High-fat diet amplifies renal renin angiotensin system expression, blood pressure elevation, and renal dysfunction caused by Ceacam1 null deletion

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Li C, Culver SA, Quadri S, Ledford KL, Al-Share QY, Ghadieh HE, Najjar SM, Siragy HM. High-fat diet amplifies renal renin angiotensin system expression, blood pressure elevation, and renal dysfunction caused by Ceacam1 null deletion. Am J Physiol Endocrinol Metab 309: E802–E810, 2015. First published September 15, 2015; doi:10.1152/ajpendo.00158.2015.—Carcinoembryonic antigen-related cell adhesion molecule 1 (CECAM1), a substrate of the insulin receptor tyrosine kinase, regulates insulin action by promoting insulin clearance. Global null mutation of Ceacam1 gene (Cc1−/−) results in features of the metabolic syndrome, including insulin resistance, hyperinsulinemia, visceral adiposity, elevated blood pressure, and albuminuria. It also causes activation of the renal renin-angiotensin system (RAS). In the current study, we tested the hypothesis that high-fat diet enhances the expression of RAS components. Three-month-old wild-type (Cc1+/+) and Cc1−/− mice were fed either a regular or a high-fat diet for 8 wk. At baseline under regular feeding conditions, Cc1−/− mice exhibited higher blood pressure, urine albumin-to-creatinine ratio (UACR), and renal expression of angiotensinogen, renin/prorenin, angiotensin-converting enzyme, (pro)renin receptor, angiotensin subtype AT1 receptor, angiotensin II, and elevated PI3K phosphorylation, as detected by p85 (Tyro808) immunostaining, inflammatory response, and the expression of collagen I and collagen III. In Cc1+/+ mice, high-fat diet increased blood pressure, UACR, the expression of angiotensin-converting enzyme and angiotensin II, PI3K phosphorylation, inflammatory response, and the expression of collagen I and collagen III. In Cc1−/− mice, high-fat intake further amplified these parameters. Immunohistochemical staining showed increased p-PI3K p85 (Tyro808) expression in renal glomeruli, proximal, distal, and collecting tubules of Ceacam1−/− mice fed a high-fat diet. Together, this demonstrates that high-fat diet amplifies the permissive effect of Ceacam1 deletion on renal expression of all RAS components, PI3K phosphorylation, inflammation, and fibrosis.

MATERIALS AND METHODS

Animals. Cc1−/− mice were generated and back-crossed 12 times onto the C57BL/6 (BL6) genetic background, as described previously (31). Mice were kept on a 12-h dark-light cycle and fed a standard chow ad libitum. The University of Virginia Animal Care and Use Committee approved all procedures. Three-month-old male Cc1−/− mice were fed ad libitum either a regular diet (RD; 12% fat content) or a HF diet (45% fat content, catalog no. D12451; Research Diets, New Brunswick, NJ) for 2 mo. One day before euthanasia, body weight, blood glucose, plasma insulin, systolic blood pressure, and 24-h urinary levels of albumin and creatinine were evaluated. Systolic blood pressure was measured in nonanesthetized mice using a tail-cuff noninvasive multichannel blood pressure system (ITTC Life Sciences, Woodland Hills, CA), as done previously (35). Renal interstitial fluid (RIF) was collected using a microdialysis technique to measure renal levels of II, as we have described previously (49, 50). Renal tissues were harvested for expression of renin, angiotensinogen, and ACE as well as morphological examinations.

Urine albumin-to-creatinine ratio. For urine collection, mice were placed in individual metabolic cages for a period of 24 h, and urine samples were kept at ~80°C until they were assayed. Urinary albumin was determined by commercial ELISA kit (Exocell, Philadelphia, PA), and urine creatinine was assessed by ELISA kit (Cayman Chemical, Ann Arbor, MI). Urinary albumin-to-creatinine ratio (UACR) was used as a marker for renal dysfunction.

