

Research paper

Kidney-secreted erythropoietin lowers lipidemia via activating JAK2-STAT5 signaling in adipose tissue

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ABSTRACT

Background: Dyslipidemia is commonly observed in various kidney diseases, renal specific secreted erythropoietin (EPO) may participate in this process. However, how this process is regulated remains elusive.

Method: Dyslipidemia was evaluated in chronic kidney disease and ischemia kidney injury animal model. Primary cultured adipocytes were harvested to investigate the lipid metabolic effect of EPO. Lipidemia was evaluated in EPO treated animals. Blood samples from cardiac surgery-induced kidney injury patient were collected to assess correlation between EPO and lipidemia.

Findings: We found a decrease in secreted EPO and hypertriglyceridemia in chronic kidney disease (CKD) mice. In contrast, in renal ischemia animal model, increased EPO triggered by hypoxia signaling activation, was accompanied by decreased triglyceride (TG) in serum. Mechanistically, circulating EPO modulated JAK2-STAT5 signaling, which in turn enhanced lipid catabolism in peripheral adipose tissue and contributed to dysregulated lipidemia. Delivering of recombinant EPO into both wild type and CKD mice suppressed TG in serum by accelerating lipid catabolism in adipose tissue. In a cohort of patients diagnosed with acute kidney injury after cardiopulmonary bypass surgery, the decreased TG and cholesterol negatively correlated with increased EPO in serum.

Interpretation: This study depicted a new mechanism by which renal secreted EPO controlled lipidemia in kidney diseases including chronic kidney disease. Circulating EPO stimulated lipid catabolism by targeting JAK2-STAT5 signaling in peripheral adipose tissue, providing new therapeutic target for dyslipidemia treatment.

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Research in context

Evidence before this study

Dyslipidemia in chronic kidney disease is a major contributor to cardiovascular complications. However, mechanisms underlying dysregulated lipid profile in blood are not well illustrated in the context of kidney disease. And there remains an unmet need for more effective therapies for hyperlipidemia

Added value of this study

In our study, we described a kidney specific secreted cytokine, erythropoietin (EPO), controls blood lipid in kidney disease. Mechanistically, Circulating EPO stimulates lipid catabolism through

activating JAK2-STAT5 signaling in peripheral adipose tissue. Restored EPO mitigates hyperlipidemia in chronic kidney disease. In patients with acute kidney injury, the induced EPO negatively correlated with lipidemia.

Implications of all the available evidence

These findings provide important novel insights into understanding the mechanism underlying dyslipidemia in kidney disease, and imply that activating lipid catabolism in adipose tissue may serve as a new strategy for dyslipidemia treatment in kidney disease.

1. Introduction

Dyslipidemia is commonly observed in patients with various kidney diseases. Hyperlipidemia in chronic kidney disease (CKD), including increased triglycerides (TG), total cholesterol, decreased high density lipoprotein (HDL), and varying low density lipoprotein (LDL) level,

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contributes to the cardiovascular complications [1–5]. Mechanism underlying dysregulated lipid profile in the blood is not fully understood.

Reagents reversing abnormal lipid profile prevent cardiovascular events and CKD progression [3,6–8]. Statins, drugs lower cholesterol in the blood by targeting hydroxy-methylglutaryl-coenzyme A reductase, are the first line treatment and best studied lipid lowering medicine in CKD. However, side effects including muscle aches, contraindications for statins including liver disease and history of rhabdomyolysis, limit utilization of statins in dialysis patients. Hence preferable treatment for hyperlipidemia is in drastic need [9–11].

Erythropoietin (EPO), a circulating glycoprotein mainly produced by the capillary-surrounding fibroblasts in kidney in adults, stimulates erythropoiesis in bone marrow [12–14]. The common pathological progression of CKD is characterized by excessive accumulation of extracellular matrix and dysfunction of renal cells, resulting in EPO-deficiency and anemia [15–17]. However, activation of hypoxia signaling in the kidney inactivating HIF-prolyl hydroxylase domain-containing proteins (PHDs), leading to stabilization of hypoxia-inducible factors (HIFs), including HIF-1 α and HIF-2 α , which in turn upregulates EPO expression [12,18–21].

Clinically, recombinant human EPO is used for correcting anemia. Previous studies documented that EPO not only counteracts the anemia but also influences various other pathways including inflammation, oxidative stress and metabolism [22–25]. It is reported that CKD patients with maintenance hemodialysis or peritoneal dialysis receiving EPO treatment show decreased TG and LDL levels in serum [26,27]. In mice, disrupted EPO signaling in all nonerythroid tissues promotes obesity and increased TG in serum [28], suggesting that impaired EPO secretion caused by kidney dysfunction may influence lipid metabolism in peripheral tissues, and finally contribute to dyslipidemia in CKD.

EPO binds exclusively to its receptor (EPOR), leading to the recruitment of nonreceptor tyrosine kinase janus kinase 2 (JAK2), and activation of downstream signaling including signal transducer and activator of transcription (STAT5). Lipid catabolism related genes are upregulated when JAK2-STAT5 signaling is activated, including PPAR gamma coactivator 1 α (*Pgc-1 α*), uncoupling protein 1 (*Ucp-1*), and carnitine palmitoyltransferase 1 (*Cpt1*), adipose triglyceride lipase (*ATGL*) [29–34]. *EPOR* gene is highly expressed in the white adipose tissue (60% of that in spleen, a hematopoietic tissue in mice) [32,35]. Specific disruption of JAK2-STAT5 signaling in adipose tissue impairs lipolysis, leading to increased adiposity and body weight in mice [34,36]. The beneficial effects of EPO may be ascribed to the browning of white adipose tissue, characterized by increased lipid catabolism [28,32]. However, the effects of altered EPO expression in the injured kidney have not been documented.

In the present study, we found that CKD suppressed renal EPO secretion whereas hypoxia induced renal EPO expression and secretion. Altered EPO gave rise to change in lipid profile in serum through modulating JAK2-STAT5 signaling in the adipose tissue. Administration of EPO suppressed TG in serum by strengthening lipid catabolism in the adipose tissue in both the wild type and CKD mice. In serum from patients with ischemia induced kidney injury, the augmented EPO was negatively correlated with diminished TG and cholesterol concentration. These observations provided the evidence of the essential role of renal EPO excretion in controlling serum lipid profile. In addition, JAK2-STAT5 signaling in adipose tissue may serve as a new therapeutic target for treatment of dyslipidemia in the kidney injury.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Institutional Animal Care Committee of Nanfang Hospital. Male C57BL/6 wild type mice (Guangdong Medical Laboratory Animal Center, Guangzhou, China)

were housed in cages at 24 ± 1 °C, 12 h light-dark cycle condition with ad-libitum access to water and chow diet or high fat diet (HFD) consisted of 60% energy by fat.

2.2. CKD mice model

To prepare a CKD model with severe kidney injury, 7 to 8-week-old mice were bilateral kidney clamped for 45 min and sacrificed at day 1, 7, 14 or 28 after surgery. Blood, kidney, liver, and inguinal adipose tissue were collected for various analyses.

Briefly, after the mice fasted overnight were anesthetized, a mid-line abdominal incision was made and the renal bilateral pedicles were clamped for 45 min using atraumatic microvascular clamps to block blood flow. After removal of the clamps, reperfusion of the kidneys was confirmed by observing the color change from dark black to red. The incision was closed in two layers with 4–0 silk braided suture. The mice received sham operation without clamping the renal pedicles were used as control.

2.3. Renal ischemia animal model

For renal ischemia model, 7 to 8-week-old mice were bilateral kidney clamped shortly for 20 min and the incision was closed. Mice were sacrificed at day 1, 7 or 14 after surgery. Blood, kidney, liver, and inguinal adipose tissue were collected for subsequent analyses.

To inhibit JAK2-STAT5 signaling, mice were intraperitoneally injected with JAK2 inhibitor AG490 (Sigma) at 10 mg/kg body weight one time, immediately after 20 min renal clamp surgery. 1 day after surgery, mice were sacrificed and tissue were collected for further analyses.

2.4. EPO treatment in mice

For studying the effect of EPO, 7 to 8-week-old chow diet mice were intraperitoneally injected with 3000 IU/kg recombinant human EPO (Kyowa Hakko Kirin Pharmaceutical Co., Ltd. Shanghai, China) once every two days for 14 days. Vehicle groups were received an equivalent volume of saline. The HFD mice were fed with high fat diet 1 week before and during EPO treatment (3000 IU/kg recombinant human EPO, once every two days for 14 days). The CKD mice were treated with recombinant human EPO on the day of surgery and once every two days for 14 days. After 2-week EPO treatment, the body weight of mice was measured. Then inguinal, epididymal, anterior, mesenteric and interscapular adipose tissue, blood, kidney and liver were collected for further analyses.

2.5. HIF signaling activation and JAK2-STAT5 signaling inhibition in mice

For HIF signaling activation in mice, wild type mice were intraperitoneally injected with HIF activator, JNJ-42041935 (Sigma), at dose of 100 μ M/kg once every two days for 6 days. Then mice were sacrificed, blood and tissues were collected for further molecular analyses.

For JAK2-STAT5 signaling inhibitor, 7 to 8-week-old wild type mice receiving EPO treatment were intraperitoneally injected with JAK2 inhibitor AG490 (Sigma) at 10 mg/kg body weight every day for 1 week. Then mice were sacrificed, blood and tissues were collected for further molecular analyses.

2.6. Cell culture and treatment

Isolation and differentiation of adipocyte and hepatocyte are processed as previously described [37,38].

Briefly, subcutaneous adipose tissue were collected from male C57BL/6 wild type mice, and digested with collagenase D, dispase II

to collected stromal vascular fractions. Primary isolated preadipocytes were cultured and differentiated in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 ham (DMEM/F-12) (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with indomethacin (125 mM; Sigma), dexamethasone (5 mM; Sigma), insulin (0.5 mg/ml), isobutylmethylxanthine (0.5 mM; Sigma), rosiglitazone (1 mM; Sigma) and T3 (1 nM; Sigma) for 2 days. Next, the cells were maintained in DMEM/F-12 medium containing insulin, T3, rosiglitazone and 10% (vol/vol) FBS for another 4 days. 4 h before EPO treatment, the culture medium was replaced by DMEM/F-12.

