High-Fat Diet Amplifies Renal Renin Angiotensin System Expression,

Blood Pressure Elevation and Renal Dysfunction Caused by Ceacam1 Null Deletion

Caixia Li¹, Silas A. Culver¹, Syed Quadri¹, Kelly L. Ledford², Qusai Y. Al-Share², Hilda E. Ghadieh², Sonia M. Najjar²*, Helmy M. Siragy¹, *

1 Division of Endocrinology and Metabolism, University of Virginia Health System, Charlottesville, VA 22903;
2 Center for Diabetes and Endocrinology Research, University of Toledo College of Medicine and Life Sciences, Toledo, OH 43614.

Running Title: CEACAM1 and diet-induced renal dysfunction

* Authors contributed equally to this work

Address for correspondence:

Dr. Helmy M. Siragy,
P.O. Box 801409
University of Virginia Health System
Charlottesville, VA 22908
Telephone: 434-924-5629
Fax: 434-982-3626
Email: hms7a@virginia.edu

Manuscript word count: 5881
Abstract word count: 241
Number of figures: 8

Copyright © 2015 by the American Physiological Society.
Abstract

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM 1), 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-months old wild-type (Cc1\(^{+/+}\)) and Cc1\(^{-/-}\) mice were fed either a regular or a high-fat diet for 8 weeks. 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 p85α (Tyr508) immunostaining, inflammatory response, and the expression of collagen I and collagen III. In Cc1\(^{+/+}\) mice, high-fat 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 p85α (Tyr508) expression in renal glomeruli, proximal, distal and collecting tubules of Cc1\(^{-/-}\) mice fed a high-fat diet. Together, this demonstrates that high-fat amplifies the permissive effect of Ceacam1 deletion on renal expression of all RAS components, PI3K phosphorylation, inflammation and fibrosis.
Keywords: Kidney; CEACAM1; RAS; high-fat diet; PI3K
Introduction

The role of renin-angiotensin system (RAS) in the regulation of blood pressure and organ function has been well documented. Local RAS components are synthesized and secreted in many tissues (27). All RAS components are produced by the kidney, including angiotensinogen (AGT), renin, (pro)renin receptor (PRR), angiotensin I (Ang I), angiotensin-converting enzymes (ACE), angiotensin II (Ang II), the angiotensin subtype 1 receptor (AT1R), and subtype 2 receptor (AT2R) (10, 32). Increased local tissue RAS activity was linked to the development of kidney disease, hypertension, diabetes, metabolic syndrome, and heart disease (9, 48).

Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a substrate of the insulin receptor kinase (37, 45), is ubiquitously expressed. It exerts several functions, including regulating tumor development (6, 17, 25, 47), and cell growth (1). In a phosphorylation-dependent manner, it promotes receptor-mediated insulin endocytosis and degradation (46) that constitutes the main mechanism of insulin clearance, which occurs mostly in liver and to a lower extent, in kidney (36). Consistently, mice lacking the Ceacam1 gene (Cc1−/−) develop insulin resistance resulting from impaired insulin clearance and hyperinsulinemia (16). In addition, they exhibit an increase in blood pressure with activation of RAS components including PRR (22). The increase in PRR expression is mediated by a positive cell autonomous effect of Ceacam1 deletion on phosphatidylinositol-3′ kinase (PI3K) activity, as demonstrated by siRNA-mediated downregulation of Ceacam1 in murine renal inner medullary collecting duct (IMCD) epithelial cells (22).

Increased energy supply causes insulin resistance through several mechanisms, including
induction of inflammatory pathways, activation of PKC delta and others (38). Thus, the current study evaluated whether subjecting $CcI^{-/-}$ mice to a high-fat (HF) diet amplifies the permissive effect of $Ceacam1$ deletion on blood pressure and renal RAS expression, and whether this involves changes in PI3K activation.
Animals

Cc1−/− mice were generated and backcrossed twelve times onto the C57BL/6 (BL6) genetic background, as previously described (31). Mice were kept in a 12-hour 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 regular diet (RD, 12% fat content) or high-fat diet (HF, 45% fat content, Research Diets, Catalog #D12451, New Brunswick, NJ) for 2 months. One day before sacrifice, body weight (BW), blood glucose (BG), plasma insulin, systolic blood pressure (BP), and 24-h urinary levels of albumin and creatinine were evaluated. Systolic blood pressure (SBP) was measured in non-anesthetized mice using a tail-cuff noninvasive multichannel blood pressure system (IITC Life Sciences, Woodland Hills, CA), as previously done (35). Renal interstitial fluid (RIF) was collected using a microdialysis technique to measure renal levels of angiotensin II (Ang II), as we have previously described (49-50). Renal tissues were harvested for expression of renin, angiotensinogen, and angiotensin converting enzyme (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 assayed. Urinary albumin was determined by commercial ELISA kit (Exocell, Philadelphia, PA, USA), and urine creatinine was assessed
by ELISA kit (Cayman Chemical, Ann Arbor, MI, USA). Urinary albumin-to-creatinine ratio (UACR) was used as a marker for renal dysfunction.

