Adiponectin Upregulates SHBG Production: Molecular Mechanisms and Potential Implications

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Epidemiological studies have shown that plasma SHBG levels correlate with plasma adiponectin levels, both in men and women. There are no reports describing any molecular mechanism by which adiponectin regulates hepatic SHBG production. The aim of the present study is to explore whether adiponectin regulates SHBG production by increasing HNF-4α levels through reducing hepatic lipid content. For this purpose, in vitro studies using human HepG2 cells, as well as human liver biopsies, were performed. Our results show that adiponectin treatment increased SHBG production via AMPK activation in HepG2 cells. Adiponectin treatment decreased the mRNA and protein levels of enzymes related to hepatic lipogenesis (ACC) and increased those related to fatty acid oxidation (ACOX and CPTI). These adiponectin-induced changes in hepatic enzymes resulted in a reduction of total TG and FFA and an increase of HNF-4α. When HNF-4α expression was silenced by using siRNA, adiponectin-induced SHBG overexpression was blocked. Furthermore, adiponectin-induced upregulation of SHBG production via HNF-4α overexpression was abrogated by the inhibition of fatty acid oxidation or by the induction of lipogenesis with a 30mM glucose treatment in HepG2 cells. Finally, adiponectin levels correlated positively and significantly with both HNF-4α and SHBG mRNA levels in human liver biopsies. Our results suggest for the first time that adiponectin increases SHBG production by activating AMPK, which reduces hepatic lipid content and increases HNF-4α levels. (Endocrinology 155: 2820–2830, 2014)

Sex hormone-binding globulin (SHBG) modulates the bioavailability of sex steroids in blood and hormone-target tissues (1). Genetic, hormonal and lifestyle-related factors determine SHBG levels. Low SHBG levels are a risk factor for developing metabolic syndrome, type 2 diabetes, and cardiovascular diseases (2–6).

Body mass index (BMI) is considered a major determinant of SHBG plasma concentrations (7, 8). Obese individuals of all ages have low plasma SHBG levels (7, 8) whereas high serum SHBG levels are present in children (9) and women (10) with anorexia nervosa. Recent evidence suggests that elevated plasma levels of proinflammatory cytokines rather than insulin account for the low plasma SHBG levels in obese subjects (11, 12). In this regard, we have shown clear evidence that elevated plasma levels of tumor necrosis factor alpha (TNFα) or interleukin 1β (IL1β) are factors downregulating SHBG production (11–13).

Orphan receptor hepatocyte nuclear factor 4 α (HNF-4α) is a highly conserved member of the nuclear receptor superfamily of ligand-dependent transcription factors. It is known as a master regulator of liver-specific gene expression (14). It has been reported that HNF-4α has a ligand-binding pocket that binds fatty acyl Coenzyme A thioesters and linoleic (15, 16). Previous studies have described HNF-4α as the major transcription factor regu-
lating hepatic SHBG gene expression (17). In addition, we have previously reported that SHBG gene expression is downregulated by an increase in de novo lipogenesis induced by high carbohydrate diets that reduce HNF-4α levels (18). On the other hand, thyroid hormones increase SHBG gene expression by increasing hepatic HNF-4α levels and this effect is abrogated by inhibiting β-oxidation (19). Therefore, depending on the metabolic status of the liver SHBG production can be modulated by inducing or inhibiting lipogenesis or β-oxidation. This means that activation of lipogenesis or inhibition of β-oxidation will decrease hepatic SHBG production and, by contrast, inhibition of lipogenesis or activation of β-oxidation will increase SHBG production.

