The evolving relationship between adiponectin and insulin sensitivity in hepatitis C patients during viral clearance

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ABSTRACT

Background: The evolution of the relationship between adiponectin and insulin sensitivity in hepatitis C virus (HCV) patients during viral clearance is unclear and warrants investigation.

Methods: A prospective study including 747 consecutive chronic hepatitis C (CHC) patients, of whom 546 had completed a course of anti-HCV therapy and underwent pre-, peri- and post-therapy surveys for anthropomorphic, viral, metabolic and hepatic profiles and adiponectin levels, was conducted in a tertiary care center.

Results: Multivariate analyses indicated associations of sex, triglyceride levels and hepatic steatosis with adiponectin levels and of triglyceride levels and interferon λ3 (IFNL3) genotype with homeostasis model assessment-estimated insulin resistance (HOMA-IR) levels before anti-HCV therapy. In patients with a sustained virological response (SVR; n = 546), at 24 weeks post-therapy, sex, BMI, aspartate aminotransferase to platelet ratio index (APRI), HOMA-IR and steatosis were associated with adiponectin levels, and IFNL3 genotype was associated with HOMA-IR levels. GEE analysis demonstrated that SVR affected longitudinal trends in adiponectin levels. Compared with pre-therapy levels, adiponectin and APRI levels decreased 24 weeks post-therapy in SVR patients, regardless of baseline insulin resistance (IR). However, HOMA-IR levels decreased in SVR patients with baseline IR but increased in those without baseline IR. Compared with controls, immunohistochemical studies showed that pre-therapy CHC patients had higher hepatic adiponectin expression associated with hepatic fibrosis.

Conclusions: During HCV infection, adiponectin may affect insulin sensitivity through triglycerides. After viral clearance, adiponectin levels were directly associated with insulin sensitivity and decreased upon improved hepatic fibrosis; with a link to the IFNL3 genotype, insulin sensitivity improved only in patients with baseline IR.

KEYWORDS
adiponectin; HCV; HOMA-IR; insulin sensitivity; triglycerides

Introduction

Hepatitis C virus (HCV), a human pathogen responsible for acute and chronic liver disease, has variants classified into 7 major genotypes and infects an estimated 130–170 million individuals worldwide. HCV causes cardiometabolic alterations including hepatic steatosis, dyslipidemia, insulin resistance (IR), diabetes, obesity and cardiovascular events in addition to liver cirrhosis and hepatocellular carcinoma (HCC). Much of the HCV life cycle is closely associated with lipid metabolism in the host. Additionally, HCV down-regulates glucose transporters and inhibits insulin receptor substrate function to alter host glucose metabolism. Although most HCV infections are currently curable using potent direct-acting anti-viral agents, not all HCV-associated metabolic and oncogenic complications are reversible after viral clearance, especially among those with baseline diabetes and cirrhosis.

As an important endocrine organ, adipose tissue regulates metabolism through adipokines. Adiponectin, a 30-kDa adipokine, is highly expressed in adipocytes and is also expressed in hepatocytes. Increased visceral adipose tissue stores reduce the abundance of circulating adiponectin. Several IR-associated hormones such as insulin and catecholamines might dysregulate adiponectin expression. Post-translational adiponectin modifications result in the secretion of oligomers of 90-kDa trimers, which are found in the circulation as low molecular weight (LMW) and high molecular weight (HMW) adiponectins. HMW adiponectin is more closely correlated with insulin sensitivity than LMW adiponectin. Adiponectin mediates...
its effects on target cells via at least 2 adiponectin receptors, adiponectin receptor I (AdipoR1) and receptor II (AdipoR2). AdipoR1 is abundantly expressed in skeletal muscle and the liver, whereas AdipoR2 is primarily expressed in the liver. Adiponectin and its receptors might protect hepatocytes from triglyceride accumulation by increasing β-oxidation, decreasing the de novo synthesis of fatty acids, and promoting the uptake and inhibiting the production of glucose in the liver. Therefore, adiponectin has anti-inflammatory, anti-atherosclerotic and anti-apoptotic properties. Paradoxically, circulating adiponectin has been positively correlated with heart failure, coronary artery disease and all-cause mortality. Because both HCV infection and adiponectin are critically involved in metabolism, their precise relationship might aid to probe the therapeutic targets for HCV-associated cardiometabolic complications but remains inconclusive. For example, compared with controls, serum adiponectin levels have been reported to be higher, lower or not different in chronic hepatitis C (CHC) patients. All studies but one failed to correlate HCV viral load with adiponectin levels. Low adiponectin levels in CHC patients have been linked to poor anti-HCV immune response and the interferon α3 (IFNL3) CC genotype than non-

Moreover, an inconsistent association between hyperadiponectinemia and HCV-associated fibrosis, HCC and liver-unrelated mortality has been noted. In addition to various HCV genotypes and the pleiotropic function of adiponectin, these tremen-

dous obscurities are primarily due to individual bias, which is difficult to completely eliminate from case-controlled, retrospective or prospective studies with small sample sizes or with limited adjusting confounders.

