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Impact of Direct Acting Antiviral (DAA) Treatment on Glucose Metabolism and Reduction of Pre-diabetes in Patients with Chronic Hepatitis C

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ABSTRACT

Background & Aim: With the development of direct acting antiviral agents (DAA) chronic hepatitis C virus (HCV) infection has become curable in most patients. Since HCV infection is known to have direct and/or indirect effects on glucose metabolism, successful HCV treatment may have an impact in reducing glucose level, pre-diabetes, the need of treatment for diabetes, and ultimately diabetes-associated morbidity. We investigated the association of DAA treatment and glucose metabolism in the context of development or resolution of hepatic fibrosis in a large cohort of HCV- infected patients.

Methods: In this retrospective single-center observational study, we investigated 281 patients receiving all-oral DAA therapy for fasting plasma glucose, HbA1c, liver enzymes and general clinical chemistry, measured during a 52-week follow-up. In addition, elastography, FIB-4- and APRI-calculation were used to assess hepatic fibrosis non-invasively.

Results: Successful elimination of HCV through DAA treatment was associated with a significant drop in fasting glucose level and a reduced rate of impaired fasting plasma glucose (FPG). Interestingly, this metabolic change was BMI-independent. In addition, long-term glucose levels also decreased after successful DAA treatment. A significant APRI-score reduction was associated with a persistent improvement of FPG. However, DAA did not have an impact on glucose metabolism in patients suffering from liver cirrhosis.

Conclusion: This study highlights the beneficial impact of successful HCV therapy on glucose metabolism and identifies patients with liver cirrhosis as a collective in need of intensified surveillance with regard to diabetes progression despite HCV eradication.

Key words: Hepatitis C – direct acting antiviral – diabetes – metabolism.

Abbreviations: DAA: direct acting antiviral agents; FPG: fasting plasma glucose; HCV: hepatitis C virus; IFG: impaired fasting glucose; SVR: sustained virological response.

INTRODUCTION

Chronic infection with hepatitis C virus (HCV) is one of the major causes of chronic liver disease, cirrhosis and its complications as well as hepatocellular carcinoma. Affecting approximately 170 million people worldwide with an increasing morbidity and mortality, HCV is a major issue for global health [1, 2]. Yielding sustained virological response (SVR) rates between 79% and 100% for the combination of Simeprevir and Sofosbuvir [3], 93% to 99% for Sofosbuvir plus Ledipasvir [4, 5], 91% to 96% in compensated cirrhosis for the regimen containing Ombitasvir, Paritaprevir, Ritonavir and Dasabuvir [6], and 92% to 100% for Daclatasvir and Sofosbuvir [2], the phase II and III studies demonstrated chronic HCV infection to be a curable disease in most patients.

Achieving SVR leads to a decrease in mortality and morbidity in line with a reduction of the complications associated with liver disease progression, such as cirrhosis, liver failure, or hepatocellular carcinoma [7]. Comparing paired liver biopsies obtained before and after achieved SVR by interferon-based therapies, an improvement in hepatic inflammation was demonstrated in about 90% of patients [7]. This finding was accompanied by a reduction of histologic fibrosis scores. However, the reported rate of fibrosis regression ranges from 25% [8] to 56% [9] with lower rates found in larger study populations. Using non-invasive assessments of

hepatic fibrosis, this change seems to be slow, leading to a small reduction of the number of patients progressing to cirrhosis [10]. Adding the DAAs Boceprevir and Telaprevir to the eradication regimen, results in a rapid and significant drop in serum markers of liver fibrosis [11]. Beside promoting hepatic fibrosis and carcinogenesis, chronic inflammation caused by the HCV has been associated with an impairment of the liver central role in glucose homeostasis, leading to an increased incidence of prediabetes, diabetes and hepatic steatosis [12, 13]. Eradication of the HCV virus, achieved by interferon-based therapies, was first linked to an improvement of the markers of glucose metabolism in a hospital-based study in a relatively small Chinese population [14]. In line with this observation, Pavone et al. [15] were the first to describe a rapid improvement of fasting plasma glucose (FPG) following SVR achieved by interferon-free regimens. Hitherto, these data have been further substantiated by additional smaller reports on this issue [16-19]. However, correlation to body mass index (BMI) and/or liver cirrhosis remained unclear in most of these studies.

Therefore, we analyzed these aspects of DAA-based hepatitis C therapy on the metabolic function of the liver in a retrospective single-center observational study.

PATIENTS AND METHODS

HCV cohort

This retrospective single-center observational study group comprised 281 patients treated with DAA ± Ribavirin between March 2013 and June 2016 in the outpatient department of the Mannheim University Medical Center. The analysis included treatment-naïve and treatment-experienced patients with and without cirrhosis. HCV genotypes included genotypes 1 – 5. Follow-up data were available 4 to 52 weeks post-treatment. There were no specific inclusion or exclusion criteria for this analysis. Table I describes patient baseline characteristics by genotype. There were no significant differences regarding gender (p=0.8827), BMI (p=0.8897) or frequency of liver cirrhosis (p=0.8391) for the different genotypes. The baseline prevalence of diabetes mellitus type 2 (DMT2) was 10% (n=28). Further data regarding DMT2 patients is provided in Suppl. Table I. Impaired fasting plasma glucose (IFG) could be detected in 22 of the 129 patients (17%) with sufficient data on FPG.