**Real-time Reverse Transcription Polymerase Chain Reaction**

Total RNA was extracted from isolated whole kidney using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) per manufacturer instructions. Aliquots of total RNA (1 µg) were reverse-transcribed into cDNA, using the first strand cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). 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, Hercules, CA, USA). mRNA levels were normalized to GAPDH mRNA levels. Results are expressed in fold change as mean ± SEM. Primers (Table 1) were synthesized by Operon (Huntsville, AL, USA).

**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 proteins were 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 include anti-AGT (1:200, sc-7419, Santa Cruz), anti-PRR (1:1000, ab40790, Abcam), anti-renin (1:200, sc-22752, Santa Cruz), anti-ACE (1:200, sc-23908, Santa Cruz), anti-AT1R (1:200, sc-31181, Santa Cruz), a custom-made CEACAM1 antibody (16) or anti-β-actin (1:5000, A5441, Sigma); with the latter being used in reprobing to normalize per total amount of loaded proteins.
Following incubation with horseradish peroxidase-labeled IgG (1:5000), 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 the Image J software (NIH, Bethesda, MD, USA).

**Measurement of Renal Angiotensin II Production**

Angiotensin II levels in renal interstitial fluid were measured by Angiotensin II EIA kit per manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI).

**Immunohistochemical Staining**

Immunohistochemical staining was performed to determine renal cellular expression of phospho-PI3K p85α (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 10mM sodium citrate (pH 6.0). Endogenous peroxide activity was suppressed by 0.3% peroxide-methanol solution. VECTASTAIN® ABC KIT (Vector Laboratories, Burlingame, CA) was used for blocking and color reaction. Immunostaining was performed by incubating overnight at 4°C with polyclonal rabbit anti-phospho-PI3K p85α (Tyr508) antibody (Santa Cruz Biotechnology, Santa Cruz, CA), collagen I and collagen III (Abcam, Cambridge, MA) followed by 1 hr of incubation with a secondary antibody conjugated with biotin at room temperature (Vector Laboratories, Burlingame, CA).

**Statistical Analysis**
Data analysis was carried out using STATISTICA version 5.0 (StatSoft, Tulsa OK). Values are expressed as mean ± SEM. Significant differences among multiple groups were examined using two-way analysis of variance (ANOVA) with repeated measures, and the Bonferroni correction method as a post-hoc test. P<0.05 was considered statistically significant.
Results

Blood pressure and urine albumin-to-creatinine ratio in response to high-fat diet

At baseline and under standard feeding conditions, Ccl1−/− mice exhibited higher BP (Figure 1A, p<0.05) and urine albumin-to-creatinine ratio (UACR) (Figure 1B, P<0.01) than Ccl1+/+ mice (KORD versus WTRD). HF diet significantly increased blood pressure (Figure 1A, p<0.01) and UACR (Figure 1B, p<0.01) in both Ccl1+/+ and Ccl1−/− mouse groups, causing UACR to remain higher in HF-fed Ccl1−/− than HF-fed Ccl1+/+ mice (Figure 1B, p<0.01).

Renal AGT, renin, pro-renin and ACE expression in response to high-fat diet

mRNA levels of AGT (Figure 2A, p<0.001) and renin (Figure 2C, p<0.01) were significantly higher in RD-fed Ccl1−/− than RD-fed Ccl1+/+ mice. Similarly, protein levels of AGT (Figure 2B, p<0.01) and renin (Figure 2A, p<0.01) were significantly increased in RD-fed Ccl1−/− mice compared to RD-fed Ccl1+/+ mice. HF intake did not affect either renal AGT or renin mRNA (Figure 2A and 2C) or protein levels (Figure 2C and 2D).

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

ACE mRNA (Figure 4A, p<0.01) and protein (Figure 4B, p<0.01) levels were significantly higher in the kidney of Ccl1+/+ than Ccl1+/+ mice. HF caused a further increase in
ACE mRNA (Figure 4A, p<0.01) and protein (Figure 4B, p<0.05) levels in Cc1−/− mice.

