OBJECTIVE—Haptoglobin (Hp) is upregulated in both inflammation and obesity. The low chronic inflammatory state, caused by massive adipose tissue macrophage (ATM) infiltration found in obesity, and low adiponectin have been implicated in the development of insulin resistance and hepatosteatosis. The aim of this work was to investigate whether and how Hp interferes with the onset of obesity-associated complications.

RESEARCH DESIGN AND METHODS—Hp-null (Hp−/−) and wild-type (WT) mice were metabolically profiled under Chow-food diet (CFD) and high-fat diet (HFD) feeding by assessing physical parameters, glucose tolerance, insulin sensitivity, insulin response to glucose load, liver triglyceride content, plasma levels of leptin, insulin, glucose, and adiponectin. ATM content was evaluated by using immunohistochemistry (anti-F4/80 antibody). Adiponectin expression was measured in Hp-treated, cultured 3T3-L1 and human adipocytes.

RESULTS—No genotype-related difference was found in CFD animals. HFD-Hp−/− mice revealed significantly higher glucose tolerance, insulin sensitivity, glucose-stimulated insulin secretion, and adiponectin expression and reduced hepatomegaly/steatosis compared with HFD-WT mice. White adipose tissue (WAT) of HFD-Hp−/− mice showed higher activation of insulin signaling cascade, lower ATM, and higher adiponectin expression. Hp was able to inhibit adiponectin expression in cultured adipocytes.

CONCLUSIONS—We demonstrated that in the absence of Hp, obesity-associated insulin resistance and hepatosteatosis are attenuated, which is associated with reduced ATM content, increased plasma adiponectin, and higher WAT insulin sensitivity.

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Haptoglobin (Hp) is a circulating tetrameric glycoprotein and is considered a classic clinical marker of the liver acute phase response to inflammation. Diversified functions have been attributed to this circulating protein, including angiogenic capacity, the ability to bind free hemoglobin, and, as recently demonstrated by the authors, an important chemotactic activity for monocytes in vitro (1–3). Hp is also abundantly expressed by white adipose tissue (WAT) (4,5), which is one of those few inflammatory molecules specifically produced by the adipocyte and not present in the stromal vascular fraction (6,7). Obesity has been recently defined as a low chronic inflammatory state, and this has been implicated in the development of common medically important complications, including hepatic steatosis, insulin resistance, and atherosclerosis (8–10). Classic markers of the obesity-induced inflammatory state include the augmented circulating levels of proinflammatory proteins, procoagulant factors, cytokines, and chemokines. The molecular and cytologic alterations taking place in WAT on obesity play a determinant role in this phenomenon. Obesity is in fact associated with increased infiltration of macrophages in WAT, and this certainly contributes to the inflammatory-like gene expression pattern displayed by the WAT of obese individuals. The mechanisms underlying macrophages recruitment are still a matter of investigation and likely involve increased secretion of chemotactic molecules by the adipocytes. Monocyte chemoattractant protein 1 (MCP-1) and its receptor C-C chemokine receptor 2 (CCR2) have been considered the main players in this process (11,12). Recent work by the authors demonstrates that Hp induces CCR2 internalization and that pharmacologic inhibition of CCR2 abolishes monocytes migration toward Hp (2).

As we previously reported, WAT Hp expression is induced in obesity and the circulating levels of the glycoprotein are significantly related to the degree of adiposity in humans (4,5). The depicted scenario thus far led to the consideration of Hp as a novel adipokine (5) and a further intersection between obesity and inflammation. However, if its role in the latter condition has long been established and characterized, its role in metabolism and WAT has yet to be fully elucidated.

In the current study, we investigated how variations in Hp expression might be relevant to metabolism and to WAT expression and inflammatory profile. These issues were investigated in mice by using the Hp-null (Hp−/−) model, for which no metabolic characterization had been carried out (13). Our findings indicate that on the onset of obesity, Hp deficiency confers a partial protection against impaired glucose homeostasis and hepatomegaly/steatosis and that these features are associated with increased adiponectin and reduced WAT macrophage infiltration.

