AMP-activated protein kinase activation ameliorates eicosanoid dysregulation in high-fat–induced kidney disease in mice

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Running title: AMPK ameliorates eicosanoids in HFD-induced CKD

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Keywords: Obesity, eicosanoids, chronic kidney disease, high fat diet, AMPK, AICAR
**Abstract**

High-fat diet (HFD) causes renal lipotoxicity that is ameliorated with AMPK activation. Although bioactive eicosanoids increase with HFD and are essential in regulation of renal disease, their role in the inflammatory response to HFD-induced kidney disease and their modulation by AMPK activation remain unexplored. In a mouse model, we explored the effects of HFD on eicosanoid synthesis and the role of AMPK activation in ameliorating these changes. We used targeted lipidomic profiling with quantitative mass spectrometry to determine PUFA and eicosanoid content in kidneys, urine, renal arterial and venous circulation. HFD increased phospholipase expression as well as the total and free pro-inflammatory AA and anti-inflammatory DHA in kidneys. Consistent with the parent PUFA levels, the AA-and DHA-derived lipoxygenase (LOX), cytochrome P450, and nonenzymatic degradation (NE) metabolites increased in kidneys with HFD, while EPA-derived LOX and NE metabolites decreased. Conversely, treatment with AICAR, AMPK activator, reduced the free AA and DHA content and the DHA-derived metabolites in kidney. Interestingly, both kidney and circulating AA, AA metabolites, and EPA-derived LOX and NE metabolites are increased with HFD; whereas, DHA metabolites are increased in kidney in contrast to their decreased circulating levels with HFD. Together, these changes showcase HFD-induced pro- and anti-inflammatory eicosanoid dysregulation and highlight the role of AMPK in correcting HFD-induced dysregulated eicosanoid pathways.
INTRODUCTION

The prevalence of obesity has continued to rise over the past few decades and was 36.5% among US adults between the years 2011 to 2014 (1). Obesity serves as a significant risk factor both for the initiation and progression of kidney disease independent of hypertension and diabetes (2-4). Excessive intake of calorie-dense lipids leads to organ dysfunction both by direct lipotoxicity and inflammation. In fact, the western diet enriched in saturated animal fats has been shown to increase albuminuria and cause a faster decline in renal function (5). In our previous studies, we established that HFD induced kidney disease is characterized by renal hypertrophy, increased albuminuria and elevated markers of renal fibrosis and inflammation (6). These HFD-induced markers of inflammation, oxidative stress, and fibrosis are reversed by AMP-activated protein kinase (AMPK) activation (6, 7).

HFD alters the activity of crucial lipid metabolism enzymes Acetyl-CoA carboxylase (ACC) and HMG-CoA reductase (HMGCR) contributing to lipid accumulation in the kidney (6). Total cholesterol esters and phosphatidylcholine content in the kidney are elevated while the fatty acid and triglyceride content is unchanged. Phospholipid accumulation in the proximal tubules is associated with lysosomal dysfunction, stagnant autophagic flux, mitochondrial dysfunction and inflammasome activation (8). High-fat feeding for long periods causes recruitment of macrophages, switch to macrophage pro-inflammatory phenotype and increased inflammatory mediators like TNFα, MCP-1, IL-6, COX-2 and IL-1β (9-12). Diets rich in polyunsaturated fatty acids (PUFAs) are known to change plasma lipids, renal phospholipid content, and subsequently PUFA-derived eicosanoid inflammation in a rat model of nephrotic syndrome (13). However, the influence of renal
Eicosanoid synthesis and eicosanoid derived inflammation in HFD induced kidney disease is unknown.

Eicosanoids are oxylipins derived from arachidonic acid (AA) or related PUFAs and are inextricably related to inflammation in the kidney. The primary PUFAs for the n-6 series and n-3 series, Linoleic acid (LA) and alpha-linolenic acid (ALA) respectively, are both derived from the diet. These 18-carbon PUFAs are then metabolized by various desaturase and elongase enzymes in a stepwise fashion. However, both LA or ALA are acted on by the same enzymes, resulting in a competition between the n-3 and n-6 series (14). LA is metabolized through multiple steps to dihomo-γ-linolenic acid (DGLA, 20:3n6) and, ultimately, to AA (20:4n6). On the other hand, ALA is metabolized to eicosapentaenoic acid (EPA, 20:5 n-3) and subsequently to docosahexaenoic acid (DHA, 22:6 n-3) (15). These PUFAs are incorporated into membrane phospholipids and released by phospholipase A2 (PLA2) under the influence of various stimuli. In subsequent reactions, cyclooxygenases (COX), lipoxygenases (LOX), cytochrome P450 (P450) enzymes act on free PUFAs to form eicosanoids. Some eicosanoids can also be formed from PUFAs via non-enzymatic reactions (NE), e.g., isoprostanes.

Eicosanoids play an essential role in the regulation of renal physiology and disease by modulating renal blood flow, glomerular filtration rate, autoregulation, tubular glomerular feedback, excretion of renal water and sodium, and release of renin and erythropoietin. HFD feeding causes an increase in circulating eicosanoids. In the kidney, these eicosanoids are produced by all different cell types – mesangial cells, renal microvessels, and tubular cells. This makes it difficult to pinpoint the actual origin of these autacoids without actual profiling of the various compartments. Local production in the
kidney will be reflected in the kidney tissue, renal venous compartment, and urine. Recent advances in eicosanoid analysis using highly sensitive mass spectrometry (MS) has enabled us to profile over 150 different eicosanoid metabolites reliably in all tissues, enabling us to systematically profile the changes in the metabolic pathways with HFD and AICAR therapy.

AMPK is a ubiquitous, heterotrimeric kinase that acts as a cellular energy sensor that responds to changes in the intracellular AMP/ATP ratio (16). AICAR (5-aminoimidazole-4-carboxamide-1-β-D-furanosyl 5’-monophosphate) acts as a specific AMPK agonist (17). AMPK activation leads to inhibition of energy-requiring biochemical processes like fatty acid synthesis and stimulation of energy-producing biochemical pathways like beta-oxidation to improve energy efficiency (18). Metabolic stress such as diabetes or obesity impair the activity of AMPK, and AMPK activation reduces the initial and sustained inflammatory response in the kidney of the HFD-induced kidney disease model (6). Along with lipid accumulation, the markers of inflammation were modulated with AICAR use (7). AMPK signaling has been shown to influence the secretory PLA2 expression in vascular smooth muscle cells (19) and control triglyceride content in adipocytes (20). AMPK activation also decreases the formation of 15-LOX metabolites of AA in macrophages (21). While AMPK activation is beneficial in lipid and eicosanoid metabolism in other tissues, the effect of HFD and AMPK activation on eicosanoid pathways in the kidney is unknown. We hypothesized that the high-fat exposure triggers inflammation involving the eicosanoid pathway and that eicosanoid production is ameliorated with AMPK activation. We used a targeted lipidomic platform to systematically investigate the HFD-associated eicosanoid synthesis induced in mice consuming HFD with or without AMPK activation in
order to better understand the pathophysiological processes involved in the HFD-induced kidney disease.

**RESULTS**

*HFD increased phospholipase expression and free levels of polyunsaturated fatty acids and this action is reversed by AMPK activation.*

Both cytosolic and secretory PLA2 act on membrane phospholipids to release PUFA’s-the precursor of various eicosanoids. Levels of cytosolic phospho-PLA2 (Group IV) and secretory PLA2 were both increased with HFD in the kidney, and AICAR treatment decreased their levels (Figure 1).

Targeted lipidomic analysis using MS of kidney tissue revealed unique patterns in the total, and free ω3 and ω6 PUFA series that are demonstrated in Figure 2. The total fatty acid (FA) (esterified and unesterified FA) and free FA (unesterified, FFA) profiles generated from the kidney of mice fed a low fat, standard chow (STD), HFD or HFD with the AMPK activator AICAR (HFD+AICAR) are displayed in Supplemental Table 1. The total and free EPA are decreased in the kidneys with the HFD (Figure 2A and B, p<0.05) and further decreased with AICAR, while both total and free DHA, a n3 fatty acid similar to EPA, is increased with HFD and reversed with the use of AICAR (Figure 2C and D, p<0.05). While DHA and EPA levels are modulated with HFD, the parent PUFA- ALA (total and unesterified) is unchanged.

Free and total n6 fatty acid DGLA is decreased in the kidney with HFD but unchanged with AICAR (Figure 2E and F). Total and free AA in the kidney are increased
by HFD and the free AA decreased with AICAR (Figure 2G and H). Free and total levels of LA, the precursor for both DGLA and AA are unchanged by the diet and AICAR in the kidney. Delta-6 desaturase (D6D) and delta-5 desaturase (D5D) catalyze desaturations at specific positions of FA substrates. Elongases extend the fatty acid carbon chains by two carbons. The levels of both desaturases were not different between the three groups (Supplementary Figure 1).

HFD dysregulates kidney eicosanoid metabolism that is ameliorated by AMPK activation.

The eicosanoid metabolites of AA, DHA and EPA are regulated with HFD in the kidney (Table 1), and specific metabolites are modulated with AICAR (Figure 3). HFD seems to create a clear increase in COX products of AA in the kidney and decrease in COX products of EPA. 12- hydroxyl-hexadecatrienoic acid (12HHT) which is a COX / P450 product is increased with HFD and decreased with AICAR (Figure 3A). P450 products of DHA – 16, 17-epoxy-docosapentaenoic acid (16,17-EpDPE) and its product 16-hydroxy-docosahexaenoic acid (16-HDoHE), and 19,20-epoxy-docosapentaenoic acid (19,20-EpDPE) and its product 19,20-dihydroxy-docosapentaenoic acid (19,20-DiHDPa) are increased with HFD, but only the end products 19,20-DiHDPa and 16-HDoHE are decreased with AICAR (Figure 3B and C). LOX products of DHA- hydroxy-docosahexaenoic acids (HDoHE): 4-HDoHE, 7-HDoHE, 11-HDoHE, and 17-HDoHE and its product -15 (t)-protectin D1 (15 (t)-PD1) are increased with HFD (Figure 3D, 3E, and 3F). NE degradation products of DHA- 8-HDoHE, 10-HDoHE, 13-HDoHE, and 20-HDoHE are increased with HFD and decreased with AICAR (Figure 3G and 3H).
**HFD and AMPK activation alter the urinary eicosanoid profiles.**

HFD induced changes in urine metabolites are illustrated in Table 2 and the metabolites that are altered with AICAR in Figure 4. The overall trend of the kidney continues in the urine with predominant increase of COX products of AA in the urine and decrease of COX products of EPA. Adrenic acid and its COX metabolite dihomo-prostaglandin PGF$_{2\alpha}$ (dihomo-PGF$_{2\alpha}$) are both increased with HFD in the urine but only dihomo-PGF$_{2\alpha}$ is decreased with AICAR (Figure 4A). Dihomo-15- deoxyprostaglandin D$_2$ (dihomo-15d-PGD$_2$) which is a NE byproduct of another Adrenic acid COX metabolite is decreased with HFD and increased with AICAR (Figure 4B).

Urine AA (Figure 4C) is increased with HFD and decreased with AICAR which is consistent with the trend in the kidney. Downstream COX metabolites of AA and prostaglandin H$_2$ (PGH$_2$) are altered with the HFD and AICAR. P450 metabolite of prostaglandin E$_2$ (PGE$_2$) metabolites - 13,14-dihydro-15-keto-prostaglandin E$_2$ (PGEM, Figure 4D) is increased with HFD and decreased with AICAR. Another PGH$_2$ metabolite-13,14-dihydro-5-keto-prostaglandin F$_{2\alpha}$ (PGFM, Figure 4E) and prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$, Figure 4F) both the products of COX are increased with HFD and decreased with HFD with AICAR. Prostaglandin D$_2$ (PGD$_2$) metabolite -prostaglandin J$_2$ (PGJ$_2$) is increased with HFD, while urinary 13,14-dihydro-15-keto-prostaglandin D$_2$ (dhk-PGD$_2$; Figure 4G) both COX metabolites of AA is increased with HFD and decreased with HFD with AICAR following the similar pattern in the kidney AA metabolite 5-iso-prostaglandin F$_{2\alpha}$-VI(5-iso-PGF$_{2\alpha}$-VI) (Figure 4H) which is NE product is increased with HFD and decreased with AICAR. Prostaglandin E$_3$ (PGE$_3$) is an EPA metabolite of COX action is
increased with HFD and decreased with AICAR (Figure 4I). COX metabolites of EPA - Thromboxane B$_3$ (TXB$_3$) is decreased with HFD and increased with AICAR (Figure 4J).

**HFD and AMPK activation modulate circulating eicosanoid levels**

In order to delineate the origin of the eicosanoids in the kidney, both the renal artery (Table 3) and vein (Table 4) were sampled after the mice were fed STD, HFD, and HFD with AICAR. The eicosanoid profile of the arterial and venous samples revealed that AA and its metabolites- 8,9, epoxy-eicosatrienoic acid (8,9 EET) -a product of P450 were both increased with HFD. 5,6- EET and 11,12-EET are increased in the venous circulation but not in the arterial circulation. Since these metabolites are also elevated in the kidney, this suggested the possible production of the metabolites in the kidney and released into the venous circulation. AA COX metabolite 6-keto-prostaglandin F$_{1\alpha}$ is elevated in the venous circulation with HFD – this is in line with the elevation of the downstream metabolite 6-keto-prostaglandin E$_1$ in the urine.

