1 ± 1.2% (65 ± 14 mmol/mol), median ALT 23 U/L (range 5–126), AST 20 U/L (7–72). Group A had biopsy-proven NAFLD with a median Activity Score of 5 and fibrosis stage 3. Presence of NAFLD was accompanied by a significant decline in adiponectin ($p < 0.001$), which was negatively correlated with the degree of ALT in all groups (Spearman's correlation, $r_s = -0.644$, $p < 0.001$). In the subgroup intervention trial, adiponectin was significantly raised in both groups B and C (t-Student for paired samples, $p = 0.001$) by $\Delta = +24.2\%$ (interquartile range 14.8–53.2%). This elevation was not associated with the type of intervention but with weight loss, glycemic control and reduction of C-reactive protein (one-way ANCOVA).

Conclusion: Supplementation of exenatide to glargine insulin compared to standard insulin was: (i) effective in inducing weight loss, (ii) non-inferior in lowering HbA1c and (iii) non-inferior in increasing circulating adiponectin. Higher adiponectin was associated with lower ALT, suggesting a hepato-protective role for this cytokine.

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http://dx.doi.org/10.1016/j.diabres.2015.12.003
0168-8227/

1. Introduction

The global epidemic of obesity and diabetes mellitus type 2 (DMT2) affects over 300 million individuals worldwide, while prevalence of DMT2 has doubled over the past 30 years, and estimations suggest no reversal of this trend in the foreseeable future [1–3]. Insulin resistance has already been proved the ''missing link'' that obesity, DMT2, dyslipidemia and non-alcoholic fatty liver disease (NAFLD) have in common [4–7]. Furthermore, adiponectin has emerged as an important hormone/cytokine derived from adipose tissue, that mediates peripheral insulin action and being remarkably down-regulated in all states of insulin resistance [6–8]. Experimental studies have shown that replenishment of hypo-adiponectinemia is able to restore insulin sensitivity and to reverse weight gain, hyperglycemia and fatty liver [9–11]. Apart from improving insulin sensitivity, adiponectin exerts further anti-inflammatory and anti-atherogenic properties that, especially in DMT2, have been associated with reduced risk for both cardiovascular disease and non-alcoholic steatohepatitis [7,8].

It has been suggested that adiponectin-targeted interventions are essential in the treatment of DMT2 [8]. Several reports have shown that weight loss and exercise, both strong determinants for successful anti-diabetic therapy [3], led to an increase of circulating adiponectin levels. Furthermore, thiazolidinediones widely used hypoglycemic medications, act as agonist ligands for peroxisome proliferator activated receptor gamma (PPAR-γ) by inducing insulin sensitivity via enhanced production and signal transduction of adiponectin [8]. Nevertheless, screening evaluation of adiponectin levels has never been used in everyday clinical practice.

Over the past few years, a new class of pharmacological agents with anti-diabetic properties—namely glucagon-like peptide-1 (GLP-1) receptor agonists—have been shown to improve glucose homeostasis in several ways [12–14]. One of their essential advantages is that they reduce food uptake and result in weight loss [3,11,15–17]. Exenatide is a synthetic form of exendin-4, a peptide secreted in the saliva of Gila monster that activates the known mammalian GLP-1 receptor. Apart from its established glucoregulatory properties, there is ongoing research challenging the primary belief that the liver is not directly influenced by its action [18]. Experimental observations suggest that GLP-1 agonists may improve liver biochemistry and hepatic steatosis [18–28], an effect that may be linked to their extra-pancreatic action of inducing adiponectin expression and increasing its plasma levels [29–31]. However, strong evidence of exenatide to adiponectin interaction on clinical grounds is still lacking. Limited results from the few and heterogeneous clinical studies are conflicting [2,11,23–28].

Aim of this study was: (1) to compare adiponectin levels and evaluate its possible determinants in patients with DMT2 and normal aminotransferases ($n = 110$) and patients with DMT2 and concomitant NAFLD ($n = 17$), and (2) to assess whether 6-month exenatide supplementation on glargine insulin ($n = 55$) or intense insulin alone ($n = 48$) would affect circulating adiponectin levels. Differences in serum adiponectin during intervention were recorded, and associations with several possible determinants, like weight loss and glycemic control, were investigated.