RESEARCH DESIGN AND METHODS

Experimental animals. Hp−/− mice were generated previously (13). Mice were fed a chow-food diet (CFD) (2018 Teklad Global Diet; Harlan, Indianapolis, IN) or high-fat diet (HFD) (Diet F3282, 19% protein, 36% fat, and 35% carbohydrate [gram per weight]; Bio-Serve, Frenchtown, NJ) for 12 weeks (Supplementary Appendix A1). Serum, epididymal (EPI) WAT, subcutaneous
**RESULTS**

**Hp release by mouse WAT.** According to Fain et al. (7), explants of human WAT release Hp into the medium. We wanted to verify whether mouse WAT displayed this same capacity. The release of Hp by EPI WAT explants was fairly linear over the incubation time (Fig. 1A). We then wanted to compare the release of Hp from WAT with that from liver, considered the main source for this glycoprotein (18), and from kidney, which conversely exhibits very low Hp expression (4). Over 24 h, liver was the greatest producer of Hp, and Hp production in WAT was significantly higher than in kidney (Fig. 1B). These results rule out the possibility that what was detected in the medium was due to the spill-over of Hp contained in excised vessels, because a comparable amount of released Hp would be expected from all examined tissues. On the basis of these data, we calculated that the average daily release of Hp from visceral WAT and liver is 256 ± 92 and 660 ± 88 ng/g tissue per day, respectively. By taking into account that visceral WAT represents ~13% of the BW of an animal (the average BW of these animals is 26.5 g) and that the average liver weight is 1.5 g, the total Hp daily production can be estimated to attain 0.88 μg for visceral WAT and 0.99 μg for liver. Such an estimate for WAT contribution to circulating Hp is in the lower range, not including the Hp production by SC WAT.

![FIG. 1. Hp release into the medium by mouse tissue explants.](image-url)
To rule out the possibility that the Hp release measured in mouse WAT explants was due to other cell types or to continuous and progressive spill-over from vessels trapped in the tissue, Hp concentration was assessed in the medium where primary mouse adipocytes had been cultured for 6 h. Hp was present in this medium at a concentration of 22.2 ± 1.9 ng/mL/10^6 cells. Although this value cannot be easily transformed into Hp release per gram of tissue, because a variable number of cells are lost during cell isolation and preparation, this result definitely establishes that adipocytes themselves are able to release Hp.

**HFD results in increased Hp production in mice.** We previously demonstrated that serum Hp is directly related to adiposity in humans (5). To investigate this aspect in mice, a group of WT mice was exposed to an HFD on which animals gained ~88% of their initial BW. Serum Hp was then assessed before and after HFD treatment, and a significant increase of serum Hp of approximately threefold was observed (Fig. 2A). Hp gene expression, as assessed by real-time PCR, was significantly higher in the EPI WAT but not in the liver of obese mice compared with lean mice (Fig. 2B and C). Taken together, these results suggest that in murine obesity Hp gene expression is specifically induced in WAT.

**Physical parameters and plasma biochemistry of Hp^{−/−} mice.** To investigate the role of Hp in metabolism, an in vivo loss of function approach was undertaken. Adult Hp^{−/−} mice and WT controls were analyzed under two different diet regimens, namely, CFD and HFD. HFD resulted in a significant increase in BW in both Hp^{−/−} and WT animals compared with CFD-treated mice. No genotype effect was observed: WT and Hp^{−/−} mice showed similar BW on a regular diet and did not differ in their susceptibility to gain weight when fed an HFD (Table 1). Weights of EPI and perirenal fat pads consistently were affected by diet but not by genotype (Fig. 3A and B). Likewise, BAT size was increased (independently of genotype) in HFD-treated mice (Fig. 3C). Histologic examination revealed that interscapular BAT of HFD-treated mice was composed mainly of hypertrophic unilocular cells as opposed to lean mice in which typical multilocular cells predominate (Fig. 3D), which is in line with other investigations (19–21). When concentrations of fasting glucose, insulin, and leptin were assessed, a diet effect was observed, but not a genotype effect (Table 1).

**Hp deficiency results in ameliorated glucose homeostasis in obesity.** Animals were challenged with an intraperitoneal glucose tolerance test. No genotype effect was observed in CFD-treated animals. (Fig. 4A). On HFD, WT mice exhibited an important glucose intolerance with no sign of glycemia decreasing at 120 min after glucose stimulus. This effect was attenuated in Hp^{−/−} mice, for which a decrease, albeit moderate, was observed at 120 min. This is mirrored in the significantly lower value of the area under the curve exhibited by HFD-Hp^{−/−} mice compared with WT mice (Fig. 4B). Of importance, HFD-Hp^{−/−} mice displayed a clear glucose-stimulated insulin secretion, as opposed to WT mice, which showed no response (Fig. 4C).