DHA metabolites- 20-HDoHE, 10-HDoHE, and 13-HDoHE -products of NE degradation are all decreased in the venous circulation. 11-hydroxy-docosahexaenoic acid (11-HDoHE) and 4-HDoHE -products of the LOX pathway while 16-HDoHE the product of P450 are all decreased with HFD in the venous circulation. 4-HDoHE and 20-HDoHE are also decreased with HFD in the arterial circulation.

EPA levels are decreased in both the arterial and venous circulation. EPA produces hydroxy-eicosapentaenoic acid’s (HEPE) –NE products, 18-HEPE, 11-HEPE, and 9-HEPE were decreased in the arterial and venous circulation. 5-HEPE, 12-HEPE, and 15-HEPE are produced from their respective LOXs and were decreased with HFD in both
the arterial and venous circulation. 15-HEPE is decreased further with AICAR therapy in the arterial circulation. P450 metabolite-14(15)-epoxy-eicosatetraenoic acid (14(15)-EpETE) is decreased with HFD diet in both arterial and venous circulation and further with AICAR in the arterial circulation.

LA metabolites of P450 -9,10-dihydroxy-octadecanoic acid (9,10-diHOME), 12,13-dihydroxy-octadecaenoic acid (12,13-diHOME), and 12,13-epoxy-octadecaenoic acid (12,13-EpOME) are decreased with HFD and are increased with AICAR in the arterial circulation (Figure 5A-C). Venous sampling revealed that 9-hydroxy-octatrienoic acid (9-HOTrE) is a product of LOX derived from ALA is decreased with HFD but increased with AICAR (Figure 5D). 5-HEPE is LOX metabolites of EPA is decreased with HFD and increased with AICAR treatment (Figure 5E).

Specific eicosanoids are altered by AMPK activation

When taken as a whole, there were significant changes in eicosanoid pathways in arterial and venous circulation, urine and kidney compartments (Supplementary Table 2). Figure 6 depicts the changes influenced by AICAR therapy on HFD mice. To further detect trends in the changes, the highly correlated eicosanoids derived from a parent PUFA and acted on by a specific enzymatic pathways were collapsed in to principal components and analyzed for trends and statistical significance. Table 5 represents the trends in the kidney and urine of these highly correlated metabolites. In the kidney, HFD decreased EPA metabolites across all enzymatic pathways, while the COX metabolites alone were reversed by AICAR. Meanwhile AA metabolites of urine were mostly elevated with HFD, whereas the COX EPA metabolites are decreased with HFD and this is
reversed with AICAR therapy. COX metabolites of Adrenic acid are decreased with HFD in the urine and this was reversed with AICAR. **Table 6** reveals that EPA, DHA and ALA metabolites were all decreased with HFD in the arterial and venous blood.

**DISCUSSION**

This is the first study to systematically explore the effects of HFD on eicosanoid synthesis in the kidney and the role of AMPK activation in ameliorating these changes. We find that HFD increases phospholipase A2 expression and activity (through phosphorylation) in the kidney that is largely corrected by AMPK activation. HFD increases both the pro-inflammatory AA and the anti-inflammatory DHA, and their respective downstream products in the kidney and urine and these changes are reversed with AICAR therapy. However, HFD decreases EPA, DGLA, and LOX and NE metabolites of EPA, while AICAR therapy decreases free EPA levels further in the kidney. P450 and NE of LA were decreased in the arterial circulation with HFD are returned to pre-HFD levels with AICAR. These changes highlight the changes in eicosanoid pathways in the kidney with HFD that are ameliorated by AICAR therapy.

Obesity and metabolic syndrome patients have demonstrated altered fatty acid and eicosanoid metabolism compared to lean subjects. Increased serum DGLA levels and low delta-5 desaturase activity was associated with hepatic steatosis over and above conventional risk factors in metabolic syndrome subjects (22). Like our HFD mouse model, COX, LOX, and P450 metabolites of AA and other PUFA’s are elevated in the plasma of obese subjects compared to lean subjects (23). Increased TXA$_2$ can cause vasoconstriction and induce transcription of collagen in the glomerular matrix. In contrast,
PGI$_2$ counteracts the deleterious TXA$_2$ effects associated with progressive glomerular damage by acting as a vasodilator. Decreased urinary PGI$_2$/TXA$_2$ ratio has been demonstrated in diabetic humans compared to controls (24). In the Japanese population, urinary 8-iso-PGF$_{2\alpha}$ which is a AA metabolite predicts metabolic risks like obesity, hypertension, and glucose tolerance (25). Meanwhile, low serum lipoxin A4 levels (AA metabolite) are associated with metabolic syndrome risk in Chinese population, similar to the elevated kidney levels of lipoxin A4 and its metabolite in our study (26).

Obesity-induced kidney disease is characterized by glomerular enlargement, podocytes loss, and proteinuria eventually leading renal failure (2, 27, 28). In animal models, HFD increased kidney weight and glomerular area with Periodic–Acid-Schiff (PAS) positive matrix and increased Oil red-O positive vacuolated cells. HFD also caused albuminuria, increased glomerular inflammation via the NF$\kappa$B pathway and increased urinary monocyte chemoattractant protein (MCP-1) and hydrogen peroxide (6, 29). Increased fat intake increases kidney triglyceride content via increased sterol regulatory element proteins -1 and 2 and lipogenesis along with reduced AMPK and increased ACC expression and activity in mice (30) (7, 10). Also, HFD decreased renal lipolysis along with decreased carnitine palmitoyl acyl-CoA transferase-1 expression (31). Mice fed with western diet demonstrated vacuolization of the proximal tubule enriched with multi-lamellar lysosomal bodies with phospholipids the phenomenon was confirmed with human biopsies (32). Human studies have described the accumulation of ectopic lipid in obesity-induced kidney disease (4, 33). Mice lacking the innate immune receptor Nlrp3 did not develop the pathological markers of HFD induced kidney disease indicating
pathways regulated by Nlrp3 could control the development of HFD induced renal pathology (34). Clearly, HFD causes dysregulation of mainstream lipid metabolism in the kidney, but the gateway for many inflammatory mediator’s eicosanoid generation with HFD has not been clearly examined.

“The first and rate-limiting step of eicosanoid synthesis is the liberation of PUFAs from their esterified form in the sn-2 position of membrane phospholipids by cytosolic phospholipase. In our study, both the cytosolic (Group IV) and secretory phospholipase A2 were elevated with HFD and decreased with AICAR. Cytosolic PLA2 is essential for adipocyte differentiation in obesity models (35). Cytosolic PLA2 (Group IV) deficient mice are resistant to the hepatic effects of high-fat diet due to the decrease in the production of downstream products confirming the crucial role of this enzyme in high induced fat disease (36). Cytosolic PLA2 is also central to oxidative signaling in renal epithelial cells releasing arachidonic acids and its downstream products (37). Isoenzymes of phospholipase A2 are differentially expressed in various tissues in response to inflammation; for example, expression of group VII phospholipase A2 gene is increased in livers of Zucker obese rats, while expression of group IVA and VIA phospholipase A2 was inhibited (38). Phospholipase A2 isoenzymes also demonstrate substrate selectivity to certain forms of phospholipids in membranes and specific PUFA’s (39). Kidney desaturases and elongases that are responsible for conversion between the various PUFA’s are unaffected by HFD or AMPK activation in our study. However, livers of obese Zucker rats demonstrated increased expression of delta-6 desaturase and elongase-6 (38). Diets in the HFD and STD groups had similar n6 to n3 ratios, and the pattern in the
circulation and the kidney do not reflect this ratio. AA is increased in the circulation, kidney, and urine with HFD in our study and this is consistent with increased circulating PUFA's demonstrated with HFD feeding in Sprague-Dawley rats (40). In excess nutrient states, phospholipase D suppresses AMPK activity through the mammalian/mechanistic target of rapamycin (mTOR); in return, AMPK activation decreased phospholipase D activity similar to the effect of AMPK activation on other phospholipases in vascular smooth muscles, and endothelial cells (19, 41, 42). Thus, the effect of AMPK activation on phospholipases in our study could be responsible for the changes to the PUFA levels with AICAR therapy.

Both COX-1 and COX-2 and their downstream products PGD₂, PGE₂, and PGI₂ are involved in renal vasodilation. PGE₂ is the central prostaglandin synthesized in the kidney tubules and mesangial cells- and activation of its receptors cause contraction of vascular muscle cells, increased calcium in mesangial cells and inhibits sodium reabsorption at the thick ascending limb of the loop of Henle (43). In our study, PGE₂ is decreased in the kidney with HFD, but its metabolites- PGA₂, PGB₂, and PGEM are increased in the urine with HFD. PGI₂ metabolites- 6,15 dk-, dh- PGF₁α and 6k-PGE₁ are increased in the urine with HFD similar to PGD₂ metabolites – PGJ₂ and dhkPGD₂. Urinary AA-derived eicosanoids, primarily downstream of COX metabolite-PGH₂ were increased with HFD and decreased with AICAR therapy. Increased flux in these pathways possibly results in decreased bioavailability of the renal vasodilators. In line with our findings, COX-2 expression was increased in renal cortex, blood vessels and in the urine in obese Zucker rats (44). Increased urinary thromboxane B₂ (TXB₂) and 6-keto PGF₁α and
decreased PGE\(_2\) excretion rates were evident in obese Zucker rats. Similarly, decreased urinary PG\(_{12}/\)TXA\(_2\) ratio has been demonstrated in diabetic humans as well as in diabetic animal models. Rofecoxib, a COX-2 inhibitor, decreases vascular and glomerular damage when administered to obese Zucker rats (24). Decreased PGF\(_{2\alpha}\) and 8- iso-PGF\(_{2\alpha}\) levels contribute to the decreased glomerulosclerosis in obese Zucker rats treated with rofecoxib. COX-2 inhibition and decreases in 8- iso-PGF\(_{2\alpha}\) levels have also been shown to ameliorate renal injury associated with hypertensive rats (45). This pattern is also reflected in the urinary levels of 8- iso-PGF\(_{2\alpha}\) in our mouse model of HFD induced kidney disease.

Renal P450 epoxidation generates epoxyeicosatrienoic acids (EETs) that are metabolized by soluble epoxide hydrolase (sEH) to less active dihydroxyeicosatrienoic acids (DHETEs). Decreased renal expression of CYP2C epoxygenase enzymes has been observed in diabetes, high fat diet fed insulin resistance rats, and the kidney and mesenteric blood vessels of obese Zucker rats (46). Additionally, sEH expression is increased in blood vessels of obese rats and could further contribute to the decrease in EET bioavailability implicating impaired endothelial dilator responses in obesity and diabetes. Cytokines- IL-6 and TNF-\(\alpha\) decrease expression of P450 epoxygenase enzymes. TNF-\(\alpha\) and EET’s act via NF\(\kappa\)B pathway causing elevated MCP-1 levels that have been observed in HFD induced kidney disease in mice (6). Obese Zucker rats have increased renal vascular CYP4A expression and increased generation of 20-HETE (47). However, renal tubular CYP4A expression was decreased in Sprague-Dawley rats on HFD (48). AA metabolites -20-HETE, EET, and DHETE are decreased with HFD in the
kidney and associated with the diet-induced change in blood pressure and renal function of Sprague-Dawley rats (48, 49). In our study, the kidney P450 products of AA-9-HETE and EET’s are increased with HFD just as many of the EET’s are also increased in the circulation and urine. Pharmacological inhibition or transgenic deletion of sEH, the dominant enzyme in degrading EETs, have been shown to protect mice from various adverse effects induced by obesity (50). Thus, protective eicosanoid metabolites of AA-EET are increased with HFD in the kidney. In HFD fed mice, plasma profiling revealed increases in circulating P450 metabolite of LA-EpOME’s (51) which is a different trend to the one observed in the arterial circulation of our HFD mice maintained on HFD for a longer period.

Central adipose tissue in obese Zucker rats shows increased LOX expression along with inflammatory markers (52). 5-LOX products induce NF-kappaB expression and secretion of inflammatory cytokines like MCP-1, TNF-α, macrophage inflammatory protein-1gamma, and IL-6 by the adipose tissue. The 5-LOX pathway plays a major role in obesity-induced fatty liver disease (53). In our study, LOX metabolites of AA, AA metabolites- 6(R),15 (R)-LXA<sub>4</sub> and 6(S)-LXA<sub>4</sub> are increased in the kidney with HFD in our study, and LXA<sub>4</sub> has been shown by our group to attenuate inflammation in HFD related kidney disease (10). Similarly, LOX metabolites of DHA – HDoHE were elevated in the kidney, whereas LOX metabolites of EPA –HEPE’s were all decreased in the kidney and the circulation.

Omega 3 fatty acids – DHA and EPA supplementation in ob/ob mice induced AMPK phosphorylation and alleviated hepatic steatosis. Omega 3 supplementation is
anti-inflammatory, promotes insulin sensitivity and raises n3 fatty acid-derived resolvins and protectins (54). Obesity significantly decreased DHA-derived 17-hydroxydocosahexaenoic acid (17-HDHA, resolvin D1 precursor) and protectin D1 (PD1) levels in murine adipose tissue. Notably, 17-HDHA treatment reduced adipose tissue expression of inflammatory cytokines, increased adiponectin expression, and improved glucose tolerance parallel to insulin sensitivity in obese mice (55). A concentrated formulation of n3 PUFAs attenuates albuminuria, renal function, SREBP-1 expression and triglyceride levels in the kidney of type 2 diabetic obese db/db mice (56). DHA and DHA metabolites in the kidney, the various HDoHE’s, EpDPE and DiHDPA produced by P450, LOX and NE in the kidney are all increased with HFD in our study. While the kidney and urine DHA metabolites were increased, the circulatory DHA metabolites were decreased- suggesting local production in the kidney. With HFD exposure, DHA metabolites -15 (t)- PD$_1$ and PDX are both increased in the kidney and urine respectively. In our study, n3 EPA metabolites – HEPE’s were all decreased in the kidney, urine, and circulation. EPA metabolites - PGH$_3$ and its hydroxyl metabolites were all decreased in the circulation with HFD. Downregulation of HEPE’s in our study represents suppression of anti-inflammatory signals.