2. Patients and methods

2.1. *Patient selection: identifying presence of concomitant NAFLD*

The study population included white Caucasian patients diagnosed with DMT2 that were followed-up at the Medical Center of Diabetes Mellitus in ''Papageorgiou'' University Hospital of Thessaloniki from January 2010 to December 2012. Hospital's databases were accessed to collect data referring to gender, age, history of diabetes mellitus, medications and liver biochemistry. Patients with continuously abnormal liver function tests—defined as aminotransferases above the upper limit of normal on two separate occasions at least 6 months apart—were recorded and referred to the Hepatology Outpatients' Clinic, where they were further screened for presence of NAFLD (Fig. 1). Diagnostic work-up included upper abdomen ultrasonography for exclusion of intrahepatic lesions or extra-hepatic cholestasis. Furthermore, patients with excess alcohol consumption (cut-off limit 120 g per week), seropositive for viral hepatitis B and C or anti-nuclear auto-antibodies, under drugs with potential hepatotoxicity (e.g. statins, amiodarone, herbal remedies, etc.) and those with severe co-morbidities like end-stage renal disease or heart failure were excluded. Finally, patients were asked to give written informed consent for: (i) liver biopsy in order to confirm and stage NAFLD, and (ii) serum blood samples for additional laboratory testing.

2.2. *Intervention study protocol*

The second part of the study was referred to a prospective intervention trial testing the efficacy of short-term exenatide supplementation to basal glargine insulin (group B) versus intense insulin therapy alone (short-acting insulin three times daily before meals plus basal glargine insulin) (group C) (Fig. 1).

Inclusion criteria were:

- Age above 18 years old,
- Prior treatment with >20 Units per day of glargine insulin plus metformin for at least the past 6 months,
- Suboptimal glucose control with HbA1c > 8.0% (>64 mmol/mol),
- Refused history of previous administration of thiazolidinediones, GLP-1 receptor agonists or dipeptidyl peptidase-4 (DDP-4) inhibitors,
- Denied alcohol consumption in excess 120 g per week,
- Severe comorbid conditions including end-stage renal disease, recent cardiovascular events, chronic heart failure, hypo- or hyperthyroidism, history of acute or chronic pancreatitis,
- Written willing to participate.

According to the study protocol, all eligible patients were first assigned to receive treatment with optimally titrated metformin and glargine insulin, while sulfonylureas were discontinued. After this 5-week run-in period, patients were randomized to receive either exenatide (group B) or short-acting insulin three times daily prior to the meals