During an intraperitoneal insulin tolerance test, HFD-Hp^{−/−} mice were slightly more insulin-sensitive than HFD-WT mice (Fig. 4D). Of interest, an insulin-induced glucose decrease persisted for a longer period in the former.

**Insulinsation in liver, WAT, and skeletal muscle of HFD-Hp^{−/−} mice.** To better understand how global Hp deficiency contributes to define glucose homeostasis and

![FIG. 2. HFD treatment results in increased Hp production. A: Hp plasma levels in lean (n = 7) and obese (n = 7) WT mice. B: Hp WAT mRNA abundance in lean and obese WT mice. C: Hp liver mRNA abundance in lean and obese WT mice. Student t test: *P < 0.05, **P < 0.01. Data are expressed as means ± SEM.](diabetes.diabetesjournals.org)
insulin sensitivity in HFD-treated mice, we evaluated insulin-stimulated phosphorylation of Akt Ser-473 in WAT, liver, and skeletal muscle (gastrocnemius). This was significantly enhanced in WAT and showed a trend toward increase in liver and skeletal muscle of HFD-Hp2/2 mice compared with HFD-WT mice (Fig. 4E–G).

Taken together, these data suggest that Hp deficiency constitutes a partial protection against the onset of obesity-associated glucose intolerance, dampened glucose-stimulated insulin production, and insulin resistance. The latter is at least partially explained by a better preserved insulin response in the WAT.

Liver in lean and obese Hp−/− mice. Hp is abundantly expressed in the liver (18), and hepatosteatosis/hepatomegaly often characterizes obesity (22). Indeed, HFD-WT mice showed an important (35%) increase of their liver weight

| Physical and metabolic parameters in WT and Hp−/− mice under CFD and HFD treatment |

|                  | CFD (WT n = 15) | Hp−/− (n = 23) | HFD (WT n = 24) | Hp−/− (n = 30) |
|------------------|-----------------|----------------|-----------------|----------------|
| Body weight (g)  | 27.36 ± 0.56    | 27.12 ± 0.30   | 42.29 ± 0.98    | 41.96 ± 0.54   |
| Insulin (ng/mL)  | 0.88 ± 0.08     | 0.82 ± 0.09    | 2.35 ± 0.23     | 2.49 ± 0.26    |
| Glucose (mg/dL)  | 148.78 ± 7.31   | 135.67 ± 8.02  | 235.23 ± 12.23  | 212.37 ± 7.02  |
| Leptin (ng/mL)   | 2.8 ± 0.5       | 3.6 ± 0.9      | 74.1 ± 9.0*     | 89.7 ± 16.0†   |

Data are expressed as means ± SEM. Body weight: two-way ANOVA diet effect P < 0.0001. Insulin: two-way ANOVA diet effect P < 0.0001. Glucose: two-way ANOVA diet effect P < 0.0001. Leptin: two-way ANOVA diet effect P < 0.0001. *n = 18. †n = 22.
compared with CFD WT mice. HFD treatment resulted in a less pronounced (15%, not significant) liver weight increase in Hp+/− mice (Fig. 5A).

Histological analysis revealed a lower lipid accumulation in the liver of Hp+/− mice (Fig. 5B). In line with this, triglyceride content was significantly lower in HFD-Hp+/− mice compared with HFD-WT mice (Fig. 5C and D).

To investigate the mechanisms underlying the reduced triglyceride accumulation observed in the liver of obese Hp+/− mice, we measured the abundance of transcripts for 1) genes involved in lipid biosynthesis and metabolism, including the enzyme fatty acid synthase (FAS), the transcription factor sterol regulatory element-binding protein (SREBP), and the mitochondrial negative regulator of fatty acids oxidation glycerol 3-phosphate acyltransferase (mtGPAT); and 2) two gluconeogenesis rate-limiting enzymes, namely, PEPCK and glucose-6 phosphatase (G6Pase). FAS, SREBP, mtGPAT, PEPCK, and G6Pase were downregulated by 66, 13, 53, 32, and 38%, respectively, in the liver of HFD-Hp+/− mice (Fig. 5E) compared with HFD-WT mice, which is consistent with decreased lipid synthesis, increased fatty acids β oxidation (23), and diminished gluconeogenesis (24).