AMPK is a nutrient sensor and is inhibited by nutrient excess states. In our prior studies, the effects of HFD induced kidney disease were reversed with AMPK activation with AICAR therapy. AMPK activation reversed the inflammatory profile, lipid accumulation, and low adiponectin levels that were caused by HFD (9, 10, 57). Diet reversal alone was unable to improve the renal inflammation and apoptosis in HFD induced kidney damage, emphasizing the persistence of renal injury even after eight
weeks of dietary control (58). However, AMPK activation with metformin treatment was able to reduce the levels of inflammatory and apoptotic markers that remain residual despite diet reversal. Also, metformin ameliorated HFD induced glomerular injury, renal fatty acid oxidation, and serum adipokine levels (58-60). Metformin reduces fat content by decreasing sterol regulatory element-binding protein 1 (SREBP-1), fatty acid synthase (FAS), and acetyl-CoA carboxylase (ACC) expression in the kidney (61). In our study, AICAR reversed the upregulation of phospholipase and free PUFA’s. The influence of AICAR is evident in reversing the increased hydroxyl DHA metabolites in the kidney and COX metabolites of AA in the urine. In the venous circulation, AICAR reverses the downregulation of P450 metabolite of LA- 9-HOTrE and LOX metabolite of LA- 5-HEPE. HFD has been previously shown to increase leukotoxins- EpOME, while in our study, EpOME metabolites are decreased (51). AICAR also reverses the downregulation of 9,10 DiHOME, 12, 13-DiHOME, and 12,13-EpOME in the arterial circulation in our study. Thus, AMPK activation is able to reverse key pathways in eicosanoid metabolism and helps ameliorate changes resulting from HFD.

In this current study, we demonstrate modulation of PUFA’s and their metabolites after HFD as well as AMPK activation in the kidney, but we cannot delineate the origin of the various eicosanoids in the circulation and urine which could be from the diet, liver, muscle, pancreas and/or gut microbiota. Again, the changes in the eicosanoids might be influenced by the upstream action of phospholipases on specific PUFA’s whose pattern might be reflective of the specific diet; Although both the diets had a similar n6: n3 ratio, the specific composition of HFD might have influenced the eicosanoid changes. Also, as the influence of AICAR is not restricted to one specific oxygenase or PUFA, its influence
on phospholipases might influence its action to follow the pattern of the predominant PUFA availability in various compartments. Though we have independently demonstrated the effects of HFD on the kidney pathology, lipid, and inflammatory profile, we cannot conclusively provide evidence that the eicosanoid changes are the sole cause of the HFD induced kidney disease or the reason for the AICAR induced amelioration. Also, eicosanoids might have differential changes based on temporal exposure to HFD, and this was not conclusively studied in our study. It is also difficult to tease out the source of inflammation in such models as both HFD and AICAR therapy, in addition to altering inflammation also influence body weight. Other factor to account for is that pharmacologic AMPK inhibition might not be complete, and genetic manipulation of AMPK might provide a clearer picture. Genetic manipulation of the pathways clearly influenced by AMPK will help us study downstream and upstream changes and their interaction with the eicosanoid pathways and link them to renal pathology.

In summary, in this study using a targeted lipidomic approach, we demonstrate the dysregulation of eicosanoid synthesis and metabolism in the kidney with HFD and its amelioration with AMPK activation. Our work sheds light on the mechanism behind HFD related toxicity and the eicosanoid pathways under the control of AMPK activation in the kidney. We also expose the various pro- and anti-inflammatory mechanisms in HFD mediated kidney disease that are altered with AMPK activation. Future studies manipulating these dysregulated pathways might open therapeutic avenues in the management of high fat induced kidney disease.
**CONCISE METHODS**

**Animals.** All animal procedures were approved by the Institutional Animal Care and Use Committee of University of California, San Diego. Male six week old C57BL/6 mice were purchased from Jackson Laboratory (Bar Harbor, ME) were fed a standard diet or a high-fat diet (STD - 5% fat (PUFA 2.1%; n6 1.9%; n3 0.2%), 24.5% protein, 40% carbohydrate) [HFD - 60% of total calories from fat (90% lard + 10% soybean oil, PUFA 16.9%; n6 15.1%; n3 1.7%), 20% protein, 20% carbohydrate] (D12492, Research Diets, New Brunswick, NJ) for 14 weeks. The mice on the STD diet were treated with phosphate buffered solution (PBS) and mice on HFD were treated with 5-Aminoimidazole-4-carboxamide-1-ȫ-D-ribofuranoside (AICAR, 0.5 mg/g body weight, Toronto Chemicals) or PBS via intraperitoneal injections for five days a week for a total of 14 weeks (6). Mice were placed in metabolic cages for 24-h urine collection before the start of the diets and after 14 weeks. Mice were sacrificed after 14 weeks of diet plasma was collected from the renal vein, and arterial blood was obtained from direct cardiac puncture. After perfusion with PBS, kidneys were snap-frozen in liquid nitrogen for further analysis.

**Quantitative total and free fatty acids analysis:** Total and free fatty acids were analyzed by gas chromatography/mass spectrometry (GC-MS) as previously described (62, 63). Briefly, kidney tissue (~10 mg of tissue) was homogenized in 1 mL of PBS containing 10% methanol. For the analysis of total (esterified and free) fatty acids, kidney homogenates (50µL) were spiked with deuterium-labeled fatty acids, acidified with 200 µL of 0.1 N hydrochloric acid and extracted with 500 µL methanol. Phase separation was achieved by the addition of 500 µL of dichloromethane (CH₂Cl₂), and the organic phase
was removed. The extraction with dichloromethane was repeated, and the combined extracts were dried under argon gas. For saponification, the dried lipids were resuspended in 250 μL methanol and 250 μL 4N potassium hydroxide, vortexed for 30 sec and incubated at 37°C for 1 hr. The lipid hydrolysates were then neutralized with 260 μL 4N HCl and the free fatty acids were extracted twice with 2 mL isoctane and the combined extracts containing the hydrolyzed fatty acids were dried under argon before analysis with GC/MS.

Free fatty acids were extracted from 50 μL of the kidney tissue homogenates that was supplemented with a set of deuterated fatty acids that served as internal standards. The extraction was initiated by the addition of 25 μL 1N hydrochloric acid, and 500 μL methanol and a bi-phasic solution were formed by the addition of 1 mL isoctane. The isoctane phase containing the free fatty acid fraction is removed, the extraction is repeated once more, and the combined extracts are dried under argon before analysis with the GC/MS.

In preparation for GC-MS analysis, the fatty acids—both total fatty acid after saponification and free fatty acid extracted from samples and the quantitative standards were taken up in 25 μL of 1% diisopropylethylamine in acetonitrile and derivatized with 25 μL 1% pentafluorobenzyl bromide. The fatty acid esters were analyzed on an Agilent 6890N gas chromatograph equipped with an Agilent 5973 mass selective detector (Agilent, Santa Clara, CA) operated in the negative chemical ionization mode. Fatty acid quantitation was achieved by the stable isotope dilution method.
**Quantitative eicosanoid analysis:** Eicosanoids were analyzed by liquid chromatography-MS (LC-MS), as described before (63, 64). For tissue analysis, eicosanoids were isolated from 0.9 mL of kidney homogenate supplemented with a set of internal standards consisting of 26 deuterated eicosanoids (Cayman Chemical) by solid phase extraction (Strata-X, Phenomenex, Torrance, CA) and eluted into 1 mL methanol. For eicosanoid analysis in urine, 600 μL of sample were processed identically. The extracted samples were evaporated, reconstituted in 100 μL and a 40 μL aliquot was separated by reverse phase liquid chromatography (LC) using a Synergy C18 column (2.1 x 250 mm, 4 μm; Phenomenex, Torrance, CA). Eicosanoids were analyzed using a tandem quadrupole mass spectrometer (MDS SCIEX 4000 Q Trap; Applied Biosystems, Foster City, CA) via scheduled multiple-reaction monitoring (MRM) in the negative ionization mode. Eicosanoids were identified in samples by matching the MRM signal and LC retention times with those of pure standards. Data analysis was performed using MultiQuant 2.1 software (Applied Biosystems). Quantitative eicosanoid determination was performed using the stable isotope dilution method.

**Immunoblot.** Kidney tissue homogenates were electrophoretically separated on NuPAGE bis-Tris gels (Life Technologies, USA) and transferred onto nitrocellulose membrane (Life Technologies, USA). Antibodies to ELOLV5, cytosolic phospho-phospholipase A2 and secretory phospholipase 2 (Abcam, USA), FADS1/FADS2 (Biorbyt, UK) and actin (Sigma, USA) were used on the blots. Detection was performed with ECL Plus detection reagents (GE Healthcare, USA) after treatment with appropriate secondary antibody treatment.
Statistical analysis. Results are presented as mean values ± standard deviation. The kidney values were normalized with tissue weight and plasma were normalized to volume. Urinary metabolites are all normalized to urine creatinine values. All metabolites were log transformed and auto-scaled before undergoing statistical analysis using the MetaboAnalyst version 3.0 software, Canada (65). Figures were generated using GraphPad Prism Software version 4.03, California, USA. Difference between data groups was evaluated for significance using two-way ANOVA and Fisher’s least significant difference method (Fisher’s LSD) post-hoc tests for multiple comparisons. A p-value less than 0.05 was defined as statistically significant in the non-mass spectrometry experiments. To account for multiple comparisons with a large number of lipid metabolites, false discovery rate (FDR, q value) of <0.05 was considered statistically significant. The highly correlated metabolites were reduced based on eicosanoids derived from a single enzyme pathway from specific parent fatty acid in each tissue compartment were separately aggregated into one secondary variable representative of the corresponding pathway using principal component (PC) analysis. We have then used the PC explaining the highest variance in the variable for further analysis to detect trends using SPSS Version 25, IBM corp (66).

ACKNOWLEDGMENTS
The authors would like to acknowledge the support of grants from the Back to Belgium Grant from the Belgian Federal Science Policy (AED, BELSPO, Belgium); NHLBI K08HL130944 (AVM); DK082841 (KS), DP3DK094532 award (KS); R01DK105961(EAD); and P30DK063491 (EAD, OQ); NIGMS R01GM20501(EAD).
COMPETING INTERESTS

Nothing to declare

AUTHOR CONTRIBUTION

All authors have approved the final manuscript. Among the authors, AED and KS have conceived the experiments; AMA, OQ, and AED have performed the experiments; SP, ED, OQ, and KS have overseen the experiments which were performed in their respective laboratories; AVM and AED conducted all statistical analysis; KS, AVM, and AED have prepared the manuscript.

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### Table 1: Eicosanoid metabolites altered with high-fat diet in the kidney