Treatment regimens

Therapeutic regimens included Daclatasvir (Bristol-Myers Squibb S.r.l., Contrada Fontana del Ceraso, Italy), Ledipasvir (Gilead Sciences Ireland UC, Carrigtohill, Ireland), Simeprevir (Janssen-Cilag, Borgo San Michele, Italy), Sofosbuvir (Gilead Sciences Ireland UC, Carrigtohill, Ireland), the combination of Paritaprevir, Ritonavir and Ombitasvir (AbbVie Deutschland, Ludwigshafen, Germany) with or without Dasabuvir (AbbVie Deutschland, Ludwigshafen, Germany) and Velpatasvir (Gilead Sciences Ireland UC, Carrigtohill, Ireland) and Ribavirin (Roche Pharma, Grenzach-Wyhlen, Germany). All medications were administered as recommended by the manufacturer. The

Table I. Baseline Characteristics (available information*)

Median age [years] (281)								56		
Mean BMI [kg/m^2] (205) (SD[1])								26 (5.11)		
Baseline log10 HCV RNA titre [IU/ml] (122)			Mean (SD[1])					2 (3)		
			Median (IQR[2])					1 (2)		
Genotype (281*)		n (%)	1[3] (%)	1a (%)	1a/b (%)	1b (%)	2 (%)	3a (%)	4	5a
			203 (72)	71 (25)	2 (1)	118 (42)	8 (3)	53 (19)	16 (6)	1 (1)
Sex (281*)	m	154 (55)	102 (36)	36 (13)	2 (1)	59 (21)	3 (1)	39 (14)	9 (3)	-
	f	127 (45)	101 (36)	35 (13)	-	59 (21)	5 (2)	14 (5)	7 (2)	1(1)
Prediabetes (129*)		22 (17)	15 (12)	2 (2)	-	11(9)	1 (1)	3 (2)	3 (2)	-
Diabetes (281*)		28 (10)	21 (7)	9 (3)	-	9 (3)	1 (1)	3 (1)	3 (1)	-
Cirrhosis (267*)		98 (37)	75 (28)	28 (10)	-	44 (16)	4(1)	16 (6)	4 (1)	-
Child-Pugh Class (95*)	A	83 (87)	63 (66)	23 (24)	-	38 (40)	4 (4)	14 (15)	3 (3)	-
	B	8 (8)	7 (7)	3 (3)	-	2 (2)	-	-	1 (1)	-
	C	4 (4)	3 (3)	-	-	3 (3)	-	1 (1)	-	-
Mean MELD (98*)		10	10	10	-	10	16	9	9	-
Fibrosis (210*)	F0-1	71 (34)	55 (26)	20 (10)	2 (1)	28 (13)	2 (1)	10 (5)	2 (1)	1 (1)
	F2	40 (19)	26 (12)	4 (2)	-	21 (10)	1 (1)	7 (3)	7 (3)	-
	F3	35 (17)	25 (12)	10 (5)	-	15 (7)	-	8 (4)	2 (1)	-
	F4	64 (30)	51 (24)	22 (10)	-	26 (12)	3 (1)	9 (4)	1 (1)	-
Treatment history (123*)	Naïve	77 (63)	35 (28)	12 (10)	-	21 (17)	4 (3)	7 (6)	1 (1)	-
	Int[4]	13 (11)	10 (8)	1 (1)	2 (2)	7 (6)	-	2 (2)	-	-
	Int +Rv[5]	56 (46)	40 (33)	9 (7)	-	26 (21)	1 (1)	5 (4)	3 (2)	-
	Int + Rv + PI[6]	19 (15)	16 (13)	3 (2)	-	9 (7)	-	-	-	-

*Values marked with a star represent the number of patients with available information regarding the parameter in question. Percentages are calculated using this value.[1] Standard deviation; [2] Inter quartile range; [3] Containing patients without further subgenotyping; [4] Interferon; [5] Ribavirin; [6] Protease inhibitor

therapeutic regimen was selected by a board of hepatologists based on the current state of scientific information or followed the recommendations of the European Association for the Study of the Liver and the German Gastroenterological Association DGVS, once available. The most frequent therapeutic regimens used consisted of Sofosbuvir and Ledipasvir with or without Ribavirin (26%), Sofosbuvir and Daclatasvir with or without Ribavirin (26%), but a significant subset was treated with Ombitasvir, Paritaprevir, Ritonavir and Dasabuvir (17%).

Quantification of liver fibrosis

Hepatic fibrosis was estimated using the FIB-4 test combining alanine- and aspartate aminotransferase (ASAT) levels, platelet count and age according to Sterling et al. [20], the ASAT to platelet ratio index (APRI) according to Wai et al. [21] or transient elastography measured by FibroScan® or Acoustic Radiation Force Impulse (ARFI) imaging. Cutoffs used were ≤ 7/1.215 (F0-1), 7.1-9.5/1.216-1.54 (F2), 9.6-12.5/1.55-1.94 (F3), ≥12.6/1.94 (F4) for FibroScan and ARFI, respectively. Sert et al. [22] described a 70% higher APRI value in insulin-resistant patients compared with healthy controls. Therefore, we examined whether a reduction of the APRI score by this amount could be associated with an improvement of glucose metabolism in the same individual.

Evaluation of metabolic changes

Fasting plasma glucose levels were determined within one hour using lithium heparin as an anticoagulant. Impaired fasting glucose was defined as a blood glucose level ranging from 100 – 125 mg/dl.