As occurs with SHBG, it is well established that adiponectin plasma levels are negatively correlated with BMI and low plasma adiponectin levels are found in obese patients (20, 21). Adiponectin is a 244-amino acid protein secreted predominantly by white adipose tissue (22). Two different adiponectin receptors have been described in the literature: AdipoR1 that is expressed ubiquitously but abundantly in skeletal muscle and endothelial cells; and AdipoR2 that is predominantly expressed in the liver (23). Adiponectin receptors have been shown to mediate direct effects on liver and muscle insulin sensitivity (24). Most of the adiponectin actions in the liver have been attributed to the activation of adenosine monophosphate-activated protein kinase (AMPK) (25). The AMPK serves as an essential cellular monitor of energy charge; an elevated AMP/ATP ratio activates the kinase and results in a decrease in anabolic and an increase in catabolic pathways (26). Once activated, AMPK phosphorylates target proteins such as acetyl-CoA carboxylase (ACC) and CREB-regulated transcription coactivator in order to restore the energy status through different mechanisms including increased fatty acid oxidation and decreased hepatic lipogenesis (25–28).

Several epidemiological studies have reported a positive relationship between adiponectin and SHBG plasma levels (29, 30), and both have shown a negative correlation with BMI (7, 8, 20, 21). The downregulation of adiponectin levels that occurs in obese patients seems to participate in the development of fatty liver disease that is associated with low SHBG levels. On this basis we wanted to test the hypothesis that adiponectin could play a role in regulating hepatic SHBG production. To shed light on this issue, we have explored whether adiponectin regulates SHBG production by increasing HNF-4α levels through reducing hepatic lipid content.

Materials and Methods

Cell culture experiments

Cell culture reagents were from Life Technologies Inc (Invitrogen SA), HepG2 hepatoblastoma cells (catalog no. HB-8065; ATCC) were maintained in DMEM (5 mmol/L glucose) supplemented with 10% FBS and antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml). For experiments, HepG2 cells were cultured to 50–70% confluence prior to the addition of vehicle (PBS or DMSO), adiponectin (Servicios Hospitalarios), compound C (Merck Millipore), glucose (10, 20 or 30 mM), etomoxir (Sigma-Aldrich SL), or AICAR (Sigma-Aldrich SL). All experiments were performed at least twice in triplicate.

The siRNA experiments were carried out using HiPerfect Transfection Reagent together with either a control siRNA (catalog 1022076) or a HNF-4α siRNA (catalog 00161546) obtained from QIAGEN (IZASA). Three days after transfection media was collected for SHBG measurements and cells were harvested for RNA and protein analysis.

Transient transfections of human SHBG promoter-driven luciferase reporter plasmids together with a pCMRenilla control plasmid were performed using Lipofectamine 2000 (Invitrogen SA) in HepG2 cells treated previously for 3 days with vehicle (PBS) or adiponectin (50 ng/ml). Two days after transfection, luciferase and renilla activity were measured using the Dual-Luciferase Reporter Assay System (Promega).

SHBG measurements

Human SHBG levels from human samples and media from HepG2 cells were measured using an ELISA (Demeditec Diagnostics GmbH).

RNA analysis

Total RNA was extracted from HepG2 cells and human liver biopsies using TRIzol reagent (Invitrogen SA). Reverse transcription was performed at 42°C for 50 minutes, using 3 μg of total RNA and 200 U of Superscript II together with an oligo-dT primer and reagents provided by Invitrogen. An aliquot of the reverse transcription product was amplified in a 25-μl reaction real time PCR using SYBRGreen (Invitrogen SA) with appropriate oligonucleotide primer pairs corresponding to human HNF-4α, SHBG, ACC, ACOX1a, ACOX1b, CPT-1, and 18S (Supplemental Table 1). Results were analyzed using the 7000 SDS program.

Western blot analysis

After treatments, HepG2 cells were homogenized in RIPA buffer with Complete™ protease inhibitor cocktail (Roche Diagnostics). Protein extracts were used for Western blotting with antibodies against human HNF-4α (C-19; sc-6556), CPT-1 (A-14; sc-31128), and ACOX (H-140; sc-98499) (Santa Cruz Biotechnology Inc), ACC (ab45174) (Abcam) and P-ACC (#3661) (Cell Signaling Technology Inc), and human PPIA (BIOMOL Int). Specific antibody-antigen complexes were identified using an HRP-labeled goat antirabbit IgG, rabbit antimouse IgG, or rabbit antigoat IgG and chemiluminescent substrates (Pierce BioTechnology Inc) by exposure to x-ray film.