For EPO incubation, recombinant human EPO was added to the DMEM/F-12 culture medium for indicated doses (0, 0.625, 1.25, 2.5, 5, 10 IU/mL for 360 min) and times (0, 15, 30, 360, 720 min at 2.5 IU/mL).

For JAK2-STAT5 inhibition, in cultured adipocyte, 10 μ M AG490 or 47 μ M CAS 285986-31-4 (Sigma) were added to the culture medium 4 h before EPO treatment to deprive JAK2 or STAT5 activity, respectively.

2.7. Quantitative polymerase chain reaction analysis

Total RNA was isolated using the Trizol reagent according to the manufacturer's instructions. The cDNA was synthesized from 1 μ g total RNA using Reverse Transcription Kit and quantitative PCR (qPCR) was performed on 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR-Green fluorescent dye. The mRNA levels of various genes were calculated after normalizing with β -actin using the comparative Ct method. Primer pairs were used for analysis as follows:

FAS	forward 5'-CGGCGAGTCTATGCCACTAT-3'
	reverse 5'-ACACAGGGACCGAGTAATGC-3'
LPL	forward 5'-TATGGCACAGTGGCTGAAAG-3'
	reverse 5'-CTGACCAGCGGAAGTAGGAG-3'
Cpt1	forward 5'-GGTCTCTCGGGTCAAAGC-3'
	reverse 5'-TCCTCCACCAGTCACTAC-3'
Acox1	forward 5'-CITGGATGGTAGTCCGGAGA-3'
	reverse 5'-TGGCTTCGAGTGAGGAAGT-3'
Acox2	forward 5'-TACCAACGCCT GTTTGAGTG-3'
	reverse 5'-TTTCCAGCTTTGCATCAGTG-3'
ATGL	forward 5'-GCCACTCATCTACGGAGC-3'
	reverse 5'-GTTGAAGGAGGGATGCAGAG-3'
EPO	forward 5'-CATCTCGCAGCTCGAGTTCTG-3'
	reverse 5'-CACAAACCATCGTGACATTTTC-3'

2.8. Western blot analysis

Tissue or cells were lysed in ice-cold RIPA lysis buffer supplemented with protease inhibitor and phosphatase inhibitor cocktails. Western blots were probed with the following antibodies: total JAK2, total STAT5, phospho-JAK2 (Tyr1007/1008) phospho-STAT5 (Tyr694), ATGL, HSL and phospho-HSL (Ser563) purchased from Cell Signaling Technology (Danvers, MA, USA); EPOR (R&D Systems, Minneapolis, MN, USA); HIF-1 α (Sigma-Aldrich, Saint Louis, MO, USA); HIF-2 α (R&D Systems) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.9. Human study

All clinical study were approved by the Medical Ethics Committee of Nanfang Hosipital (Guangzhou, China). The participants in the study provided written informed consent. A total of thirty-seven patients with cardiac surgery of cardioplummonary bypass (CPB) from Nanfang Hosipital were included in our analysis. We exclude patients with diseases such as CKD, diabetes, cancer, chronic obstructive pulmonary disease, cerebral ischemia stroke and hyperlipidemia with statin treatment before surgery. Clinical characteristics of the patients are shown in Supplementary Table 1.

2.10. Serum parameter analysis

Serum was separated from solidified blood by centrifugation (1000 rpm, 10 min, 4 $^{\circ}$ C), and stored at -80° C. Serum creatinine (CR), blood urea nitrogen (BUN), triglyceride, cholesterol, HDL and LDL levels were measured using an automated chemistry analyzer (Beckman coulter AU480). Human and mice EPO in serum were determined by ELISA kit (R&D Systems, Minneapolis, MN, USA).

2.11. Histology staining

The kidney sections were cut into 2 μ m thickness for staining. Masson's trichrome staining were used to assess renal fibrosis by the areas of blue staining from a light microscope under a 40 \times magnification objective lens. Haematoxylin and eosin (H&E) staining which is the blue staining of nucleus and pink color of cytoplasm were carried out to observe morphology under a 40 \times magnification objective lens.

2.12. Statistical analysis

All data are presented as means \pm S.E.M. Statistical analysis of data were carried out using IBM SPSS Statistics 20. For multiple comparisons, one-way ANOVA was applied. For comparison of two groups, Student's *t*-test was used. Pearson correlation analyzed the associations between EPO and lipid profile levels in serum. Differences between groups were considered to be statistically significant at $*P \leq 0.05$ and $**P \leq 0.0001$.

3. Results

3.1. CKD mice manifest hyperlipidemia with reduced EPO in serum

Patients with CKD undergo hyperlipidemia [39]. To construct animal model of chronic kidney disease manifesting hyperlipidemia, male C57/BL6 mice were given a 45-minute kidney ischemia followed by reperfusion. Serum creatinine (CR) and blood urea nitrogen (BUN) were measured at day 1, 7, 14 and 28 after ischemia to assess kidney function. 1 day after reperfusion, 12.1-fold and 8.2-fold increase was observed for CR and BUN in serum, respectively, indicating severe kidney injury. At day 28, CR and BUN remained upregulated comparing to sham operated mice, indicating successful establishment of CKD model (Fig. 1a and b). Kidney H&E staining showed dilatation of renal tubules (Fig. 1c). From day 7 to day 28, increasingly accumulated collagen in the interstitial region was observed in the Masson's trichrome stained kidney (Fig. 1c), along with elevated TG in serum (Fig. 1d). Fibrosis impairs renal functions including secretion of EPO. We found that serumal EPO of CKD mice decreased from 311.1 pg/mL to 50.0 ng/mL at day 1. Albeit partially recovered, EPO concentration was constantly lower in CKD mice at day 7, 14, 28 (Fig. 1e), suggesting that EPO deficiency occurs and may hence be mechanistically linked to hyperlipidemia in CKD.

3.2. Hypoxia induces renal EPO biogenesis and secretion

CKD impairs renal EPO excretion whereas hypoxia induces EPO production [40]. To clarify the regulation of EPO in response to renal hypoxemia, we constructed renal ischemia model by clamping bilateral kidney for 20 min. Though a relatively moderate but significant 2.25- and 2.91-fold increase was observed for serumal CR and BUN in mice respectively, the accumulated CR and BUN in the serum were eliminated at day 7 and day 14, indicating recovery of kidney function (Fig. 2a and b). No obvious renal morphologic injury or fibrosis was detected during the whole experimental period (Fig. 2c). However, we found a significant decrease in the serumal TG at the first day after renal ischemia, whereas EPO in the serum was increased by 70%

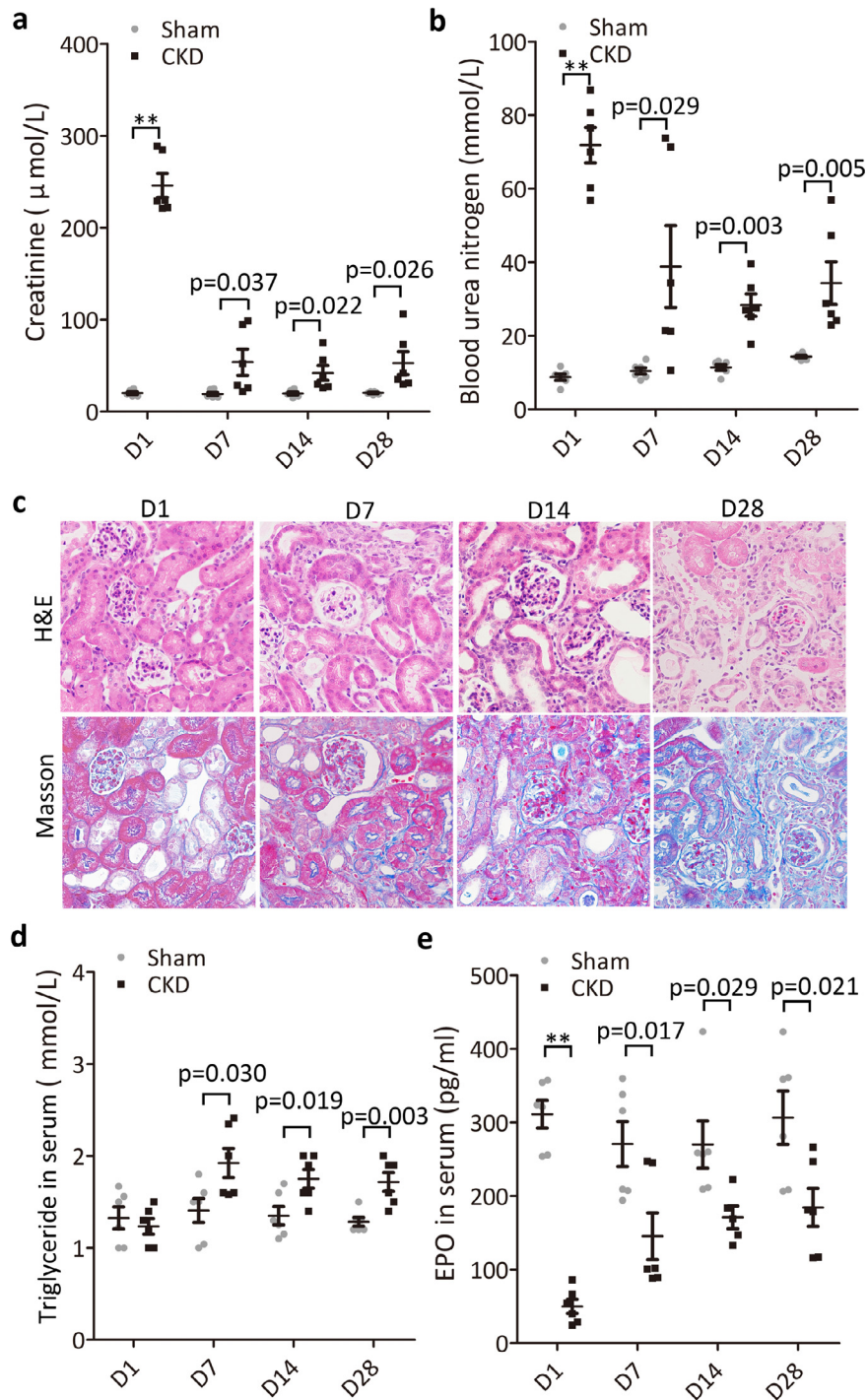


Fig. 1. Serumal EPO decreased in the CKD mice. (a, b) Creatinine (a) and blood urea nitrogen (b) in the blood from CKD mice at day 1, day 7, day 14, day 28. ($n = 6$). (c) Representative image of H&E staining and Masson's trichrome staining in kidney sections from kidney injured mice at indicated time points. (d, e) Triglyceride (d) and EPO (e) in serum from mice above. ($n = 6$). Data are expressed as means \pm S.E.M. Student's t -test was used for the comparison of two groups. $p \leq 0.05$ defined as significant different, $** \leq 0.0001$.