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Real-time reverse transcription polymerase chain reaction. Total RNA was extracted from isolated whole kidney using TRIzol reagent (Invitrogen, Carlsbad, CA) per the manufacturer’s instructions. Aliquots of total RNA (1 μg) were reverse transcribed into cDNA using the first-strand cDNA synthesis kit (Bio-Rad, Hercules, CA). Equal amounts of the reverse transcriptional products were subjected to PCR amplification, using SYBR Green as the fluorescence indicator on a Bio-Rad iCycler system (Bio-Rad). mRNA levels were normalized to GAPDH mRNA levels. Results are expressed in fold change as means ± SE. Primers (Table 1) were synthesized by Operon (Huntsville, AL).

Western blot analysis. Western blot analysis was performed as described previously (32). In brief, whole kidney homogenates were lysed in the presence of protease inhibitors, and 40 μg of protein was subjected to 4–12% gradient SDS-PAGE, transferred onto a PVDF membrane, and subjected at 4°C to an overnight incubation with primary antibodies that included anti-AGT (1:200, sc-7419; Santa Cruz Biotechnology), anti-PRR (1:1,000, ab40790; Abcam), anti-renin (1:200, sc-22752; Santa Cruz Biotechnology), anti-ACE (1:200, sc-23908; Santa Cruz Biotechnology), anti-AT1R (1:200, sc-31181; Santa Cruz Biotechnology), a custom-made CEACAM1 antibody (16), or anti-β-actin (1:5,000, A5441; Sigma), with the latter being used in reprobing to normalize per total amount of loaded proteins. Following incubation with horse-radish peroxidase-labeled IgG (1:5,000), immunoreactive bands were detected by chemiluminescence methods and visualized on Kodak Omat X-ray films. Densitometric analysis of the images obtained from X-ray films was performed using ImageJ software (National Institutes of Health, Bethesda, MD).

Measurement of renal Ang II production. Ang II levels in RIF were measured by Ang II EIA kit per the manufacturer’s instructions (Cayman Chemical).

Immunohistochemical staining. Immunohistochemical staining was performed to determine renal cellular expression of phospho-PI3K p85( Tyr508) as well as that of collagen I and collagen III. To this end, 4-μm-thick sections were cut, deparaffinized, and rehydrated. Heat-induced antigen retrieval was conducted in 10 mM sodium m-thick sections were cut, deparaffinized, and rehydrated.

Plate 1. Primers used for real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5'-3')</th>
<th>Reverse (5'-3')</th>
</tr>
</thead>
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<tr>
<td>AGT</td>
<td>AAAGAGCAGCTGTGAGGAGGAGCAGTGC</td>
<td>CTGGAAAGGAAGGAGGAGGAGTGC</td>
</tr>
<tr>
<td>PRR</td>
<td>TTTGAGGATCAGCTGCCGAGGCAGG</td>
<td>CCAAGACAGGCTGTGGCCAGATTG</td>
</tr>
<tr>
<td>Renin</td>
<td>TGGCTGACGAGCAAGAGGAGGAG</td>
<td>AGCGATCAGGCTTGCCAGATG</td>
</tr>
<tr>
<td>ACE</td>
<td>GGAGGACTTCCCAAGCCAGCTCA</td>
<td>GCCAGTGGCCATCTAGCTCC</td>
</tr>
<tr>
<td>AT1R</td>
<td>TAGATTAAAGATGAGGAGGAGGCGG</td>
<td>TGAGAGAAGGGAGGAGGAGGAGGAGG</td>
</tr>
<tr>
<td>TNFα</td>
<td>ACCGTGATGATGATGATGATGATGATG</td>
<td>CGAGCTGGCGGAGGAGGAGGAGGAGG</td>
</tr>
<tr>
<td>IL-1β</td>
<td>CCTTGGAGTGGAGTGGAGTGGTGG</td>
<td>TATTGCAGGCTTTGAGGCTGGAGG</td>
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<tr>
<td>IL-6</td>
<td>GGTCTTGCAGTCCGAGCAGTCA</td>
<td>ATTTCCGAGGCTTTGAGGCTGGAGG</td>
</tr>
<tr>
<td>PRR</td>
<td>ATGAAGCGCTACACTACACTACACTAC</td>
<td>CCTCCTTTGAGGAGGAGGAGGAGGAGG</td>
</tr>
<tr>
<td>Smad7</td>
<td>TGGTGGGACTTCCTGAG</td>
<td>GCTTCCTTGGGACTTCCTGAG</td>
</tr>
<tr>
<td>α-SMA</td>
<td>GGTGAGGCTGTGGAGGAGGAGGAGG</td>
<td>ATGAGGCTGTGGAGGAGGAGGAGGAGG</td>
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<tr>
<td>Collagen I</td>
<td>TGCGCTTGAGGCTTGAGGAGGAGGAGG</td>
<td>GGTGAGGCTGTGGAGGAGGAGGAGGAGG</td>
</tr>
<tr>
<td>Collagen III</td>
<td>GGGAGAGGCTGTGGAGGAGGAGGAGG</td>
<td>GGGAGAGGCTGTGGAGGAGGAGGAGGAGG</td>
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<tr>
<td>Gapdh</td>
<td>CAGAGGAGGCTGTGGAGGAGGAGGAGG</td>
<td>ATACCGAGAAGGAGGAGGAGGAGGAGGAGG</td>
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</table>