Angiotensin II in renal interstitial fluid and renal AT1R in response to high-fat diet

RIF Angiotensin II (Ang II) levels were significantly higher in RD-fed Cc1−/− than RD-fed Cc1+/+ mice (Figure 5, p<0.01). HF significantly increased RIF Ang II in Cc1+/+ (Figure 5, p<0.05), but not Cc1−/− mice.

In Cc1+/+ mice, HF had no significant effect on renal AT1R mRNA (Figure 6A) and protein (Figure 6B) levels. In contrast, Cc1−/− mice exhibited significant elevation in AT1R mRNA (Figure 6A, p<0.01) and protein (Figure 6B, p<0.01) compared to Cc1+/+ mice regardless of diet. HF did not increase significantly renal AT1R mRNA (Figure 6A) or protein (Figure 6B) levels in Cc1−/− mice.

Renal PI3K p85α phosphorylation in response to high-fat diet

Immunohistochemical staining showed higher basal phosphorylation of PI3K p85α (Tyr 508) in the renal glomeruli, proximal tubules, distal tubules and collecting ducts of RD-fed Cc1−/− than RD-fed Cc1+/+ mice. HF intake increased PI3K p85α phosphorylation in all kidney segments of Cc1+/+ and Cc1−/− mice (Figure 7).

Hyperinsulinemia in response to high-fat 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 reduction in hepatic CEACAM1 protein content, followed by impaired insulin clearance, as assessed by steady-state C-peptide-to-insulin molar ratio (Table 2). As previously published (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 regular diet (16), HF diet caused fasting hyperglycemia (Table 2), 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 high-fat diet

RD-fed Cc1−/− mice exhibited an increase in the mRNA content of inflammatory markers, such as TNF-α, IL-1β, IL-6 and IFN-γ (Table 3). Despite the increase in the mRNA levels of the anti-fibrogenic gene IFN-γ (7), mRNA (Table 3) and protein content of collagen I (Figure 8A) and collagen III (Figure 8B) were markedly elevated, while 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, as assessed by the higher production of TNF-α, α-SMA, collagen I and collagen III relative to RD-fed Cc1−/− mice (Table 3 and Figure 8). IL-6 and TGF-β mRNA levels in Cc1−/− mice were not further modified by HF feeding.

In Cc1+/+ wild-type mice, HF elevated renal mRNA levels of TGF-β and TNF-α (Table 3), without altering IL-6 mRNA levels (Table 3). This translated into a minor increase in
collagen I mRNA levels without a significant change in the mRNA levels of α-SMA or collagen III (Table 3). HF diet increased collagen staining in the kidney of both mouse groups (Figure 8).
As in our previous report (22), we herein observed high fat 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 high-fat 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 Cc1–/– 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 Cc1–/– mice, this suggests that other factors play an additional role(s) to 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 Cc1–/– mice (27-fold versus 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 \textit{Cecl}^{-/} mice.

Moreover, high-fat diet amplified the effect of \textit{Ceacam1} deletion on renal dysfunction and RAS expression, in particular as it pertains to elevated renal ACE and PRR levels. Consistent with the key role of PI3K activation in mediating the up-regulatory effect of \textit{Ceacam1} deletion on renal PRR expression (22), HF diet further induced PI3K phosphorylation in mice devoid of \textit{Ceacam1} and caused PI3K activation in wild-type mice in parallel to repressing its renal CEACAM1 content. This suggests that PI3K activation contributes mechanistically to diet-induced potentiation of PRR and ACE induction by \textit{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 level.

In addition to renal ACE, HF diet elevated Ang II in RIF of wild-type mice together with blood pressure and UACR, as was previously observed (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 \textit{Ceacam1} null deletion on the levels of Ang II and other RAS components (22).