Statistical analysis

Statistical analysis was conducted using SAS 9.4 and Graphpad Prism 4.0. The Shapiro-Wilk test was performed to assess normal distribution, a two-sided Student´s *t*-Test was used for normally distributed data, Sign- and the Wilcoxon signed-rank test for non-parametric data. To compare different therapeutic regimens and genotypes, One-Way ANOVA and Holm-Sidak´s multiple comparisons test were employed. A p-value less than 0.05 was regarded as statistically significant in all analyses.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards, as confirmed by the Ethics Committee of the Medical Faculty Mannheim, Germany. Written informed consent was obtained from all individual participants included in the study.

RESULTS

DAA treatment efficacy, virological outcome

In our "real world" cohort, general SVR rates were high, with an overall SVR4 rate of 99% (n=281) and an overall SVR12 rate of 94% (n=265). Regarding the treatment failure group, male gender, overweight, IFG and patients with genotype 3a were overrepresented (Suppl. Table II).

Effect of HCV treatment on plasma glucose levels

The effect of the SVR on the liver's metabolic function was assessed by determining the FPG level before treatment, at the end of the treatment as well as during an up to 48 weeks follow-up period. Sufficient baseline and follow-up data was available for 129 patients. SVR was associated with a significant reduction in fasting glucose level (p<0.0001, n=129). This effect was maintained until SVR24 (p=0.0005, n=109). At SVR48, however, the mean FPG value regained the starting level (Fig. 1a, Table II).

The effect on FPG became apparent in patients with impaired fasting glucose levels (data not shown) or manifest DMT2 (Table II) as well as in patients with normal fasting glucose levels (data not shown). In all cases in which a reduction in fasting plasma glucose was shown, a significant drop of serum transaminases became apparent (ASAT: p<0.0001 81 ± 6 U/l vs. 33 ± 3 U/l, ALAT: p<0.0001 106 ± 9 U/l vs. 37 ± 3 U/l, n=98).

Impaired fasting glucose was present in 17% (n=22) of the patients before the initiation of therapy. After completion of therapy, IFG was observed only in 6% (n=8) of the patients (p=0.0017, n=129). During the same period of observation, body mass index (BMI) did not change (Fig. 1b).

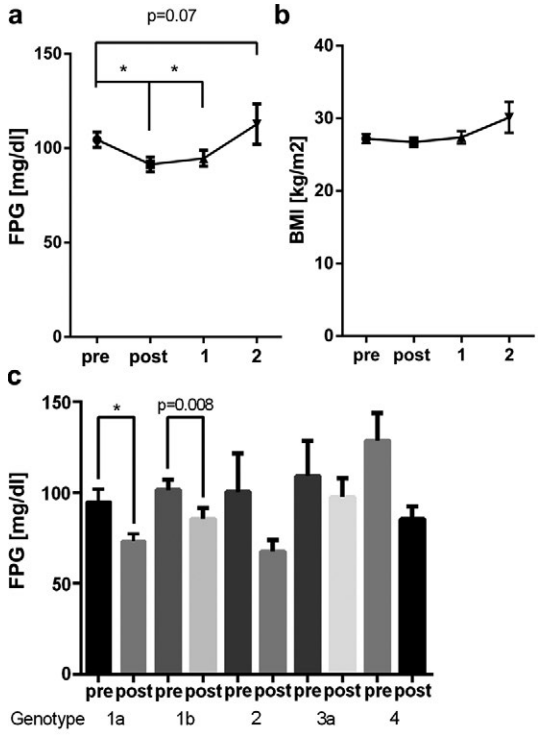

Fig. 1. Impact of SVR on fasting plasma glucose levels. SVR leads to a consistent drop in FPG levels, sustained until SVR24 (a). Absolute values in mg/dl ± SEM post-treatment (SVR4), at SVR 24 (1) and 48 (2). SVR is not associated with a significant change in BMI (b). Absolute values in kg/m2 ± SEM. A significant reduction in plasma fasting glucose levels could be shown in patients infected with genotype 1a and 1b (c).