Accordingly, we sought to elucidate the impact of HCV infection on adiponectin levels and associated metabolic alterations after adjusting for crucial confounders in a prospective study of CHC patients before, during and after anti-HCV therapy.

### Results

**Baseline characteristics**

The baseline characteristics of the CHC patients are listed in Table 1. Of 747 patients, 407 (54.5%) and 295 (39.5%) were infected with genotype 1 (G1) and G2 HCV, respectively. The SVR patients had lower levels of HCV RNA and homeostatic model assessment for insulin resistance (HOMA-IR) and lower rates of G1 HCV infection and cirrhosis but higher rates of G2 infection and the interferon α3 (IFNL3) CC genotype than non-

#### Table 1. Baseline characteristics of all the enrolled chronic hepatitis C patients.

|                          | Total, n = 747 (treated and untreated) | SVR (+), n = 455 | SVR (−), n = 91 | p values |
|--------------------------|---------------------------------------|------------------|-----------------|----------|
| Male, n (%)              | 404 (54.1)                            | 257 (57.8)       | 47 (52)         | 0.394    |
| Age (yr)                 | 55.04±12.08                           | 53.04±12.93      | 57.5±12.47      | 0.160    |
| BMI                      | 24.93±3.81                            | 24.79±3.68       | 25.84±4.28      | 0.057    |
| HCV RNA (Log10 IU/ml)    | 3.97±1.12                             | 5.84±1.18        | 6.46±0.74       | <0.001   |
| HCV genotype (G), n (%)  |                                       |                  |                 |          |
| G1                       | 407 (54.5)                            | 214 (47.1)       | 72 (79.1)       | <0.001   |
| G2                       | 295 (39.5)                            | 214 (47.1)       | 17 (18.7)       | <0.001   |
| G3                       | 17 (2.3)                              | 12 (2.6)         | 0 (0)           |          |
| G6                       | 12 (1.6)                              | 6 (1.3)          | 2 (2.2)         |          |
| G1+G2                    | 7 (1.0)                               | 6 (1.3)          | 0 (0)           |          |
| G1+G3                    | 1 (0.1)                               | 1 (0.2)          | 0 (0)           |          |
| unidentified             | 8 (1.0)                               | 2 (0.8)          | 0 (0)           |          |
| HOMA-IR                  | 3.24±5.33                             | 3.02±6.56        | 5.08±8.47       | 0.043    |
| Hepatic steatosis, n (%) | 347 (46.5)                            | 218 (48)         | 40 (43.75)      | 0.373    |
| Liver cirrhosis, n (%)   | 176 (23.5)                            | 100 (22)         | 40 (44.1)       | 0.001    |
| ALT (U/L)                | 93.41±100.63                          | 104.7±96.0       | 85.6±80.0       | 0.116    |
| APRI                     | 1.66±2.11                             | 1.479±1.92       | 1.758±1.986     | 0.21     |
| Platelets count (10^3/μL) | 176.77±65.08                         | 182.2±58.5       | 155.6±57.2      | 0.001    |
| TC (mg/dL)               | 171.74±34.30                          | 168.05±32.08     | 176.29±27.00    | 0.735    |
| TGs (mg/dL)              | 105.34±55.44                          | 98.53±44.58      | 116.71±70.32    | 0.123    |
| Adiponectin (μg/mL)      | 9.56±7.09                             | 10.1±7.48        | 8.04±5.23       | 0.097    |
| HMW Adiponectin (μg/mL)  | 4.74±4.16                             | 6.01±4.04        | 4.85±2.56       | 0.641    |
| eGFR                     | 90.54±36.35                           | 82.75±34.89      | 84.39±35.82     | 0.983    |
| IFNL3-rs12979860         | 634 (84.9)                            | 392 (88.2)       | 61 (67)         | 0.003    |

Notes. *Chi-square test; SVR: sustained virological response; BMI: body mass index; G: genotype; Log: logarithmic; *: p < 0.05; G: genotype; HOMA-IR: homeostasis model assessment-estimated insulin resistance; ALT: alanine aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; TC: total cholesterol; TGs: triglycerides; HMW: high-molecular weight; eGFR:estimated glomerular filtration rate; IFNL3: interferon-α3.
SVR patients. Before anti-HCV therapy, male sex, triglyceride (TG) levels and hepatic steatosis were negatively associated with adiponectin levels (Table 2 and Fig. 1). TG levels and IFNL3 genotype were associated with HOMA-IR levels (Table 2 and Fig. 1). Subgroup analyses showed that the IFNL3 CC genotype [95% confidence interval (CI) of β: −13.53 to −5.24, estimated β: 9.38, p < 0.001] was the only factor associated with HOMA-IR levels among those with baseline IR (n = 321); whereas body mass index (BMI) (95% CI of β: 0.021 to 0.093, estimated β: 0.057, p = 0.002), and TG (95% CI of β: 0.001 to 0.006, estimated β: 0.004, p = 0.01) levels were associated with HOMA-IR levels among those without baseline IR (n = 426). HMW and total adiponectin levels were highly correlated (Pearson’s correlation coefficient: 0.903, p < 0.001).