As previously reported (22), the current data showed that \textit{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. (32) 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 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), while increased PRR production enhanced PI3K phosphorylation (33). These results suggested PI3K activation contributes to up-regulation of PRR, which may mediate further increased of PI3K phosphorylation Prorenin 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 pro-fibrogenic effect of IL-6 and TGF-β (7), which were elevated in the null mouse under normal feeding conditions. The mRNA level of Smad7, which inhibits TGF-β activation (52), was reduced in RD-fed Ceacam1−/− mice, likely owing to the countervailing effect of TNF-α (52). Sustained HF feeding exacerbated these inflammation and fibrogenic changes in Ceacam1−/− mice, suggesting HF induced kidney damage through both Ceacam1-dependent and -independent signaling pathways, such as oxidative stress (3) and inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) (43). Surprisingly, IL-6 and
TGF-β levels were not further induced by HF feeding in Cc1−/− mice, attributing the pro-fibrogenic effect to increased levels of TNF-α, which mediates 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 high-fat diet. To our knowledge, this is the first report demonstrating the involvement of CEACAM1 in regulating blood pressure and renal dysfunction in response to high-fat 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.
Source of Funding

This study was supported by National Institutes of Health grants DK078757 and HL091535 to H.M. Siragy, and DK054254, DK083850, HL112248 and HL036573-26 to S.M. Najjar.

Disclosure

None.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5’-3’)</th>
<th>Reverse (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGT</td>
<td>AATGGAGTCGACAGGGGTG</td>
<td>CTGTGAAGGAGGGAGACTGC</td>
</tr>
<tr>
<td>PRR</td>
<td>TTTGGATGCACCTGGAGAAGG</td>
<td>CACAAGGGATGTGCTCAATG</td>
</tr>
<tr>
<td>Renin</td>
<td>GTGAATCCCAACAGGAAGGTT</td>
<td>AGGCCCTCTGGAGCCAATCT</td>
</tr>
<tr>
<td>ACE</td>
<td>GGAGCTCTCCACACGGGTCA</td>
<td>GCCTGGCTTCATGCTACTCT</td>
</tr>
<tr>
<td>AT1R</td>
<td>TAATAGATGATGGCTGCCGCC</td>
<td>TGGAAATCAAAGGAGAAGGGG</td>
</tr>
<tr>
<td>TNF-α</td>
<td>ACGGCATGGATCTCAAGAC</td>
<td>CGGACTCAGCAAAGTCTAAG</td>
</tr>
<tr>
<td>IL-1β</td>
<td>CCCTGCAGCTGGGAGAGTTG</td>
<td>TATTCTGTCCATTGGAGGTGAG</td>
</tr>
<tr>
<td>IL-6</td>
<td>GCCCTTCCCTACTTCACAGAG</td>
<td>ATTTCCAGAATCTCCAGAG</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>ATGAACGCATACACACGTGCTC</td>
<td>CCATCTTTTGGCAAGATGTCTC</td>
</tr>
<tr>
<td>TGF-β</td>
<td>CAACATATCTCCTGGACTTTG</td>
<td>GAAACCGCTGTATCGGCTTCTCTTT</td>
</tr>
<tr>
<td>Smad7</td>
<td>TCAGGCTGGCCATCTCA</td>
<td>GGTGATCTGCCAGTACTTC</td>
</tr>
<tr>
<td>α-SMA</td>
<td>GTGATGACGTGCTCTGGGACTTTGA</td>
<td>ATGAAGATGCTGGAGAGGTC</td>
</tr>
<tr>
<td>Collagen I</td>
<td>TGCCGTGACCTCAAGATG</td>
<td>CACAAGGCGCTTGACTTGA</td>
</tr>
<tr>
<td>Collagen III</td>
<td>GCGGATCCGAGACCAGGGGTG</td>
<td>GCGGGATCCGAGACCAGTTCCCAT</td>
</tr>
<tr>
<td>Gapdh</td>
<td>CCAGGTTTGCTCCTGGCACT</td>
<td>ATACCAAGAAATGAGCTTGACAAAGT</td>
</tr>
</tbody>
</table>
**TABLE 2. Plasma and Blood Biochemistry**

<table>
<thead>
<tr>
<th></th>
<th>Cc1+/+</th>
<th></th>
<th>Cc1−/−</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RD</td>
<td>HF</td>
<td>RD</td>
<td>HF</td>
</tr>
<tr>
<td><strong>Fasting Plasma Insulin, pM</strong></td>
<td>76.7 ± 9.03</td>
<td>144. ± 10.0 *</td>
<td>123. ± 4.00 †</td>
<td>287. ± 30.0 *†</td>
</tr>
<tr>
<td><strong>Fasting C/I molar ratio</strong></td>
<td>9.28 ± 0.50</td>
<td>6.02 ± 0.23 *</td>
<td>5.12 ± 0.60 †</td>
<td>5.45 ± 0.25</td>
</tr>
<tr>
<td><strong>Fasting Blood glucose (mg/dl)</strong></td>
<td>70.2 ± 1.11</td>
<td>72.4 ± 3.13</td>
<td>68.3 ± 2.53</td>
<td>104. ± 4.43 *†</td>
</tr>
<tr>
<td><strong>Fed Blood Glucose (mg/dl)</strong></td>
<td>83.4 ± 4.05</td>
<td>120. ± 5.32 *</td>
<td>115. ± 4.70 †</td>
<td>125. ± 6.62</td>
</tr>
</tbody>
</table>

Male mice (n>7; 3 months of age) were fed RD or HF for 2 months. Values are expressed as mean ± SEM.