Microarray analysis was used to better define the expression profile of HFD-Hp+/− versus HFD-WT mice. Hierarchical clustering identified WT and Hp+/− as two distinct groups. A total of 611 genes were differentially expressed, of which 573 were upregulated and 38 were downregulated in the HFD-Hp+/− (Fig. 5F). Among them, we chose to present those (Fig. 5F, right; Supplementary Appendix A6) more specifically related to liver functions/pathology and displaying more than a twofold change. Real-time PCR performed on two representative genes (cell death-inducing DNA fragmentation factor α [CIDEC] and acetyl-CoA carboxylase β [ACACB]) confirmed microarray results (Supplementary Appendix A7).

If we exclude renin, glutathione S-transferases, and cytochrome P450 CYP4I, the other nine genes could be placed in three functional categories, including 1) apoptosis-related genes that include members of the serpin family (upregulated in HFD-Hp+/−) and members of the CIDE family (downregulated in HFD-Hp+/−), the former being inhibitors and the latter inducers of apoptosis; 2) genes implied in lipid metabolism (downregulated in HFD-Hp+/−); and 3) transcription factors Forkhead box A2 (FOXA2) and hepatocyte nuclear factor 6 (HNF6) both associated with improved insulin sensitivity (25,26) when overexpressed and both upregulated in the absence of Hp (A6).

**Adiponectin is increased in HFD-Hp+/− mice.** Absence of Hp results in a more benign prognosis on the onset of obesity, because hepatomegaly/steatosis and insulin resistance are attenuated. For further insights into this matter, we searched for systemic or local factors that might explain such phenotype.

Adiponectin is a key factor in determining insulin sensitivity (27) because its plasma levels are inversely associated with glucose intolerance (28,29). Further, recent studies reported that hypoadiponectinemia enhances and...
adiponectin administration prevents steatohepatitis progression in mice (30). We investigated this parameter in our models. Although plasma adiponectin was similar in CFD-WT and CFD-Hp\textsuperscript{−/−} mice, HFD-Hp\textsuperscript{−/−} exhibited significantly higher levels of adiponectin compared with HFD-WT mice (Fig. 6A). We also observed a significant increase of EPI WAT adiponectin mRNA in HFD-Hp\textsuperscript{−/−} versus HFD-Hp-WT mice (Fig. 6B).

**Effect of Hp on adiponectin expression in vitro.** To investigate whether Hp plays a direct role on adiponectin expression, we performed in vitro experiments using 3T3-L1 adipocytes and purified Hp. Treatment of terminally

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**FIG. 5.** Weight, triglyceride content, and gene expression in the liver of Hp\textsuperscript{−/−} mice. A: Liver weight of WT and Hp\textsuperscript{−/−} mice after CFD or HFD (n = 15). Two-way ANOVA: diet effect P < 0.001, genotype effect P < 0.05. B: Oil Red O staining of liver sections (8 μm) from HFD-WT and HFD-Hp\textsuperscript{−/−} mice. C: Liver triglyceride content expressed per milligram of tissue. Two-way ANOVA: diet effect P < 0.01, genotype effect P < 0.05. D: Total liver triglyceride content in WT and Hp\textsuperscript{−/−} mice. Two-way ANOVA: diet effect P < 0.0001, genotype effect P < 0.01, interaction P < 0.05. Bonferroni post hoc tests referred to A, C, and D: HFD-WT vs. HFD-Hp\textsuperscript{−/−} *P < 0.05, **P < 0.01, ***P < 0.001. Bonferroni post hoc test CFD-WT vs. HFD-WT §§§ P < 0.001. Data are expressed as means ± SEM. E: Relative expression of genes involved in liver metabolism (FAS, SREBP, mtGPAT, PEPCK, and G6Pase) in HFD-WT and Hp\textsuperscript{−/−} mice (n = 8). Student t test: HFD-WT vs. HFD-Hp\textsuperscript{−/−} *P < 0.05, **P < 0.01. Data are expressed as means ± SEM. F: Microarray analysis of hepatic mRNA expression in HFD-WT and HFD-Hp\textsuperscript{−/−} mice (n = 4). Left: Hierarchic clustering of 611 genes differentially expressed in the two genotypes. Right: Magnification showing 13 selected genes (see text). Nine of them were grouped in functional categories according to their biological function: apoptosis (in blue), transcription factors (in red), and lipid metabolism (in green). (A high-quality digital representation of this figure is available in the online issue.)
FIG. 6. Hp affects adiponectin expression in vivo and in vitro. A: Plasma adiponectin levels in HFD-WT and Hp−/− mice (n = 15). Student t test: *P < 0.05. B: Relative expression of adiponectin in EPI WAT of HFD-WT and Hp−/− mice. Student t test: *P < 0.05. C: Adiponectin and αP2 relative gene expression in terminally differentiated 3T3-L1 adipocytes treated for 24 h with Hp as indicated. One-way ANOVA for adiponectin: P < 0.01. Bonferroni post hoc test: Hp 1 mg/mL vs. control *P < 0.01. D: Adiponectin release after Hp treatment for 24 h (doses as indicated) in terminally differentiated 3T3-L1 adipocytes. One-way ANOVA: P < 0.05. Bonferroni post hoc test: Hp 1 mg/mL vs. control *P < 0.05. E: Relative expression of adiponectin in SC human adipocytes, treated or not (control) with Hp for 24 h. Student t test: *P < 0.05. Data are expressed as means ± SEM.