Factors associated with the longitudinal trend of adiponectin levels

The factors affecting the longitudinal trend in adiponectin levels are listed in Table S1. Sex, hepatic steatosis, SVR, age, BMI, and HOMA-IR, platelet, total cholesterol (TC), TG and estimated glomerular filtration rate (eGFR) levels were associated with the longitudinal trends of adiponectin levels. The effects of categorical variables including sex, hepatic steatosis and SVR on adiponectin levels were further analyzed as shown in Fig. 2. Throughout therapy, (1) male patients had lower adiponectin levels than female patients (Fig. 2A); (2) patients with hepatic steatosis had lower adiponectin levels than patients without hepatic steatosis (Fig. 2B); (3) only SVR patients had a trend of decreased adiponectin levels, whereas the non-SVR patients showed fluctuating adiponectin levels (Fig. 2C).

Factors associated with adiponectin and HOMA-IR levels in SVR patients at 24 weeks post-therapy

Among the SVR patients at 24 weeks post-therapy, male sex, levels of BMI and HOMA-IR, and hepatic steatosis were negatively associated with adiponectin levels, whereas aspartate transaminase to platelet ratio index (APRI) levels were positively associated with adiponectin levels (Table 3 and Fig. 1). For post-therapy HOMA-IR levels, the IFNL3 genotype was the only independent factor. Subgroup analyses showed that the IFNL3 genotype was particularly important for post-therapy HOMA-IR levels among those with baseline IR (95% CI of B: −7.76 to −3.23, estimated B: −5.49, p < 0.001).

Table 2. Univariate and multivariate analyses of factors associated with pre-therapy HOMA-IR and adiponectin levels in all 744 enrolled chronic hepatitis C patients.

| Variants                     | HOMA-IR Univariate analysis: 95% CI of estimated β (p values) | Multivariate analysis: 95% CI of estimated β (p values) | Adiponectin (µg/mL) Univariate analysis: 95% CI of estimated β (p values) | Multivariate analysis: 95% CI of estimated β (p values) |
|------------------------------|---------------------------------------------------------------|--------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------|
| Sex (Male)                   | −0.366 to −1.168 (0.305)                                       | −6.1 to −2.53 (0.001)                                   | −5.39 to −1.8 (−3.65) (0.001)                                            | −5.39 to −1.8 (−3.65) (0.001)                             |
| Age                          | 0.001 to 0.063 (0.467)                                         | −0.61 to −0.106 (0.041)                                 | 32.8 to 194.7 (0.006)                                                   | 32.8 to 194.7 (0.006)                                     |
| BMI                          | 0.16 to 0.369 (0.001)                                          | −0.251 to −0.276 (0.097)                               | −0.71 to −0.23 (0.001)                                                  | −0.71 to −0.23 (0.001)                                   |
| HCV genotype                 | −0.75 to 1.07 (0.14)                                          | −0.69 to 0.91 (0.789)                                  | 0.467 to −0.037 (−0.215)                                               | 0.467 to −0.037 (−0.215)                                 |
| HCV RNA (Log_{10} IU/mL)     | 0.016 to −0.733 (0.061)                                       | −0.83 to 0.74 (0.801)                                  | −0.83 to 0.74 (0.801)                                                   | −0.83 to 0.74 (0.801)                                   |
| ALT (U/L)                    | −0.02 to 0.006 (0.352)                                         | 0.01 to 0.107 (0.569)                                  | −0.39 to −0.34 (0.264)                                                  | −0.39 to −0.34 (0.264)                                   |
| APRI                         | −0.69 to 0.313 (0.211)                                         | 10.1 to 18.1 (0.138)                                  | −0.30 to −0.05 (0.01)                                                   | −0.30 to −0.05 (0.01)                                   |
| Platelets count (10^3/µL)    | −0.01 to 0.011 (0.092)                                         | 0.00 to 0.041 (0.02)                                  | 0.007 to 0.052 (0.138)                                                 | 0.007 to 0.052 (0.138)                                   |
| Tg (mg/dL)                   | 0.008 to 0.021 (0.001)                                         | 0.001 to 0.042 (0.036)                                 | −0.056 to −0.19 (0.001)                                                | −0.056 to −0.19 (0.001)                                 |
| HOMA-IR                      | NA                                                            | −0.33 to −0.018 (0.029)                                 | −0.279 to 0.02 (0.093)                                                  | −0.279 to 0.02 (0.093)                                   |
| Adiponectin (µg/mL)          | −0.424 to −0.015 (0.029)                                       | −0.234 to −0.051 (−0.09)                               | NA                                                                       | NA                                                       |
| Hepatic steatosis            | 0.298 to 1.702 (0.005)                                         | 1.66 to 3.25 (1.064)                                   | −0.53 to 1.63 (0.001)                                                  | −0.53 to 1.63 (0.001)                                   |
| Liver cirrhosis              | 0.219 to 1.889 (0.013)                                         | −0.11 to 6.54 (2.64)                                   | −4.31 to −0.151 (−0.21)                                               | −4.31 to −0.151 (−0.21)                                 |
| eGFR                         | −0.012 to 0.006 (0.495)                                        | −1.9 to 2.24 (0.909)                                  | −0.025 to −0.034 (0.758)                                               | −0.025 to −0.034 (0.758)                                 |
| IFNL3-rs12979860 (CC)        | −3.28 to 6.609 (0.004)                                         | −4.82 to −1.06 (−2.94)                                 | −2.47 to 1.42 (0.723)                                                  | −2.47 to 1.42 (0.723)                                   |