Table II. Results of the investigations in our patients

	pre[1]	post	SVR24	SVR48	p-value	n
FPG (whole cohort)						
pre vs. post	108.7 ± 4 mg/dl	92.9 ± 4 mg/dl			<0.0001	129
pre vs. SVR24			93.47 ± 4 mg/dl		0.0005	109
FPG (DMT2)						
pre vs. post	168 ± 8.7 mg/dl	146 ± 9.2 mg/dl			0.04	28
pre vs. SVR24			139 ± 9 mg/dl		0.01	25
FPG GT1a						
pre vs. post	95 ± 7 mg/dl	73 ± 4 mg/dl			< 0.0001	20
FPG GT1b						
pre vs. post	101 ± 6 mg/dl	85 ± 6 mg/dl			0.008	50
FPG GT2						
pre vs. post	100 ± 21 mg/dl	67 ± 6 mg/dl			0.0625	5
FPG GT3a						
pre vs. post	109 ± 20 mg/dl	97 ± 10 mg/dl			0.5	12
FPG GT4						
pre vs. post	129 ± 15 mg/dl	85 ±7,mg/dl			0.125	5
HbA1c in Patients without Ribavirin						
pre vs. t=6-12 months	7.08 ± 0.27%		6.79 ± 0.48%		0.0156	9
Hb[2] in Patients with Ribavirin						
pre vs. t=0-6 months	13.36 ± 0.9 g/dl	12.08 ± 0.9 g/dl			0.12	7
Elastography value [% of pre]						
pre vs. 6 months	100%		76.50 ± 6.18%		<0.0001	13
pre vs. 12 months				17.3 ± 9%		2
FIB-4 value						
pre vs. post	4.51 ± 0.53	2.8 ± 0.36			<0.0001	104
pre vs. SVR24			2.42 ± 0.29		<0.0001	100
pre vs. SVR48				2.66 ± 0.5	<0.0001	56
APRI value						
pre vs. post	1.56 ± 0.18	0.68 ± 0.1			<0.0001	104
pre vs. SVR24			0.57 ± 0.08		<0.0001	100
pre vs. SVR48				0.59 ± 0.12	<0.0001	60
Reduction FIB-4 (Δ pre/post)						
No cirrhosis vs. cirrhosis	0.67 ± 0.33*	2.57 ± 0.71**			0.0014	*56,**62
Reduction APRI (Δ pre/post)						
No cirrhosis vs. cirrhosis	0.36 ± 0.095	1.38 ± 0.29			0.0017	*56,**62
FPG DMT2 APRI drop > 70%						
pre vs. post	197.6 ± 19.86 mg/dl	135.3 ± 18.96 mg/dl			0.037	9
pre vs. SVR24			128.9 ± 21.24 mg/dl		0.032	8
pre vs. SVR48				113.0 ± 26.47 mg/dl	0.033	4
FPG DMT2 APRI drop < 70%						
pre vs. post	154.8 ± 7.26 mg/dl	152.2 ± 10.25 mg/dl			0.835	19
pre vs. SVR24			143.8 ± 9.49 mg/dl		0.35	17
pre vs. SVR48				177.3 ± 22.56 mg/dl	0.23	7

Table II (continued)

	pre[1]	post	SVR24	SVR48	p-value	n
FPG IFG APRI drop > 70%						
pre vs. post	125.2 ± 7 mg/dl*	93.60 ± 9 mg/dl**			0.0007	*13,**12
pre vs. SVR24			84.00 ± 5 mg/dl		0.0003	6
FPG IFG APRI drop < 70%						
pre vs. post	138.9 ± 16.6 mg/dl	104.1 ± 14.4 mg/dl			0.006	9
pre vs. SVR24			107 ± 10.4 mg/dl		0.059	8
pre vs. SVR48				120.3 ± 21.8 mg/dl		4
FPG no IFG/DMT2 APRI drop > 70%						
pre vs. post	82.77 ± 1.4 mg/dl	72.06 ± 2 mg/dl			<0.0001	35
FPG no IFG/ DMT2 APRI drop < 70%						
pre vs. post	79.56 ± 2 mg/dl	71.82 ± 3 mg/dl			0.0055	39

[1] Depicted are the mean values ± standard error of the mean, before(pre), after (post) at SVR24 and SVR48; [2] Hemoglobin concentration. DMT2: diabetes mellitus 2; FPG: fasting plasma glucose; IFG: impaired fasting glucose.

An obvious trend towards a reduction of fasting glucose level could be found in all HCV genotypes (Table II). In genotype 1a and 1b, this difference was significant (Table II). Again, BMI remained unchanged (p>0.5, Suppl. Fig. 1a-c).

Effect of HCV treatment on long-term parameters of glucose metabolism (HbA1c)

With regard to changes in long-term parameters of glucose metabolism, HbA1c was assessed only in patients with manifest DMT2. Of the 28 patients with DMT2, baseline values were available from 22 patients, 12 of whom received a follow-up 6 months after therapy termination. The 12- and 18-month data were available for 15 individuals and 24-month follow-up data for 9 patients.

As for the plasma glucose levels, HbA1c demonstrated a significant decrease following HCV eradication in patients with manifest DMT2 (p=0.0367 n=12, Fig. 2a). In this subgroup, the decrease of HbA1c was accompanied by a non-significant reduction in BMI immediately after therapy (pre vs. post: 30.35 ± 2 kg/m² n=17 vs. 27.18 ± 2 kg/m² p=0.1, n=5). In the non-cirrhotic subgroup, we observed a significant decrease of the HbA1c values up to 12 months after therapy (pre vs. t=0-6 months: p=0.09 n=6; pre vs. t=6-12 months: p=0.03 n=6; Fig. 2b). However, in patients with liver cirrhosis this effect was not observed (pre vs. t=0-6 months: p=0.4 n=7; pre vs. t=6-12 months: p=0.1 n=9; Fig. 2c). The highest impact on HbA1c improvement had the presence of steatosis. In those cases, a trend for prolonged reduction of HbA1c became apparent (n=8, Fig. 2d), whereas there was no effect in patients with DMT2 without steatosis (n=11, Fig. 2e). As Ribavirin-associated hemolytic anemia may lead to an artificially low measurement of HbA1c [23], we performed the same analysis in patients not receiving Ribavirin. In this subpopulation, we also detected a significant reduction in HbA1c 6-12 months after therapy (Suppl. Figure 1d, Table II). In patients, receiving