(Fig. 2d and f). Both serum TG and EPO traced back to the base level after 14 days when kidney function was fully recovered (Fig. 2d and f). No significant decline in cholesterol, another key lipid component in the serum, was detected during the 14 days period (Fig. 2e). Oxygen deprivation induced hypoxia signaling may contribute to the elevated EPO in serum. Results showed that HIF-1 α and HIF-2 α protein were induced in ischemia kidney injured mice at day 1 (Fig. 2g). Consistent with previous reports, elevation of HIF-1 α and HIF-2 α promoted the

EPO gene transcription in kidney (Fig. 2h). To further validate the effect of HIF signaling in EPO transcription and secretion *in vivo*, we treated wild type mice with HIF activator, JNJ-42041935, and found that induction of HIF-1 α and HIF-2 α protein in kidney did accelerate renal EPO biogenesis and secretion (Fig. 2i–k). Serumal TG was suppressed in the HIF signaling activated mice (Fig. 2l). These results suggested that renal hypoxia induced EPO mRNA expression in kidney and EPO secretion into circulation, which may correlated with suppressed TG in serum.

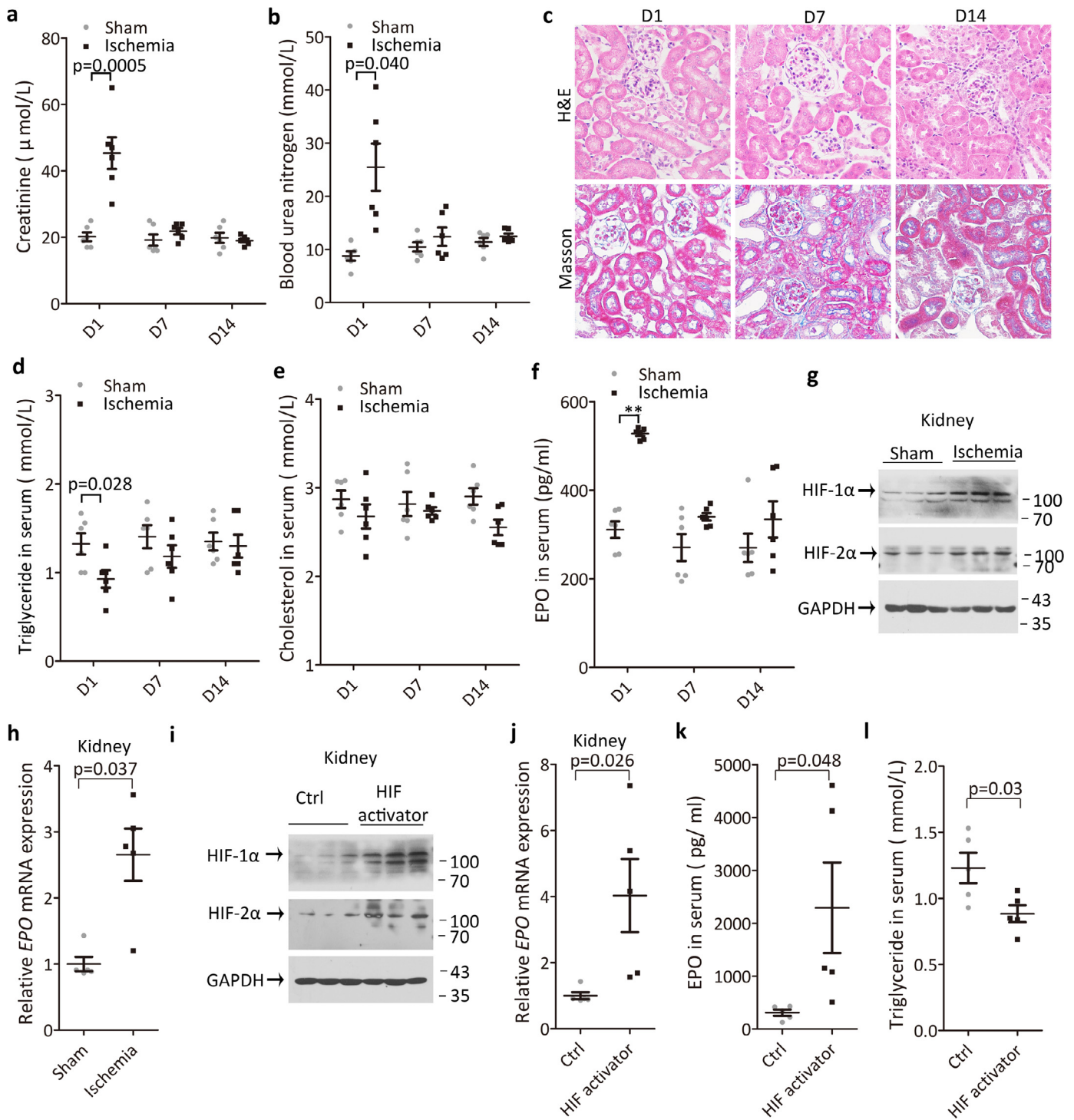


Fig. 2. Serumal EPO increased upon hypoxia signaling activation in kidney. (a, b) Creatinine (a) and blood urea nitrogen (b) in the serum from 20 min renal ischemia mice followed by reperfusion at day 1, day 7, day 14. (n = 6). (c) Representative image of H&E staining and Masson's trichrome staining in kidney sections from renal ischemia mice at indicated time points. (d–f) Triglyceride (d), total cholesterol (e) and EPO (f) concentration in the serum from mice above. (n = 6). (g) Western blot for HIF-1α and HIF-2α in the kidney from renal ischemia mice at day 1. (h) Relative EPO mRNA expression in kidney from renal ischemia mice at day 1. (n = 6). (i) Western blot for HIF-1α and HIF-2α in kidney from HIF activator treated mice. (j) Relative EPO mRNA expression in kidney from HIF activator treated mice, (n = 5). (k, l) Serumal EPO (k), TG (l) in HIF activator treated mice. (n = 5). Data are expressed as means ± S.E.M. Student's *t*-test was used for the comparison of two groups. *p* ≤ 0.05 defined as significant different, ** ≤ 0.0001.

3.3. Altered JAK2-STAT5 signaling in adipose tissue leads to dysregulated blood lipid in kidney injury

Adipose tissue and liver are the major organs involve in lipid metabolism *in vivo* [41,42]. So we harvested subcutaneous adipose tissue, which is more sensitive to extrinsic signal than visceral adipose tissue [37], and liver to investigate whether altered lipidemia is

influenced by modulated lipid metabolism in peripheral organs. Results showed that in adipose tissue of CKD mice, JAK2-STAT5 signaling was inhibited (Fig. 3a). Moreover, the downstream lipid catabolic genes, including *ATGL* (adipose triglyceride lipase), *Acox1*, *Acox2* (acyl-coenzyme A oxidase 1, 2) and *Cpt1* (carnitine palmitoyltransferase 1) were downregulated as well (Fig. 3b). Unexpectedly, lipogenic-related genes, *FAS* (fatty acid synthesis) and *LPL* (lipoprotein lipase)