Notes: CI: confidence interval; OR: odds ratio; *: p < 0.05; NA, not accessible; HCV: hepatitis C virus; SVR: sustained virological response; BMI: body mass index; Log: logarithmic; **: p < 0.05; HOMA-IR: homeostasis model assessment-estimated insulin resistance; Alt: alanine aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; HsCRP: high sensitivity C-reactive protein; WBC: white blood cells; C3: complement component 3; C6: complement component 4; TC: total cholesterol; Tg: triglyceride; NA: not accessible; eGFR: estimated glomerular filtration rate; IFNL3: interferon-β3.
Changes in adiponectin and HOMA-IR levels in SVR patients at 24 weeks post-therapy

Compared with pre-therapy levels, paired t-tests demonstrated that APRI (1.479+/−1.92 vs. 0.418+/−0.297, p < 0.001) and adiponectin (10.1+/−7.48 vs. 8.13+/−5.92 μg/mL, p < 0.001) levels decreased in SVR patients at 24 weeks after therapy, regardless of the HCV genotype (APRI: G1, p < 0.001, G2, p < 0.001; adiponectin: G1, p = 0.001, G2, p < 0.001). None of the aforementioned variables changed significantly in non-SVR patients (Table S2). Interestingly, when we stratified the SVR patients by baseline IR, although adiponectin decreased after SVR regardless of baseline IR (Fig. 2D), HOMA-IR levels decreased in patients with baseline IR but increased in patients without baseline IR (Fig. 2E).

To elucidate the factors that independently affect SVR, the impacts of sex, age, HCV and IFNL3 genotype, BMI, HCV viral load, levels of HOMA-IR, APRI, TGs, TC, adiponectin and eGFR, liver cirrhosis and fatty liver in SVR were surveyed by multivariate analyses. Among the surveyed factors, only BMI [95% CI of odds ratio (OR): 0.763−0.997], HCV (95% CI of OR: 2.72−5.31) and IFNL3 (95% CI of OR: 1.29−7.19) genotypes as well as liver cirrhosis (95% CI of OR: 0.061−0.742) independently affected SVR.

Figure 1. The cross-sectional adiponectin and homeostasis model assessment-estimated insulin resistance (HOMA-IR)-centered associations between dependent and independent factors before (pre-therapy) and 24 weeks after anti-hepatitis C virus (anti-HCV) therapy (post-therapy). Tips of black arrowheads: dependent factors; bases of black arrowheads: independent factors; FL: fatty liver, i.e., hepatic steatosis; TGs: triglycerides; IR: insulin resistance; IFNL3: interferon, λ3; BMI: body mass index; APRI: aspartate aminotransferase to platelet ratio index; pre-therapy: levels of variables before anti-HCV therapy; SVR: sustained virological response. Red arrows indicate post-therapeutic increases in HOMA-IR (baseline IR = 0) levels, while blue arrows indicate post-therapeutic decreases in HOMA-IR (baseline IR = 1) and adiponectin levels.