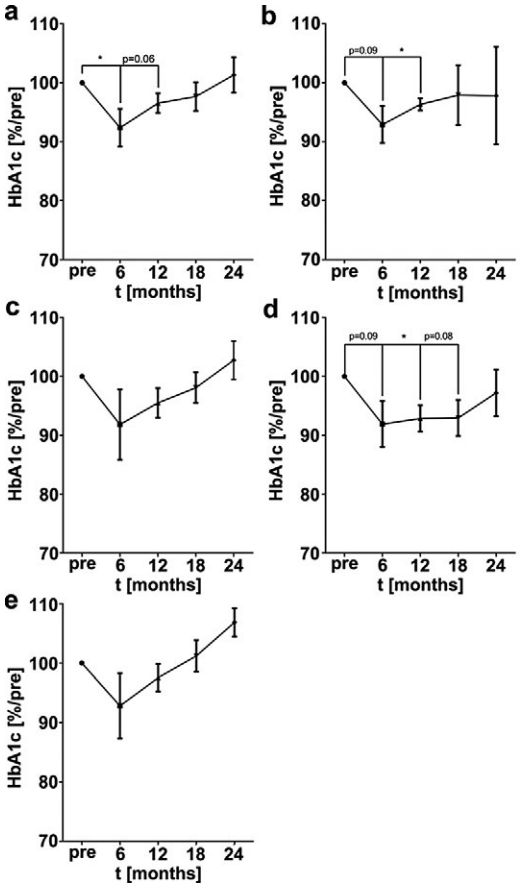

Fig. 2. Effect of HCV treatment on HbA1c. The relative HbA1c values ± SEM, normalized on the pretreatment value (pre) at 6, 7-12 (12), 13-18 (18) and 19-24 (24) months post treatment of all patients suffering from DMT2 (a), DMT2 without liver cirrhosis (b) and patients suffering from DMT2 and manifest cirrhosis (c). In the presence of steatosis (d), a trend for a prolonged effect could be shown (6: n=6, 12: n=5, 18: n=5, 24: n=4), while there was no effect detectable in patients without steatosis (e) (6: n=6, 12: n=8, 18: n=8, 24: n=3).

Ribavirin no significant change in HbA1c could be detected (Suppl. Figure 1e), albeit a non-significant decrease in Hb was observed (Suppl. Figure 1f, Table II). Notably, in this subgroup 5 of 7 patients suffered from liver cirrhosis.

Effect of DAA treatment on hepatic fibrosis

To investigate whether a correlation of glucose metabolism and liver fibrosis can be detected, we investigated the course of development or reversal of fibrosis during and after DAA treatment. Therefore, we applied the non-invasive fibrosis assessment modalities FIB-4 and APRI and performed elastography before treatment initiation in 55% (154/281) of all patients.

Performing elastography 4 to 12 weeks after termination of therapy, no difference to the baseline elasticity values was detected (p=0.203, mean difference of the Fibroscan value pre and post treatment: 2.29 ± 4.23; n=31). However, after 6 and 12 months, a significant reduction was observed (pre vs. 6 months: p<0.0001, n=13; pre vs. 12 months: n=2; Fig. 3a). Overall, 40% of the patients were shown to have a decrease in liver fibrosis, another 48% were stable with respect to fibrosis and only 12% progressed within 12 months after treatment (Fig. 3b). Of note, in patients with genotype 3a no significant reduction in any fibrosis score could be detected.

Evaluating FIB-4 value pre- and post-treatment, a decrease in the frequency of calculated advanced fibrosis by 19% was observed, accompanied by a significant reduction in absolute FIB-4 values (pre vs. post: p<0.0001, n=104; pre vs. SVR24: p<0.0001, n=100; pre vs. SVR48: p<0.0001, n=56) (Fig. 3c, Table II). The same effect was demonstrated calculating the APRI score pre- and post-treatment (Fig. 3d, Table II).

Regarding the reduction of the two fibrosis scores, the effect was significantly more pronounced in cirrhotic livers (Fig. 3e&f, Table II).

Correlation of plasma fasting glucose and liver fibrosis in patients with DMT2

Fasting plasma glucose levels of patients with manifest DMT2 and IFG were significantly changed depending on the underlying fibrosis status (Fig. 4). In patients with manifest diabetes and a decrease in APRI of more than 70%, a significant decrease of FPG was observed throughout treatment and follow-up (Fig. 4a), while this decrease did not become apparent in patients with an APRI drop smaller than 70% (Fig. 4b, Table II).

Correlation of plasma fasting glucose and liver fibrosis in patients with IFG

Similar changes were observed in patients with impaired fasting glucose and an APRI drop of more than 70%, where a decrease in FPG was observed up to week 24 post treatment (pre vs. post: p=0.0007, n=13 vs. n=12; pre vs. SVR24: p=0.0003, n=13 vs. n=6; Fig. 4c). However, in patients with IFG and an APRI drop of less than 70% no significant effect on the FPG level was observed at SVR24 and SVR48 (Fig. 4d, Table II).

Correlation of plasma fasting glucose and liver fibrosis in patients with preserved glucose metabolism

In patients not suffering from diabetes or IFG, the changes described above were only moderate and occurred immediately after therapy termination (Fig. 4e, f, Table II). In these patients, FPG even slightly increased back to base levels after termination of treatment.