| Name | Acronym | MECH | STD | HFD | p value | q value |
|------|---------|------|-----|-----|---------|---------|
| **Up with HFD** | | | | | | |
| **Arachidonic acid metabolites** | | | | | | |
| Arachidonic acid | AA | PUFA | 243.4±19.2 | 312.7±40.9 | 0.010 | 0.037 |
| 13,14-dihydro-15-keto-prostaglandin D₂ | dhk-PGD₂ | COX | 0.04 ± 0.01 | 1.20 ± 0.02 | 0.008 | 0.035 |
| 5,6-epoxy-eicosatrienoic acid | 5,6-EET | P450 | 1.59 ± 0.74 | 8.64 ± 5.64 | 0.014 | 0.045 |
| 11,12-epoxy-eicosatrienoic acid | 11,12-EET | P450 | 0.02 ± 0.01 | 0.18 ± 0.13 | 0.015 | 0.045 |
| 5-oxo-eicosatetraenoic acid | 5-oxoETE | LOX | 0.22 ± 0.11 | 1.56 ± 1.21 | 0.013 | 0.043 |
| 6(R),15(R)-lipoxin A₄ | 6(R),15(R)-LXA₄ | LOX | 0.02 ± 0.01 | 0.05 ± 0.02 | 0.006 | 0.032 |
| 6(S)-lipoxin A₄ | 6SLXA₄ | LOX | 0.02 ± 0.01 | 0.05 ± 0.02 | 0.015 | 0.045 |
| 5-oxo-eicosatetraenoic acid | 5-oxoETE | LOX | 0.22 ± 0.11 | 1.56 ± 1.21 | 0.013 | 0.043 |
| **Docosahexaenoic acid metabolites** | | | | | | |
| 16,17-epoxy-docosapentenoic acid | 16,17-EpDPE | P450 | 0.42±0.07 | 0.65±0.08 | 0.001 | 0.010 |
| 16-hydroxy-docosahexaenoic acid | 16-HDoHE | P450 | 0.60±0.07 | 1.20±0.24 | <0.001 | 0.003 |
| 19,20-epoxy-docosapentenoic acid | 19,20-EpDPE | P450 | 1.03±0.19 | 1.63±0.22 | 0.001 | 0.010 |
| 19,20-dihydroxy-docosapentenoic acid | 19,20-DiHDP | P450 | 0.55±0.10 | 1.32±0.43 | 0.001 | 0.008 |
| 4-hydroxy-docosahexaenoic acid | 4-HDoHE | LOX | 1.22±0.20 | 3.17±1.36 | 0.007 | 0.032 |
| 7-hydroxy-docosahexaenoic acid | 7-HDoHE | LOX | 0.17±0.02 | 0.32±0.09 | 0.004 | 0.021 |
| 11-hydroxy-docosahexaenoic acid | 11-HDoHE | LOX | 0.25±0.03 | 0.45±0.06 | <0.001 | 0.001 |
| 17-hydroxy-docosahexaenoic acid | 17-HDoHE | LOX | 1.04±0.13 | 1.84±0.31 | <0.001 | 0.005 |
| Protectin DX | PDX | LOX | 0.02±0.01 | 0.07±0.02 | 0.009 | 0.037 |
| 15 (t)-protectin D₁ | 15(t)-PD₁ | LOX | 4.14±0.89 | 12.68±3.76 | 0.001 | 0.008 |
| 8-hydroxy-docosahexaenoic acid | 8-HDoHE | NE | 0.67±0.08 | 1.16±0.24 | 0.001 | 0.009 |
| 10-hydroxy-docosahexaenoic acid | 10-HDoHE | NE | 0.26±0.03 | 0.45±0.07 | 0.001 | 0.007 |
| 13-hydroxy-docosahexaenoic acid | 13-HDoHE | NE | 0.51±0.10 | 0.78±0.11 | 0.003 | 0.017 |
| 20-hydroxy-docosahexaenoic acid | 20-HDoHE | NE | 1.61±0.22 | 2.46±0.40 | 0.002 | 0.013 |
| **Eicosapentaenoic acid metabolites** | | | | | | |
| 11-hydroxy-eicosapentaenoic acid | 11-HEPE | NE | 0.28±0.10 | 2.0±1.50 | 0.010 | 0.037 |
| **Down with HFD** | | | | | | |
| **Arachidonic acid metabolites** | | | | | | |
| Prostaglandin E₂ | PGE₂ | COX | 2.88±1.02 | 1.43±0.21 | 0.011 | 0.040 |
| Dihomo-Y-linoleic acid metabolites | | | | | | |
| 15-keto-Prostaglandin F₁₀ | 15-ketoPGF₁₀ | COX | 0.43±0.09 | 0.23±0.08 | 0.007 | 0.032 |
| 15-hydroxy-eicosatrienoic acid | 15-HETe | LOX | 0.46±0.07 | 0.34±0.04 | 0.009 | 0.037 |
| **Eicosapentaenoic acid metabolites** | | | | | | |
| 5-hydroxy-eicosapentaenoic acid | 5-HEPE | LOX | 0.24±0.03 | 0.09±0.02 | <0.001 | 0.001 |
| 12-hydroxy-eicosapentaenoic acid | 12-HEPE | LOX | 4.23±0.31 | 1.17±0.26 | <0.001 | <0.001 |
| 15-hydroxy-eicosapentaenoic acid | 15-HEPE | LOX | 0.15±0.02 | 0.05±0.01 | <0.001 | <0.001 |
| 18-hydroxy-eicosapentaenoic acid | 18-HEPE | NE | 0.22±0.03 | 0.07±0.01 | <0.001 | <0.001 |
MECH- mechanism; STD – standard diet; HFD- high-fat diet; PUFA – polyunsaturated fatty acid; COX –cyclooxygenase; LOX –lipoxygenase; P450-cytochrome P450; NE- non-enzymatic degradation. All values are pmol/ mg renal tissue expressed as mean ± SD.
Table 2: Eicosanoid metabolites altered with high-fat diet in the urine

| Column Name | Acronym | MECH | STD | HFD | p value | q value |
|-------------|---------|------|-----|-----|---------|---------|
| **Up with HFD** | | | | | | |
| Adrenic acid metabolites | Adrenic acid Adrenic Acid PUFA | 0.018±0.009 | 0.27±0.27 | 0.001 | 0.011 |
| Dihomo-prostaglandin F₂α | dihomo-PGF₂α COX | 0.009±0.003 | 0.62±0.18 | <0.001 | <0.001 |
| Arachidonic acid metabolites | | | | | | |
| Arachidonic acid AA AA PUFA | 1.768±0.619 | 14.7±8.9 | <0.001 | 0.003 |
| Prostaglandin A₂ | PGA₂ COX | 0.018±0.018 | 0.09±0.03 | 0.001 | 0.011 |
| Prostaglandin B₂ | PGB₂ COX | 0.03±0.009 | 0.09±0.09 | <0.001 | 0.005 |
| 13,14-dihydro-15- keto- prostaglandin E₂ | PGEM COX | 0.35±0.035 | 1.23±0.17 | <0.001 | <0.001 |
| Prostaglandin J₂ | PGJ₂ COX | 0.002±0.002 | 0.009±0.004 | 0.002 | 0.017 |
| 13,14-dihydro-15-keto-prostaglandin D₂ | dhk-PGD₂ COX | 0.008±0.003 | 0.026±0.004 | <0.001 | <0.001 |
| 6,15-diketo-13,14-dihydro-prostaglandin F₁α 6,15 dk-,dh- PGF₁α COX | 0.27±0.09 | 2.12±0.79 | <0.001 | 0.001 |
| Prostaglandin F₂α | PGF₂α COX | 0.01±0.001 | 0.09±0.026 | 0.004 | 0.023 |
| 13,14-dihydro-15- keto-prostaglandin F₂₀ | PGFM COX | 1.59±0.26 | 7.25±1.76 | <0.001 | <0.001 |
| 6-keto-prostaglandin E₁ | 6kPGE₁ COX | 0.09±0.009 | 0.09±0.017 | 0.002 | 0.013 |
| 8,9-dihydroxy-eicosatrienoic acid | 8,9-diHETrE P450 | 0.002±0.0009 | 0.006±0.0017 | <0.001 | 0.001 |
| 11,12-epoxy-eicosatrienoic acid | 11,12-EET | 0.002±0.0009 | 0.005±0.002 | 0.002 | 0.013 |
| Eoxin D₄ | EXD₄ LOX | 20.69±13.26 | 192.5±69.2 | <0.001 | 0.001 |
| Eoxin E₄ | EXE₄ LOX | 0.088±0.088 | 2.56±1.6 | 0.009 | 0.045 |
| 5-isoprostaglandin F₂₀ VI 5-isop-PGF₂₀ VI NE | 0.026±0.007 | 0.09±0.09 | <0.001 | 0.001 |
| 8-isoprostaglandin F₂₀ | 8-isop-PGF₂₀ NE | 0.08±0.009 | 0.17±0.09 | 0.008 | 0.042 |
| Docosahexanoic acid metabolites | | | | | | |
| Protectin DX | PDX LOX | 11.22±13.96 | 27.5±8.7 | 0.008 | 0.042 |
| 19,20-dihydroxy-docosapentenoic acid | 19,20-DiHDP A P450 | 0.008±0.006 | 0.026±0.017 | 0.009 | 0.046 |
| Eicosapentaenoic acid metabolites | | | | | | |
| Prostaglandin E₃ | PGE₃ COX | 0.18±0.09 | 3.62±2.12 | <0.001 | 0.000 |
| Linoleic acid metabolites | | | | | | |
| 9-oxo-octadecadienoic acid | 9-oxoODE COX | 2.3±0.97 | 54±2.1 | 0.001 | 0.011 |
| **Down with HFD** | | | | | | |
| Eicosapentaenoic acid metabolites | | | | | | |
| Prostaglandin D₃ | PGD₃ COX | 0.71±0.26 | 0.17±0.17 | 0.003 | 0.021 |
| Prostaglandin F₃α | PGF₃α COX | 73739.8±3560.8 | 18813.4±1562 | 0.001 | 0.011 |
| Thromboxane B₃ | TXB₃ COX | 0.04±0.009 | 0.026±0.009 | 0.008 | 0.042 |

MECH- mechanism; STD – standard diet; HFD- high-fat diet; PUFA – polyunsaturated fatty acid; COX –cyloxygenase; LOX –lipoygenase; P450-cytochrome P450; NE- non-enzymatic degradation. All values are ng per mg creatinine expressed as mean ± SD.
### Table 3: Eicosanoid metabolites altered with high-fat diet in the arterial circulation

| Column1 | Name                          | Acronym | MECH | STD        | HFD               | p value | q value |
|---------|-------------------------------|---------|------|------------|-------------------|---------|---------|
| **Up with HFD** |                               |         |      |            |                   |         |         |
| **Arachidonic acid metabolites** |                               |         |      |            |                   |         |         |
| Arachidonic acid                | AA     | PUFA   | 8771.3±1930.9 | 14906.2±2726.5 | 0.002  | 0.011  |
| 8,9-epoxy-eicosatrienoic acid   | 8,9-EET| P450   | 0.10±0.14   | 3.77±3.41    | 0.001  | 0.005  |
| **Down with HFD** |                               |         |      |            |                   |         |         |
| **Alpha Linoleic acid metabolites** |                               |         |      |            |                   |         |         |
| 9-hydroxy-octatrienoic acid     | 9-HOTrE| LOX    | 2.97±0.90   | 0.99±0.31    | <0.001 | 0.003  |
| **Docosahexaenoic acid metabolites** |                               |         |      |            |                   |         |         |
| 4-hydroxy-docosahexaenoic acid  | 4-HDoHE| LOX    | 8.93±1.43   | 3.45±0.91    | <0.001 | 0.004  |
| 11-hydroxy-docosahexaenoic acid | 11-HDoHE| LOX  | 3.03±1.13   | 1.35±0.80    | 0.014  | 0.044  |
| 16-hydroxy-docosahexaenoic acid | 16-HDoHE| P450  | 1.49±0.55   | 0.58±0.17    | 0.002  | 0.012  |
| 10-hydroxy-docosahexaenoic acid | 10-HDoHE| NE   | 2.56±0.92   | 1.05±0.52    | 0.009  | 0.033  |
| 13-hydroxy-docosahexaenoic acid | 13-HDoHE| NE   | 3.62±0.27   | 1.94±0.81    | 0.010  | 0.031  |
| 20-hydroxy-docosahexaenoic acid | 20-HDoHE| NE   | 1.49±0.62   | 0.11±0.10    | 0.002  | 0.011  |
| **Eicosapentaenoic acid metabolites** |                               |         |      |            |                   |         |         |
| Eicosapentaenoic acid           | EPA    | PUFA   | 12331.9±4377.1 | 4870.6±1329.8 | 0.003  | 0.016  |
| 5-hydroxy-eicosapentaenoic acid | 5-HEPE | LOX    | 2.53±0.28   | 0.38±0.13    | <0.001 | 0.004  |
| 12-hydroxy-eicosapentaenoic acid| 12-HEPE| LOX   | 33.76±22.64 | 4.46±3.22    | 0.004  | 0.011  |
| 15-hydroxy-eicosapentaenoic acid| 15-HEPE| LOX   | 1.65±0.45   | 0.20±0.13    | 0.004  | 0.011  |
| 14(15)-epoxy-eicosatetraenoic acid| 14,15-EpETE| P450 | 0.56±0.18   | 0.18±0.05    | <0.001 | 0.005  |
| 9-hydroxy-eicosapentaenoic acid | 9-HEPE | NE     | 3.56±1.84   | 0.11±0.13    | <0.001 | 0.005  |
| 11-hydroxy-eicosapentaenoic acid| 11-HEPE| NE    | 1.45±0.18   | 0.18±0.07    | <0.001 | <0.001 |
| 18-hydroxy-eicosapentaenoic acid| 18-HEPE| NE    | 1.51±0.26   | 0.19±0.11    | <0.001 | <0.001 |
| **Linoleic acid metabolites** |                               |         |      |            |                   |         |         |
| 9,10-dihydroxy-octadecenoic acid | 9,10-diHOME| P450 | 16.71±6.03 | 7.38±4.10    | 0.013  | 0.042  |
| 12,13- epoxy-octadecenoic acid  | 12,13-EpOME| P450 | 12.65±3.65 | 5.52±2.11    | 0.002  | 0.015  |
| 12,13-dihydroxy-octadecenoic acid | 12,13-diHOME| P450 | 38.64±13.16 | 10.86±6.82   | 0.001  | 0.004  |
| 13-hydroxy-octadecadienoic acid | 13-HODE | NE    | 61.13±20.15 | 23.91±8.93   | 0.005  | 0.011  |