AGT, angiotensinogen; PRR, (pro)renin receptor; ACE, angiotensin-converting enzyme; AT1R, angiotensin subtype 1 receptor; TGFβ, transforming growth factor-β; α-SMA, α-smooth muscle actin.
RESULTS

Blood pressure and UACR in response to HF diet. At baseline and under standard feeding conditions, Ccl1−/− mice exhibited higher blood pressure (P < 0.05; Fig. 1A) and UACR (P < 0.01; Fig. 1B) than Ccl1+/+ mice [knockout-type mice fed regular diet (RD) vs. WT mice fed RD]. HF diet significantly increased blood pressure (P < 0.01; Fig. 1A) and UACR (P < 0.01; Fig. 1B) in both the Ccl1+/+ and Ccl1−/− mouse groups, causing UACR to remain higher in HF-fed Ccl1+/+ mice (P < 0.01; Fig. 1B).

Renal AGT, renin, prorenin, and ACE expression in response to HF diet. mRNA levels of AGT (P < 0.001; Fig. 2A) and renin (P < 0.01; Fig. 2C) were significantly higher in RD-fed Ccl1−/− than in RD-fed Ccl1+/+ mice. Similarly, protein levels of AGT (P < 0.01; Fig. 2B) and renin (P < 0.01; Fig. 2A) were significantly increased in RD-fed Ccl1−/− mice compared with RD-fed Ccl1+/+ mice. HF intake did not affect either renal AGT or renin mRNA (Fig. 2, A and C) or protein levels (Fig. 2, C and D).

HF diet had no significant effect on renal PRR mRNA (Fig. 3A) or protein levels (Fig. 3B) in Ccl1+/+ mice. In Ccl1−/− mice, however, it induced a significant increase in PRR mRNA (P < 0.01; Fig. 3A) and protein (P < 0.05; Fig. 3B) levels. This caused PRR levels to be significantly higher in HF-fed Ccl1−/− than in HF-fed Ccl1+/+ mice (P < 0.05).

ACE mRNA (P < 0.01; Fig. 4A) and protein (P < 0.01; Fig. 4B) levels were significantly higher in the kidneys of Ccl1−/− than Ccl1+/+ mice. HF diet caused a further increase in ACE mRNA (P < 0.01; Fig. 4A) and protein (P < 0.05; Fig. 4B) levels in Ccl1−/− mice.

Ang II in RIF and renal AT1R in response to HF diet. RIF (Ang II) levels were significantly higher in RD-fed Ccl1−/− than in RD-fed Ccl1+/+ mice (P < 0.01; Fig. 5). HF significantly increased RIF Ang II in Ccl1+/+ (P < 0.05; Fig. 5) but not Ccl1−/− mice.