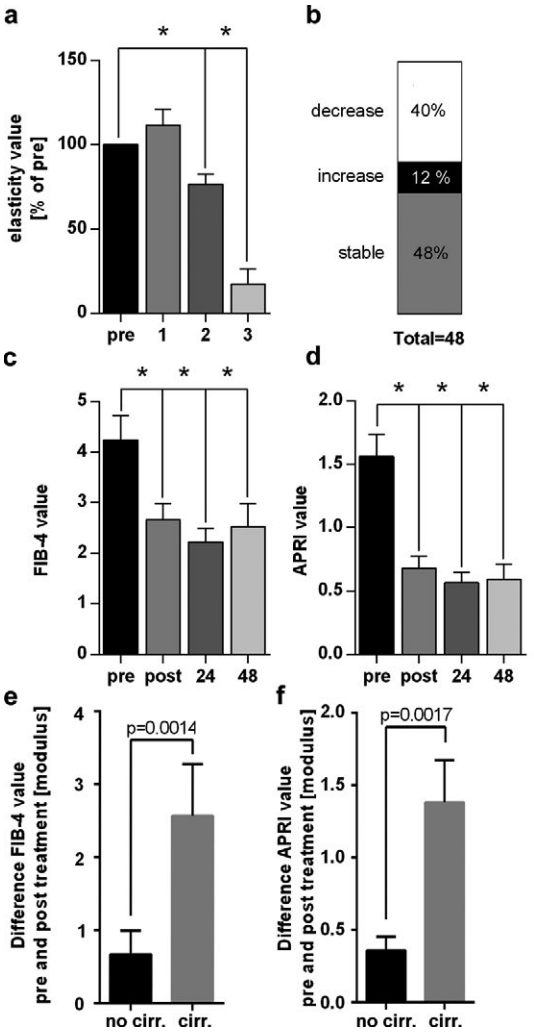

Fig. 3. SVR leads to a significant reduction of hepatic stiffness, preceded by a significant drop of FIB-4 and APRI values. (a) The elasticity values measured by ARFI and Fibroscan in percent of the value attained before initiation of treatment. First measurement (1) was performed within 6 weeks after termination of therapy, second measurement (2) within 6 months and third measurement (3) within 12-14 months. (b) A course of hepatic fibrosis following SVR. (c-d) SVR led to a significant and lasting reduction in FIB-4 and APRI values: depicted are the absolute values calculated pre- and post-treatment at SVR 24 and 48 ± SEM. (e-f). The reduction of the FIB-4 and APRI values was more pronounced in patients with liver cirrhosis.

DISCUSSION

Hepatocytes are crucial for maintenance of plasma glucose homeostasis. In a constant adjustment between glucose production and utilization via the gluconeogenic and glycolytic pathways, these cells are at the center of plasma glucose level regulation. Throughout the past decade, multiple molecular mechanisms of the HCV virus interfering at different levels with glucose metabolism have been established. Among these

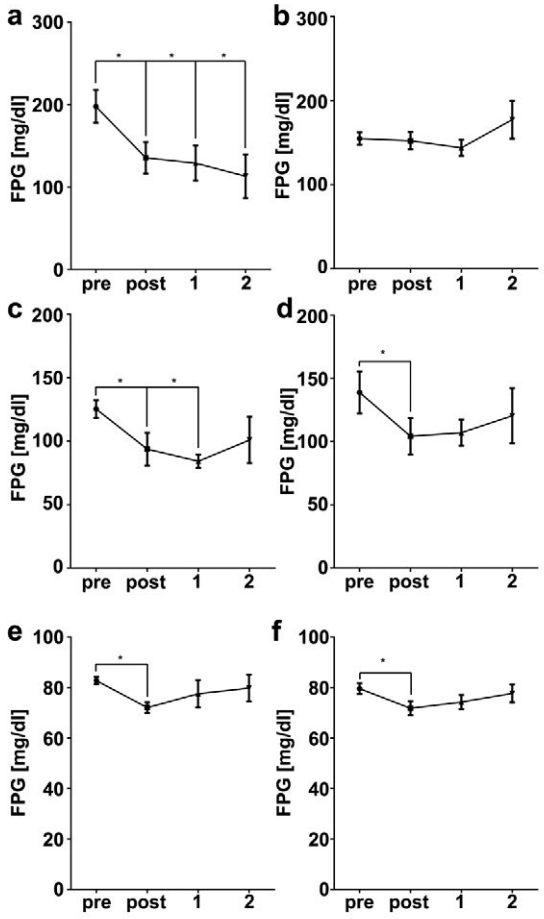

Fig. 4. Correlation of plasma fasting glucose and liver fibrosis. The absolute FPG values ± SEM in patients with a drop in APRI >70% (a, c, e), <70% (b, d, f) and DMT2 (a, b), IFG (c,d) and with normal fasting plasma glucose levels (e, f), pre and post treatment as well as at SVR24 (1) and 48 (2).

are effects of HCV replication on cellular glucose uptake through down-regulation of cell surface expression of glucose transporter 2 (GLUT2) [24] or hepatic gluconeogenesis via an NS5A-mediated, FoxO1-dependent pathway [25]. These molecular changes translate into a close link between HCV and DMT2. The large cross-sectional NHANES III study, surveying 9841 U.S. adults, demonstrated that HCV-positive U.S. patients above 40 years had a threefold increased risk for diabetes mellitus [26]. Numerous other studies have further substantiated these findings [27, 28]. An IFG rate of 18% detected in our patients is in line with these reports.

The availability of DAA-based therapies started a new era of HCV treatment with high efficacy and acceptable side effects even under "real-world" conditions [29]. In line with these published data, we achieved in our cohort overall SVR4 and SVR12 rates of 98% and 94%, respectively. Likewise, with 98% SVR12 rate being accomplished by using a regimen based on Daclatasvir or Ledipasvir (SVR12: 93% n=70), the treatment of our patients with DAA was very successful. Treatment failure most often occurred in the genotype 3a subgroup, which has already been shown to be the subpopulation most difficult to treat with a DAA-containing regimen [30-32].