Figure 2. The longitudinal trends of adiponectin levels (μg/mL) and homeostasis model assessment-estimated insulin resistance (HOMA-IR). The trends were stratified by sex (A), steatosis (B) and SVR (C). Blood drawing time points: 1, 2 weeks before therapy; 2, after 4 weeks of therapy; 3, after 12 weeks of therapy; 4, after 24 weeks of therapy; 5, after 36 weeks of therapy; 6, after 48 weeks of therapy; 7, after 60 weeks of therapy; and 8, after 72 weeks of therapy. 1: yes (or male for A); 0: no. (or female for A) D-E, Alterations of levels of adiponectin (μg/mL) (D) and HOMA-IR (E) in SVR patients. Red lines: SVR patients with baseline IR; black lines: SVR patients without baseline IR. Pre-therapy: levels of variables before anti-hepatitis C virus therapy; post-therapy: levels of variables at 24 weeks post-therapy.
CHC patients exhibited higher pre-therapy hepatic adiponectin levels than controls

Before anti-HCV therapy, the CHC patients displayed higher hepatic adiponectin (Fig. 3A) levels than the controls (Fig. 3B) (29.88+/−13.21% vs. 11.02+/−5.64%, p = 0.011). In the controls, most adiponectin-positive cells were endothelial cells. By contrast, in CHC patients, both endothelial cells and some hepatocytes were

![Image A](image1.png)
![Image B](image2.png)
![Image C](image3.png)
![Image D](image4.png)

**Figure 3.** Immunohistochemical studies of adiponectin (A and B, 200X) and adiponectin receptor II (C and D, 200X) in representative liver's sections from chronic hepatitis C patients before anti-hepatitis C virus therapy (A and C) and in controls (B and D). Arrows: adiponectin-positive biliary and endothelial cells; arrow heads: adiponectin-positive hepatocytes.

Table 3. Univariate and multivariate analyses of factors associated with post-therapy HOMA-IR and adiponectin levels in the 455 chronic hepatitis C patients with SVR.

| Variants                  | HOMA-IR: Univariate analysis: 95% CI of estimated β (p values) | HOMA-IR: Multivariate analysis: 95% CI of estimated β (estimated β)(p values) | Adiponectin (µg/mL): Univariate analysis: 95% CI of estimated β (p values) | Adiponectin (µg/mL): Multivariate analysis: 95% CI of estimated β (estimated β)(p values) |
|---------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Sex (Male)                | −0.79−0.403 (0.523)                                              | −6.3−3.2 (0.001)                                                            | −5.66−2.6 (−4.1|< 0.001)                                                                |
| Age                       | 0.0−0.050 (0.053)                                                | −0.052−0.092 (0.584)                                                        | −0.54−0.096 (−0.28|0.011)                                                                |
| BMI                       | 0.127−0.302 (< 0.011)                                            | −0.79−0.34 (< 0.001)                                                        | −0.10−0.005 (0.077)                                                        |
| ALT (U/L)                 | 0.0−0.042 (0.048)                                                | −0.034−0.033 [−0.001|0.096)                                                | 1.73−5.35 (< 0.011)                                                        |
| APRI                      | −6.0−20.1 (0.045)                                                | 1.00−0.005 (0.195)                                                          | 1.40−4.76 [2.6|< 0.001)                                                                |
| Platelets count (10^12/µL)| −0.09−0.002 (0.263)                                              | −0.024−0.005 (0.195)                                                        | −0.037−0.008 (0.215)                                                        |
| TC (mg/dL)                | −0.007−0.01 (0.679)                                              | −0.003−0.012 (< 0.001)                                                      | −0.028−0.006 (0.003)                                                        |
| TGs (mg/dL)               | 0.003−0.012 (< 0.001)                                            | −0.07−0.1 (0.002|0.666)                                                | −0.61−0.11 (0.005)                                                          |
| HOMA-IR                   | NA                                                               | −0.149−0.033 (0.002)                                                         | −0.61−0.11 (0.005)                                                          |
| Adiponectin (µg/mL)       | −0.017−0.019 [−0.049|0.158)                                      | −0.149−0.033 (0.002)                                                         | −0.47−0.017 [−0.024|0.041)                                                   |
| Hepatic steatosis         | 0.411−1.648 (0.011)                                              | −0.475−0.539[0.532|0.299)                                                  | −5.18−2.0 (< 0.001)                                                         |
| Liver cirrhosis           | −0.227−1.277 (0.171)                                             | −3.0−0.81 [−1.92|0.001)                                                    | −3.7−0.66 [−2.43|0.002)                                                      |
| eGFR                      | −0.018−0.033 (0.188)                                             | −2.6−0.63 (0.001)                                                            | −2.16−2.07 (0.966)                                                          |
| IFNL3-rs12979860 (CC)     | NA                                                               | −2.6−0.63 (0.001)                                                            | −2.16−2.07 (0.966)                                                          |

Notes: CI: confidence interval; OR: odds ratio.
*: p < 0.05; NA, not accessible; HCV: hepatitis C virus; SVR: sustained virological response; BMI: body mass index; Log: logarithmic; “: p < 0.05; HOMA-IR: homeostasis model assessment-estimated insulin resistance; NA: not accessible; ALT: alanine aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; TC: total cholesterol; TGs: triglycerides; eGFR:estimated glomerular filtration rate; IFNL3: interferon-λ3.
adiponectin-positive. Adiponectin-positive hepatocytes were surrounded by hepatic inflammation and fibrosis foci. No difference in hepatic AdipoR2 expression (Fig. 3C and D) was noted between the CHC patients and controls (39.28%/+/−8.36 vs. 42.65%/+/−10.12%, p = 0.898). Almost all AdipoR2-positive cells were hepatocytes, regardless of HCV infection. No visible hepatic expression of AdipoR1 could be demonstrated regardless of HCV infection (data not shown).