In Ccl1+/+ mice, HF diet had no significant effect on renal AT1R mRNA (Fig. 6A) or protein (Fig. 6B) levels. In contrast, Ccl1−/− mice exhibited significant elevation in AT1R mRNA (P < 0.01; Fig. 6A) and protein (P < 0.01; Fig. 6B) compared with Ccl1+/+ mice, regardless of diet. HF diet did not increase renal AT1R mRNA (Fig. 6A) or protein (Fig. 6B) levels significantly in Ccl1−/− mice.

Renal PI3K p85α phosphorylation in response to HF diet. Immunohistochemical staining showed higher basal phosphorylation of PI3K p85α (Tyr508) in the renal glomeruli, proximal tubules, distal tubules, and collecting ducts of RD-fed Ccl1−/− mice.
than of RD-fed Cc1+/+ mice. HF intake increased PI3K p85 phosphorylation in all kidney segments of Cc1+/+ and Cc1−/− mice (Fig. 7).

Hyperinsulinemia in response to HF diet. Because HF-mediated reduction of CEACAM1 is associated with hyperinsulinemia caused by impaired insulin clearance (2), we then examined whether hyperinsulinemia develop in HF-fed mice. As expected, HF caused hyperinsulinemia in wild-type mice (Table 2), likely owing to a reduction in hepatic CEACAM1 protein content, followed by impaired insulin clearance, as assessed by steady-state C-peptide/insulin molar ratio (Table 2). As published previously (16, 19), Cc1−/− mice exhibited impaired insulin clearance and hyperinsulinemia under both feeding conditions (Table 2). Sustained HF intake induced fed hyperglycemia in wild types (Table 2), consistent with the development of insulin resistance in wild-type mice under HF feeding conditions. In Cc1−/− mutants that already manifested hyperinsulinemia (Table 2) on a RD (16), HF diet caused fasting hyperglycemia (Table 2), which was likely due to increased hepatic glucose production, as occurred in L-SACC1 mice with liver-specific inactivation of CEACAM1 (29).

Renal inflammation and fibrosis in response to HF diet. RD-fed Cc1+/+ mice exhibited an increase in the mRNA content of inflammatory markers such as TNFα, IL-1β, IL-6, and IFNγ (Table 3). Despite the increase in the mRNA levels of the anti-fibrogenic gene IFNγ (7), mRNA (Table 3) and protein content of collagen I (Fig. 8A) and collagen III (Fig. 8B) were markedly elevated, whereas the increase in α-SMA mRNA did not reach statistical significance. The mRNA levels of Smad7 are reduced in RD-fed Cc1+/+ mice. Sustained HF feeding exacerbated these fibrogenic changes in Cc1−/− mice, exacerbated these fibrogenic changes in Cc1−/− mice.
IL-6 and TGF-

Because HF diet induced plasma leptin acts centrally to activate renal sympathetic nerve and cause relative to RD-fed mice (19). Among its multiple effects, leptin, a cytokine released from white adipose tissue, in HF-fed secondary factors could include the rise in the levels of plasma CEA CAM1 in amplifying these hemodynamic changes. These observations confirmed that CEACAM1 prevents elevation in components, PI3K activation, inflammation, and fibrosis. Thus, consistent with reports on induction of blood pressure and renal dysfunction, in mice with null mutation of Ceacam1 together with an underlying increase in the expression of all renal RAS components, PI3K activation, inflammation, and fibrosis. Thus, these studies confirmed that CEACAM1 prevents elevation in blood pressure and UACR, and this is mediated by curtailing renal PI3K p85α phosphorylation, RAS activity, and inflammation and kidney fibrosis.