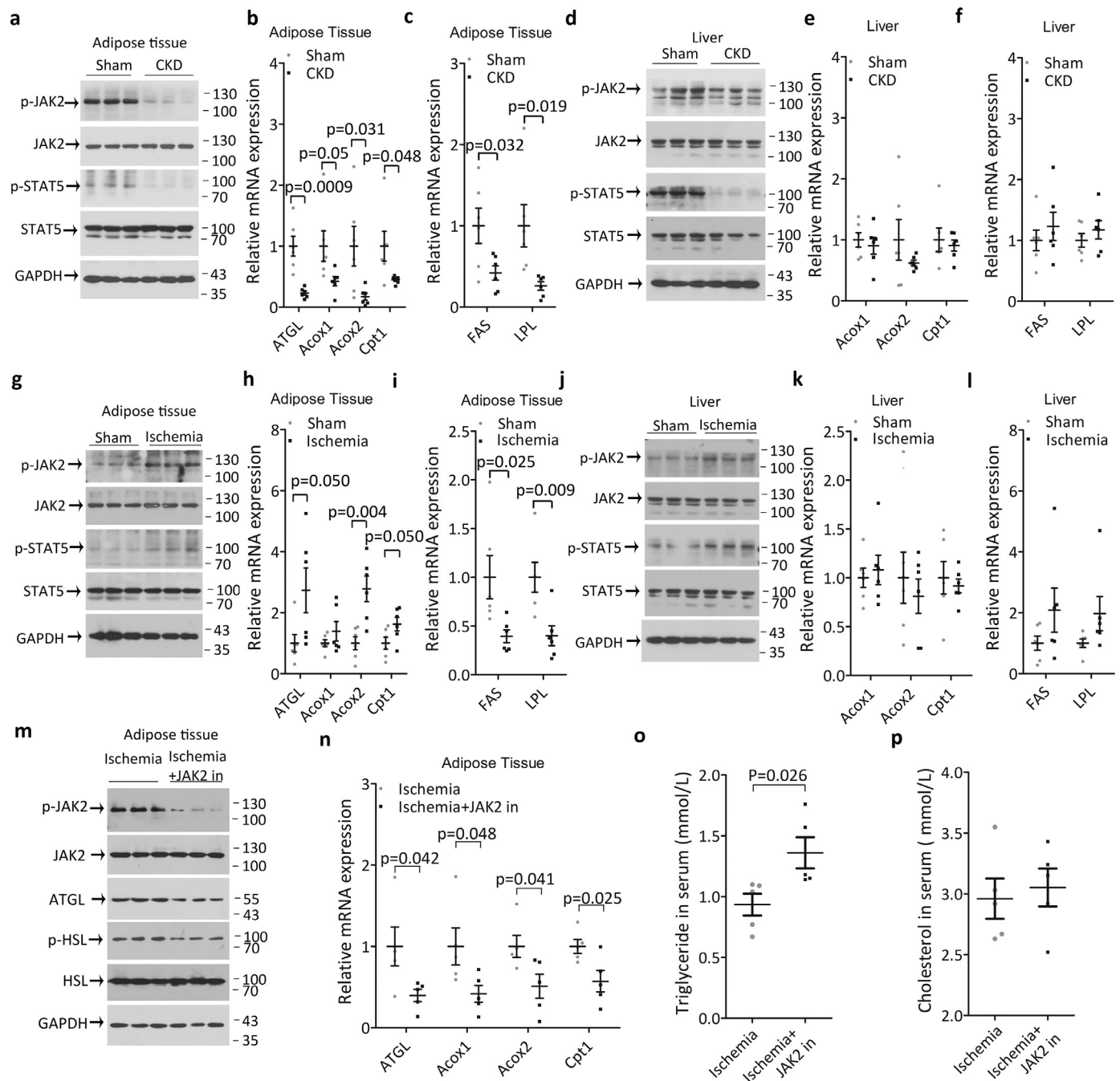


Fig. 3. Dysregulated JAK2-STAT5 signaling in adipose tissue modulated TG in serum. (a) Western blot for phosphorylated JAK2 and STAT5 in adipose tissue from CKD mice at day 28 post surgery. (b, c) Relative lipid catabolic (b) and synthetic (c) mRNA expression in adipose tissue from CKD mice at day 28 post surgery. ($n = 6$). (d) Western blot for phosphorylated JAK2 and STAT5 in liver tissue from CKD mice at day 28 post surgery. (e, f) Relative lipid catabolic (e) and synthetic (f) mRNA expression in liver from CKD mice at day 28 post surgery. ($n = 6$). (g) Western blot for phosphorylated JAK2 and STAT5 in adipose tissue from renal ischemia mice at day 1 after surgery. (h, i) Relative lipid catabolic (h) and synthetic (i) mRNA expression in adipose tissue from renal ischemia mice at day 1 after surgery. ($n = 6$). (j) Western blot for phosphorylated JAK2 and STAT5 in liver tissue from renal ischemia mice at day 1 after surgery. (k, l) Relative lipid catabolic (k) and synthetic (l) genes expression in liver from renal ischemia mice at day 1 after surgery ($n = 6$). (m) Western blot for phosphorylated JAK2, ATGL and phosphorylated HSL in adipose tissue from renal ischemia mice treated with JAK2 inhibitor or saline at day 1. (n) Relative lipid catabolic mRNA expression in adipose tissue from renal ischemia mice treated with JAK2 inhibitor or saline. ($n = 5$ for ischemia. $n = 5$ for ischemia+JAK2 inhibitor). (o, p) Triglyceride (o) and cholesterol (p) in serum from renal ischemia mice treated with JAK2 inhibitor or saline. ($n = 5$ for ischemia. $n = 5$ for ischemia+JAK2 inhibitor). Data are expressed as means \pm S.E.M. Student's t-test was used for the comparison of two groups. $p \leq 0.05$ defined as significant different.

which contribute to lipid storage in adipocyte, also showed decreased transcription in adipose tissue (Fig. 3c). Although, JAK2-STAT5 signaling was also weakened in liver tissue, genes related to lipolysis and liposynthesis remained unchanged (Fig. 3d–f).

Far from the case of CKD, in the ischemia kidney mice with magnified renal EPO secretion, augmented JAK2-STAT5 signaling accompanied by induced lipid catabolism and reduced liposynthesis was

observed in the adipose tissue (Fig. 3g–i). In liver, however, moderately enhanced JAK2-STAT5 signaling failed to trigger lipid catabolism or to suppress liposynthesis (Fig. 3j–l), consistent with the previous report that acute kidney injury led to hepatic steatosis due to decreased constitutive androstane receptor [43]. These results suggested that the dysregulated JAK2-STAT5 signaling in adipose tissue contributed to the altered blood lipid. To further testify the causality

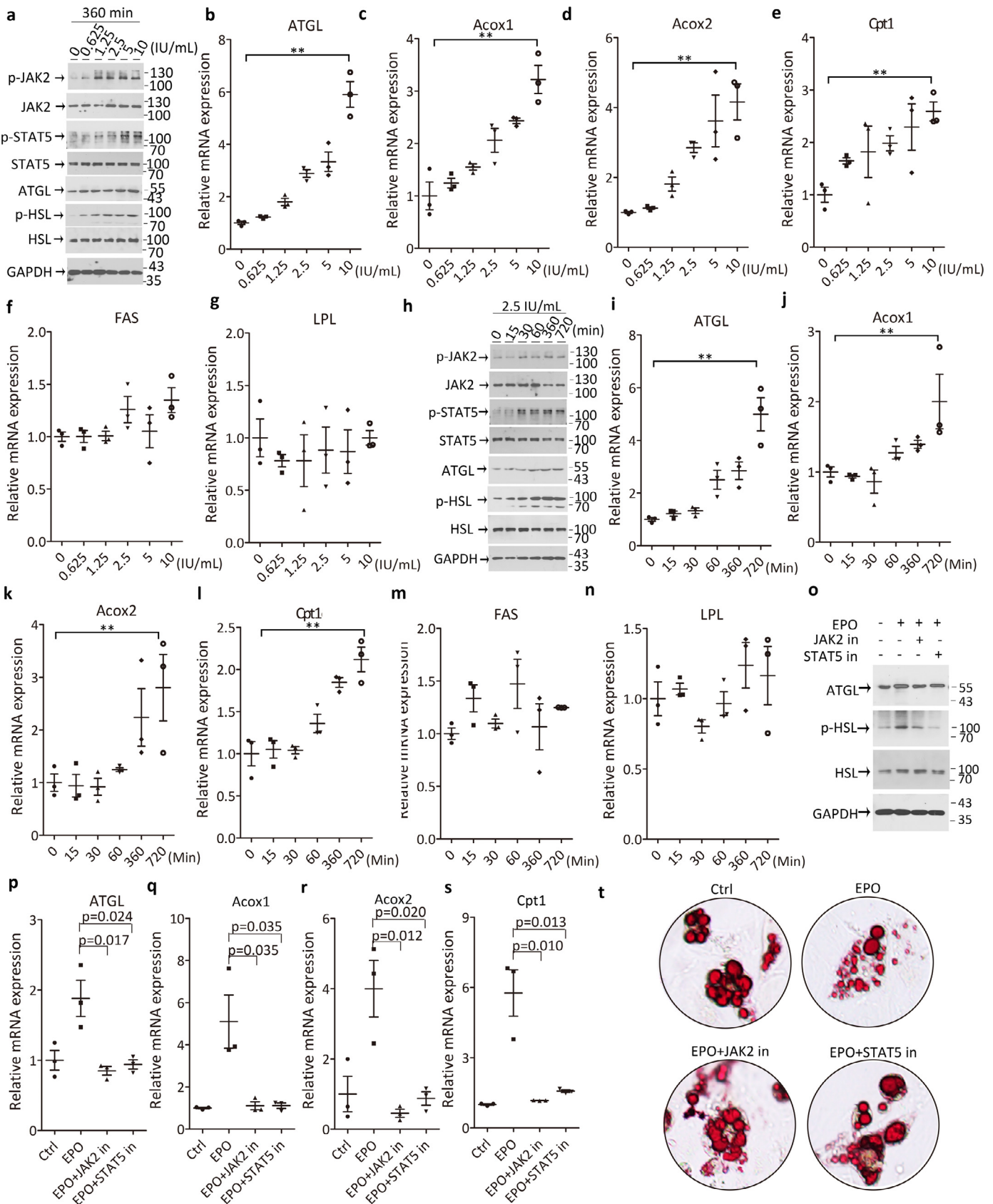


Fig. 4. EPO stimulated lipid catabolism in a time- and dose- dependent manner in adipocytes. (a) Western blot for phosphorylated JAK2, STAT5, HSL and ATGL protein expression in primary isolated adipocytes treated with EPO at 0, 0.625, 1.25, 2.5, 5, 10 IU/mL for 360 min. (b–g) Relative mRNA expression of ATGL (b), Acox1 (c), Acox2 (d), Cpt1 (e), FAS (f), LPL (g) in primary isolated adipocytes treated with EPO at various dose for 360 min. (h) Western blot for phosphorylated JAK2, STAT5, HSL and ATGL in primary isolated adipocytes treated with EPO (2.5 IU/mL) for 0, 15, 30, 60, 360, 720 min. (i–n) Relative mRNA expression of ATGL (i), Acox1 (j), Acox2 (k), Cpt1 (l), FAS (m), LPL (n) in primary isolated adipocytes treated with EPO for different minutes. (o) Western blot for phosphorylated HSL and ATGL protein in primary isolated adipocytes treated with EPO, EPO + JAK2 inhibitor, or EPO + STAT5 inhibitor. (p–s) Relative ATGL (p), Acox1 (q), Acox2 (r), Cpt1 (s) mRNA expression in primary isolated adipocytes treated with saline (Ctrl), EPO, EPO + JAK2 inhibitor

between JAK2-STAT5 signaling and dysregulated lipid profile in serum, we blocked JAK2-STAT5 signaling in the ischemia kidney injury animal model. Results showed that after depriving JAK2 activity (JAK2 in) in adipose tissue (Fig. 3m), the lipolytic protein including ATGL, and phosphorylated HSL (hormone sensitive lipase) in adipose tissue was suppressed, with reduced gene expression related to lipid catabolism (Fig. 3m and n). In addition, the renal ischemia suppressed TG but not cholesterol in serum was resumed by the JAK2 inhibition (Fig. 3o and p). These results showed that modulation of JAK2-STAT5 signaling in adipose tissue contributed to the dysregulated serumal TG in kidney injured mice.