*P< 0.05 HF vs. RD; †P < 0.05 Cc1−− vs. Cc1+/+ in the same feeding group.

**Abbreviations:** Cc−−, global Ceacam1 null mouse; Cc+/+, wild type mouse from the same genetic background as Cc−− mice; RD, regular diet; HF, high-fat diet; C/I molar ratio, C-peptide-to–Insulin molar ratio at a steady-state is a measure of insulin clearance.
### TABLE 3. Effect of high-fat intake on the expression of selected genes related to inflammation and fibrosis in kidney

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cc1+/+</th>
<th>Cc1−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RD</td>
<td>HF</td>
</tr>
<tr>
<td>TNF-α (x10^−1)</td>
<td>0.08 ± 0.01</td>
<td>0.25 ± 0.02 *</td>
</tr>
<tr>
<td>IL-1β (x10^−1)</td>
<td>0.82 ± 0.16</td>
<td>1.63 ± 0.11 *</td>
</tr>
<tr>
<td>IL-6 (x10^−1)</td>
<td>0.05 ± 0.00</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>IFN-γ (x10^−1)</td>
<td>0.06 ± 0.00</td>
<td>0.11 ± 0.02 *</td>
</tr>
<tr>
<td>TGFβ</td>
<td>0.55 ± 0.04</td>
<td>0.80 ± 0.30 *</td>
</tr>
<tr>
<td>Smad7</td>
<td>0.60 ± 0.08</td>
<td>0.40 ± 0.03 *</td>
</tr>
<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>
</table>

Male mice (n>4; 3 months of age) were fed RD or HF for 2 months. mRNA was analyzed by semi-quantitative real time-PCR in duplicate. Values are normalized to Gapdh and expressed as mean ± SEM. 

*P< 0.05 HF vs. RD; †P < 0.05 Cc1−/− vs. Cc1+/+ in the same feeding group.

**Abbreviations:** Cc−/−, global Ceacam1 null mouse; Cc+/+, wild type mouse from the same genetic background as Cc−/− mice; RD, regular diet; HF, high-fat diet; TNF-α, tumor necrosis factor-α; TGFβ, transforming growth factor β; IFN-γ, interferon gamma; IL, Interleukins; α-SMA, α-smooth muscle actin


3. Alcala M, Sanchez-Vera I, Sevillano J, Herrero L, Serra D, Ramos MP, and Viana M. Vitamin E reduces adipose tissue fibrosis, inflammation, and oxidative stress and improves metabolic profile in obesity. Obesity (Silver Spring) 2015.


17. Estrera VT, Chen DT, Luo W, Hixson DC, and Lin SH. Signal transduction by the CEACAM1 tumor


Figures Legends

Figure 1. Effect of HF diet on blood pressure and urine albumin to creatinine ratio in 
*Cc1*+/+ and *Cc1*−/− mice. A. Blood pressure; B. urine albumin to creatinine ratio. WT:
CEACAM1 wild type (*Cc1*+/+); KO: CEACAM1 knockout mice (*Cc1*−/−); RD: Regular diet;
HF: High fat. n= 6 mice per group. Values are mean ± SEM.

Figure 2. Effect of HF diet on mRNA and protein levels of AGT and Renin in the kidney 
of *Cc1*+/+ and *Cc1*−/− mice. A. Real time PCR analysis of AGT mRNA expression in whole 
kidney lysates; B. Western blot analysis of AGT protein expression in whole kidney lysates;
C. Real time PCR analysis of renin mRNA expression in whole kidney lysates; D. Western 
blot analysis of renin protein in whole kidney lysates. WT: CEACAM1 wild type (*Cc1*+/+);
KO: CEACAM1 knockout mice (*Cc1*−/−); RD: Regular diet; HF: High fat; AGT:
Angiotensinogen; REN: Renin. n= 6 mice per group. Values are mean ± SEM.