**MECH**- mechanism; **STD** – standard diet; **HFD**- high-fat diet; **PUFA** – polyunsaturated fatty acid; **COX** – cyclooxygenase; **LOX** – lipoxygenase; **P450**-cytochrome P450; **NE**- non-enzymatic degradation. All values are pmol/ml plasma expressed as mean ± SD.
### Table 4: Eicosanoid metabolites altered with high-fat diet in the venous circulation

| Column1 | Name                                      | Acronym   | MECH | STD       | HFD          | p value | q value |
|---------|-------------------------------------------|-----------|------|-----------|--------------|---------|---------|
| **Up with HFD**                        | Arachidonic acid metabolites              |           |       |           |              |         |         |
|         | Arachidonic acid                          | AA        | PUFA | 7763.6±1110.5 | 13307.3±2881.1 | 0.001   | 0.009   |
|         | 8,9-epoxy-eicosatrienoic acid             | 8,9-EET   | P450 | 0.04±0.08  | 0.51±0.41     | 0.012   | 0.049   |
|         | 5,6-epoxy-eicosatrienoic acid             | 5,6-EET   | P450 | 8.26±4.66  | 20.49±6.51    | 0.006   | 0.031   |
|         | 11,12-epoxy-eicosatrienoic acid           | 11,12-EET | P450 | 1.82±0.65  | 3.89±0.97     | 0.002   | 0.015   |
|         | 6-keto-prostaglandin F1α                  | 6k-PGF₁₀  | COX  | 1.11±0.44  | 2.64±1.14     | 0.005   | 0.027   |
|         | **Down with HFD**                         | Arachidonic acid metabolites              |           |       |           |              |         |         |
|         | 4-hydroxy-docosahexaenoic acid            | 4-HDoHE   | LOX  | 9.87±2.09  | 3.26±0.61     | <0.001  | 0.001   |
|         | 20-hydroxy-docosahexaenoic acid           | 20-HDoHE  | NE   | 0.89±0.33  | 0.13±0.22     | 0.004   | 0.027   |
|         | 9-hydroxy-octatrienoic acid               | 9-HOTrE   | LOX  | 1.87±0.68  | 0.69±0.21     | 0.001   | 0.007   |
|         | Eicosapentaenoic acid metabolites         |           |      |           |              |         |         |
|         | Eicosapentaenoic acid                     | EPA       | PUFA | 10459.9±4175.6 | 4259.2±1425.1 | 0.008   | 0.035   |
|         | 9-hydroxy-eicosapentaenoic acid           | 9-HEPE    | NE   | 4.50±2.72  | 0.45±0.60     | 0.009   | 0.037   |
|         | 11-hydroxy-eicosapentaenoic acid          | 11-HEPE   | NE   | 1.06±0.22  | 0.21±0.10     | <0.001  | 0.001   |
|         | 18-hydroxy-eicosapentaenoic acid          | 18-HEPE   | NE   | 1.11±0.22  | 0.14±0.06     | <0.001  | 0.001   |
|         | 12-hydroxy-eicosapentaenoic acid          | 12-HEPE   | LOX  | 42.94±25.80| 7.48±9.07     | 0.005   | 0.028   |
|         | 5-hydroxy-eicosapentaenoic acid           | 5-HEPE    | LOX  | 2.25±0.64  | 0.17±0.11     | <0.001  | 0.001   |
|         | 15-hydroxy-eicosapentaenoic acid          | 15-HEPE   | LOX  | 1.36±0.74  | 0.07±0.10     | 0.001   | 0.009   |
|         | 14(15)-epoxy-eicosatetraenoic acid        | 14,15-EpETE | P450 | 0.72±0.23  | 0.18±0.10     | 0.001   | 0.007   |

MECH- mechanism; STD – standard diet; HFD- high-fat diet; PUFA – polyunsaturated fatty acid; COX – cyclooxygenase; LOX – lipoxygenase; P450- cytochrome P450; NE- non-enzymatic degradation. All values are pmol/ml plasma expressed as mean ± SD.
Table 5 represents the trends in the kidney and urine of highly correlated metabolites using principal component (PC) analysis.

| Variable                     | PC variables | PC Variance | ANOVA value | p-Value | Post Hoc p-values |  |
|------------------------------|--------------|-------------|-------------|---------|-------------------|---|
|                              |              | All         | STD vs. HFD | HFD vs. |                   |   |
| **KIDNEY**                   |              |             |             |         | HFD vs. DICAIR    |   |
| Arachidonic acid             | LOX 15       | 64.2        | 0.02        | 0.03    | 1                 |   |
|                              | COX 15       | 45.0        | 0.81        | 1       | 1                 |   |
|                              | P450 17      | 49.7        | 0.09        | 0.11    | 1                 |   |
|                              | 13 PGR 2     | 60.6        | 0.06        | 0.11    | 1                 |   |
|                              | NE 3         | 42.2        | 0.20        | 0.34    | 1                 |   |
| Eicosopentaenoic acid        | LOX 3        | 93.0        | 0.00002     | 0.00008 | 1                 |   |
|                              | NE 2         | 77.9        | 0.0000003   | 0.000001| 1                 |   |
| Docosahexaenoic acid         | LOX 4        | 77.2        | 0.007       | 0.006   | 0.28              |   |
|                              | COX 2        | 96.0        | 0.001       | 0.001   | 0.03              |   |
|                              | P450 2       | 97.5        | 0.003       | 0.003   | 0.08              |   |
|                              | NE 4         | 95.8        | 0.008       | 0.007   | 0.12              |   |
| Dihomo-γ-linolenic acid      | LOX 2        | 92.5        | 0.03        | 0.06    | 1                 |   |
| Linoleic acid                | LOX 2        | 98.0        | 0.07        | 0.40    | 1                 |   |
|                              | NE 2         | 77.4        | 0.06        | 0.42    | 1                 |   |
| Alpha-Linolenic acid         | LOX 3        | 82.4        | 0.26        | 0.89    | 0.33              |   |
| **URINE**                    |              |             |             |         |                   |   |
| Arachidonic acid             | LOX 28       | 46.3        | 0.06        | 0.42    | 0.95              |   |
|                              | COX 27       | 33.6        | 0.0005      | 0.004   | 1                 |   |
|                              | P450 19      | 53.9        | 0.12        | 0.70    | 1                 |   |
|                              | 13 PGR 3     | 47.7        | 0.03        | 0.04    | 1                 |   |
|                              | PGDH 5       | 45.6        | 0.00000002  | 0.0000001| 0.00003           |   |
|                              | NE 3         | 48.5        | 0.002       | 0.001   | 0.09              |   |
| Eicosopentaenoic acid        | LOX 3        | 96.8        | 0.14        | 0.214   | 0.33              |   |
|                              | COX 5        | 50.8        | 0.00022     | 0.000161| 0.03              |   |
|                              | CYP 2        | 82.0        | 0.85        | 1       | 1                 |   |
|                              | NE 3         | 82.2        | 0.05        | 0.24    | 0.06              |   |
| Docosahexaenoic acid         | LOX 6        | 55.0        | 0.49        | 0.94    | 1                 |   |
|                              | COX 2        | 96.0        | 0.83        | 1       | 1                 |   |
|                              | NE 4         | 71.3        | 0.85        | 1       | 1                 |   |
AMPK ameliorates eicosanoids in HFD induced-CKD  

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| LOX  | 2  | 97.1 | 0.02 | 0.03 | 1       |
|------|----|------|------|------|---------|
| Linoleic acid | | | | | |

| LOX  | 2  | 98.0 | 0.07 | 0.4  | 1 |
|------|----|------|------|------|---|
| NE   | 2  | 77.4 | 0.04 | 0.36 | 0.72 |

| Alpha-Linolenic acid | | | | | |

| LOX  | 3  | 82.4 | 0.26 | 0.88 | 0.33 |
|------|----|------|------|------|------|
| Adrenic acid | | | | | |

| COX  | 2  | 71.3 | 0.00002 | 0.00002 | 0.006 |
|------|----|------|--------|---------|-------|

PC principal components; PC variables - number of eicosanoids in the principal component; PC variance - amount of variance explained by the principal component; ANOVA p-value - p-value generated comparing all three groups; STD – standard diet; HFD- high-fat diet; HFD+AICAR - high-fat diet and AICAR therapy; COX – cyclooxygenase; LOX – lipoxygenase; P450-cytochrome P450; 13 PGR - delta13-15-ketoprostaglandin reductase; PGDH-15-hydroxyprostaglandin dehydrogenase; NE- non-enzymatic degradation; Red highlight elevated with HFD; Green highlight decreased with HFD; Yellow highlight elevated with AICAR; Blue highlight decreased with AICAR.
Table 6 represents the trends in the arterial and venous blood of highly correlated metabolites using principal component (PC) analysis.

| Variable            | PC variables | PC Variance | ANOVA value | Post Hoc p-values |
|---------------------|--------------|-------------|-------------|-------------------|
|                     |             |             |             | STD vs. HFD       | HFD vs. HFDAICAR  |
| **ARTERY**          |             |             |             |                   |
| Arachidonic acid   | 9           | 40.9        | 0.73        | 1                 | 1                 |
| LOX                 |             | 70.3        | 0.42        | 0.60              | 1                 |
| 13 PGR              | 2           | 59.9        | 0.90        | 1                 | 1                 |
| COX                 |             | 49.3        | 0.13        | 0.43              | 0.16              |
| Eicosopentaenoic    | 3           | 78.9        | 0.000003    | 0.00004           | 0.72              |
| acid                |             |             |             |                   |
| LOX                 |             | 79.9        | 0.001       | 0.003             | 1                 |
| P450                | 2           | 78.1        | 0.01        | 0.03              | 1                 |
| NE                  |             | 77.1        | 0.01        | 0.01              | 1                 |
| Linoleic acid       | 4           | 92.8        | 0.32        | 0.43              | 0.97              |
| NE                  |             |             |             |                   |
| Alpha-Linolenic acid| 3           | 75.7        | 0.04        | 0.03              | 0.37              |
| **VEIN**            |             |             |             |                   |
| Arachidonic acid   | 8           | 59.4        | 0.87        | 1                 | 1                 |
| LOX                 |             | 50.5        | 0.06        | 0.06              | 0.47              |
| 13 PGR              |             | 67.2        | 0.84        | 1                 | 1                 |
| COX                 |             | 47.9        | 0.12        | 1                 | 0.19              |
| Eicosopentaenoic    | 3           | 78.0        | 0.000002    | 0.00003           | 0.60              |
| acid                |             |             |             |                   |
| LOX                 |             | 82.4        | 0.000004    | 0.00003           | 1                 |
| P450                |             |             |             |                   |
| Linoleic acid       |             | 58.0        | 0.0004      | 0.002             | 1                 |
| NE                  |             | 73.9        | 0.08        | 0.09              | 0.76              |
| Alpha-Linolenic acid|             |             |             |                   |
| LOX                 |             | 90.4        | 0.11        | 0.79              | 0.11              |
| **Table continues** |             |             |             |                   |
PC principal components; PC variables - number of eicosanoids in the principal component; PC variance - amount of variance explained by the principal component; ANOVA p-value - p-value generated comparing all three groups; STD - standard diet; HFD - high-fat diet; HFD+AICAR - high-fat diet and AICAR therapy; COX - cyclooxygenase; LOX - lipoxygenase; P450-cytochrome P450; 13 PGR - delta13-15-ketoprostaglandin reductase; PGDH-15-hydroxyprostaglandin dehydrogenase; NE - non-enzymatic degradation; Green highlight demonstrates decreased with HFD.
Figure 1. High-fat diet increases cytosolic phospholipase A2 and secretory-phospholipase A2 levels in the mouse kidney that is improved with AICAR treatment. Western blot analysis of the effect of standard (STD), high-fat diet (HFD) and HFD with AICAR (HFD+AICAR) treatment on cytosolic phospho-phospholipase A2 (pPLA2) and secretory-phospholipase A2 (sPLA2) in mice kidney. Relative densitometry of the immunoblots were normalized with beta-actin. Values are means ± SD. N=5 in each group. *p<0.05.
Figure 2. High fat diet increases free and total polyunsaturated fatty acid levels in the mouse kidneys that is rescued by AMPK activation.

Total and free levels of $\omega_3$ fatty acids eicosapentaenoic acid (EPA, panels A and B) and docosahexaenoic acid (DHA, panels C and D) and $\omega_6$ fatty acids – dihomo gamma-linolenic acid (DGLA, panels E and F) and arachidonic acid (AA, panels G and H) in the mice fed standard (STD), high fat diet (HFD), and HFD with AICAR. Values are means ± SD. N=6 in each group. *p<0.05.
Figure 3.

Profile of eicosanoid species modulated by high-fat diet and AMPK activation in mouse kidneys.

Eicosanoids profiles show changes in the kidneys of mice fed standard (STD), high-fat diet (HFD), and HFD with AICAR. Values are means ± SD. N=6 in each group. Statistical analyses were performed by one-way ANOVA followed by Fisher post hoc tests. All p values corrected for Bonferroni FDR correction of *q ≤ 0.05. Arachidonic acid metabolite of cytochrome P450 12-hydroxy-hexadecatrienoic acid (12HHT; Panel A); Docosahexaenoic acid metabolites of cytochrome P450: 19,20-dihydroxy-
docosapentanoic acid (19,20-DHDPA, Panel B); 16-hydroxy-docosahexaenoic acid (16-HDoHE; Panel C); Lipoxygenase metabolites: 15 (t) Protectin D1 (15(t)-PD1, Panel D), 7-hydroxy-docosahexaenoic acid (7-HDoHE; Panel E), and 11-hydroxy-docosahexaenoic acid (11-HDoHE; Panel F); and non-enzymatic process: 8-hydroxy-docosahexaenoic acid (18-HDoHE; Panel G) and 13-hydroxy-docosahexaenoic acid (13-HDoHE; Panel H).
Figure 4

A. Dihomo-prostaglandin F₂α

B. dihomo-15d-Prostaglandin D₂

C. Arachidonic acid

D. 13,14-dihydro-15-keto-prostaglandin E₂

E. 13,14-dihydro-15-keto-prostaglandin F₂α

F. Prostaglandin F₂α

G. dhk-prostaglandin D₂

H. 5-iso-prostaglandin F₂α

J. Thromboxane B₃

I. Prostaglandin E₃
Figure 4. Profile of urinary eicosanoid species in mice on high-fat diet and AICAR.

Eicosanoid species in urine were normalized for concentration with creatinine levels in standard diet (STD), high-fat diet (HFD) and HFD with AICAR therapy. Values are means ± SD of original data or normalized data (log transformed, auto scaled) represented as box plots with whiskers (median, Interquartile interval). N=6 in each group. Statistical analyses were performed by one-way ANOVA by Fisher post hoc tests. All p values corrected for Bonferroni FDR correction of *q ≤ 0.05. Adrenic acid metabolites- dihomo-prostaglandin PGF$_{2\alpha}$ (dihomo-PGF$_{2\alpha}$, Panel A), dihomo-15-deoxyprostaglandin D$_2$ (dihomo-15d-PGD$_2$, Panel B). Urine arachidonic acid (AA, Panel C) and its cyclooxygenase metabolites- 13,14-dihydro-15-keto-prostaglandin E$_2$ (PGEM, Panel D); 13,14-dihydro-5-keto-prostaglandin F$_{2\alpha}$ (PGFM, Panel E); Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$, Panel F); 13,14-dihydro-15-keto-prostaglandin D$_2$ (dhk-PGD$_2$, Panel G); non-enzymatic product 5-iso-prostaglandin F$_{2\alpha}$-VI (5-iso-PGF$_{2\alpha}$-VI, Panel H). Cyclooxygenase metabolites of eicosapentaenoic acid- Prostaglandin E$_3$ (PGE$_3$, Panel I) and Thromboxane B$_3$ (TXB$_3$, Panel J).
Figure 5. Profile of eicosanoid species in the circulation regulated by high-fat disease and AICAR.