Consistent with reports on induction of blood pressure and renal dysfunction by HF feeding (13, 18, 21), we have herein observed that prolonged HF intake caused elevation in blood pressure and UACR in wild-type as well as Ccl1−/− mice. In support of the role for insulin resistance and leptin in enhancing renal sodium reabsorption and promoting obesity-related hemodynamic changes (4, 12, 28), HF elevated blood pressure and caused kidney dysfunction in wild-type mice in parallel to insulin resistance and repression of renal CEACAM1 level. Because HF induced blood pressure and UACR to a much higher extent in wild-types than Ccl1−/− mice, this suggests that other factors play an additional role(s) in the loss of CEACAM1 in amplifying these hemodynamic changes. These secondary factors could include the rise in the levels of plasma leptin, a cytokine released from white adipose tissue, in HF-fed relative to RD-fed mice (19). Among its multiple effects, leptin acts centrally to activate renal sympathetic nerve and cause hypertension (34). Because HF diet induced plasma leptin more strongly in wild-type than in Ccl1−/− mice (27-fold vs. 9-fold in mutants) (19), it is likely that leptin contributes to the differential potentiating effect of HF diet on blood pressure and kidney function in wild-type and Ccl1−/− mice.

Moreover, HF diet amplified the effect of Ceacam1 deletion on renal dysfunction and RAS expression, particularly as it pertains to elevated renal ACE and PRR levels. Consistent with the key role of PI3K activation in mediating the upregulatory effect of Ceacam1 deletion on renal PRR expression (22), HF diet further induced PI3K phosphorylation in mice devoid of Ceacam1 and caused PI3K activation in wild-type mice in parallel with repressing its renal CEACAM1 content. This suggests that PI3K activation contributes mechanistically to diet-induced potentiation of PRR and ACE induction by Ceacam1 deletion. This notion is supported at least in part by the observation that HF diet induced renal ACE expression in wild-type mice while markedly reducing their CEACAM1 levels.

**DISCUSSION**

As in our previous report (22), we herein observed that a HF diet increased blood pressure and UACR, a marker of renal dysfunction, in mice with null mutation of Ceacam1 together with an underlying increase in the expression of all renal RAS components, PI3K activation, inflammation, and fibrosis. Thus, these studies confirmed that CEACAM1 prevents elevation in blood pressure and UACR, and this is mediated by curtailing renal PI3K p85α phosphorylation, RAS activity, and inflammation and kidney fibrosis.

Consistent with reports on induction of blood pressure and renal dysfunction by HF feeding (13, 18, 21), we have herein observed that prolonged HF intake caused elevation in blood pressure and UACR in wild-type as well as Ccl1−/− mice. In support of the role for insulin resistance and leptin in enhancing renal sodium reabsorption and promoting obesity-related hemodynamic changes (4, 12, 28), HF elevated blood pressure and caused kidney dysfunction in wild-type mice in parallel to insulin resistance and repression of renal CEACAM1 level. Because HF induced blood pressure and UACR to a much higher extent in wild-types than Ccl1−/− mice, this suggests that other factors play an additional role(s) in the loss of CEACAM1 in amplifying these hemodynamic changes. These secondary factors could include the rise in the levels of plasma leptin, a cytokine released from white adipose tissue, in HF-fed relative to RD-fed mice (19). Among its multiple effects, leptin acts centrally to activate renal sympathetic nerve and cause hypertension (34). Because HF diet induced plasma leptin more strongly in wild-type than in Ccl1−/− mice (27-fold vs.

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**Fig. 5. Effect of HF diet on renal interstitial fluid (RIF) angiotensin (Ang) levels in Ccl1++/+ and Ccl1−/− mice. A: RIF Ang II. Values are means ± SE; n = 6 mice/group.**

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**Fig. 6. Effect of HF diet on angiotensin subtype 1 receptor (AT1R) mRNA and protein levels in the kidneys of Ccl1++/+ and Ccl1−/− mice. A: real-time PCR analysis of AT1R mRNA expression in whole kidney lysates. B: Western blot analysis of AT1R protein expression in whole kidney lysates. Values are means ± SE; n = 6 mice/group.**
In addition to renal ACE, HF diet elevated Ang II in RIF of wild-type mice together with blood pressure and UACR, as was observed previously (5). The importance of RAS in the regulation of blood pressure and development of albuminuria is well recognized (20, 26, 41–42). Ang II is the principal RAS peptide regulating blood pressure and contributing to increased urinary albumin. Both PRR and ACE can increase Ang II production (39), ACE activation causes more Ang I to Ang II conversion (9, 27). Although the cause-effect relationship was not established in this study, it is intriguing that Ang II elevation by HF diet in wild-type mice was associated with a marked loss of renal CEACAM1 content, simulating the established effect of Ceacam1 null deletion on the levels of Ang II and other RAS components (22).