Lipid measurements

Total triglycerides and free fatty acid content from HepG2 cells were measured using a triglyceride assay kit (Cat.#K622-100 BioVision) and a free fatty acid kit (Cat.#K612-100 BioVision) following the manufacturer’s instructions.
Human Samples
We recruited 15 obese male subjects [BMI median 42.27 kg/m² (range 32.61–52.31 kg/m²)] of Caucasian origin who underwent bariatric surgery at the University Hospital Vall d’Hebron (Barcelona, Spain). The exclusion criteria were: 1) Elevated transaminases. We defined normal transaminases as values within the 95th percentile of healthy subjects (AST: 12–40 UI/l and ALT: 8–44 UI/l); 2) Hepatitis C virus infection. For this purpose all patients with positive anti-hepatitis C virus antibodies were excluded; and 3) Presence of diabetes. This was defined on the basis of a history of therapy with oral hypoglycemic agents, GLP1 analogues or insulin at the time of inclusion. In all patients not previously diagnosed, the criteria recommended by the Expert Committee on the Diagnosis and Classification of Diabetes were used (31).

Blood samples were collected before surgery from the antecubital vein, plasma was separated immediately by centrifugation, and aliquots were frozen at −80°C for subsequent analysis. Liver biopsies were obtained using a fine needle. All biopsies were at least 2 cm in length and contained at least eight portal tracts. Samples were frozen at −80°C for subsequent analysis.

Informed written consent was obtained from all participants, and the study was approved by the human ethics committee from the Hospital Vall d’Hebron.

Statistical analyses
Normal distribution of the variables was evaluated using the Kolmogorov-Smirnov test. Comparison of quantititative variables was performed by either the Student’s t test or Mann-Whitney test according to the data distribution. All data are presented as means ± standard deviation. Spearman’s correlation coefficients were used to establish the association between SHBG levels and the other parameters. For graphics a linear regression test was applied. In addition, a multiple regression analysis was performed (dependent variable: SHBG; independent variables: adiponectin and BMI). Significance was accepted at the level of $P < .05$.

Statistical analyses were performed with the SPSS statistical package (SPSS Inc).

Results
Adiponectin increases SHBG production through AMPK activation in HepG2 cells
We first examined the effects of daily supplementation of two doses

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**Figure 1.** Daily treatment with adiponectin increases SHBG production via AMPK over 3 days in HepG2 cells. A, SHBG accumulation in the medium was measured using an ELISA in HepG2 cells treated daily with vehicle or 2 concentrations of adiponectin (25 and 50 ng/ml) for 3 days. Data points are mean ± SD of triplicate measurements. ***, $P < .01$ when compared with the control. B, Analysis of SHBG mRNA levels in HepG2 cells treated as in (A). Human 18S (18S) mRNA was amplified as a control. Data points are shown as mean ± SD of triplicates. **, $P < .01$ when compared with the control. C, Adiponectin (50 ng/ml) treated HepG2 cells over 3 days showed increased SHBG promoter activity in luciferase reporter gene assays when compared with vehicle treated cells. Data points are mean ± SD of triplicate measurements. *, $P < .05$ when compared with the control. D, SHBG accumulation in the medium was measured using an ELISA in HepG2 cells treated daily with vehicle or AICAR (100 µM) for 3 days. Data points are mean ± SD of triplicate measurements. **, $P < .01$ when compared with the control. E, Analysis of SHBG mRNA levels in HepG2 cells treated as in (D). Human 18S (18S) mRNA was amplified as a control. Data points are shown as mean ± SD of triplicates. **, $P < .01$ when compared with the control. F, SHBG accumulation in the medium was measured using an ELISA in HepG2 cells treated daily with vehicle, adiponectin (50 ng/ml) alone or in the presence of compound C (1 µM or 5 µM) over the course of 3 days. Data points are mean ± SD of triplicate measurements. ***, $P < .01$ when compared with the control. G, Analysis of SHBG mRNA levels in HepG2 cells treated as in (F). Human 18S (18S) mRNA was amplified as a control. Data points are shown as mean ± SD of triplicates. **, $P < .01$ when compared with the control.
of adiponectin (25 or 50 ng/ml) on SHBG production by HepG2 cells over the course of 3 days by comparing medium SHBG concentrations on days 1 and 3 of HepG2 cells treated with vehicle and 25 or 50 ng/ml of adiponectin. Adiponectin treatment increased SHBG protein levels (Figure 1A) and SHBG mRNA levels (Figure 1B) when compared with the vehicle treated HepG2 cells.