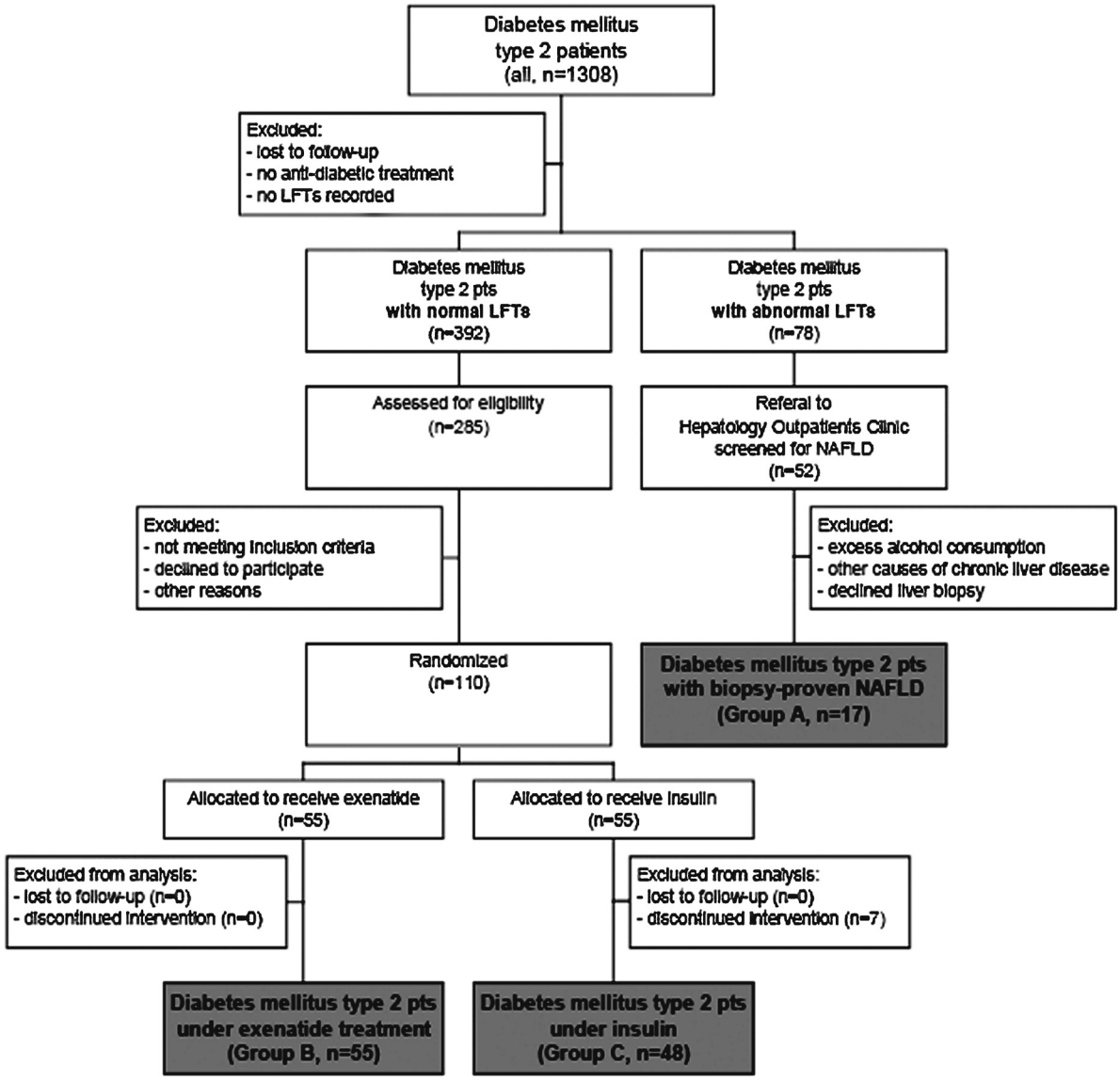

Fig. 1 – Flowchart of the study protocol according to CONSORT criteria.

according to self-monitoring of glucose levels and self-titrations of insulin (group C). Randomization was done according to patients' name initials. Exenatide was supplied through subcutaneous injections in the upper arm, thigh or abdomen with prefilled pens of 5 μg twice daily for the first 4 weeks and 10 μg (forced titration) twice daily thereafter. Patients were self-injected within 60 min before morning and evening meals. None of the patients received special recommendations on diet and exercise. Experienced medical staff was available for counseling on a 24 h basis. Compliance and adverse events were recorded on scheduled visits every 2 weeks. The study duration was set to 6 months.

The study protocol was approved by both the Scientific Board of ''Papageorgiou'' University Hospital and the Bioethics Committee of the Medical School of Aristotle University of Thessaloniki and was undertaken according to the CONSORT criteria [32].

2.3. *Anthropometric evaluation*

Anthropometric evaluations included measurements of weight in kilograms (kg), height in meters (m), body-mass index (BMI) calculated as weight divided by height squared in kg/m^2, waist (widest area between the lower rib margin and the iliac crest) and hip (widest area over the greater trochanters) circumference in centimeters (cm), as well as waist to hip ratio (WHR). Obesity was defined by a BMI $> 30\ kg/m^2$.

2.4. *Laboratory evaluation*

Venous blood samples were drawn after an overnight 12-hour fasting to determine levels of glucose, glycated hemoglobin (HbA1c), total cholesterol, low-(LDL) and high-density lipoprotein (HDL), triglycerides (TG), aspartate (AST) and alanine (ALT) aminotransferases, high-sensitivity C-reactive protein (CRP)

and adiponectin. Atherogenic index was calculated by using the formula log (TG/HDL) [33].

Serum adiponectin levels were determined in duplicate by an enzyme-linked immunosorbent assay (ELISA) kit (Quintikine) obtained from R&D Systems (Wiesbaden-Nordenstadt, Germany). Each serum sample was diluted 100-fold, according to the manufacturer's instructions [34]. All other biochemical tests were performed using conventional automated analyzers within the Biochemistry Laboratory at ''Papageorgiou'' University Hospital. All biochemical analyses were performed in a ''blinded'' manner.

2.5. Liver biopsy

DMT2 patients with concomitant NAFLD (group A) were subjected to a needle liver biopsy that was fixed in formalin and processed for paraffin-embedding. The sections were stained with hematoxylin/eosin and Masson trichrome. Histologic examination was performed by a ''blinded'' liver pathologist. Steatosis was semi-quantitatively assessed as the percentage of hepatocytes containing fat droplets, and was graded as absent (<5% of hepatocytes affected), mild (5% to 33%), moderate (33% to 66%) and severe (>66%) [35]. In addition to steatosis, lobular inflammation and ballooning of hepatocytes were scored, to obtain the NAFLD activity score (NAS) according to Kleiner et al. [36]. Once the diagnosis of NAFLD was confirmed histologically, fibrosis was scored by the 5-point scale proposed by Brunt et al. [35]. Briefly, this included F0 = absence of fibrosis; F1 = perisinusoidal fibrosis; F2 = perisinusoidal and portal/periportal fibrosis; F3 = bridging fibrosis; and F4 = cirrhosis.