Discussion

To the best of our knowledge, this prospective study is the first to comprehensively analyze the evolving relationship between adiponectin levels and insulin sensitivity in CHC patients during viral clearance. The most compelling results are as follows: (1) Before anti-HCV therapy, male sex, TG levels and hepatic steatosis were negatively associated with adiponectin levels. The IFNL3 genotype and TG levels were associated with HOMA-IR levels. The subgroup analyses showed that the IFNL3 CC genotype was associated with HOMA-IR levels particularly in CHC patients with baseline IR; BMI and TG levels were associated with HOMA-IR, particularly in those without baseline IR. (2) Sex, hepatic steatosis and SVR were independent factors for the longitudinal trend in adiponectin levels. (3) In SVR patients at 24 weeks post-therapy, sex, levels of BMI, APRI, HOMA-IR, and hepatic steatosis were associated with adiponectin levels, whereas the IFN-L3 genotype was associated with HOMA-IR levels particularly in CHC patients with baseline IR; BMI and TG levels were associated with HOMA-IR, particularly in those without baseline IR. Neither pre-therapy HOMA-IR nor adiponectin levels independently affected SVR. (4) Compared with pre-therapy levels, adiponectin and APRI levels decreased in SVR patients 24 weeks after therapy, regardless of viral genotype. The subgroup analyses showed that HOMA-IR levels decreased in SVR patients with baseline IR but increased in those without baseline IR. (5) Compared with controls, CHC patients exhibited significantly higher pre-therapy hepatic adiponectin expression levels, which were associated with hepatic fibrosis.

Hypoadiponectinemia was reported to be a positive predictor for SVR in G4 HCV infection, but whether pre-therapy HOMA-IR levels determine SVR remains controversial. In the current study, neither HOMA-IR nor adiponectin levels independently affected the therapeutic response in patients mainly infected with G1 or G2 HCV. Differences in HCV genotypes and baseline glucose metabolism among the patients in these studies might account for these discrepancies. By contrast, all factors associated with pre-therapy adiponectin levels in the current study have been consistently reported, regardless of HCV infection. Moreover, the negative effects of male sex and hepatic steatosis on adiponectin levels were constant and existed during pre-, peri- and post-therapy. These effects seem to be independent of HCV infection. In addition, before anti-HCV therapy, adiponectin levels were not associated with HCV RNA levels, consistent with the results of most studies. Combined, these results indicate that the impact of HCV infection on adiponectin levels, if any, does not occur directly through viral RNA modulation but through alterations subsequent to HCV infection, probably in metabolic or hepatic aspects. The high correlation noted between HMW adiponectin and adiponectin (total) confirmed the representativeness of adiponectin in surveying insulin sensitivity. Although adiponectin is regarded as an insulin-sensitizing adipokine that metabolically mimics insulin, the connection between pre-therapy adiponectin and HOMA-IR levels was not a direct association but rather was mediated through TGs (Fig. 1). Consistently, a previous study of untreated CHC patients showed that HCV-associated IR is likely an adipokine-independent effect. Furthermore, the association between HCV clearance and IR improvement was considered independent of adiponectin levels in CHC patients. Adiponectin regulates the hepatic secretion of very low density lipoprotein, which accounts for 85% of TGs and is secreted from the liver to carry TGs into the bloodstream. Thus, TGs seem to be a feasible linker between adiponectin and insulin sensitivity in the CHC patients.