3.4. EPO induces lipid catabolism in adipocytes through activation of JAK2-STAT5 signaling

Serumal EPO was influenced in kidney disease, and altered JAK2-STAT5 in adipose tissue contributed to the dysregulated TG in serum. To investigate whether EPO is the reason for altered JAK2-STAT5 signaling in adipocyte, we treated primary isolated adipocyte from subcutaneous adipose tissue with recombinant EPO. Results showed that exogenous EPO at 0.625 IU/mL was sufficient to drive the phosphorylation of JAK2 (Fig. 4a). Lipolytic protein including ATGL and phosphorylated HSL protein as well as gene expression of *ATGL*, *Acox1*, *Acox2*, *Cpt1* were upregulated by exogenous EPO in a dose-dependent manner (Fig. 4a–e). However, liposynthesis genes were not influenced by the EPO treatment (Fig. 4f and g). Moreover, a time-dependent activation of JAK2-STAT5 signaling was observed with increased lipid catabolic and unchanged liposynthetic genes expression (Fig. 4h–n). To further validate that EPO mediates lipid catabolism dependent on JAK2-STAT5 signaling. We pre-treated adipocytes with specific JAK2 inhibitor (JAK2 in, AG490) or STAT5 inhibitor (STAT5 in, CAS 285986-31-4) before 24-h EPO treatment. Results showed that depriving either JAK2 or STAT5 abrogated EPO stimulated increase in ATGL, phosphorylated-HSL protein and genes related to lipid catabolism in adipocyte (Fig. 4o–s). Enhanced lipid catabolism impairs lipid accumulation in adipocytes. Oil red O staining showed that EPO suppressed lipid accumulation in adipocyte whereas inhibition of JAK2 or STAT5 restored lipid accumulation in adipocyte (Fig. 4t). Of note, though EPO did stimulate JAK2-STAT5 signaling in a time- and dose-dependent manner in hepatocyte (Supplementary Fig. 1a and b), the minimum effective concentration of EPO was 5 IU/mL, approximately eight times the concentration in adipocyte. The relative low expression of EPO receptor (EPOR) in liver could explain why hepatocyte and adipocyte had different sensitivity to EPO (Supplementary Fig. 1c). Collectively, we found that EPO stimulated lipid catabolism in a time- and dose- dependent manner in the adipocyte by targeting JAK2-STAT5 signaling.

3.5. Excessive EPO suppresses serumal TG via activating lipid catabolism in adipose tissue

Next, we examined the physiological function of EPO in regulating lipid metabolism. For wild type mice treated with EPO for 2 weeks, adipose tissues, except for classic brown adipose tissue, shrank upon EPO stimulation, including subcutaneous adipose tissue located in the inguinal and anterior region and visceral adipose tissue surrounding the epididymis and mesentery. However, due to the small proportion of adipose tissue weight to whole body weight, we did not observe a decrease in body weight in EPO treated wild type mice and the blood glucose remained unaffected (Supplementary Fig. 2a–c). However, we found a significant decrease in the TG in serum,

without alteration in total cholesterol, LDL and HDL (Fig. 5a–d). We postulated that the low TG in the serum and downsizing adipose tissue may result from activated JAK2-STAT5 signaling in the adipose tissue. Results showed that phosphorylation of JAK2 and STAT5 went up, leading to increased ATGL and phosphorylated HSL protein (Fig. 5e). Subsequently, lipid catabolism related gene expression were upregulated whereas fatty acid synthetic genes were suppressed in adipose tissue upon EPO treatment (Fig. 5f). As expected, once we blocked JAK2-STAT5 signaling with JAK2 inhibitor, EPO treatment failed to triggered the lipid catabolism in adipose tissue (Fig. 5g and h). In addition, the EPO suppressed serumal TG was resumed upon JAK2-STAT5 inhibition without significant alteration of serumal cholesterol and HDL. Although LDL did not decrease in the EPO treated wild type mice, the deprivation of JAK2 activity upregulates LDL in serum (Fig. 5i–l). These results indicated that EPO treatment indeed enhanced lipid catabolism through sensitizing JAK2-STAT5 signaling in adipose tissue, leading to down-regulated serumal TG.

To further investigate whether EPO was capable of scavenging high blood lipid, we challenged mice with high fat diet (HFD) 1 week before EPO administration. Results showed that EPO inhibited the elevated TG but not cholesterol, LDL and HDL in serum (Fig. 5m–p). Although HFD-induced body weight gain was not rescued by the EPO treatment, the elevation of adipose tissue weight was slowed down by the EPO injection (Supplementary Fig. 2g and h). Increased JAK2-STAT5 activation and lipid catabolism, suppressed liposynthesis were observed in the adipose tissue from EPO treated HFD mice (Fig. 5q and r).

Interestingly, EPO was reported to suppress inflammation in adipose tissue. Our results showed that in both the wild type and HFD mice, exogenous EPO inhibited TNF α protein and pro-inflammatory factor gene expression including TNF α , IL-1 β (interleukin-1 β), IL-6 (interleukin-6) in adipose tissue (Fig. 5e and q, Supplementary Fig. 2d and i). In liver tissue, for both chow diet and HFD mice, though the 2-weeks EPO treatment activated JAK2-STAT5 signaling, the lipogenic and lipolytic genes expression were not significantly regulated (Supplementary Fig. 2e, f, j, k). These results suggested that EPO suppressed lipid profile in serum through activating lipid catabolism in adipose tissue *in vivo*.

3.6. Restoration of EPO rectifies hypertriglyceridemia in CKD

In CKD mice, the EPO synthesis was deficient due to impaired kidney function. We recovered EPO by intraperitoneal injection of recombinant EPO into CKD mice. Results showed that restoration of EPO in the CKD mice restrained the hypertriglyceridemia. Strikingly, the total cholesterol and LDL but not the HDL level were also suppressed by the EPO rectification (Fig. 6a–d). EPO treatment did not affect serumal CR, BUN, blood glucose and body weight in the CKD mice (Supplementary Fig. 3a–d). Kidney injury reduced the adipose tissue weight and the EPO treatment further contributed to the reduction of inguinal, and epididymal adipose tissue weight (Supplementary Fig. 3e). Activated JAK2-STAT5 in subcutaneous adipose tissue triggered the lipid catabolism without modulating liposynthesis (Fig. 6e and f). Pro-inflammatory factors expressions were also inhibited in the adipose tissue by the EPO administration (Supplementary Fig. 3f). In liver of EPO restored CKD mice, the JAK2-STAT5 signaling was mildly induced without significantly alteration in genes related to lipid metabolism (Supplementary Fig. 3g and h). These results suggested that restoration of EPO in CKD mice improved hyperlipidemia mainly by activating lipid catabolism in adipose tissue.

(EPO + JAK2 in), or EPO + STAT5 inhibitor (EPO + STAT5 in). (t) Representative images of oil red O staining in primary isolated adipocytes treated with saline (Ctrl), EPO, EPO + JAK2 inhibitor (EPO + JAK2 in), or EPO + STAT5 inhibitor (EPO + STAT5 in). Data are expressed as means \pm S.E.M, one-way ANOVAs were used for the comparison of two groups and multiple groups, respectively. $p \leq 0.05$ defined as significant different, $** \leq 0.0001$.