As reported previously (22), the current data showed that Ceacam1 deletion caused PI3K activation, pointing to a role for loss of renal CEACAM1 in diet-induced activation of PI3K p85α (Tyr508) in renal glomeruli, proximal, distal, and collecting tubules in RD-fed wild-type mice. This agrees with other reports showing reduction of PI3K/Akt pathways by CEACAM1 in response to different signals (22, 45, 53). Poy et al. (45) showed that upon its phosphorylation by the insulin receptor tyrosine kinase, CEACAM1 binds to Shc and positions it to compete more efficiently with the insulin receptor substrate 1 for the receptor-binding, downregulating, downstream PI3K/Akt pathway. Activation of PI3K/Akt pathway by Ceacam1 deletion increased PRR production via CREB family and NF-kB transcription factors (22), whereas increased PRR production enhanced PI3K phosphorylation (33). These results suggest that PI3K activation contributes to upregulation of PRR, which may mediate an additional increase in PI3K phosphorylation of prorenin that binds to PRR, leading to increased Ang II formation both in vitro and in vivo (15, 40).

Activation of RAS induces tissue inflammation and fibrosis (14, 23, 24, 44, 51), which are important contributors to development of hypertension and renal damage (8). Like the liver (19), Ceacam1 null mutation leads to higher inflammation response and fibrosis in the kidney. This is probably due to the profibrogenic effect of IL-6 and TGFβ (7), which were elevated in the null mouse under normal feeding conditions. The mRNA level of Smad7, which inhibits TGFβ activation (52), was reduced in RD-fed Cecl−/− mice, likely owing to the countervailing effect of TNFα (52). Sustained HF feeding exacerbated these inflammation and fibrogenic changes in Cecl−/− mice, suggesting HF induced kidney damage through both Ceacam1-dependent and -independent signaling pathways such as oxidative stress (3) and inducible nitric oxide synthase-derived nitric oxide (43). Surprisingly, IL-6 and

Table 2. Plasma and blood biochemistry

<table>
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<tr>
<th></th>
<th>Cecl+/+</th>
<th>Cecl−/−</th>
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<tr>
<td></td>
<td>RD</td>
<td>HF</td>
</tr>
<tr>
<td>Fasting plasma insulin, pM</td>
<td>76.71 ± 9.034</td>
<td>144.1 ± 10.04*</td>
</tr>
<tr>
<td>Fasting C/I molar ratio</td>
<td>9.282 ± 0.501</td>
<td>6.023 ± 0.233*</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>70.22 ± 1.112</td>
<td>72.42 ± 3.133</td>
</tr>
<tr>
<td>Fed blood glucose, mg/dl</td>
<td>83.42 ± 4.052</td>
<td>120.3 ± 3.532*</td>
</tr>
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</table>

Values are expressed as means ± SE. Cecl−/−, global Ceacam1 null mouse; Cecl+/+, wild-type mouse from the same genetic background as Cecl−/− mice; RD, regular diet; HF, high-fat diet; C/I molar ratio, C-peptide-to-insulin molar ratio at a steady state is a measurement of insulin clearance. Male mice (n = 7, 3 mo of age) were fed RD or HF for 2 mo. *P < 0.05, HF vs. RD; †P < 0.05, Cecl−/− vs. Cecl+/+ in the same feeding group.