Of note, SVR patients showed a gradual decrease in adiponectin throughout therapy, with a significant decrease 24 weeks post-therapy compared with pre-therapy levels, regardless of viral genotype. Whether viral clearance leads to increased or decreased adiponectin levels in CHC patients remains unclear and may differ among various HCV genotypes. This lack of clarity may arise from the heterogeneous hepatic pathologies and metabolic conditions of these patients, as fibrosis and steatosis are associated with hyperadiponectinemia and hypoadiponectinemia, respectively. Thus, after SVR, the decrease in adiponectin in G4 CHC patients might reflect the reversal of hepatic fibrosis, whereas the increase in adiponectin in G3 CHC patients might indicate an improvement in hepatic steatosis, which is most evident in G3 CHC and regarded as “viral steatosis.” By contrast, steatosis appears to be secondary to IR and is regarded as “metabolic steatosis” in G1, G2 or G4 CHC, which may explain why patients in the current study (mainly G1 and G2 patients) had adiponectin alteration patterns similar to those noted in G4 CHC. In addition, adiponectin regulates immune responses in HCV infection. The decreased adiponectin levels after SVR might indicate that eliciting an immune response is unnecessary to expel HCV. After HCV clearance, namely, without viral interference, the levels of adiponectin were
directly and negatively associated with levels of HOMA-IR as reported.\textsuperscript{5} Compared with pre-therapy levels, sex, hepatic steatosis and levels of HOMA-IR remained unchanged (Table S2). After being counterbalanced by BMI (Table 3, $\beta = -0.28$), the decreased adiponectin levels seemed to follow decreased levels of APRI (Table 3, $\beta = 2.6$). Concordantly, IHC studies showed higher hepatic adiponectin expression in CHC patients than in controls, and most adiponectin-positive hepatocytes were surrounded by hepatic fibrosis with inflammatory cell infiltration. The major driving force for decreasing adiponectin after SVR was thus directed to, at least partly, attenuated hepatic fibrosis in many aspects. However, the current study did not support the connection between adiponectin alterations and adiponectin resistance,\textsuperscript{19} as IHC studies failed to demonstrated a significant difference in hepatic adiponectin receptor expression between CHC patients and the controls. In addition, after SVR, the HCV-associated hypolipidemia was reversed (Table S2).\textsuperscript{2} As mentioned previously, hypolipidemia is positively associated with adiponectin levels.\textsuperscript{12} Consistent with the link between TGs and adiponectin levels during HCV infection, SVR-associated hyperlipidemia may also contribute to the decreased adiponectin levels after viral clearance, probably through negative transcriptional regulation. Moreover, because of the pleiotropic functions of adiponectin mentioned above,\textsuperscript{5-10, 12-15} the alteration pattern of adiponectin might serve as a feasible reference to monitor co-morbidities in CHC patients after SVR.\textsuperscript{14-15, 28-30}

A trend toward an inverse correlation between the change in adiponectin and in IR, although not statistically significant, has been noted in CHC patients.\textsuperscript{35} Thus, the opposing trends of HOMA-IR levels between those with and without baseline IR after SVR were particularly notable. Because adiponectin enhances insulin sensitivity and counteracts IR in animal studies,\textsuperscript{10, 38} the decreased adiponectin after SVR should cause the increase in HOMA-IR levels as observed in patients without baseline IR. By contrast, a comprehensive review failed to demonstrate modulation of adiponectin in human insulin sensitivity.\textsuperscript{39} In CHC patients with baseline IR, the concurrent decreases in adiponectin and HOMA-IR levels after viral clearance suggest a paradoxical homeostasis in glucose metabolism. Adiponectin is stably present in plasma with little evidence of being an acutely regulated protein. The major physiologic role of adiponectin is to adapt to long-term metabolic dysregulation,\textsuperscript{38-39} which might explain why adiponectin increased after SVR, when metabolism was altered, regardless of baseline IR. However, the baseline IR may be a consequence of HCV infection, which impairs host glucose metabolism.\textsuperscript{2} These effects likely explain why viral clearance led to decreased HOMA-IR levels (i.e., improved IR) in CHC patients with baseline IR, even when adiponectin levels decreased. More specifically, pre-therapy anthropometric (i.e., BMI) and metabolic factors (i.e., TGs) were associated with pre-therapy HOMA-IR levels among those without baseline IR. By contrast, among those with baseline IR, the IFNL3 genotype, a genetic factor associated with anti-HCV therapeutic responses,\textsuperscript{2, 40-42} was more important than anthropometric and metabolic factors. This finding indicates that the physiologic regulation between adiponectin and insulin sensitivity was primarily preserved in patients without baseline IR as determined by the associations among the anthropometric and metabolic factors, which are crucial for homeostasis. Whether IR is associated with the IFNL3 genotype on HOMA-IR levels would not be evident unless analyzed in a large-scale study including many patients with baseline IR or even diabetes.