Figure 3. Effect of HF diet on PRR mRNA and protein level in the kidney of *Cc1*+/+ and
*Cc1*−/− mice. A. Real time PCR analysis of PRR mRNA expression in whole kidney lysates; B.
Reconstructed western blot analysis as representative of PRR protein expression in whole 
kidney lysates, one band was chosen randomly from each treatment group; WT: CEACAM1 
wild type (*Cc1*+/+); KO: CEACAM1 knockout mice (*Cc1*−/−); RD: Regular diet; HF: High fat;
PRR: (pro)renin receptor. n= 6 mice per group. Values are mean ± SEM.

Figure 4. Effect of HF diet on ACE mRNA and protein levels in the kidneys of *Cc1*+/+ 
and *Cc1*−/− mice. A. Real time PCR analysis of ACE mRNA expression in kidney lysates; B.
Western blot analysis of ACE protein expression in whole kidney lysates; WT: CEACAM1 wild type (Cc1+/+); KO: CEACAM1 knockout mice (Cc1−/−); RD: Regular diet; HF: High fat; ACE: angiotensin-converting enzymes. n= 6 mice per group. Values are mean ± SEM.

Figure 5. Effect of HF diet on RIF Ang levels in of Cc1+/+ and Cc1−/− mice. A. RIF Ang II. WT: CEACAM1 wild type (Cc1+/+); KO: CEACAM1 knockout mice (Cc1−/−); RD: Regular diet; HF: High fat; Ang II: Angiotensin II; RIF: Renal interstitial fluid. n= 6 mice per group. Values are mean ± SEM.

Figure 6. Effect of HF diet on AT1R mRNA and protein level in the kidney of Cc1+/+ and Cc1−/− 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; WT: CEACAM1 wild type (Cc1+/+); KO: CEACAM1 knockout mice (Cc1−/−); RD: Regular diet; HF: High fat; AT1R: angiotensin II type 1 receptor. n= 6 mice per group. Values are mean ± SEM.

Figure 7. Immunostaining of phospho-PI3K p85α in the kidney of Cc1+/+ and Cc1−/− mice. A. Immunostaining of Phospho-PI3K p85α (Tyr508) in the kidneys of Cc1+/+ and Cc1−/− mice fed regular or high fat diet. WT: CEACAM1 wild type (Cc1+/+); KO: CEACAM1 knockout mice (Cc1−/−); RD: Regular diet; HF: High fat; PT: Proximal tubule; DT: Distal tubule; CD: Collecting ducts. n= 6 mice per group.
Figure 8. Immunostaining of collagen I and collagen III in the kidney of male $Cc1^{+/+}$ and $Cc1^{-/-}$ mice. A. Immunostaining of collagen I in the kidneys of $Cc1^{+/+}$ and $Cc1^{-/-}$ mice fed regular or high fat diet. B. Typical immunostaining of collagen III in the kidneys of $Cc1^{+/+}$ and $Cc1^{-/-}$ mice fed regular or high fat diet. WT: CEACAM1 wild type ($Cc1^{+/+}$); KO: CEACAM1 knockout mice ($Cc1^{-/-}$); RD: Regular diet; HF: High fat; PT: Proximal tubule. n=6 mice per group.
The graph illustrates the levels of Ang II in RIF (pg/ml) across different conditions: WTRD, WTHF, KORD, and KOHF. The bars indicate the concentration levels with error bars for variability. Significant differences are marked with P-values: P<0.05, P<0.01. The data shows a trend with KOHF having the highest Ang II levels compared to the other conditions.
Immunostaining of Collagen I and III in Mice Kidney

A

<table>
<thead>
<tr>
<th></th>
<th>WT-RD</th>
<th>WT-HF</th>
<th>KO-RD</th>
<th>KO-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
<td><img src="image4" alt="Image" /></td>
</tr>
<tr>
<td>PT</td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
<td><img src="image7" alt="Image" /></td>
<td><img src="image8" alt="Image" /></td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th></th>
<th>WT-RD</th>
<th>WT-HF</th>
<th>KO-RD</th>
<th>KO-HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomeruli</td>
<td><img src="image9" alt="Image" /></td>
<td><img src="image10" alt="Image" /></td>
<td><img src="image11" alt="Image" /></td>
<td><img src="image12" alt="Image" /></td>
</tr>
<tr>
<td>PT</td>
<td><img src="image13" alt="Image" /></td>
<td><img src="image14" alt="Image" /></td>
<td><img src="image15" alt="Image" /></td>
<td><img src="image16" alt="Image" /></td>
</tr>
</tbody>
</table>