2.6. Statistical analysis

Table entries represent total numbers with percentages (%), means ± standard deviations (SD) or medians with 25th to 75th interquartile range (IQR), as appropriate. Comparisons between groups were performed with χ^2 test for categorical variables, Student's *t* test for normally distributed continuous variables and Mann–Whitney *U* test for non-parametrical continuous variables. Differences within groups during intervention (Δ table entries) were calculated as the final minus the baseline value of each variable, and comparisons were performed with t-Student test for paired samples or Wilcoxon signed ranks test, while associations between continuous variables were tested with Spearman's correlation.

Power analysis tables provided *f* values in order to define the sample size needed for the intervention controlled trial to detect differences between groups with high power at the 0.05 level of significance. According to the study protocol, a sample size of 100 patients was needed to warrant detection of differences between groups with high power of 0.80 at the 0.05 alpha level of significance (power analysis, *f* value = 7.9).

Finally, analysis of covariance (ANCOVA) was performed to test if differences of adiponectin during intervention were attributable to similar differences in other variables like weight loss, glucose control, systemic inflammation, etc.

Statistical analyses were performed with SPSS version 11.5. All *p* values were two-sided and considered to be significant at the 0.05 level.

3. Results

3.1. Baseline patient characteristics

A total of 1308 DMT2 patients had been recorded in the databases of the Medical Center of Diabetes Mellitus. As shown in Fig. 1, continuously normal aminotransferases were found in 392 patients, while 78 patients were found with ALT and/or AST above the upper limit of normal for at least 6-month duration. This latter group of patients was referred to the Hepatology Outpatient's Clinic for further diagnostic work-up. After exclusion for other possible causes of chronic liver disease—mainly excess alcohol consumption, seropositivity for hepatitis B surface antigen and statin use—the majority of the remaining patients with DMT2 and abnormal aminotransferases refused liver biopsy. Finally, histological evaluation in 17 patients confirmed presence of non-alcoholic steatohepatitis (NASH) as fatty infiltration plus parenchymal inflammation plus ballooning degeneration in all 17 liver specimens. Table 1 summarizes the histologic findings. The majority of patients (70.6%) had ''definite'' NASH (NAS > 5), while the remaining patients had features ''suggestive'' of NASH (NAS 3 to 4). Advanced fibrosis (F3 and F4/cirrhosis) was evident in 64.7% of patients.

On the other hand, DMT2 patients with normal aminotransferases were further assessed for eligibility criteria (already mentioned in Section 2) and finally 110 patients were randomized to receive either exenatide or intense insulin. Table 1 summarizes baseline patient characteristics for both group A (DMT2 patients with concomitant NAFLD) and for groups B and C (DMT2 patients with normal aminotransferases before intervention). No statistical significant differences were recorded in gender, age and somatometric measurements (including weight, BMI or waist circumference). All patients had a similar history of DMT2 (duration, anti-diabetic treatment and glycemic control). Apart from aminotransferases, which were expected to be elevated in group A, statistical significant differences were also recorded in the lipidemic profile. This result however may be confounded by the wide use of statins in groups B and C, as NAFLD patients under prior use of statins were excluded from the study.

3.2. Serum adiponectin concentration and its associations

Adiponectin levels ranged between 4.15 and 10.77 μg/mL (mean 5.77 ± 2.52) in the total of patients, with 39.4% of all patients having an adiponectin concentration below the lowest levels of normal (range of normal values reported in the literature 5–30 μg/mL). Presence of NAFLD (group A) was associated with significant hypoadiponectinemia when compared to group B and C patients (mean adiponectin concentration 2.87 ± 1.28 vs. 6.21 ± 2.38 μg/mL, $p < 0.001$).

Further statistical analysis revealed a strong association of adiponectin with gender (4.97 ± 2.39 μg/mL in males vs. 6.26 ± 2.48 μg/mL in females, $p = 0.001$). Adiponectin was found not to be correlated with age, BMI, waist circumference or WHR, while it correlated negatively with total cholesterol (Spearman's *rho* correlation coefficient $r_s = -0.306$, $p < 0.001$),

Table 1 – Patient baseline characteristics[a].