To determine if adiponectin treatment was increasing the transcriptional activity of the SHBG gene we next performed luciferase reporter gene assays using the proximal SHBG promoter in HepG2 cells in the presence or absence of adiponectin (50 ng/ml). The results showed that adiponectin treatment increased SHBG transcriptional activity when compared with control treated HepG2 cells (Figure 1C).

In order to explore whether adiponectin-induced increase of SHBG production was mediated by AMPK activation, we next treated HepG2 cells with AICAR (100 μM), a well-known AMPK activator (32). We also treated HepG2 cells with compound C (1 and 5 μM), an AMPK inhibitor in the presence or absence of adiponectin (50 ng/ml), over the course of 3 days. The results showed that AICAR treatment increased SHBG protein levels (Figure 1D) and SHBG mRNA levels (Figure 1E) when compared with the vehicle treated HepG2 cells. The compound C cotreatment was able to block the adiponectin-induced increase of SHBG protein levels (Figure 1F) and SHBG mRNA levels (Figure 1G).

Adiponectin treatment reduces hepatic triglyceride and free fatty acid by inhibiting lipogenesis and inducing fatty acid oxidation that results in an increase of HNF-4α in HepG2 cells

The hepatic effects of adiponectin in the liver are well described in the literature and it is well known that adiponectin reduces lipogenesis and increases fatty acid oxidation in the liver (24, 33). To explore if this was the case in our HepG2 cell cultures,

Figure 2. Adiponectin reduces lipogenesis and increases fatty acid oxidation in HepG2 cells. A, Analysis of ACC, ACOX1, ACOX2, and CPT-I mRNA levels in HepG2 cells treated daily with vehicle and two concentrations of adiponectin (25 and 50 ng/ml) for 3 days. Human 18S (h18S) mRNA was amplified as a control. Data points are shown as mean ± SD of triplicates. **, \( P < .01 \) when compared with the control. B, Western blot of ACC, phospho-ACC, and PPIA in total protein extracts from HepG2 cells treated as in (A). Data points are shown as mean ± SD of triplicates. *, \( P < .05 \) and **, \( P < .01 \) when compared with the control. C, Western blot of ACOX, CPT-I, and PPIA in total protein extracts from HepG2 cells treated as in (A). Data points are shown as mean ± SD of triplicates. *, \( P < .05 \) and **, \( P < .01 \) when compared with the control. D, Triglyceride content in HepG2 cells treated as in (A). Data points are shown as mean ± SD of triplicates. **, \( P < .01 \) when compared with the control.

E, Free fatty acid content in HepG2 cells treated as in (A). Data points are shown as mean ± SD of triplicates. **, \( P < .01 \) when compared with the control.
we analyzed the mRNA levels of ACC as a lipogenic enzyme and peroxisomal acyl-coenzyme A oxidase (ACOX) and carnitine palmitoyl transferase-I (CPT-I) as fatty acid oxidation enzymes after 3 days of treatment with adiponectin (25 or 50 ng/ml). Our results showed that adiponectin treatment reduced mRNA levels of ACC and increased mRNA levels of ACOX1a, ACOX1b, and CPT-I when compared with vehicle treated HepG2 cells (Figure 2A). Moreover, a decrease in total ACC protein levels and an increase in ACC phosphorylation were detected after adiponectin treatment when compared with vehicle treated HepG2 cells (Figure 2B). In addition, an increase in ACOX and CPT-I protein levels was detected in adiponectin treated HepG2 cells when compared with vehicle treated cells (Figure 2C).