Profile of eicosanoid species in arterial and venous circulation in mice fed a standard diet (STD), high-fat diet (HFD) or HFD with AICAR therapy. Values are means ± SD. N=6 in each group. Statistical analyses were performed by one-way ANOVA followed by Fisher post hoc tests. All p values corrected for Bonferroni FDR correction of *q ≤ 0.05. Linoleic acid metabolites of cytochrome P450 in the artery- 9,10-dihydroxy-octadecenoic acid (9, 10-diHOME, Panel A), 12,13-epoxy-octadecenoic acid (12,13-EpHOME, Panel B), and 12,13-dihydroxy-octadecenoic acid (12,13-diHOME, Panel C). Venous Lipoxygenase metabolite of alpha-linolenic acid- 9-hydroxy-octatrienoic acid (9-HOTrE, Panel D) and eicosapentaenoic acid- 5-hydroxy-eicosapentaenoic acid (5-HEPE, Panel E).
Figure 6. Schematic diagram of the eicosanoid metabolites regulated by AMPK activation to reverse the effects of high-fat diet in the kidney, urine, arterial and venous circulation.

Adrenic acid metabolites—dihomo-prostaglandin PGF$_{2\alpha}$ (dihomo-PGF$_{2\alpha}$) and dihomo-15-deoxy prostaglandin D$_2$ (dihomo-15d-PGD$_2$); Arachidonic acid (AA) metabolites—13,14-dihydro-15-keto-prostaglandin E$_2$ (PGEM) and 13,14-dihydro-5-keto-prostaglandin F$_{2\alpha}$ (PGFM), and Prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$); Eicosapentaenoic acid (EPA) metabolites—Prostaglandin E$_3$ (PGE$_3$), Thromboxane B$_3$ (TXB$_3$) and 5-hydroxy-eicosapentaenoic acid (5-HEPE); Alpha – Linolenic acid (ALA) metabolite—9-hydroxy-octatrienoic acid (9-HOTrE); Docosahexaenoic acid (DHA) metabolites—19,20-dihydroxy-docosapentenoic acid (19,20-DiHDPA), 16-hydroxy-docosahexanoic acid (16-HDoHE), 15 (t)- Protectin D1(15(t)-PD1), 7-hydroxy-docosahexanoic acid (7-HDoHE), 11-hydroxy-docosahexanoic acid (11-HDoHE), 8-hydroxy-docosahexanoic acid (8-HDoHE); 13-hydroxy docosahexaenoic acid (13-HDoHE); and finally Linoleic
acid metabolite-9,10-dihydroxy-octadecaenoic acid (9,10-diHOME); 12,13, Epoxy-octadecaenoic acid (12,13-EpOME) and, 12,13,dihydroxyoctadecenoic acid (12,13-diHOME). P450- cytochrome P450; LOX – lipoxygenases; COX – cyclooxygenase; NE –non-enzymatic. Red font indicates that high-fat diet increases these metabolites and AICAR reverses this, while blue font indicates the metabolite is decreased with high-fat diet and AICAR reverses this trend.
| Name                        | Acronym | STD         | HFD         | HFD+AICAR    | p.value | FDR | p.value | FDR |
|----------------------------|---------|-------------|-------------|--------------|---------|-----|---------|-----|
| Lauric acid                | 12:0    | 113.8±43.7  | 107.32±68.64| 136.06±107.07| 0.623   | 0.688 | 0.880   | 0.939 |
| Myristic acid              | 14:0    | 509.26±325.48| 377.07±201.08| 279.34±100.17| 0.703   | 0.750 | 0.620   | 0.709 |
| Pentadecanoic acid         | 15:0    | 116.02±22.47| 91.2±29.13  | 74.96±21.75  | 0.148   | 0.237 | 0.082   | 0.197 |
| Palmitic acid              | 16:0    | 11296.42±1178.66| 11037.4±1518.56| 11327.56±38.66| 0.749   | 0.773 | 0.952   | 0.952 |
| Palmitoleic acid           | 16:1    | 673.58±454.33| 794.32±1092.33| 352.16±264.06| 0.592   | 0.676 | 0.415   | 0.531 |
| Margaric acid              | 17:0    | 215.78±17.30 | 191.92±29.28| 219.98±71.14 | 0.156   | 0.237 | 0.620   | 0.709 |
| Heptadecanoic acid         | 17:1    | 38.2±19.69  | 73.92±80.87 | 38.16±23.63  | 0.532   | 0.631 | 0.665   | 0.734 |
| Stearic acid               | 18:0    | 7072.54±480.45| 7533.62±1106.65| 7712.4±2610.78| 0.514   | 0.631 | 0.916   | 0.945 |
| Oleic acid                 | 18:1    | 2103.92±295.41| 2720.78±395.47| 3291.42±998.03| 0.024   | 0.065 | 0.031   | 0.111 |
| Linoleic acid              | 18:2    | 6441.18±992.92| 5556.98±437.79| 7099.84±2237.18| 0.115   | 0.197 | 0.309   | 0.430 |
| α-linolenic acid           | 18:3 N3 | 55.2±20.59  | 50.12±12.24 | 80.16±34.36  | 0.793   | 0.793 | 0.216   | 0.340 |
| γ-linolenic acid           | 18:3 N6 | 338.16±87.45| 231.02±79.04| 272.04±110.77| 0.062   | 0.142 | 0.219   | 0.340 |
| Steardioinonic acid        | 18:4    | 3.76±0.89   | 3.06±0.56   | 8.12±5.76   | 0.180   | 0.250 | 0.157   | 0.279 |
| Arachidic acid             | 20:0    | 12125.58±615.62| 41.7±5.51  | 47.78±26.86  | 0.001   | 0.003 | 0.144   | 0.272 |
| Eicosanoic acid            | 20:1    | 83.32±42.52 | 73.92±52.95 | 53.58±31.50  | 0.531   | 0.631 | 0.494   | 0.608 |
| Eicosadienoic acid         | 20:2    | 276.74±48.97| 381.55±96.99| 336.8±93.59  | 0.083   | 0.176 | 0.223   | 0.340 |
| Eicosatrienoic acid        | 20:3 N3 | 9.04±4.79   | 3.83±1.47   | 2.9±1.24    | 0.045   | 0.110 | 0.025   | 0.100 |
| Bishomo-γ-linolenic acid   | 20:3 N6 | 645.14±75.59| 474.05±65.27| 494.36±139.85| 0.006   | 0.020 | 0.044   | 0.141 |
| 5,8,11-Eicosatrienoic acid| 20:3 N9 | 30.36±6.24  | 86.63±16.89 | 72.8±17.91  | <0.001  | <0.001 | <0.001  | <0.001 |
| Arachidonico acid          | 20:4    | 12125.58±615.62| 15756.66±1501.57| 15817.94±44 | 0.001   | 0.003 | 0.055   | 0.160 |
| Eicosapentaenoic acid      | 20:5    | 224.94±29.74| 104.02±42.67| 73.26±22.30  | 0.001   | 0.005 | <0.001  | 0.001 |
| Behenic acid               | 22:0    | 30.44±24.46 | 0          | 4.12±5.15   | <0.001  | <0.001 | <0.001  | <0.001 |
| cis-Erucic acid            | 22:1    | 0.56±0.69   | 0          | 0           | 0.104   | 0.196 | 0.086   | 0.197 |
| Docosadienoic acid         | 22:2    | 0.14±0.15   | 0.02±0.04  | 0.02±0.04   | 0.097   | 0.194 | 0.101   | 0.202 |
| Docosadienoic acid         | 22:4    | 12.0±2.53    | 30.33±8.23 | 26.82±7.41  | <0.001  | <0.001 | <0.001  | <0.001 |
| Docosapentaenoic ω-3 acid  | 22:5 N3 | 38.38±5.66  | 111.42±32.16| 99.64±27.51 | <0.001  | <0.001 | <0.001  | <0.001 |
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| Name                              | Acronym | STD     | HFD     | HFD+AICAR | p.value | FDR    | p.value | FDR    |
|-----------------------------------|---------|---------|---------|-----------|---------|--------|---------|--------|
| Lauric acid                       | 12:0    | 15.6±15.50 | 7.18±8.33 | 57.07±55.86 | 0.623   | 0.688  | 0.278   | 0.569  |
| Myristic acid                     | 14:0    | 38.4±24.04 | 24.62±15.85 | 43.52±39.63 | 0.703   | 0.750  | 0.755   | 0.872  |
| Pentadecanoic acid                | 15:0    | 7.92±6.12  | 4.35±2.52  | 12.23±4.75  | 0.148   | 0.237  | 0.071   | 0.269  |
| Palmitic acid                     | 16:0    | 959.04±315.02 | 795.55±133.79 | 884.17±248.61 | 0.749   | 0.773  | 0.752   | 0.872  |
| Palmitoleic acid                  | 16:1    | 29.1±7.16  | 22.4±9.92  | 30.08±19.80 | 0.592   | 0.676  | 0.624   | 0.872  |
| Margaric acid                     | 17:0    | 11.12±6.11 | 12.31±7.29 | 14.55±6.71  | 0.156   | 0.237  | 0.696   | 0.872  |
| Heptadecenoic acid                | 17:1    | 2.66±0.70  | 2.96±1.39  | 4.72±1.94   | 0.532   | 0.631  | 0.230   | 0.522  |
| Stearic acid                      | 18:0    | 602±326.84 | 348.12±168.69 | 671.9±219.04 | 0.514   | 0.631  | 0.103   | 0.323  |
| Oleic acid                        | 18:1    | 360.26±83.69 | 397.6±99.28 | 399±120.81  | 0.024   | 0.065  | 0.821   | 0.872  |
| Linoleic acid                     | 18:2    | 613±117    | 486.4±72.3 | 552.6±148   | 0.115   | 0.197  | 0.351   | 0.628  |
| α-linolenic acid                  | 18:3 N3 | 4.6±1.2 | 5.4±1.1 | 6.3±3.3 | 0.062 | 0.142 | 0.810 | 0.872 |
| γ-linolenic acid                  | 18:3 N6 | 35.7±10.6 | 25.5±5.3 | 37±15 | 0.793 | 0.793 | 0.325 | 0.613 |
| Stearidonic acid                  | 18:4    | 0.9±0.4    | 0.9±0.3   | 1.3±0.8    | 0.180   | 0.250  | 0.885   | 0.912  |
| Arachidic acid                    | 20:0    | 6.6±3.4    | 3±1.5     | 6.9±3.1    | 0.001   | 0.003  | 0.067   | 0.269  |
| Eicosanoic acid                   | 20:1    | 4.1±1.4    | 3.6±1     | 5.3±2      | 0.531   | 0.631  | 0.483   | 0.775  |
| Eicosadienoic acid                | 20:2    | 20.6±3     | 27.1±6.5  | 25±5.7     | 0.083   | 0.176  | 0.201   | 0.522  |
| Eicosatrienoic acid               | 20:3 N3 | 0.4±0.3 | 0.2±0.2 | 0.1±0.2 | 0.045 | 0.110 | 0.147 | 0.418 |
| Bishomo-γ-linolenic acid          | 20:3 N6 | 34.2±5.5 | 24.6±5.4 | 22.2±3 | 0.006 | 0.020 | 0.005 | 0.035 |
| 5,8,11-Eicosatrienoic acid        | 20:3 N9 | 2.4±0.9 | 4.4±1.3 | 3.9±1.4 | <0.001 | <0.001 | 0.068 | 0.269 |
| 20 | Arachidonic acid 20:4 | 391.6±32 | 481.7±45.4 | 384.1±14.2 | 0.001 | 0.003 | 0.001 | 0.009 |
| 21 | Eicosapentaenoic acid 20:5 | 47.5±3.3 | 19.3±2.9 | 14.9±2.6 | 0.001 | 0.005 | <0.001 | <0.001 |
| 22 | Behenic acid 22:0 | 3.5±1.6 | 2.8±1.1 | 4.8±2.2 | <0.001 | <0.001 | 0.451 | 0.767 |
| 23 | cis-Elaric acid 22:1 | 6.2±3.3 | 3±1.7 | 2.4±1.2 | 0.104 | 0.196 | 0.216 | 0.522 |
| 24 | Docosadienoic acid 22:2 | 1.9±0.5 | 2±0.3 | 1.9±0.7 | 0.097 | 0.194 | 0.760 | 0.872 |
| 25 | Docosatrienoic acid 22:3 | 0.2±0.3 | 0.1±0.1 | 0.2±0.3 | 0.820 | 0.924 | 0.958 | 0.958 |
| 26 | Aradonic acid 22:4 | 16.3±5.3 | 22.4±3.3 | 19.6±4.17 | <0.001 | 0.003 | 0.105 | 0.323 |
| 27 | Docosapentaenoic ω-3/Docosapentaenoic ω-6 acid 22:5 N3 | 41.4±7.4 | 36.4±8.5 | 27.2±6.1 | 0.003 | 0.009 | 0.025 | 0.141 |
| 28 | Docosahexaenoic acid 22:6 | 412.5±60.5 | 570.8±89.5 | 396.2±66.3 | <0.001 | 0.001 | 0.005 | 0.035 |
| 29 | Tricosanoic acid 23:0 | 0.2±0.2 | 0.1±0.1 | 0.4±0.3 | 0.117 | 0.197 | 0.284 | 0.569 |
| 30 | Lignoceric acid 24:0 | 2.5±1.1 | 1.9±1.2 | 3±1.8 | 0.015 | 0.044 | 0.657 | 0.872 |
| 31 | cis-Selacholeic acid 24:1 | 0.3±0.1 | 0.4±0.1 | 0.5±0.2 | 0.283 | 0.378 | 0.816 | 0.872 |
| 32 | Cerotic acid 26:1 | 63±87 | 99±146 | 388.4±610.3 | 0.171 | 0.249 | 0.501 | 0.775 |