	Group A (NAFLD pts), $n = 17$	Group B + C (before intervention), $n = 110$	p values[b]
Demographics			
Male gender	6 (35.3%)	43 (39.1%)	0.765
Age (years)	64.4 + 9.9	62.9 + 7.1	0.453
Somatometrics			
Weight (kg)	88.3 ± 12.9	87.5 ± 11.5	0.805
Height (cm)	162.7 ± 7.3	163.5 ± 7.8	0.694
BMI (kg/m^2)	33.5 ± 5.5	32.9 ± 4.8	0.637
Obesity	14 (82.4%)	72 (65.5%)	0.165
Waist circumference (cm)	103.5 ± 14.3	104.4 ± 9.2	0.738
Hip circumference (cm)	111.1 ± 15.7	113.4 ± 10.9	0.955
WHR	0.94 (0.89–0.96)	0.95 (0.88–0.98)	0.941
Diabetes history			
Duration of DMT2 (years)	12 (11–14)	12 (9–15)	0.784
HbA1c % (mmol/mol)	8.3 ± 1.3 (67 ± 15)	8.1 ± 1.2 (65 ± 13)	0.512
Prior anti-DM therapy			0.655
Metformin + gliclazide + insulin	4 (23.5%)	18 (16.4%)	
Metformin + glimepiride + Insulin	8 (47.1%)	64 (58.2%)	
Metformin + Insulin	5 (29.4%)	28 (25.5%)	
Metformin dosage (mg)	1700 (850–1700)	1700 (1700–1700)	0.258
Lipidemic profile			
Prior statin use	0 (0%)	66 (60%)	<0.001
Total cholesterol (mmol/L)	5.5 ± 1.6	4.4 ± 1.3	0.009
LDL (mmol/L)	3.6 ± 1.4	2.5 ± 1.1	0.001
HDL (mmol/L)	1.2 ± 0.2	1.2 ± 0.3	0.439
TG (mmol/L)	2.1 ± 1.2	1.8 ± 1.4	0.049
Atherogenic index	0.21 (0.10–0.31)	0.07 (0.03–0.19)	0.103
Factors of metabolic syndrome			0.898
>3 out of 5	17 (100%)	110 (100%)	
>4 out of 5	14 (82.4%)	90 (81.8%)	
All 5	5 (29.4%)	38 (34.5%)	
Laboratory investigation			
ALT (U/L)	43 (11–126)	23 (5–34)	<0.001
AST (U/L)	38 (17–72)	18 (7–25)	<0.001
CRP (mg/dL)	6.7 ± 5.1	4.7 ± 3.2	0.056
Adiponectin (μg/mL)	2.87 ± 1.28	6.21 ± 2.37	<0.001
Histology			
Fibrosis			
F1	3 (17.6%)	NA[c]	
F2	3 (17.6%)	NA	
F3	3 (17.6%)	NA	
F4	8 (47.1%)	NA	
Steatosis			
Mild	9 (52.9%)	NA	
Moderate	2 (11.8%)	NA	
Severe	6 (35.3%)	NA	
Inflammation			
Score 1	9 (52.9%)	NA	
Score 2	8 (47.1%)	NA	
Score 3	0 (0%)	NA	
Ballooning			
Score 1	10 (58.8%)	NA	
Score 2	7 (41.2%)	NA	
NAFLD activity score			
>5	12 (70.6%)	NA	
3–4	5 (29.4%)	NA	
<3	0 (0%)	NA	

[a] Table entries represent numbers (%), means ± standard deviations or medians (interquartile range), as appropriate.
[b] Statistical analysis included χ^2 for categorical variables, t-Student for parametrical or Mann–Whitney U test for non-parametrical continuous variables.
[c] NA: not applicable.

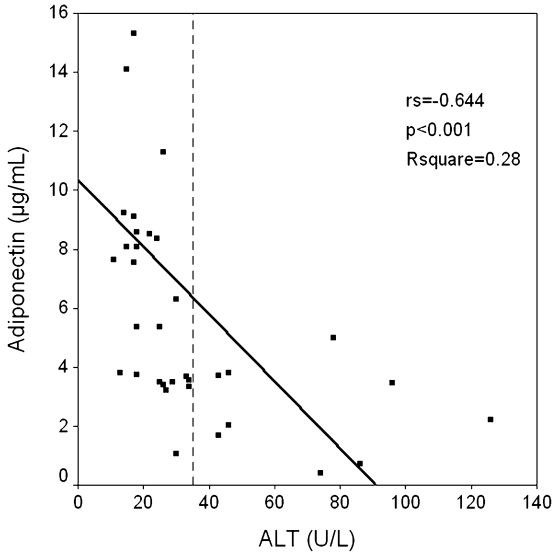

Fig. 2 – Scatter plot of adiponectin and ALT values showing negative correlation (dashed line represents cut-off limit of abnormal ALT).