Fig. 7. Immunostaining of phosphorylated (phospho) phosphatidylinositol 3’-kinase (PI3K) p85α in the kidneys of Cecl+/+ and Cecl−/− mice. Immunostaining of phospho-PI3K p85α (Tyr508) in the kidneys of Cecl+/+ and Cecl−/− mice fed RD or HF diet; n = 6 mice/group. PT, proximal tubule; DT, distal tubule; CD, collecting ducts.
TGFβ levels were not further induced by HF feeding in Ccl1−/− mice, attributing the profibrogenic effect to increased levels of TNFα, which mediate progression of fibrosis in addition to inflammation (11).

In summary, we demonstrated that Ceacam1 null mutation caused an elevation in blood pressure and increased UACR through RAS and PI3K pathway activation, which is magnified by HF diet. To our knowledge, this is the first report demonstrating the involvement of CEACAM1 in regulating blood pressure and renal dysfunction in response to HF intake. Given that hepatic CEACAM1 level is reduced in obese subjects (30) and that local RAS activation contributes to kidney dysfunction induced by hypertension, diabetes, metabolic syndrome, and heart disease (9, 48), the current studies promote an important role for CEACAM1 reduction in the development of hypertension associated with metabolic syndrome. Thus, inducing CEACAM1 activity or its signaling pathways could be beneficial in managing hypertension and renal dysfunction.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


### Table 3. Effect of HF intake on the expression of selected genes related to inflammation and fibrosis in kidney

<table>
<thead>
<tr>
<th></th>
<th>Ccl1+/+</th>
<th>Ccl1−/−</th>
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<tr>
<td></td>
<td>RD</td>
<td>HF</td>
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<tr>
<td>TNF-α (×10^−1)</td>
<td>0.08 ± 0.01</td>
<td>0.15 ± 0.02†</td>
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<tr>
<td>IL-β (×10^−1)</td>
<td>0.82 ± 0.16</td>
<td>1.63 ± 0.11*</td>
</tr>
<tr>
<td>IL-6 (×10^−1)</td>
<td>0.05 ± 0.00</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>IFNγ (×10^−1)</td>
<td>0.06 ± 0.00</td>
<td>0.11 ± 0.02*</td>
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<td>TNFβ</td>
<td>0.55 ± 0.04</td>
<td>0.80 ± 0.30*</td>
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<td>Smad7</td>
<td>0.60 ± 0.08</td>
<td>0.40 ± 0.03*</td>
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<tr>
<td>α-SMA</td>
<td>1.52 ± 0.23</td>
<td>1.63 ± 0.11</td>
</tr>
<tr>
<td>Collagen I</td>
<td>0.34 ± 0.04</td>
<td>0.50 ± 0.05†</td>
</tr>
<tr>
<td>Collagen III</td>
<td>1.82 ± 0.30</td>
<td>3.03 ± 0.84</td>
</tr>
</tbody>
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Values are normalized to Gapdh and expressed as means ± SE. Male mice (n > 4, 3 mo of age) were fed RD or HF for 2 mo. mRNA was analyzed by semiquantitative real-time PCR in duplicate. *P < 0.05, HF vs. RD; †P < 0.05, Ccl1−/− vs. Ccl1+/+ in the same feeding group.

**Fig. 8.** Immunostaining of collagen I and collagen III in the kidneys of male Ccl1+/+ and Ccl1−/− mice. A: immunostaining of collagen I in the kidneys of Ccl1+/+ and Ccl1−/− mice fed RD or HF diet. B: typical immunostaining of collagen III in the kidneys of Ccl1+/+ and Ccl1−/− mice fed RD or HF diet; n = 6 mice/group.
results of experiments: C.L., S.A.C., S.Q., K.J.L., Q.Y.A.-S., H.E.G., and S.M.N. prepared figures; C.L., S.A.C., S.Q., S.M.N., and H.M.S. drafted manuscript; S.M.N. and H.M.S. edited and revised manuscript; S.M.N. and H.M.S. approved final version of manuscript.

REFERENCES


11. Leung N. The CEACAM1 expression is decreased in the liver of severely obese patients with or without diabetes. DiaaPath 60: 40, 2011.