Because adipose tissue is the major source of the main adipocytokines,\textsuperscript{5-8} one of the major limitations of this study is the lack of pathological study of adipose tissue, which is the origin of adiponectin.\textsuperscript{5-8} Second, the precise role of hepatic fibrosis in altered adiponectin levels may not be revealed without comparing quantitative measurements of hepatic fibrosis before and after anti-HCV therapy. Third, the potential role of transcriptional regulation of adiponectin by the altered metabolism after HCV clearance, particular hyperlipidemia,\textsuperscript{2, 12} could not be evaluated in the current study. Fourth, although our previous study precluded a pro-diabetic function of resistin in CHC,\textsuperscript{1} adipokines other than adiponectin or resistin may play roles in the paradoxical glucose metabolism of CHC patients with baseline IR. For example, the HCV core protein increases reactive oxygen species, which activate nuclear factor kappa-light-chain-enhancer of activated B cells,\textsuperscript{43} subsequently increasing cytokines including tumor necrosis factor $\alpha$ (TNF$\alpha$). TNF$\alpha$ modulates adipocytes and induces a decrease in the production of adiponectin and its receptor$^{44}$ but is not investigated in the current study. Future studies of adiponectins in CHC patients with adipose tissue pathology surveys, quantitative scoring of hepatic fibrosis, in vitro studies of the transcriptional regulation of adiponectin levels and comprehensive assessment of adipokine profiles may be required to confirm our findings and elucidate the associated molecular basis.

Taken together, sex and hepatic steatosis consistently affected adiponectin levels, regardless of HCV infection. During HCV infection, adiponectin might indirectly affect insulin sensitivity through TGs. After SVR, adiponectin levels were directly associated with insulin sensitivity and decreased, likely subsequent to attenuated hepatic fibrosis. However, the HOMA-IR levels increased in patients without baseline IR but decreased in patients
with baseline IR, which was associated with the IFNL3 genotype. This alteration pattern of adiponectin levels may serve as a feasible reference to monitor associated co-morbidities in CHC patients after SVR. Moreover, the observation of the paradoxical alterations of adiponectin and insulin sensitivity in SVR patients with baseline IR might provide a basis for the investigation of therapeutic targets for HCV-associated cardiometabolic complications, especially irreversible ones.

**Patients and methods/materials**

**Patients**

The study group comprised subjects 18 y or older with CHC, defined as the presence of documented HCV antibodies and detectable HCV RNA for >24 weeks. Subjects with human immunodeficiency virus, hepatitis B infection, hemochromatosis, primary biliary cholangitis, primary sclerosing cholangitis, autoimmune hepatitis or malignancy and recipients of solid organ transplants were excluded.

**Methods/materials**

A total of 747 patients with CHC were recruited consecutively at a tertiary referral center, Chang Gung Memorial Hospital, Taoyuan, Taiwan, between July 2010 and October 2015. Of these patients, 546 received anti-HCV therapy with weight-based pegylated interferon-α-2b and ribavirin for either 24 or 48 weeks.1, 35-37 HCV RNA levels, genotypes, and single-nucleotide polymorphisms (SNPs) of IFNL3 were assessed as described previously.1, 35-37 For all included 747 patients, several baseline factors were evaluated, including sex, age, body mass index (BMI), HCV RNA and genotype, presence of hepatic steatosis and cirrhosis, eGFR, APRI, TC, TGs, HOMA-IR [fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5], alanine aminotransferase (ALT), adiponectin (i.e., total adiponectin) and HMW adiponectin (R&D Systems, MN, USA) levels. For the 546 patients who completed anti-HCV therapy, the aforementioned factors were evaluated 2 weeks before therapy; after 4, 12 and 24 weeks of therapy; at the end of therapy; and 12 and 24 weeks after the end of therapy. Abdominal ultrasound studies were performed to assess the presence of hepatic steatosis and cirrhosis. IR was defined as an HOMA-IR score ≥ 2.5. An SVR was defined as undetectable levels of HCV RNA 24 weeks after the completion of therapy.

Liver biopsy was performed in CHC patients before anti-HCV therapy (n = 20). Control liver samples were acquired from the livers of sex- and age-matched participants taken from the tissue bank of the hospital (n = 20). IHC studies of adiponectin (Novus Biologicals), AdipoR1 (Enzo Life Sciences, NY, USA) and AdipoR2 (Phoenix Pharmaceuticals, CA, USA) were performed using paraffinized liver samples according to the manufacturers’ protocols. Protein expression intensity was determined as described previously.1

**Statistics**

All statistical analyses were performed using Statistical Package for Social Science (SPSS package version 21, SPSS Inc., Chicago, IL, USA) software. Univariate and multivariate linear regression models were used to assess relationships between various dependent and independent variables. Generalized estimating equation (GEE) repeated measures tests were applied to determine the relationships between the dependent and independent variable levels longitudinally. Paired t-tests were used to compare variables before and at 24 weeks after anti-HCV therapy within individuals. Statistical significance was defined at the 5% level based on 2-tailed tests of the null hypothesis.

**Informed consent**

Written informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local institutional review board.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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Notes on contributors

MLC designed and completed the study, drafted the article and critically revised it for intellectual content. CJK collected and analyzed the data and wrote the manuscript. LHP interpreted the data and wrote the manuscript. CMH and CTC collected and analyzed the data and wrote the manuscript. All authors approved the final version of the article, including the authorship list.

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