LDL ($r_s = -0.343$, $p < 0.001$), and positively with HDL ($r_s = 0.273$, $p = 0.002$). As use of statins certainly affects lipids, it could not be determined if this association of adiponectin with lipidemic profile was confounded by the prior statin use. Furthermore, adiponectin was not associated with metformin dosage, glycemic control measured with HbA1c or CRP. Finally, a strong negative correlation was revealed between adiponectin levels and ALT in the total of the study sample ($r_s = -0.644$, $p < 0.001$), as shown in Fig. 2.

3.3. *Intervention group analysis*

From the total of 110 patients allocated to receive intervention, 7 patients (6.4% of total) dropped out, all from group C (Fig. 1). Finally, 55 patients were included in group B and 48 patients in group C. As recorded in the scheduled patient visits, compliance to treatment was high in both groups. Only mild adverse effects were reported including redness at sites of injection (both groups), morning hypoglycemic events easily reversible by oral intake of juice (both groups) and mild abdominal discomfort after exenatide injections (group B, less than 10%).

3.4. *Comparison of the two intervention arms of the study*

Table 2 summarizes baseline patient characteristics, comparisons between two groups, as well as the results of intervention presented as Δ differences. Group B and group C patients did not differ significantly in their baseline characteristics, except from the fact that group C patients were randomly selected to be heavier by approximately 5 kg (no statistical significant difference in BMI), to have a wider waist circumference by approximately 4 cm (no difference in WHR) and to have higher baseline aminotransferases (within normal range however).

During the 6-month intervention trial, patients of both groups succeeded in lowering their baseline HbA1c values by approximately 1% (−10% of baseline values, $p < 0.001$), total cholesterol ($p = 0.029$) and triglyceride levels ($p < 0.001$), CRP ($p < 0.001$) while adiponectin levels increased by +24.2% (IQR 14.8–53.2%, $p = 0.001$) (Fig. 3). As shown in Table 2, the effects of intervention in glucose control and adiponectin increase did not differ significantly between two groups. On the other hand, decreases in lipidemic profile and CRP were significantly greater in group C. Finally, intervention had a significant impact on patients' weight and waist circumference, with the results being reverse in the two groups; group B patients presented a significant weight loss of approximately 2 kg (−2.1%) with no change in their waist circumference, while group C patients had a weight gain of 3.5 kg (+4.2%) with a parallel increase in their waist of 2.25 cm (Fig. 3).

3.5. *Determinants of adiponectin changes (ANCOVA)*

Statistical analysis of one-way ANCOVA was performed in order to test if increases of adiponectin during intervention could be explained by differences in other factors after controlling the effect of baseline adiponectin. ANCOVA tests of between-subjects effects revealed no significant interaction with the type of intervention (exenatide vs. insulin group), but with weight loss ($p = 0.011$), HbA1c control ($p = 0.045$) and CRP reduction ($p = 0.047$). Even though effect sizes estimated with Eta squared were small (0.114), the observed power remained high in each multifactorial model (>0.522).

4. Discussion

Adiponectin has been proved an essential hormone/cytokine mediating peripheral insulin action and affecting both hepatic steatogenesis and cardiovascular risk in patients with DMT2, which has not been introduced in everyday clinical practice yet, even though essential therapeutic interventions in DMT2 (diet, exercise and drugs) have all proved to affect circulating adiponectin levels. In the light of recent experimental data suggesting extra-pancreatic action of the new anti-diabetic GLP-1 receptor agonists on adiponectin's expression, this study was the first to directly address the question of how GLP-1 and adiponectin interact on clinical grounds.

In the first part of the study, NAFLD—mostly NASH—was diagnosed in a small proportion of DMT2 patients by monitoring level of aminotransferases, excluding other causes of chronic liver disease and confirming diagnosis with liver biopsy (gold-standard method). The majority of patients with abnormal aminotransferases refused liver biopsy, and this remains within the limitations of our study. Even though normal aminotransferase level cannot exclude presence of NAFLD, however NASH has been repeatedly associated with abnormal ALT [37–39]. Statistical comparison of this group of patients with DMT2 patients with normal aminotransferases revealed that they did not differ in any of their baseline characteristics (gender, age, somatometrics, factors of insulin resistance, duration of diabetes, glucose control, CRP) other than their circulating adiponectin levels. Furthermore, within the results of this study was a strong negative correlation of adiponectin with ALT in the total of our patients—not only when NALFD was present—suggesting a "*hepatoprotective*" role

Table 2 – Intervention group analysis[a] (including baseline characteristics and results of intervention presented as Δ/differences).