MECH - mechanism; STD – standard diet; HFD - high fat diet; HFD +AICAR – HFD with AICAR therapy; PUFA – polyunsaturated fatty acid; COX –cyclooxygenase; LOX –lipoxygenase; CYT-cytochrome P450 oxidase; NE- non-enzymatic degradation; P value for two way ANOVA; FDR- False discovery rate; for the comparison of STD, HFD, and HFD with AICAR.
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Supplemental Figure S1. Delta-6-desaturase, delta-5-desaturase, and elongase 5 levels are unchanged in the kidney with high fat diet and AMPK activation

Western blot analysis of the effect of high fat diet (HFD) and AMPK activator -AICAR on delta-5-desaturase (D5D; FADS1), delta-6-desaturase (D6D; FADS2), and elongase 5 (ELOLV5) in standard (STD), high fat diet (HFD) and HFD+AICAR-treated mice. Relative densitometry of the immunoblots did not show a difference between groups. All figures were normalized with beta-actin. Values are means ± SD. N=5 in each group.
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Supplementary Table S2. Significant eicosanoid metabolites altered with high fat diet and AMP activation in the kidney, urine, arterial and venous circulation

| NAME                                      | ACRONYM | PARENT | MECH | STD   | HFD   | HFD+AICAR | p.value | FDR   | Fisher’s LSD |
|-------------------------------------------|---------|--------|------|-------|-------|-----------|---------|-------|---------------|
| **KIDNEY (pmol/mg)**                      |         |        |      |       |       |           |         |       |               |
| 1. Arachidonic acid                       | AA      | AA     | PUFA | 243.4±19.2 | 312.7±40.9 | 237.3±18.7 | 0.002   | 0.013 | 2 - 1; 2 - 3   |
| 2. 9-hydroxy-eicosatetraenoic acid       | 9-HETE  | AA     | NE   | 0.02±0.01 | 0.14±0.09 | 0.08±0.03 | 0.006   | 0.025 | 2 - 1; 3 - 1   |
| 3. 12-oxo-eicosatrienoic acid            | 12-oxoETE | AA | LOX  | 2.73±0.34 | 12.28±9.19 | 8.72±2.78 | 0.014   | 0.039 | 2 - 1; 3 - 1   |
| 4. Hepoxilin B3                           | HXB3    | AA     | LOX  | 0.26±0.05 | 0.94±0.60 | 0.65±0.18 | 0.021   | 0.050 | 2 - 1; 3 - 1   |
| 5. 5-oxo-eicosatetraenoic acid           | 5-oxoETE | AA     | LOX  | 0.21±0.11 | 2.55±1.70 | 1.21±0.49 | 0.004   | 0.024 | 2 - 1; 3 - 1   |
| 6. 15-oxo-eicosatrienoic acid            | 15-oxoETE | AA | LOX  | 0.51±0.21 | 2.55±1.70 | 1.71±0.58 | 0.014   | 0.039 | 2 - 1; 3 - 1   |
| 7. 6(t),12, epi-Leukotriene B4           | 6t,12-epiLTB4 | AA | LOX  | 0.01±0.00 | 0.02±0.01 | 0.02±0.00 | 0.014   | 0.039 | 2 - 1; 3 - 1   |
| 8. 6(R), 15(R) Lipoxin A4                 | 6(R),15(R)-LXA4 | AA | LOX  | 0.02±0.01 | 0.05±0.02 | 0.05±0.01 | 0.004   | 0.024 | 2 - 1; 3 - 1   |
| 9. 6(S)-lipoxin A4                        | 6(S)-LXA4 | AA | LOX  | 0.02±0.01 | 0.05±0.02 | 0.05±0.02 | 0.009   | 0.028 | 2 - 1; 3 - 1   |
| 10. 11,12-epoxy-eicosatrienoic acid      | 11,12-EET | AA | P450 | 0.02±0.01 | 0.18±0.13 | 0.13±0.05 | 0.005   | 0.025 | 2 - 1; 3 - 1   |
| 11. 5,6-epoxy-eicosatrienoic acid        | 5, 6-EET | AA | P450 | 1.59±0.74 | 8.64±5.64 | 5.72±1.95 | 0.007   | 0.025 | 2 - 1; 3 - 1   |
| 12. 8,9-epoxy-eicosatrienoic acid        | 8, 9-EET | AA | P450 | 0.04±0.01 | 0.16±0.10 | 0.12±0.04 | 0.011   | 0.032 | 2 - 1; 3 - 1   |
| 13. Prostaglandin E2                      | PGE2    | AA     | COX  | 2.88±1.02 | 1.43±0.21 | 1.32±0.49 | 0.006   | 0.025 | 1 - 2; 1 - 3   |
| 14. 12-hydroxy-heptadecatetraenoic acid  | 12-HHT   | AA     | COX  | 7.63±0.41 | 9.13±1.36 | 5.94±0.93 | <0.001  | 0.005 | 1 - 3; 2 - 3   |
| 15. 13,14-dihydro-15-keto-prostaglandin D2 | dhk-PGD2 | AA     | 13PGR | 0.04±0.01 | 0.08±0.02 | 0.09±0.02 | 0.001   | 0.008 | 2 - 1; 3 - 1   |
| 16. 15-dehydro- Prostaglandin F1α        | 15k-PGF1α | DGLA | PGDH | 0.43±0.10 | 0.23±0.07 | 0.14±0.03 | <0.001  | 0.002 | 1 - 2; 1 - 3; 2 - 3 |
| 17. 15-hydroxy-eicosatrienoic acid       | 15HETE  | DGLA   | LOX  | 0.46±0.07 | 0.34±0.04 | 0.33±0.06 | 0.009   | 0.028 | 1 - 2; 1 - 3   |
| 18. 8-hydroxy-docosahexanoic acid        | 8-HDoHE | DHA    | NE   | 0.67±0.08 | 0.36±0.24 | 0.81±0.22 | 0.006   | 0.025 | 2 - 1; 2 - 3   |
| 19. 13-hydroxy-docosahexanoic acid       | 13-HDoHE | DHA | NE   | 0.51±0.08 | 2.92±0.11 | 0.61±0.13 | 0.011   | 0.032 | 2 - 1; 2 - 3   |
| 20. 20-hydroxy-docosahexanoic acid       | 20-HDoHE | DHA | NE   | 1.61±0.22 | 2.46±0.40 | 2.05±0.50 | 0.017   | 0.042 | 2 - 1; 2 - 3   |
| 21. 10-hydroxy-docosahexanoic acid       | 10-HDoHE | DHA | NE   | 0.26±0.03 | 0.45±0.07 | 0.35±0.10 | 0.007   | 0.027 | 2 - 1; 2 - 3   |
| 22. 15(t)- Protectin D1                  | 15(t)-PD1 | DHA | LOX  | 4.14±0.89 | 12.69±3.76 | 6.22±2.11 | <0.001  | 0.007 | 2 - 1; 2 - 3   |
| 23. 7-hydroxy- docosahexaenoic acid      | 7-HDoHE | DHA | LOX  | 0.17±0.02 | 0.31±0.09 | 0.21±0.05 | 0.008   | 0.028 | 2 - 1; 2 - 3   |
| 24. 4-hydroxy- docosahexanoic acid       | 4-HDoHE | DHA | LOX  | 1.22±0.19 | 3.17±1.36 | 2.57±0.63 | 0.005   | 0.025 | 2 - 1; 3 - 1   |
| 25. 10(S), 17(S)-dihydroxy-docosahexanoic acid | 10(S),17(S)-DiHDoHE | DHA | LOX  | 0.02±0.01 | 0.07±0.02 | 0.04±0.01 | 0.008   | 0.028 | 2 - 1; 2 - 3   |
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| NAME | ACRONYM     | PARENT | MECH | STD     | HFD     | HFD+ACTAR | p.value | FDR   | Fisher’s LSD |
|------|-------------|--------|------|---------|---------|-----------|---------|-------|---------------|
| 1    | Arachidonic | AA      | AA   | NE      | 0.03±0.01 | 0.12±0.04 | 0.075±0.012 | <0.001 | <0.001 | 2 - 1; 3 - 1 |
| 2    | 5-iso-Prostaglandin F_{2α}-VI | 5-isOPGF_{2α}-VI | AA | NE | 1.77±0.62 | 14.69±8.94 | 5.75±1.68 | <0.001 | 0.001 | 2 - 1; 3 - 1 |
| 3    | 20-carboxylic-Leucotrine B₄ | 20cooh-LTB₄ | AA | LOX | 14.07±8.23 | 13.45±26.1 | 0.02±0.01 | 0.009 | 0.034 | 2 - 1; 3 - 1 |
| 4    | Eoxin D₄ | EXD₄ | AA | LOX | 20.71±13.28 | 192.67±69.30 | 401.97±300.5 | <0.001 | <0.001 | 2 - 1; 3 - 1 |
| 5    | Eoxin E₄ | EXE₄ | AA | LOX | 0.09±0.09 | 2.57±1.59 | 1.95±1.68 | 0.003 | 0.013 | 2 - 1; 3 - 1 |
| 6    | 12-hydroxy-eicosatetraenoic acid | 12-HETE | AA | LOX | 0.0062±0.0062 | 0.02±0.01 | 0.09±0.004 | 0.003 | 0.017 | 2 - 1; 3 - 1 |
| 7    | Leuchotriene E₄ | LTE₄ | AA | LOX | 0.0009±0.0009 | 0.002±0.0009 | 0.004±0.002 | 0.004 | 0.018 | 2 - 1; 3 - 1 |
| 8    | 5-hydroxy-eicosatetraenoic acid | 5-HETE | AA | LOX | 0.03±0.04 | 0.06±0.04 | 0.06±0.03 | 0.009 | 0.034 | 2 - 1; 3 - 1 |