Given the well-established correlation between HCV and glucose metabolism and the high rate of viral clearance utilizing the novel DAAs, we aimed to investigate the impact of DAA treatment and viral clearance on glucose metabolism. Our findings regarding reduced plasma fasting glucose levels and HbA1c values were in line with published literature on this issue [16-19]. In addition to these reports, we demonstrated in our more comprehensive cohort that these differences in glucose metabolism were independent of changes in body mass index (BMI). Our cohort comprised a constant high number of overweight patients and a mean BMI of 25.27 kg/m^2. Thus, the observed changes in glucose levels might be an effect of HCV clearance [24, 25]. The observation that even in patients with preserved glucose metabolism a reduction of FPG can be obtained, indicates a subclinical insulin resistance associated with HCV infection. This conclusion is in line with reports of reduced plasma insulin and HOM-IR following SVR [17]. Given our study's prolonged observation period, we were able to characterize this effect as only transient. While in all subgroups a significantly reduced FPG level could be seen only up to SVR24, patients with DMT2 and a >70% drop of the APRI value achieved a sustained improvement of FPG. As both scores highly weight transaminases in their calculation, these results may stress the importance of hepatic inflammation in HCV-induced insulin resistance, rather than the presence of fibrosis, especially in light of the observation of the delayed improvement in hepatic fibrosis, determined by elastography. Of note, 4 (14%) patients with DMT2 developed a new insulin dependency in the immediate aftermath of the therapy, of whom no patient achieved a >70% drop of APRI at the end of the therapy.

Finally, we were concerned that Ribavirin may induce hemolytic anemia, subsequently lowering HbA1c artificially. This was ruled out by analyzing HbA1c values in Ribavirin-free patients only, yielding similar results. In addition, FPG levels, not affected by hemolytic anemia, reflected HbA1c measurements.

Our data on changes in glucose metabolism in patients with DMT2 and liver cirrhosis are of considerable interest regarding their clinical implications. In contrast to other publications, we demonstrate that this subgroup of patients does not benefit from DAA treatment with respect to improvement of glucose levels. One reason for this lack of benefit might be that an important part of the pro-diabetic potential of chronic HCV-infection is due to peripheral insulin resistance caused e.g. by direct impairment of glucose transporter 4 expression by the HCV core protein in skeletal muscle and adipose tissue [33]. In cirrhotic patients, muscle tissue may exhibit impaired glucose storage because of reduced insulin sensitivity independent of HCV infection [34]. In addition, a reduced hepatic insulin clearance contributes to hyperinsulinemia and concomitant insulin resistance as well as increased plasma levels of insulin-counteracting hormones and a lack of liver-derived humoral factors with insulin-like activity, e.g. insulin-like growth factors I and II [35]. However, Gitto et al. [36] described a significant reduction in serum insulin levels after DAA-based SVR in a mainly cirrhotic group, while being unable to detect an effect on plasma glucose levels. Thus, one might explain the reduced responsiveness to DAA treatment of cirrhotic patients with regard to glucose metabolism by either a reduced effect on the peripheral glucose uptake or the decreased hepatic production

of insulin-like factors. Our data contributes to the growing evidence that DAA-based HCV eradication might result in a decreased cardiovascular risk both by the resolution of hepatic and systemic HCV-toxicity as indicated e.g. by a reduction of carotid atherosclerosis [37].

Given the retrospective approach of the current analysis, several limitations of this study have to be taken into account. The non-invasive assessment of hepatic fibrosis is limited by the lack of more specific laboratory tests such as ELF-test or Pro-C3 assessment and the absence of elastography measurements in all patients. The same applies for parameters of insulin resistance. The heterogeneity study population limits its explanatory power with regard to the possibility of correction for potential confounders such as genotype and the treatment regimen used.

CONCLUSION

Direct acting antiviral treatment was confirmed to be highly effective in a large "real-world" cohort of HCV patients and exhibited beneficial effects beyond viral elimination especially on glucose metabolism. This effect was independent of patients´ BMI. However, DAA did not have an impact on glucose metabolism in patients suffering from liver cirrhosis.

Conflicts of interest: None declared.