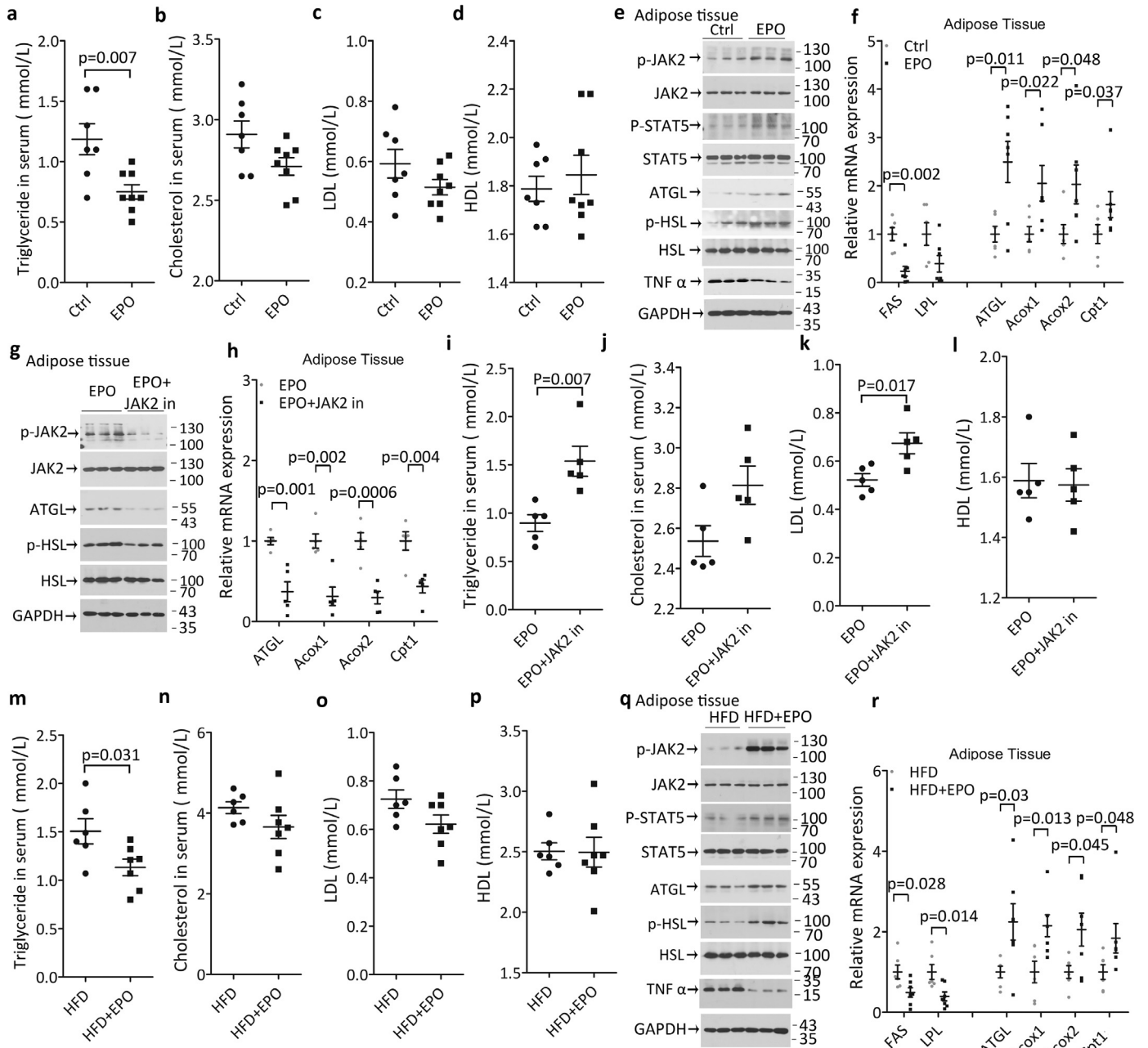


Fig. 5. EPO administration decreased lipidemia via activating JAK2-STAT5 in adipose tissue. (a–d) Triglyceride (a), total cholesterol (b), LDL (c) and HDL (d) in serum from 2-week EPO treated mice. ($n = 7$ for control, $n = 8$ for EPO mice). (e) Western blot for phosphorylated JAK2, STAT5, HSL and ATGL in adipose tissue from EPO treated mice. (f) Relative mRNA expression of lipid metabolism in adipose tissue from EPO treated mice. ($n = 7$ for control, $n = 8$ for EPO mice). (g) Western blot for phosphorylated JAK2, HSL and ATGL protein in adipose tissue from EPO administrated mice with or without JAK2 inhibitor (referred as EPO and EPO + JAK2 in). (h) Relative mRNA expression of lipid catabolism in adipose tissue from EPO and EPO + JAK2 inhibitor treated mice. ($n = 5$ for EPO, $n = 5$ for EPO+JAK2 inhibitor). (i–l) Triglyceride (i), total cholesterol (j), LDL (k) and HDL (l) in serum from EPO and EPO + JAK2 inhibitor treated mice. ($n = 5$ for EPO, $n = 5$ for EPO+JAK2 inhibitor). (m–p) Triglyceride (m), total cholesterol (n), LDL (o) and HDL (p) in serum from 2-week EPO treated mice with high fat diet feed 1 week before and during EPO treatment. ($n = 6$ for HFD, $n = 7$ for HFD + EPO mice). (q) Western blot for phosphorylated JAK2, STAT5, HSL and ATGL in adipose tissue from EPO treated mice with high fat diet above. (r) Relative mRNA expression of lipid metabolism in adipose tissue from EPO treated mice with high fat diet above ($n = 6$ for HFD, $n = 7$ for HFD + EPO mice). Data are expressed as means \pm S.E.M. Student’s *t*-test was used for the comparison of two groups. $p \leq 0.05$ defined as significant different.

3.7. EPO negatively correlates with lipid profile in serum in AKI patients

It is well accepted that EPO deficiency presents in CKD patients with hyperlipidemia [2,44,45]. However, whether lipid profile and EPO secretion are also dysregulated in patients with acute kidney injury is not well illustrated. We collected blood samples from patients developed acute kidney injury (AKI) after cardiopulmonary bypass surgery (AKI was defined as over 1.5-fold increase for serum creatinine within 7 days after surgery [KDIGO stage 1, 2, or 3], all

patients with AKI were recovered before discharge) (Supplementary Table 1). Results showed that out of 21 patients diagnosed with AKI, 17 patients showed decreased serum TG and cholesterol 1 day after surgery with increased EPO secretion (Fig. 7a–c). The other four AKI patients showed elevated TG and cholesterol and repressed EPO in serum. Pearson correlation analysis showed alteration of EPO negatively correlated with TG and cholesterol change in serum (Fig. 7d and e). Consistence with the sex difference in metabolic response to EPO in rodents [46], we observed male patients were

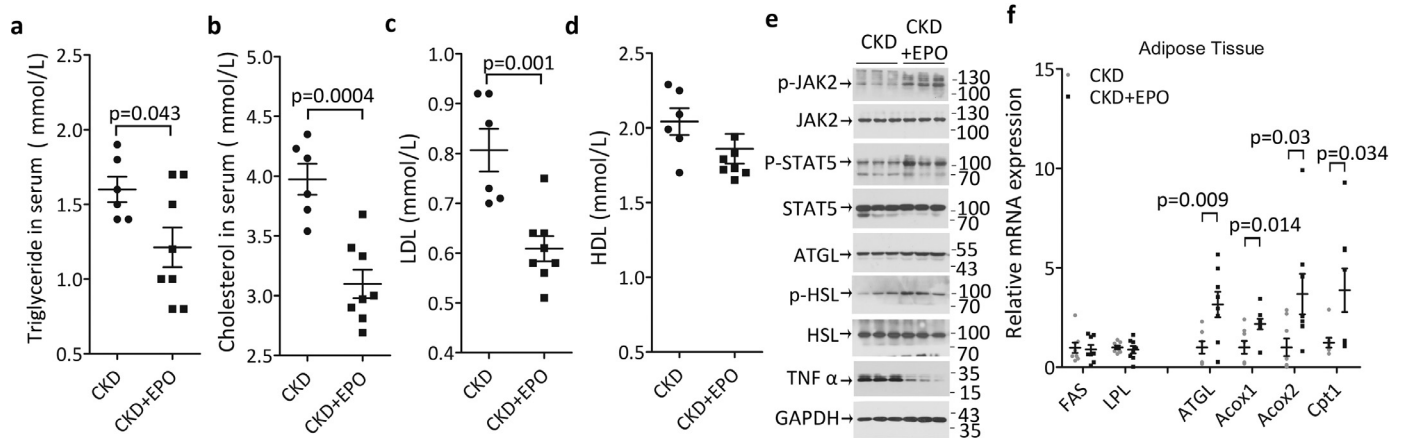


Fig. 6. Restored EPO mitigated hyperlipidemia in CKD mice. (a–d) Triglyceride (a) total cholesterol (b), LDL (c) and HDL (d) in serum from 2-week EPO treated CKD mice 2 weeks after surgery. ($n = 6$ for CKD, $n = 8$ for CKD+EPO mice). (e) Western blot for phosphorylated JAK2, STAT5, HSL and ATGL in adipose tissue from EPO treated CKD mice 2 weeks after surgery. (f) Relative mRNA expression of lipid metabolism in adipose tissue from EPO treated CKD mice 2 weeks after surgery. ($n = 6$ for CKD, $n = 8$ for CKD+EPO mice). Data are expressed as means \pm S.E.M. Student's *t*-test was used for the comparison of two groups. $p \leq 0.05$ defined as significant different, $** \leq 0.0001$.

more sensitive to EPO, which could be reflected in a sharper down-regulation of serum TG by 35% in male compared to 23% in female with-out significant difference in EPO alteration (Supplementary Fig. 4a and b). To exclude the possibility that the decreased TG and cholesterol may result from food deprivation before and after surgery, we collected blood samples from patients underwent cardiac surgery without cardiopulmonary bypass. These patients underwent the same fasting process as the patients with cardiopulmonary bypass. In

these patients, the serumal TG and cholesterol showed 9.6% and 19.6% decrease at D1 compared to D0, respectively (Supplementary Fig. 4c and d). In AKI patient, the number is 29.8% and 39.6% for TG and cholesterol, respectively. These data suggested that the more striking declined TG and cholesterol in serum from AKI patients were the consequence of altered EPO but not fasting. To further testify whether EPO secretion correlated with lipid profile in non-AKI patients underwent cardiopulmonary bypass surgery. We collected