We next explored whether this adiponectin-induced reduction in lipogenesis and increase in lipolysis would reduce the lipid content of HepG2 cells. We therefore measured total triglycerides and total free fatty acids in control and adiponectin (25 or 50 ng/ml) HepG2 cells. Our results showed that adiponectin treated cells had less triglyceride and free fatty acids than untreated HepG2 cells (Figure 2, D and E).

Given that HNF-4α plays a key role in the transcriptional activity of the human SHBG promoter (17) and we have previously shown that HNF-4α protein levels are altered by changes in hepatic lipid content induced by increasing lipogenesis (18) or β-oxidation (19), we examined HNF-4α levels in HepG2 cells after a 3-day treatment with vehicle or adiponectin (25 or 50 ng/ml). These treatments showed that cells treated with adiponectin had increased HNF-4α mRNA levels (Figure 3A) and protein levels (Figure 3B) when compared with vehicle treated cells. Moreover, AICAR treatment was also able to increase HNF-4α mRNA and protein levels when compared with vehicle treated cells. Furthermore, compound C cotreatment was able to block the adiponectin induced increase of HNF-4α mRNA levels (Figure 3E) and protein levels (Figure 3F).

**Adiponectin-induced increase of SHBG production is blocked by siRNA treatment against HNF-4α in HepG2 cells**

We next used a siRNA against HNF-4α in order to explore if we
could block the increase in SHBG production caused by adiponectin. The results showed that in HepG2 cells treated with siRNA control adiponectin was able to increase SHBG production when compared with the vehicle treated HepG2 cells (Figure 4A). However, when HepG2 cells were treated with siRNA against HNF-4α the adiponectin-induced increase in SHBG production was lost (Figure 4A). Moreover, HNF-4α mRNA and protein were increased by adiponectin treatment in siRNA control HepG2 cells when compared with vehicle treated cells (Figure 4, B and C). This effect on HNF-4α mRNA and protein was abrogated by HNF-4α siRNA treatment (Figure 4, B and C).

**Adiponectin-induced increased SHBG production is blocked by inducing lipogenesis or blocking fatty acid oxidation in HepG2 cells**

We next explored whether increasing lipogenesis or blocking fatty acid oxidation would abrogate the effects of adiponectin on SHBG production. For this purpose, we treated HepG2 cells with adiponectin alone or in the presence of glucose (30 mM), which, as we have previously shown, increases lipogenesis (18) or etomoxir (10μM), a fatty acid oxidation inhibitor (19), over the course of 3 days. As previously reported (19) our results showed that whereas treatment of etomoxir alone did not alter SHBG production, the 30 mM glucose treatment significantly reduced SHBG production by reducing HNF-4α mRNA and protein levels in HepG2 cells (Supplemental Figure 1). However, when cells were treated with glucose (30 mM) or etomoxir (10μM) in the presence of adiponectin (50 ng/ml) both cotreatments were able to block the adiponectin-induced increase of mRNA and protein levels of both HNF-4α and SHBG in HepG2 cells (Figure 5). Interestingly, lower glucose concentrations (10 mM or 20 mM) were also able to block the adiponectin-induced increase of SHBG mRNA and protein in HepG2 cells (Supplemental Figure 2).

**Plasma adiponectin levels correlate with hepatic HNF-4α mRNA levels that in turn correlate with SHBG mRNA levels in human subjects**

A positive and significant correlation ($r = 0.612$, $P = .015$) between plasma levels of SHBG and adiponectin was found (Figure 6A). In addition we found that plasma adiponectin levels correlated with liver HNF-4α mRNA levels ($r = 0.539$, $P < .038$) (Figure 6B). More-
over, we found a significant correlation between hepatic SHBG mRNA and HNF-4α mRNA levels ($r = 0.624$; $P < .013$) (Figure 6C).