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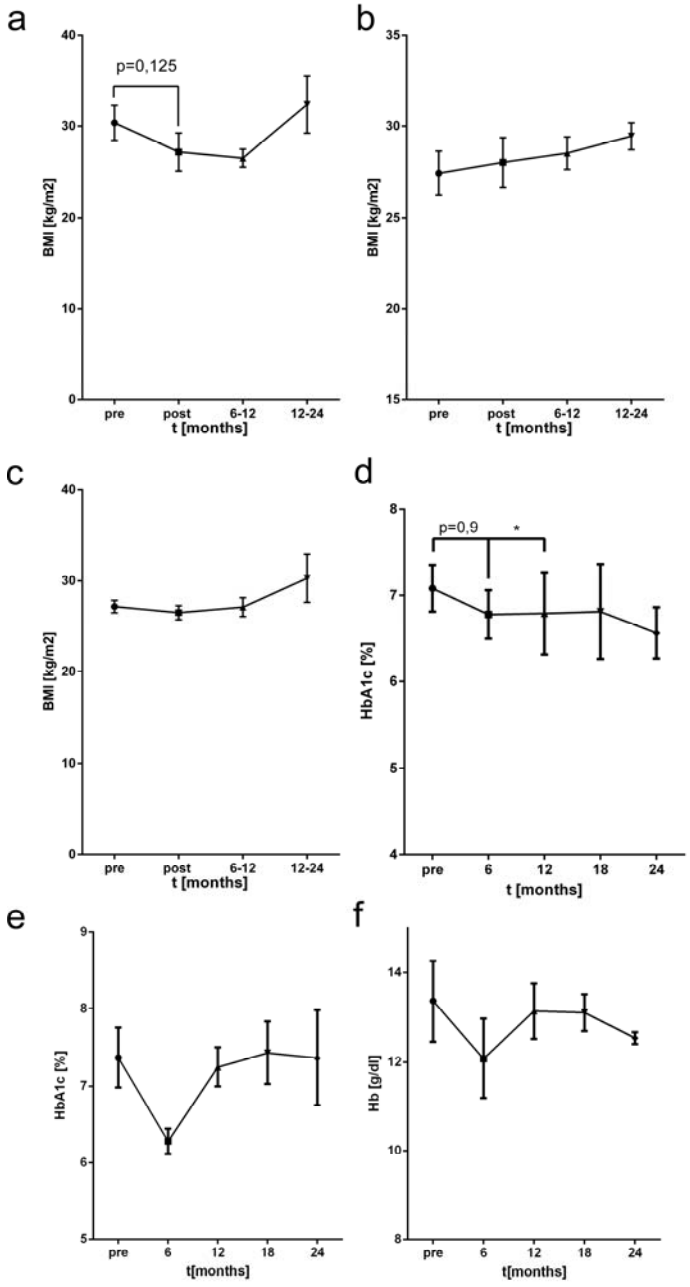
Supplementary Table I Baseline Characteristics of Patients with Type 2 Diabetes

Characteristic (available information*)																			
Median age [years] (28)*											62,5								
Mean BMI [kg/m2] (21) (STD)*											30,35 (7,4)								
Genotype (28)*		*n (%)*		*1[1] (%)*		*1a (%)*		*1a/b (%)*		*1b (%)*		*2 (%)*		*3a (%)*		*4*		*5a*	
				21 (75)		9 (32)		-		9 (32)		1 (4)		3 (11)		3 (11)		-	
Sex (28)*	*m*	16 (57)		10 (35)		4 (14)		-		5 (18)		-		3 (11)		3 (11)		-	
	f	12 (43)		11 (39)		5 (18)		-		4 (14)		1 (4)		-		-		-	
Cirrhosis (27)*		19 (70)		13 (48)		5 (19)		-		6 (22)		1(4)		3 (11)		2 (7)		-	
Child-Pugh Class (19)*	*A*	14 (74)		10 (53)		5 (26)		-		4 (21)		1 (5)		2 (11)		1 (5)		-	
	B	4 (21)		2 (11)		-		-		1 (5)		-		1 (5)		1 (5)		-	
	C	1 (5)		1 (5)		-		-		1 (5)		-		-		-		-	
Medication (28)*		I[2]	O[3]	I	O	I	O	I	O	I	O	I	O	I	O	I	O	I	O
		8 (29)	20 (71)	5 (18)	16 (57)	1 (4)	8 (29)	-	-	4 (14)	5 (18)	-	1 (4)	2 (7)	1 (4)	1 (4)	2 (7)	-	-
DAA Therapy + Ribavirin (28)*		11 (40)		5 (18)		3 (11)		-		2 (7)		-		3 (11)		3 (11)		-	

*Values marked with a star represent the number of patients with available information regarding the parameter in question. Percentages are calculated using this value.
[1] Containing patients without further subgenotyping
[2] Insulin
[3] Oral diabetic drug



Supplementary Figure 1: Correlation of BMI and characteristics of glucose metabolism: Depicted are the absolute BMI values in kg/m² in patients with DMT2 **(a)**, IFG **(b)** and with normal FPG levels **(c)**, pre and post treatment as well as after 6 to 12 and 12 to 24 months (x-axis). Data was available for 11, 5, 3, and 10 patients with diabetes (pre, post, 6-12, 12-24), 9, 6, 6 and 3 patients with IFG and 72, 37, 26 and 13 patients with preserved glucose tolerance. A trend for a change in BMI was observed in patients with DMT2, **(a).** SVR is associated with a significant reduction of HbA1c in patients not receiving Ribavirin **(d)** and receiving a Ribavirin-containing regimen **(e).** Depicted are the mean HbA1c values. Therapy with a Ribavirin-containing regimen leads to a non-significant drop of Hb **(f)**. Depicted are the mean, absolute Hb values.

Supplementary Table II. Therapy Failure – Clinical Characteristics

Genotype (16*)	*ALL*	*1a*	*1b*	*2*	*3a*
	16	3	5	1	7
Therapy experienced (9*)	6/9	0/0	4/5	0/1	2/3
Cirrhosis (16*)	8/16	3/3	4/5	1/1	0/7
BMI > 25 (6*)	6/6	3/3	2/2	1/1	0/0
IFG (15*)	6/15	0/3	2/4	0/1	4/7
Baseline log10 HCV RNA titre [IU/ml] MEAN (SD) (10*)	- -	6,86 -	0,9 (0,8)	0,17 -	8 (7,2)
Albumin (STD)(16*)	- -	36,3 -	34 (3,1)	43 -	35 (5,6)
Gender (16*)					
♂	13	1	4	1	7
♀	3	2	1	-	-

* Values marked with a star represent the number of patients with available information regarding the parameter in question.