HAPTOGLOBIN ROLE IN GLUCOSE HOMEOSTASIS

WAT macrophage content in Hp−/− mice. Another important determinant of the risk to develop obesity-associated complications resides in the WAT inflammatory status. Having previously proved that Hp acts as a chemoattractant in vitro (2) and that it is released by murine WAT (present study), we wanted to establish whether a deficiency of this glycoprotein could affect WAT macrophage infiltration in vivo.

When HFD-treated mice were considered, a significantly lower macrophage infiltration was found in both EPI and SC WAT of Hp−/− compared with WT, as assessed by macrophage-specific surface protein F4/80 immunoreactivity (Fig. 7A and B). The abundance of macrophage-specific mRNAs (F4/80 and CD68) consistently was lower in EPI WAT of obese Hp−/− mice compared with weight-matched WT mice (Fig. 7C). No genotype-related difference was observed in the ATM content of CFD-treated mice (data not shown).

These results are consistent with a role of Hp in contributing to WAT macrophage infiltration and consequent onset of altered inflammatory status at this site.

DISCUSSION

Inflammation/obesity is becoming an inseparable binomial association that has yet to be fully unraveled in terms of clinical consequences and underlying cytologic, biochemical, and molecular mechanisms. Hp, an inflammatory molecule that is upregulated in the WAT of obese subjects, constitutes an interesting tool to further explore this relationship.

We demonstrated that murine WAT not only expresses but also releases Hp at levels that equal, if not overcome, the hepatic release in terms of contribution to Hp-circulating concentration. We also prove that in obese mice, which show increased serum Hp, WAT Hp expression is increased, but not liver Hp expression, suggesting that obesity induces a specific WAT Hp upregulation. These premises should be considered in the context of the better preservation of glucose homeostasis shown by the Hp-deficient model of obesity. The concept of WAT being relevant for this phenotype is reinforced by insulin signaling studies that indicate this organ as the one with the highest increase (with respect to HFD-WT mice) in insulin sensitivity, among metabolically active tissues.

Lack of Hp attenuates the hepatomegaly/steatosis often associated with the obesity state. In addition to less-activated machinery for triglyceride accumulation, microarray analysis showed changes in HFD-Hp−/− liver expression profile that are consistent with lower activation of the apoptotic pathways. Apoptosis is a classic feature of hepatic steatosis progressing toward hepatitis and irreversible liver damage (31,32), and what we observe on Hp deficiency is, therefore,
consistent with a phenotype that is more protected against this risk. Whether such changes in liver expression are the result of local, systemic, or both Hp actions cannot be established on the basis of the present findings. The local scenario implies an Hp autocrine effect, possibly modulating deleterious gene expression programs. According to the systemic hypothesis, the liver is healthier because of surrounding conditions of improved glucose homeostasis and insulin sensitivity, determined by other organs.

In this regard, benign consequences of Hp deficiency are associated with reduced WAT macrophage infiltration and higher adiponectin levels. Both aspects imply a direct involvement of WAT, which is in line with the specific WAT Hp upregulation induced by obesity in the WT mouse. WAT dysfunction was previously associated with hepatosteatosis, and plasma Hp was defined as a prognostic marker for nonalcoholic steatohepatitis in animals exposed to HFD (33).