URINE (ng/mg Creatinine)
| No. | Eicosanoid Family                          | Eicosanoid Name          | Species | LOX     | COX     | LOX     | COX     | COX     | LOX     | COX     | LOX     | COX     | LOX     | COX     | COX     |
|-----|------------------------------------------|--------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 9   | 11(t)-Leukotriene D₄                     | 11(t)-LTD₄               | AA      | 0.0009±0.002 | 0.0004±0.0004 | 0.004±0.004 | 0.002 | 0.012 | 3 - 1; 3 - 2 |
| 10  | Leukotriene D₄                           | LTD₄                     | AA      | 8.76±3.36 | 26.28±16.4 | 97.88±111.33 | 0.007 | 0.028 | 3 - 1; 3 - 2 |
| 11  | 11,12-epoxy-eicosatrienoic acid          | 11,12-EET                | AA      | 0.002±0.000 | 0.005±0.000 | 0.006±0.002 | 0.001 | 0.007 | 2 - 1; 3 - 1 |
| 12  | 17-hydroxy-eicosatetraenoic acid         | 17-HETE                  | AA      | 0.03±0.03 | 0.11±0.05 | 0.50±0.30 | 0.003 | 0.013 | 2 - 1; 3 - 1 |
| 13  | 8,9-dihydroxy-eicosatrienoic acid       | 8, 9-diHETrE             | AA      | 0.002±0.000 | 0.006±0.000 | 0.009±0.0008 | 0.004 | 0.019 | 2 - 1; 3 - 1 |
| 14  | 13,14-dihydro-15-keto-Prostaglandin F₂α | PGFM                     | AA      | 1.59±0.26 | 7.26±1.77 | 3.01±0.44 | <0.001 | 1 | 2 - 1; 3 - 1; 2 - 3 |
| 15  | 13,14-dihydro-15-keto-Prostaglandin A₂  | PGEM                     | AA      | 0.35±0.035 | 1.24±0.18 | 0.53±0.18 | <0.001 | 1 | 2 - 1; 3 - 1; 2 - 3 |
| 16  | Prostaglandin F₂α                        | PGF₂α                    | AA      | 0.009±0.008 | 0.056±0.02 | 0.02±0.01 | 0.004 | 0.019 | 2 - 1; 2 - 3 |
| 17  | 6,15-diketo-13,14-dihydro Prostaglandin F₁α | PGF₁α                  | AA      | 0.26±0.09 | 2.12±0.8 | 2.74±1.33 | <0.001 | 1 | 2 - 1; 3 - 1 |
| 18  | Prostaglandin B₂                         | PGB₂                     | AA      | 0.04±0.009 | 0.09±0.09 | 0.2±0.02 | <0.001 | 0.001 | 2 - 1; 3 - 1 |
| 19  | Prostaglandin A₂                         | PGA₂                     | AA      | 0.02±0.02 | 0.09±0.04 | 0.18±0.09 | <0.001 | 0.001 | 2 - 1; 3 - 1 |
| 20  | Prostaglandin J₂                         | PGJ₂                     | AA      | 0.002±0.002 | 0.01±0.004 | 0.01±0.005 | <0.001 | 0.008 | 2 - 1; 3 - 1 |
| 21  | Thromboxane B₂                           | TXB₂                     | AA      | 0.035±0.009 | 0.02±0.002 | 0.08±0.03 | <0.001 | 1 | 3 - 1; 3 - 2 |
| 22  | 2,3 dioeno-11β-Prostaglandin F₂α         | 2,3-dioeno-11β-PGF₂α     | AA      | 682.3±129.4 | 1197.3±67.9 | 2510.8±1119.3 | 0.002 | 0.012 | 3 - 1; 3 - 2 |
| 23  | 13,14-dihydro-15-keto-Prostaglandin D₂   | dhkPGD₂                  | AA      | 0.01±0.002 | 0.03±0.004 | 0.01±0.005 | <0.001 | 0.002 | 2 - 1; 2 - 3 |
| 24  | Adrenic acid                             | Adrenic                  | PUFA    | 0.01±0.01 | 0.25±0.22 | 0.06±0.02 | <0.001 | 0.002 | 2 - 1; 3 - 1 |
| 25  | Dihomo-15-deoxyprostaglandin D₂          | dihomo15dPGD₂            | Adrenic | 323.2±571.7 | 77.0±77.6 | 202.4±233.8 | 0.015 | 0.047 | 1 - 2; 3 - 2 |
| 26  | Dihomo-prostaglandin F₂α                | dihomoPGF₂α              | Adrenic | 0.01±0.003 | 0.62±0.18 | 0.44±0.62 | <0.001 | <0.001 | 2 - 1; 3 - 1; 2 - 3 |
| 27  | 15-dehydro-Prostaglandin F₁α            | 15kPGF₁α                 | DGLA    | 0.02±0.005 | 0.03±0.008 | 0.04±0.02 | 0.009 | 0.034 | 2 - 1; 3 - 1 |
| 28  | 15-hydroxy-eicosatrienoic acid           | 15-HETrE                 | DGLA    | 0.003±0.004 | 0.009±0.008 | 0.006±0.003 | 0.014 | 0.047 | 2 - 1; 3 - 1 |
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| 29 | 6-keto-Prostaglandin E₁ | 6kPGE₁ | DGLA | COX | 0.05±0.01 | 0.08±0.02 | 0.16±0.08 | <0.001 | 0.003 | 2 - 1; 3 - 1; 3 - 2 |
| 30 | Thromboxane B₁ | TXB₁ | DGLA | COX | 0.02±0.006 | 0.014±0.003 | 0.11±0.04 | <0.001 | <0.001 | 1; 3 - 1; 3 - 2 |
| 31 | 10(S),17(S)-dihydroxy-docosahexanoic acid | 10(S),17(S)-DiHDoHE | DHA | LOX | 11.2±13.9 | 27.5±8.8 | 35.6±16.8 | 0.004 | 0.017 | 2 - 1; 3 - 1 |
| 32 | Protectin D₁ | PD₁ | dha | LOX | 11.2±7.4 | 13.6±11.2 | 33.9±13.5 | 0.004 | 0.019 | 3 - 1; 3 - 2 |
| 33 | 19, (20)-dihydroxy-docosapentaenoic acid | 19,20-DiHDoPA | DPA | P450 | 0.009±0.006 | 0.03±0.02 | 0.02±0.009 | 0.007 | 0.028 | 2 - 1; 3 - 1 |
| 34 | Eicosapentaenoic acid | EPA | EPA | PUFA | 19.1±18.3 | 42.4±24.5 | 49.5±7.79 | 0.011 | 0.038 | 2 - 1; 3 - 1 |
| 35 | Prostaglandin F₃α | PGF₃α | EPA | COX | 73809.3±350 | 18830.7±1 | 22977.5±1012 | 8.6 | 0.001 | 0.007 | 1 - 2; 1 - 3 |
| 36 | Thromboxane B₃ | TXB₃ | EPA | COX | 0.05±0.01 | 0.03±0.01 | 0.09±0.09 | <0.001 | 0.002 | 1 - 2; 3 - 1; 3 - 2 |
| 37 | Prostaglandin D₃ | PGD₃ | EPA | COX | 2.53±0.28 | 0.38±0.13 | 0.29±0.10 | <0.001 | <0.00 | 1; 2 - 1; 3 - 2 |
| 38 | Prostaglandin E₃ | PGE₃ | EPA | COX | 12.65±3.65 | 5.52±2.11 | 8.81±2.69 | 5.44 | 0.005 | 0.019 | 1 - 2; 3 - 2 |
| 39 | 9-oxo-octadecadienoic acid | 9-oxoODE | LA | NE | 2.39±1.06 | 6.2±2.1 | 7.9±2.8 | <0.001 | 0.002 | 1 - 2; 3 - 1 |
| 40 | 5-hydroxy-eicosatrienoic acid | 5-HETE | Mead | P450 | 0.0009±0.0009 | 0.002±0.0009 | 0.003±0.0009 | 0.002 | 0.013 | 2 - 1; 3 - 1 |

**ARTERY (pmol/ml)**

| NAME | ACRONYM | PARENT | MECH | STD | HFD | HFD+ AICAR | p.value | FDR | Fisher's LSD |
|------|---------|--------|------|-----|-----|-----------|---------|-----|-------------|
| 1    | Arachidonic acid | AA | AA | PUFA | 8771.3±193 | 0.89 | 14906.19±2726.51 | 14113.42±2755.44 | 0.001 | 0.008 | 2 - 1; 3 - 1 |
| 2    | 5-hydroxy-eicosapentaenoic acid | 12,13-EpOME | AA | P450 | 12.65±3.65 | 5.52±2.11 | 8.81±2.69 | 5.44 | 0.005 | 0.019 | 1 - 2; 3 - 2 |
| 3    | 12,13-epoxy-octadecenoic acid | 12,13-dHOME | AA | P450 | 38.65±13.16 | 10.86±6.82 | 45.12±27.10 | 2 - 1; 3 - 2 |
| 4    | 8,9-epoxy-eicosatrienoic acid | 8,9-EET | AA | P450 | 0.10±0.14 | 3.77±3.41 | 1.08±0.87 | 2 - 1; 3 - 1 |
| 5    | 9,10-dihydroxy-octadecenoic acid | 9,10-diHOME | AA | P450 | 16.71±6.03 | 7.38±4.10 | 26.75±12.49 | 0.002 | 0.008 | 1 - 2; 3 - 2 |
| 6    | 13-hydroxy-octadecenoic acid | 13-HODE | ALA | LOX | 61.13±20.15 | 23.9±8.93 | 38.27±15.92 | 0.014 | 0.046 | 1 - 2; 3 - 2 |
| 8  | 10-hydroxy-docosahexanoic acid | 10-HDoHE | DHA | NE | 2.56±0.92 | 1.05±0.51 | 1.49±0.41 | 0.007 | 0.027 | 1-2; 1-3 |
| 9  | 13-hydroxy-docosahexanoic acid | 13-HDoHE | DHA | NE | 3.62±0.27 | 1.94±0.81 | 2.13±0.74 | 0.014 | 0.046 | 1-2; 1-3 |
| 10 | 20-hydroxy-docosahexanoic acid | 20-HDoHE | DHA | NE | 1.48±0.62 | 0.11±0.10 | 0.07±0.15 | <0.001 | 0.003 | 1-2; 1-3 |
| 11 | 4-hydroxy-docosahexanoic acid  | 4-HDoHE  | DHA | LOX | 8.93±1.43 | 3.45±0.91 | 3.37±0.91 | <0.001 | 1     | 1-2; 1-3 |
| 12 | 16-hydroxy-docosahexanoic acid | 16-HDoHE | DHA | P450 | 1.49±0.55 | 0.58±0.17 | 0.82±0.28 | 0.003 | 0.014 | 1-2; 1-3 |
| 13 | Eicosapentaenoic acid          | EPA      | EPA | PUFA | 12331.95±43| 4870.59±1 | 3910.81±956.15 | <0.001 | 0.003 | 1-2; 1-3 |
| 14 | 18-hydroxy-eicosapentaenoic acid | 18-HEPE  | EPA | NE  | 1.51±0.26 | 0.19±0.11 | 0.16±0.05 | <0.001 | 1     | 1-2; 1-3 |
| 15 | 9-hydroxy-eicosapentaenoic acid | 9-HEPE   | EPA | NE  | 3.56±1.84 | 0.11±0.13 | 0.11±0.15 | 0.001 | 0.005 | 1-2; 1-3 |
| 16 | 12-hydroxy-eicosapentaenoic acid | 12-HEPE  | EPA | LOX | 33.76±22.64| 4.46±3.22 | 6.02±3.17 | 0.003 | 0.012 | 1-2; 1-3 |
| 17 | 15-hydroxy-eicosapentaenoic acid | 15-HEPE  | EPA | LOX | 1.65±0.46 | 0.20±0.13 | 0.03±0.05 | <0.001 | 0.003 | 1-2; 1-3; 2-3 |
| 18 | 14, (15)-epoxy-eicosatetraenoic acid | 14,15-EpETE | EPA | P450 | 0.56±0.18 | 0.18±0.05 | 0.11±0.10 | <0.001 | 0.001 | 1-2; 1-3; 2-3 |
| 19 | 11-hydroxy-eicosapentaenoic acid | 11-HEPE  | EPA | COX | 1.45±0.18 | 0.18±0.07 | 0.16±0.07 | <0.001 | 1     | 1-2; 1-3 |
| 20 | 9-hydroxy-octatrienoic acid    | 9-HOTrE  | LA  | LOX | 2.97±0.90 | 0.99±0.31 | 1.68±1.17 | 0.004 | 0.016 | 1-2; 1-3 |

### VEIN (pmol/ml)

| NAME | ACRONYM | PARENT | MECH | STD | HFD | HFD+AlCaR | p.value | FDR | Fisher’s LSD |
|------|---------|--------|------|-----|-----|--------|--------|-----|-----------|
| Arachidonic acid | AA | AA | PUFA | 7763.61±111 0.50 | 13307.28±2881.09 | 13715.20±3888.78 | 0.002 | 0.013 | 2-1; 3-1 |
| 5-hydroxy-eicosapentaenoic acid | 5-HEPE | AA | LOX | 2.26±0.64 | 0.17±0.11 | 0.29±0.09 | <0.001 | 1     | 1-2; 1-3; 3-2 |
| 11,12-epoxy-eicosatrienoic acid | 11,12-EET | AA | P450 | 1.82±0.65 | 3.89±0.98 | 2.95±0.34 | 0.001 | 0.009 | 2-1; 3-1 |
| 5, 6-epoxy-eicosatetraenoic acid | 5, 6-EET | AA | P450 | 8.26±4.66 | 20.49±6.51 | 15.55±2.33 | 0.002 | 0.015 | 2-1; 3-1 |
| 4-hydroxy-docosahexanoic acid | 4-HDoHE | DHA | LOX | 9.87±2.09 | 3.26±0.61 | 3.18±0.86 | <0.001 | 1     | 1-2; 1-3 |
| 7-hydroxy-docosahexanoic acid | 7-HDoHE | DHA | LOX | 0.91±0.38 | 0.34±0.42 | 0.05±0.08 | 0.008 | 0.041 | 1-3; |
| Eicosapentaenoic acid | EPA | EPA | PUFA | 10459.89±41 | 4259.23±1 | 3679.71±840.96 | 0.001 | 0.011 | 1-2; 1-3 |
| 18-hydroxy-eicosapentaenoic acid | 18-HEPE | EPA | NE  | 1.11±0.22 | 0.14±0.06 | 0.11±0.07 | <0.001 | 1     | 1-2; 1-3 |
| 15-hydroxy-eicosapentaenoic acid | 15-HEPE | EPA | LOX | 1.36±0.74 | 0.07±0.10 | 0.06±0.10 | 0.001 | 0.011 | 1-2; 1-3 |
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| Compounds                         | Code  | Diet     | COX | LOX | P450   | p-value |
|-----------------------------------|-------|----------|-----|-----|--------|---------|
| 14, (15)-epoxy-eicosatetraenoic  | EpETE | STD      | 1.06| 0.69| 0.72   | <0.001  |
| acid                             |       | 1; HFD   | 0.21| 0.69| 0.18   |         |
| 15-EpETE                         |       | HFD +AICAR| 0.11| 0.21| 0.12   | <0.001  |
| 11-hydroxy-eicosapentaenoic acid| HEPE  | STD      | 1.87| 1.33| 1.06   | 0.003   |
| acid                             |       | 1; HFD   | 0.21| 1.33| 0.21   | 0.013   |
| 9-hydroxy-octatrienoic acid      | HOTrE | STD      | 1.87| 1.33| 0.72   | 0.002   |
| acid                             |       | 1; HFD   | 0.69| 0.69| 0.69   | 0.013   |
|                                  |       | HFD +AICAR| 1.33| 1.33| 0.18   |         |

MECH- mechanism; STD – standard diet; HFD- high fat diet; HFD +AICAR – HFD with AICAR therapy; PUFA – polyunsaturated fatty acid; COX –cyclooxygenase; LOX –lipoxygenase; CYT–cytochrome P450 oxidase; NE- non-enzymatic degradation. P value for two way ANOVA; FDR- False discovery rate; Fisher’s LSD indicates posthoc tests comparing –STD (1),HFD(2), and HFD-AICAR(3) groups and represents only the significant comparisons.