Finally, in the multiple regression analysis we observed that adiponectin plasma levels were independently related to circulating SHBG ($P = .045$).

**Discussion**

Epidemiological evidence shows a positive relationship between adiponectin and SHBG plasma levels (29, 30). It is also well known that both adiponectin and SHBG plasma levels show a negative correlation with the BMI (7, 8, 20, 21). However, it is unknown whether the correlation between adiponectin and SHBG is merely a surrogate mediated by the BMI or, by contrast, there are underlying molecular mechanisms that could explain this relationship. In this study, the hypothesis that adiponectin regulates hepatic SHBG production has been tested. We provide evidence that adiponectin, at physiological doses, increases SHBG production through HNF-4α upregulation in HepG2 cells. This effect is mediated by AMPK and the consequent reduction in lipid hepatic content caused by the inhibition of lipogenesis and the activation of β-oxidation. However, it should be noted that whereas the effect of adiponectin in increasing the protein levels of SHBG and HNF-4α protein was very similar to the observed by AICAR, we detected differences at transcriptional level, the adiponectin effect being more potent than the AICAR. These results may suggest that adiponectin SHBG induction was partially exerted through an AMPK independent pathway.

Adiponectin is secreted predominantly by the white adipose tissue (22) modulating a number of metabolic processes, including glucose regulation and fatty acid oxidation (34). In the liver, adiponectin exerts its actions through AdipoR2 which activates the AMPK signaling pathway that results in the decrease of de novo lipogenesis by the phosphorylation of ACC (23, 33). On the other hand, adiponectin also upregulates CPT-I expression levels that in turn increases hepatic fatty acid oxidation (33). These adiponectin actions result in the reduction of the hepatic lipid content (33). Moreover, genetic variations in the AdipoR2 gene are associated with liver fat content (35) and AdipoR2 mRNA expression is significantly reduced in liver biopsies of patients with NASH (36). In our in vitro studies we found that HepG2 cells treated with adiponectin showed a reduction in ACC mRNA and protein levels.
and an increase in ACOX and CPTI mRNA and protein levels when compared with vehicle treated HepG2 cells. Importantly, adiponectin treatment reduced hepatic total triglyceride and free fatty acid content in HepG2 cells. These findings confirm the results of previous studies (33, 37) and provide molecular evidence supporting the results of a large multiethnic population-based cohort showing that adiponectin levels were inversely associated with hepatic steatosis, even after controlling for measures of insulin sensitivity, extrahepatic abdominal adiposity, and ethnicity (38).

It has been previously reported by Peter et al (39) that liver fat, but not visceral fat or total body fat was an independent predictor of plasma SHBG levels. Moreover, after a dietary intervention the increase in plasma SHBG levels was strongly associated with a decrease in liver fat (40). These findings are in accordance with our previous studies using HepG2 cells and the human SHBG transgenic mice that showed the molecular mechanisms by which a reduction or accumulation of hepatic lipids was able to regulate SHBG production (18, 19). In this regard, we showed that SHBG production was reduced by exogenous palmitate or endogenous palmitate accumulation from de novo lipogenesis induced by high carbohydrate diets (18). In these studies we also showed that hepatic palmitate accumulation was able to reduce HNF-4α levels (18). In contrast, we also demonstrated that thyroid hormones increased SHBG production by inducing fatty acid oxidation that was able to reduce hepatic lipid content. This reduction was able to increase hepatic HNF-4α levels (19). With this evidence we hypothesized that the mechanism by which adiponectin regulates hepatic SHBG production is based on the changes in HNF-4α due to the alterations in hepatic lipid turnover. Our results support this hypothesis since HepG2 cells treated with adiponectin increased SHBG promoter activity due to the inhibition of lipogenesis and the activation of β-oxidation.