We recently reported that Hp is a chemotactic molecule (2), which is consistent with the reduced ATM infiltration observed in the obese Hp-/- mice. Macrophage infiltration in the WAT of obese individuals is related to the low chronic inflammatory state that often characterizes the obesity status. In particular, the release of inflammatory factors by macrophages, such as interleukin-6 and tumor necrosis factor-α, contributes to the onset of insulin resistance (34). Hepatic steatosis is also generally associated with the inflamed WAT: Mechanisms postulated to explain this association include a secondary effect of the altered availability of lipids generated by the insulin resistance state and a direct consequence of the altered WAT expression profile (35). As previously reported, increased fat content in the liver is independent of the degree of obesity (36) but significantly associated with WAT inflammation, and specifically with the abundance of inflammatory markers, including CD68 and MCP-1. MCP-1 was initially described as a potent chemoattractant factor (37) implicated in the recruitment of macrophages in WAT. Supporting evidence in this direction derives from the increased infiltration of macrophages observed in lean mice overexpressing MCP-1 in WAT (11) and consistently from the lower ATM content found in obese mice deficient for this factor (11) or for its receptor (CCR2) (12). In contrast, Inouye et al. (38) reported that macrophage content in the WAT of MCP-1-/- obese mice is, if nothing, higher than that of obese controls. In no case did genetic obese models for MCP-1 or CCR2 exhibit a level of ATM normalized to that observed in lean mice, thus implying the presence of other uncovered factors in the modulation of this phenomenon. According to our previously published and currently reported evidence, Hp presents all of the features to be considered a potent chemotactic molecule (2). 

FIG. 7. ATM content in HFD-Hp−/− mice. Left: Immunohistochemical detection of F4/80 in EPI WAT (A) and SC WAT (B) of HFD-WT and Hp−/− mice. Right: Bar graphs indicating F4/80 stained cells/total nuclei in EPI WAT (A) (n = 7, 15 fields per animal) and SC WAT (B) (n = 8, 15 fields per animal). F4/80 positive macrophages from each individual depot were counted using a 40× objective. The ratio of F4/80-positive cells was calculated as the number of F4/80 positive nuclei divided by the total number of nuclei in the same field. C: EPI WAT gene expression of CD68 and F4/80 in HFD-WT and Hp−/− mice. Student t test: *P < 0.05. Data are expressed as means ± SEM. (A high-quality color representation of this figure is available in the online issue.)
sensitivity, reduced hepatic steatosis, and increased adiponectin (12). Obese Hp−/− mice show higher adiponectin expression compared with controls. This adipokine is considered an important player in the determination of insulin sensitivity. A large amount of evidence points to the lack of a sufficient amount of this factor as a key signal for the onset of insulin resistance (41) and for the development of hepatic steatosis. In HFD-treated mice, decreased adiponectin often precedes steatohepatitis (42) and adiponectin reverses steatosis in ob/ob mice (43). Obese Hp−/− mice exhibit higher circulating and local levels of adiponectin compared with matched WT controls, which may partly explain their improved glucose tolerance and reduced hepatosteatosis. Our observation regarding adiponectin in HFD-treated Hp−/− mice was independently proved by the inhibitory effect exerted by Hp on adiponectin expression and secretion in cultured adipocytes. To our knowledge, this is the first report demonstrating that adiponectin is significantly downregulated by Hp, thus introducing a new player in the regulation of this adipokine. Accordingly, similar inhibitory capacities were previously reported for other inflammatory factors, including tumor necrosis factor-α and interleukin-6 (44–47), also upregulated in the WAT of obese subjects.

In conclusion, we have established that Hp not only marks the intersection between obesity and inflammation but also actively contributes to the WAT inflammatory profile often found in obese subjects and to the downregulation of adiponectin. These features may explain the phenotype of the obese Hp−/− mouse that is partially protected from the onset of obesity-associated complications, including severe insulin resistance and hepatosteatosis/hepaticomegaly.

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S.L. researched data, analyzed data, and wrote the manuscript. O.G. and G.S. researched and analyzed data. T.V., M.F., M.Mar., and G.G. researched data. I.A. and R.B. performed microarray experiments. M.D. analyzed microarray data and contributed to discussion. A.P. contributed to discussion. F.S. contributed to experimental design and discussion. M.Maf. designed the experiments, analyzed data, and wrote the manuscript. All authors reviewed and edited the manuscript.

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