		Group B, $n = 55$	Group C, $n = 48$	p (within groups)[b]	p (between groups)[c]
Treatment duration (months)	6.7 (6.4–7.1)	6.65 (4.1–7.0)		0.425	
Demographics					
Male gender (n %)		25 (45.5%)	16 (33.3%)		0.210
Age (years)		62.2 ± 7.2	63.7 ± 7.1		0.296
Somatometrics					
Height (cm)		162.5 ± 8.2	164.7 ± 7.3		0.154
Weight (kg)	Baseline	84.6 ± 12.4	90.4 ± 9.9		0.010
	Final	82.4 ± 11.5	91.7 ± 12.5		<0.001
	Δ_weight (kg)	−2.0 (−3.2–0)	+3.5 (0.6–4.5)	0.237	<0.001
BMI (kg/m^2)	Baseline	32.2 ± 5.5	33.4 ± 3.9		0.202
	Final	31.4 ± 5.1	33.8 ± 4.0		0.009
	Δ_BMI (kg)	−0.8 (−1.3–0)	+1.3 (0.2–1.4)	0.130	<0.001
Waist circumference	Baseline	102.3 ± 11.2	106.5 ± 6.4		0.020
	Final	103.0 ± 10.7	108.5 ± 6.8		0.002
	Δ_waist (cm)	0 (−1.5–2.0)	2.3 (0–4.0)	0.001	0.079
Hip circumference (cm)		112.0 ± 11.0	114.5 ± 10.5		0.235
WHR		0.92 (0.87–1.01)	0.95 (0.92–0.97)		0.353
Glucose control					
Duration of DMT2 (years)	12 (10–15)	10.5 (9.0–15)		0.236	
HbA1c % (mmol/mol)	Baseline	8.3 ± 1.5 (67 ± 16)	8.0 ± 0.9 (64 ± 10)		0.154
	Final	7.1 ± 0.8 (54 ± 9)	6.7 ± 0.3 (50 ± 4)		<0.001
	Δ_HbA1c %	−0.8 [−1.4–(−0.6)]	−1.0 [−1.8–(−0.6)]	<0.001	0.690
Metformin dosage (mg)	1700 (850–1700)	1700 (850–2550)		0.346	
Lipidemic control					
Prior statin use (%)		30 (54.5%)	32 (66.7%)		0.210
Total cholesterol	Baseline	4.6 ± 1.3	4.3 ± 1.3		0.243
	Final	4.5 ± 1.0	4.8 ± 1.0		0.115
	Δ_cholesterol (mmol/L)	0.2 (−0.3–0.2)	0.6 (−0.4–1.6)	0.029	<0.001
LDL (mmol/L)	Baseline	2.5 ± 1.0	2.5 ± 1.6		0.791
	Final	2.4 ± 0.9	2.7 ± 0.9		0.353
	Δ_LDL (mmol/L)	0 (−0.3–0.2)	0.2 (−0.5–0.9)	0.210	0.085
HDL (mmol/L)	Baseline	1.3 ± 0.3	1.1 ± 0.1		0.085
	Final	1.3 ± 0.3	1.2 ± 0.2		0.008
	Δ_HDL (mmol/L)	0 (−0.3–0.2)	0 (0–0.1)	0.076	0.595
TG (mmol/L)	Baseline	1.9 ± 1.8	1.6 ± 0.7		0.791
	Final	1.7 ± 1.0	2.0 ± 1.2		0.289
	Δ_TG (mmol/L)	0 (−0.2–0.5)	0.4 (0.2–0.6)	<0.001	0.004
Additional laboratory investigation					
ALT (U/L)	Baseline	18 (13–19)	24.5 (23–32)		<0.001
	Final	17 (15–18)	25.5 (22–30)		<0.001
	Δ_ALT (U/L)	0 (−1–3)	−1 (−1–7)	0.112	0.175
AST (U/L)	Baseline	16 (14–21)	21 (18–25)		0.008
	Final	20 (14–23)	23.5 (18–24)		0.002
	Δ_AST (U/L)	0 (0–6)	1 (−1–6)	<0.001	0.789
CRP (mg/dL)	Baseline	3.3 (0.53–5.9)	1.7 (1.3–6.7)		0.691
	Final	1.3 (0.6–8.3)	1.1 (1.1–1.3)		0.063
	Δ_CRP (mg/dL)	0 (−1.5–0.9)	−0.5 [−3.2–(−0.1)]	<0.001	0.001
Adiponectin (μg/mL)	Baseline	6.2 ± 2.2	6.2 ± 2.6		0.994
	Final	8.2 ± 2.8	8.2 ± 3.6		0.968
	Δ_adiponectin (μg/mL)	1.5 (0.9–4.2)	1.1 (0.6–4.9)	0.001	0.965
	Δ_adiponectin (%)	24.2 (11.3–81.9)	23.6 (17.5–53.2)	0.001	0.596

[a] Table entries represent numbers (%), means ± standard deviations or medians (interquartile range), as appropriate.
[b] Comparisons within groups were performed using t-test for paired samples or Wilcoxon signed ranks test (significance indicated with p values within groups).
[c] Comparisons between groups were performed using χ^2 test for categorical variables, t-Student for parametrical or Mann–Whitney U test for non-parametrical continuous variables.