The relationship between adiponectin and HNF-4α has been analyzed previously. A study performed with primary cultures of human hepatocytes found that adiponectin treatment was able to reduce HNF-4α levels (41). These results do not agree with our results. However, several aspects of the experimental design distinguish our work from this previous study. First, they performed a 24-hour treatment whereas our studies were performed over the course of three days. Second, our studies were performed in the presence of serum in the culture media, whereas they used serum free media. Third and most important, they used an adiponectin dose of 10 μg/ml that is a nonphysiological concentration and 200 times higher than the adiponectin dose (50 ng/ml) used in our study. In addition, the results obtained by Liu et al (42)

Figure 6. Correlations between SHBG, adiponectin and HNF-4α in human plasma and liver biopsies. Positive correlation between plasma SHBG and adiponectin (A), plasma adiponectin and hepatic HNF-4α mRNA levels (B), and hepatic SHBG and HNF-4α mRNA levels (C).
after generating the adiponectin KO mouse are in agreement with our results. These studies reveal that the hepatic HNF-4α/H9251 levels in adiponectin KO mice were reduced when compared with wild-type mice. To further confirm our results we demonstrated that both treatment with specific siRNA against HNF-4α or the induction of lipid accumulation were able to block the adiponectin-induced increase in SHBG production in HepG2 cells. Furthermore, we have also found a significant correlation between plasma adiponectin levels and hepatic HNF-4α mRNA levels, as well as between SHBG and HNF-4α mRNA levels in liver biopsies from obese patients, that suggest the molecular mechanisms detected in our in vitro studies could also be present in humans. A scheme illustrating the metabolic pathway by which adiponectin regulates hepatic SHBG production is displayed in figure 7.

Figure 7. Mechanism by which adiponectin regulates hepatic SHBG production. Among others, adiponectin effects on the liver consist in reducing lipogenesis and increasing fatty acid oxidation. This will cause a reduction in the hepatic fatty acid pool increasing HNF-4α levels that in turn increase SHBG production.

Our findings have potential clinical implications. First, adiponectin could be contemplated as a new mechanism involved in SHBG regulation in the setting of obesity. In this regard, previous reports have suggested that other signals from the adipose tissue independently of adiponectin and leptin could regulate SHBG production by the liver (43). We have also reported that the proinflammatory cytokines TNFα and IL1β, which are elevated in obese patients, induce a downregulation of hepatic SHBG production. In view of our results, the low adiponectin levels reported in obesity could be added to the mechanisms involved in the obesity-induced downregulation of SHBG and total testosterone levels. In addition, it is possible that therapeutic strategies addressed to upregulating adiponectin could be useful in increasing SHBG and testosterone levels. Further studies to confirm this hypothesis seem warranted. Finally, our findings could contribute to explaining the higher plasma levels of SHBG that women present in comparison with men. It has been postulated that testosterone inhibits hepatic SHBG production but to the best of our knowledge no molecular mechanism has ever been suggested. Moreover, androgen response elements have not been found in the human SHBG promoter. Nishizawa et al (44) have recently demonstrated in vivo and in vitro that treatment with testosterone in mice is accompanied by a reduction in plasma adiponectin and, that in cultured adipocytes, testosterone reduces adiponectin secretion (44). Therefore, it is possible that testosterone could regulate indirectly hepatic SHBG production by reducing adiponectin levels. Because women have higher plasma adiponectin levels than men (45), this could be an explanation of why women have higher plasma SHBG levels than men.

In conclusion, adiponectin increases hepatic production of SHBG by upregulating HNF-4α levels via changes in the hepatic lipid content. These results suggest a new molecular mechanism by which adiponectin regulates SHBG production and they could have significant clinical implications.

Acknowledgments

We thank Lorena Ramos, Research Institute Hospital Vall d’Hebron, for her technical assistance.
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This work was supported by a grant from the Instituto de Salud Carlos III (PI12/01357 to D.M.S.) and Centro de Investigación Biomédica en Red, an initiative of Instituto de Salud Carlos III (R.S., C.S.L., A.L., F.J.M., V.R., C.H. and D.M.S.). D.M.S. is the recipient of a Miguel Servet contract.

Disclosure Summary: The authors have nothing to disclose.

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