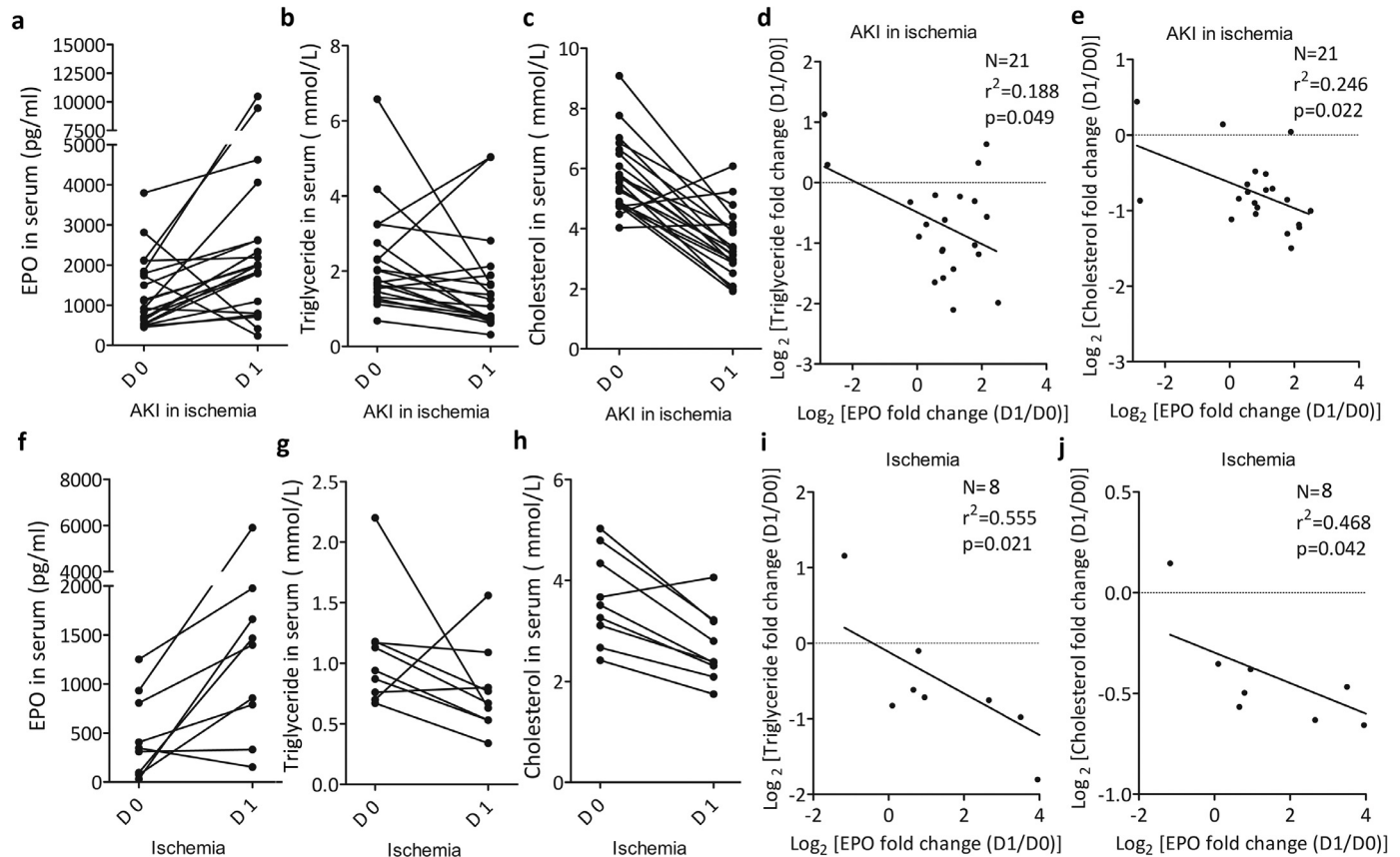


Fig. 7. EPO negatively correlated with blood lipid in patients with kidney injury. (a–c) EPO (a), triglyceride (b), and total cholesterol (c) in the serum before surgery and 1 day after surgery from patient developed ischemia-induced AKI. (d and e) Pearson correlation analysis between logarithmically (log)-transformed EPO fold change and logarithmically (log)-transformed triglyceride (d) cholesterol (e) fold change. (f–h) EPO (f), triglyceride (g) and total cholesterol (h) in the serum before surgery and 1 day after surgery from patient with ischemia alone without ischemia-induced AKI (i, j) Pearson correlation analysis between logarithmically (log)-transformed EPO fold change and logarithmically (log)-transformed triglyceride (i) cholesterol (j) fold change.

eight serum specimens from patients who went through the same surgically anoxic process with unchanged serum creatinine. Results showed that EPO elevation in the serum indeed negatively correlated with decreased TG and cholesterol in serum (Fig. 7f–j).

Though all the AKI patients recovered before discharge. There were eight patients whose serum creatinine remained 30% higher at day 7 than day 0. We classified these patients into relatively severe kidney injury because of prolonged recovery time. Strikingly, six patients within this group showed decreased serum EPO at day 1. And only two patients in this group showed suppressed TG (Supplementary Fig. 4 c and d).

Results from patients confirmed that in ischemia with or without totally recovered AKI, the EPO in serum negatively correlated with the suppressed lipid components.

4. Discussion

In this study, we demonstrated the kidney-secreted EPO functions as a negative mediator of lipid profile in blood. EPO stimulates lipolysis in peripheral adipose tissue by activating JAK2-STAT5 signaling and may serve as a potential therapeutic target.

CKD patients develop hyperlipidemia at early stage of renal dysfunction. In turn, the dyslipidemia impairs kidney function and aggravates CKD-associated cardiovascular disease. Although several molecules and pathways have been reported to participate in dyslipidemia in CKD [2,6], there remains a large gap in understanding the mechanism underlying the dyslipidemia in CKD. We provided evidence demonstrating that EPO went down and serum TG went up as early as one day after severe kidney injury. In contrast, ischemia stimulated EPO secretion through hypoxia signaling activation, led to suppressed TG in serum in both mice and human patients (Figs. 1, 2, and 7). These data indicated that dyslipidemia was caused by aberrant kidney-secreted EPO.

Either suppressing TG, LDL, cholesterol or up-regulating HDL exert beneficial effect in dyslipidemia in CKD. Clinically, Statins are the most commonly used agents to rectify dyslipidemia by inhibiting HMG CoA reductase in liver, which drives LDL receptor expression and accelerates LDL clearance from circulation. However, side effects, contraindications, and ineffectiveness in dialysis CKD patients limited the statins utilization. Ezetimibe lowers LDL by inhibiting dietary cholesterol absorption in intestine with relatively modest lipid lowering effects. Therefore, Ezetimibe is always used in combination with statins to enhance statins effects. Fibrates agitates peroxisome proliferator alpha receptor, expediting TG clearance and HDL biogenesis. However, side effects include serum creatinine accumulation limit its popularization. Our results demonstrated that physiologically, EPO administration lowered the TG content in serum. Pathologically, restoration of EPO suppressed increased TG, cholesterol and LDL in serum in CKD mice (Figs. 5 and 6). Unlike other antilipemic strategies which may cause side effects including elevation in serum creatinine, EPO treatment affected neither creatinine nor BUN in serum in the context of CKD (Supplementary Fig. 3).

EPO initially binds to its receptor EPOR, resulting in JAK2 activation. And then the phosphorylated STAT5 translocates into nucleus to upregulate lipid catabolic genes expression. Besides hematopoietic tissue, EPOR is abundant in adipose tissue. Moreover, liver and adipose tissue are the major organs that participate in lipid metabolism. It is reported that disruption of JAK2-STAT5 impairs lipolysis in adipose tissue. Our results showed that EPO administration activated JAK2-STAT5 signaling, which resulted in upregulation of lipolytic protein including ATGL and HSL phosphorylation, the rate limiting enzyme in lipolysis. What's more, lipolysis and fatty acid oxidation related gene expression including *ATGL*, *Cpt1*, *Acox1*, *Acox2* were elevated upon EPO stimulation and therefore led to enhanced lipid

catabolism both *in vitro* and *in vivo* (Figs. 3–6). Once the JAK2-STAT5 signaling was inhibited by the JAK2 inhibitor, the EPO cannot exert the lipid catabolic effect in adipose tissue or adipocytes. Although we observed JAK2-STAT5 signaling in liver was also upregulated upon EPO stimulation, the genes involved in lipid metabolism were not affected. This may be explained that EPOR is relatively mildly expressed in the liver tissue, making downstream JAK2-STAT5 signaling less relevant to lipid metabolism. Some other mechanism including reported constitutive androstane receptor may contribute to lipid metabolism in liver as well.

It was assumed that perioperative statin treatment would reduce the acute kidney injury incidence after cardiac surgery. However, double-blinded, placebo-controlled, randomized clinical trials showed that high dose perioperative statin failed to reduce the risk of AKI overall [47,48]. Our clinical data showed that in quickly fixed AKI patients, circulation EPO increased whereas TG and cholesterol declined 1 day post surgery. However, if the time for kidney to recovery increases, the EPO decreased with non-reduced TG (Fig. 7). Our data provided insights to explain the unexpected results of the clinical trial. Altered EPO not only influence lipidemia but also indicates the severity of renal injury. Therefore the hypolipidemia is a consequence of sustainable renal function, rather than the beneficial contributor to AKI development, which explains why lipid-lowering statin failed to reduce the incidence of AKI.

In conclusion, our study revealed EPO, a renal secreted cytokines, plays anti-dyslipidemia role in CKD by activating lipolysis in peripheral adipose tissue. These findings shed light on mechanisms underlying dyslipidemia in renal diseases from another facet and provide theoretical basis for potential approaches to correct dyslipidemia in CKD by targeting JAK2-STAT5 signaling in adipose tissue or EPO supplements.

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Declaration of Competing Interest

The authors declare that there is no duality of interest associated with this manuscript.

CRedit authorship contribution statement

Jinxiang Li: Experiments investigation, data curation, formal analysis, writing original draft. **Minliang Yang:** Experiments investigation, data curation, formal analysis. **Zhuo Yu:** Resources of clinical data. **Jianwei Tian:** Investigation. **Songlin Du:** Resources of clinical data. **Hanying Ding:** Supervised the project, funding acquisition, writing original draft and review & editing.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2019.11.007.

References

- 1 Wanner C, Tonelli M. Kidney disease: improving global outcomes lipid guideline development work group M. KDIGO clinical practice guideline for lipid management in CKD: summary of recommendation statements and clinical approach to the patient. *Kidney Int* 2014;85(6):1303–9.