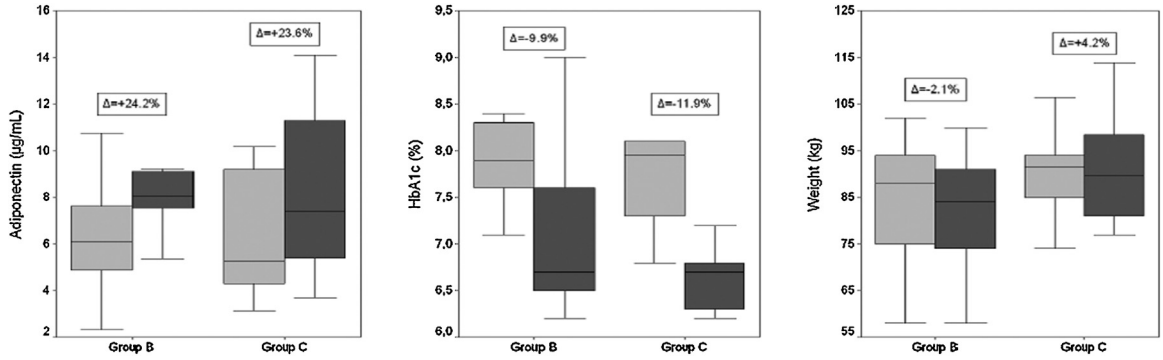

Fig. 3 – Changes of adiponectin levels, HbA1c and weight within and between groups (light-gray bars represent baseline and dark bars represent final values).

for this hormone. However, whether decreased adiponectin levels are the cause or the result of steatohepatitis remains unanswered [9].

Other findings of this first part of the study confirmed that adiponectin was higher in females, a finding that has been confirmed in other studies and that has been attributed to the absence of androgens [40–42]. On the other hand, adiponectin was found not to be associated with age nor BMI, as literature suggests [3,8,11,40]. One possible explanation may be that the majority of the patients studied were old (95% of all patients between 57.5–71 years old, no patients under 45) and overweight (92.1% of all patients had BMI $>$ 25 and 67.7% BMI $>$ 30 kg/m^2), thus making associations probably difficult to reach the level of statistical significance. Moreover, adiponectin was found to correlate with total cholesterol, LDL (positively) and HDL (negatively), but it could not be determined if these associations were confounded by the prior use of statins.

The second part of the study aimed to examine the effect of exenatide vs. standard insulin on adiponectin levels. Certain recent data derived from the literature and everyday clinical practice of treating patients with DMT2 led to the selection of these two arms of the study and the definition of inclusion/exclusion criteria; first, the combined use of exenatide with glargine insulin has recently been proved effective in achieving glucose control even in patients with longer duration of DMT2 [43]. Secondly, the withdrawal of sulfonylureas aimed in alleviating any hypoglycemic events and, finally, prior use of thiazolidinediones was excluded because of their established action in increasing adiponectin levels.

During this short-term intervention trial, both groups achieved better and, simultaneously, similar glucose control, even though decreases in HbA1c were approximately only 1% (baseline values were those after the run-in period). One significant advantage of exenatide over standard insulin was its effect on weight. Having in mind that the study lasted for only 6 months, it can be presumed that differences in weight and waist circumference could be higher if administration of the drug lasted more. Finally, there were substantial increases in circulating adiponectin levels in both groups, thus rejecting our hypothesis that exenatide might outnumber standard insulin concerning its specific impact on adiponectin. When reviewing the literature of both experimental and clinical studies, results on this interaction remain conflicting [3,11,15,16,28,44–46]. Our study showed that increases in circulating adiponectin were only associated with weight loss, glucose control and CRP reductions, but not with the type of intervention. However, taking into account that weight loss was mainly induced by exenatide, questions remain to whether the clinical interaction of exenatide to adiponectin levels would be different if weight loss was greater as shown in patients after bariatric surgery [3].

Another interesting aspect that could further be addressed in future studies is if this interaction would be different in DMT2 patients with concomitant NAFLD. The major limitation of this study protocol was that NAFLD patients were excluded from the subgroup intervention trial, thus being unable to investigate an optional effect of exenatide on liver steatosis, as recent literature suggests [18–20].

Conflicts of interest

None declared.

Funding source

None.

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