- 2 Hager MR, Narla AD, Tannock LR. Dyslipidemia in patients with chronic kidney disease. *Rev Endocr Metab Disord* 2017;18(1):29–40.
- 3 Shrestha P, van de Sluis B, Dullaart RPF, van den Born J. Novel aspects of PCSK9 and lipoprotein receptors in renal disease-related dyslipidemia. *Cell Signal* 2019;55:53–64.
- 4 Iseki K. Epidemiology of dyslipidemia in chronic kidney disease. *Clin Exp Nephrol* 2014;18(2):185–8.
- 5 Lamprea-Montealegre JA, McClelland RL, Grams M, Ouyang P, Szklo M, de Boer IH. Coronary heart disease risk associated with the dyslipidaemia of chronic kidney disease. *Heart* 2018;104(17):1455–60.
- 6 Epstein M, Vaziri ND. Statins in the management of dyslipidemia associated with chronic kidney disease. *Nat Rev Nephrol* 2012;8(4):214–23.
- 7 Ferro CJ, Mark PB, Kanbay M, Sarafidis P, Heine GH, Rossignol P, et al. Lipid management in patients with chronic kidney disease. *Nat Rev Nephrol* 2018;14(12):727–49.
- 8 Liu YH, Liu Y, Chen JY, Zhou YL, Chen ZJ, Yu DQ, et al. LDL cholesterol as a novel risk factor for contrast-induced acute kidney injury in patients undergoing percutaneous coronary intervention. *Atherosclerosis* 2014;237(2):453–9.
- 9 Wanner C, Krane V, Marz W, Olschewski M, Mann JF, Ruf G, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. *N Engl J Med* 2005;353(3):238–48.
- 10 Athyros VG, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, et al. Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek atorvastatin and coronary heart disease evaluation (GREACE) study: a post-hoc analysis. *Lancet* 2010;376(9756):1916–22.
- 11 Thompson PD, Clarkson P, Karas RH. Statin-associated myopathy. *JAMA* 2003;289(13):1681–90.
- 12 Souma T, Nezu M, Nakano D, Yamazaki S, Hirano I, Sekine H, et al. Erythropoietin synthesis in renal myofibroblasts is restored by activation of hypoxia signaling. *J Am Soc Nephrol: JASN* 2016;27(2):428–38.
- 13 Mack M, Yanagita M. Origin of myofibroblasts and cellular events triggering fibrosis. *Kidney Int* 2015;87(2):297–307.
- 14 Fisher JW, Koury S, Ducey T, Mendel S. Erythropoietin production by interstitial cells of hypoxic monkey kidneys. *Br J Haematol* 1996;95(1):27–32.
- 15 Quaggin SE, Kapus A. Scar wars: mapping the fate of epithelial-mesenchymal-myofibroblast transition. *Kidney Int* 2011;80(1):41–50.
- 16 Nangaku M. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol: JASN* 2006;17(1):17–25.
- 17 Koury MJ. Abnormal erythropoiesis and the pathophysiology of chronic anemia. *Blood Rev* 2014;28(2):49–66.
- 18 Bauer C, Kurtz A. Oxygen sensing in the kidney and its relation to erythropoietin production. *Annu Rev Physiol* 1989;51:845–56.
- 19 Gobe GC, Bennett NC, West M, Colditz P, Brown L, Vesey DA, et al. Increased progression to kidney fibrosis after erythropoietin is used as a treatment for acute kidney injury. *Am J Physiol Renal Physiol* 2014;306(6):F681–92.
- 20 Wen Y, Zhou X, Lu M, He M, Tian Y, Liu L, et al. Bclaf1 promotes angiogenesis by regulating HIF-1alpha transcription in hepatocellular carcinoma. *Oncogene* 2019;38(11):1845–59.
- 21 Zhou L, Wang Y, Zhou M, Zhang Y, Wang P, Li X, et al. HOXA9 inhibits HIF-1alpha-mediated glycolysis through interacting with CRIP2 to repress cutaneous squamous cell carcinoma development. *Nat Commun* 2018;9(1):1480.
- 22 Kokot F, Wiecek A, Grzeszczak W, Klepacka J, Klin M, Lao M. Influence of erythropoietin treatment on endocrine abnormalities in haemodialyzed patients. *Contrib Nephrol* 1989;76:257–70.
- 23 Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, et al. In vivo evidence that erythropoietin protects neurons from ischemic damage. *Proc Natl Acad Sci USA* 1998;95(8):4635–40.
- 24 Tsai PT, Ohab JJ, Kertesz N, Groszer M, Matter C, Gao J, et al. A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. *J Neurosci* 2006;26(4):1269–74.
- 25 Zhou S, Liu YG, Zhang Y, Hu JM, Liu D, Chen H, et al. Bone mesenchymal stem cells pretreated with erythropoietin enhance the effect to ameliorate cyclosporine A-induced nephrotoxicity in rats. *J Cell Biochem* 2018;119(10):8220–32.
- 26 Goto T, Saika H, Takahashi T, Maeda A, Mune M, Yukawa S. Erythropoietin supplement increases plasma lipoprotein lipase and hepatic triglyceride lipase levels in hemodialysis patients. *Kidney Int Suppl* 1999;71:S213–5.
- 27 Mak RH. Metabolic effects of erythropoietin in patients on peritoneal dialysis. *Pediatric Nephrol* 1998;12(8):660–5.
- 28 Teng R, Gavrilova O, Suzuki N, Chanturiya T, Schimmel D, Hugendubler L, et al. Disrupted erythropoietin signalling promotes obesity and alters hypothalamus proopiomelanocortin production. *Nat Commun* 2011;2:520.
- 29 Zhang YL, Radhakrishnan ML, Lu X, Gross AW, Tidor B, Lodish HF. Symmetric signaling by an asymmetric 1 erythropoietin: 2 erythropoietin receptor complex. *Mol Cell* 2009;33(2):266–74.
- 30 Shi Z, Hodges VM, Dunlop EA, Percy MJ, Maxwell AP, El-Tanani M, et al. Erythropoietin-induced activation of the JAK2/STAT5, PI3K/Akt, and RAS/ERK pathways promotes malignant cell behavior in a modified breast cancer cell line. *Mol Cancer Res: MCR* 2010;8(4):615–26.
- 31 Tsuma Y, Mori J, Ota T, Kawabe Y, Morimoto H, Fukuhara S, et al. Erythropoietin and long-acting erythropoiesis stimulating agent ameliorate non-alcoholic fatty liver disease by increasing lipolysis and decreasing lipogenesis via EPOR/STAT pathway. *Biochem Biophys Res Commun* 2019;509(1):306–13.
- 32 Wang L, Teng R, Di L, Rogers H, Wu H, Kopp JB, et al. PPARalpha and Sirt1 mediate erythropoietin action in increasing metabolic activity and browning of white adipocytes to protect against obesity and metabolic disorders. *Diabetes* 2013;62(12):4122–31.
- 33 Li T, Weng J, Zhang Y, Liang K, Fu G, Li Y, et al. mTOR direct crosstalk with STAT5 promotes de novo lipid synthesis and induces hepatocellular carcinoma. *Cell Death Dis* 2019;10(8):619.
- 34 Kaltenecker D, Mueller KM, Benedikt P, Feiler U, Themanns M, Schleder M, et al. Adipocyte STAT5 deficiency promotes adiposity and impairs lipid mobilisation in mice. *Diabetologia* 2017;60(2):296–305.
- 35 Hernandez GG. researchers ignore ethnicity in defining family caregiver burden and recommending services. *Gerontologist* 1991;31(2):271–2.
- 36 Shi SY, Luk CT, Brunt JJ, Sivasubramaniyam T, Lu SY, Schroer SA, et al. Adipocyte-specific deficiency of Janus kinase (JAK) 2 in mice impairs lipolysis and increases body weight, and leads to insulin resistance with ageing. *Diabetologia* 2014;57(5):1016–26.
- 37 Ding H, Zheng S, Garcia-Ruiz D, Hou D, Wei Z, Liao Z, et al. Fasting induces a subcutaneous-to-visceral fat switch mediated by microRNA-149-3p and suppression of PRDM16. *Nat Commun* 2016;7:11533.
- 38 Ding H, Zhang Y, Xu C, Hou D, Li J, Zhang Y, et al. Norathyriol reverses obesity- and high-fat-diet-induced insulin resistance in mice through inhibition of PTP1B. *Diabetologia* 2014;57(10):2145–54.
- 39 Vaziri ND. HDL abnormalities in nephrotic syndrome and chronic kidney disease. *Nat Rev Nephrol* 2016;12(1):37–47.
- 40 Rankin EB, Wu C, Khatri R, Wilson TL, Andersen R, Araldi E, et al. The HIF signaling pathway in osteoblasts directly modulates erythropoiesis through the production of EPO. *Cell* 2012;149(1):63–74.
- 41 Hirako S, Wada N, Kageyama H, Takenoya F, Izumida Y, Kim H, et al. Autonomic nervous system-mediated effects of galanin-like peptide on lipid metabolism in liver and adipose tissue. *Sci Rep* 2016;6:21481.
- 42 Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ilttrich H, Peldschus K, et al. Brown adipose tissue activity controls triglyceride clearance. *Nat Med* 2011;17(2):200–5.
- 43 Choi YJ, Zhou D, Barbosa ACS, Niu Y, Guan X, Xu M, et al. Activation of constitutive androstane receptor ameliorates renal ischemia-reperfusion-induced kidney and liver injury. *Mol Pharmacol* 2018;93(3):239–50.
- 44 Singh AK, Szczech L, Tang KL, Barnhart H, Sapp S, Wolfson M, et al. Correction of anemia with epoetin alfa in chronic kidney disease. *N Engl J Med* 2006;355(20):2085–98.
- 45 Toth PP, Dwyer JP, Cannon CP, Colhoun HM, Rader DJ, Upadhyay A, et al. Efficacy and safety of lipid lowering by alirocumab in chronic kidney disease. *Kidney Int* 2018;93(6):1397–408.
- 46 Zhang Y, Rogers HM, Zhang X, Noguchi CT. Sex difference in mouse metabolic response to erythropoietin. *FASEB J Off Publ Fed Am Soc Exp Biol* 2017;31(6):2661–73.
- 47 Argaliou M, Xu M, Sun Z, Smedira N, Koch CG. Preoperative statin therapy is not associated with a reduced incidence of postoperative acute kidney injury after cardiac surgery. *Anesth Analg* 2010;111(2):324–30.
- 48 FtT Billings, Hendricks PA, Schildcrout JS, Shi Y, Petracek MR, Byrne JG, et al. High-dose perioperative atorvastatin and acute kidney injury following cardiac surgery: a randomized clinical trial. *JAMA* 2016;315(9):877–88.