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Uninephrectomy and class II PI3K-C2β inactivation synergistically protect against obesity, insulin resistance and liver steatosis in mice

Samira Alliouachene[1] | Julius E. Kieswich[1] | Benoit Bilanges[2] | Kieran McCafferty[1] | Christoph Thiemermann[1] | Bart Vanhaesebroeck[2] | Muhammad M. Yaqoob[1]

[1]Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Queen Mary University of London, London, UK

[2]UCL Cancer Institute, University College London, London, UK

Correspondence
Samira Alliouachene and Muhammad M. Yaqoob, Centre for Translational Medicine and Therapeutics, William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, United Kingdom.
Email: samira.alliouachene@qmul.ac.uk (S. A.); m.m.yaqoob@qmul.ac.uk (M. M. Y.)

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Uninephrectomy (UNx) in living kidney donors for transplantation is now routine clinical practice. While chronic kidney disease, due to bilateral kidney dysfunction, is associated with insulin resistance, liver steatosis, and type 2 diabetes, the metabolic impact of UNx remains unclear. To better understand the crosstalk between the kidney and insulin target tissues, we studied the metabolic consequences of UNx and the potential involvement of class II PI3K-C2β, the inactivation of which has been reported to result in insulin sensitization. Mice underwent UNx or sham operation followed by either normal chow or high-fat diet (HFD). Seventeen weeks post-UNx, mice showed improved glucose tolerance, insulin sensitivity, and decreased HFD-induced liver steatosis. This was associated with an enhanced serum FGF21 and insulin-stimulated Akt signaling in the liver and muscle of both lean and obese mice. Remarkably, the combination of UNx and PI3K-C2β inactivation protected against HFD-induced obesity and further potentiated the metabolic improvement observed in WT UNx mice correlating with a synergistic increase in metabolic tissues of (1) insulin-stimulated Akt signaling (2) FGFR1 and βKlotho expression. We demonstrated a potential beneficial effect of kidney donation and more effectively with PI3K-C2β inactivation to protect against metabolic disorders through a mutual insulin/FGF21 sensitization

KEYWORDS
basic (laboratory) research/science, diabetes: type 2, donors and donation: donor follow-up, endocrinology/diabetology, kidney transplantation/nephrology, kidney transplantation: living donor, metabolic syndrome, signaling/signaling pathways: PI-3 kinase/Akt pathway, translational research/science

1 | INTRODUCTION

The kidney is an important organ contributing to glucose hemeostasis. Thus, most of CKD patients, with bilateral kidney dysfunction, develop metabolic disorders such as insulin resistance, type 2 diabetes, and liver steatosis leading to cardiovascular disease and increased mortality.[1-5] These observations led clinicians to question the safety of living kidney donation for transplantation which is currently the best

Abbreviations: CKD, chronic kidney disease; DKD, diabetes kidney disease; FAS, fatty acid synthase; FGF21, fibroblast growth factor 21; FGFR1, fibroblast growth factor receptor 1; G6Pase, glucose 6 phosphatase; GFR, glomerular filtration rate; HFD, high-fat diet; KI, knock-in; NCD, normal chow diet; PI3K-C2β, phosphoinositide 3-kinase-C2β; UNx, uninephrectomy; WAT, white adipose tissue.

treatment for patients suffering from end-stage renal disease. A major concern regarding the use of living donors is whether UNx predisposes the donor to develop CKD and metabolic disorders. Despite numerous experimental and clinical studies documenting the metabolic consequences of a kidney donation through UNx, only few and conflicting data have been reported.[6-14] Several recent clinical studies performed on living kidney donors have suggested that kidney donation may result in insulin resistance and type 2 diabetes even independently of changes in glomerular filtration rate (GFR).[6,7] In contrast, other clinical studies indicated that the risk factors for the development of diabetes after donation are similar to what is seen in the general population.[11,14] Moreover, a recent clinical meta-analysis found that donors had no increased risk of developing diabetes.[13] Experimental studies on rats showed that UNx leads to kidney injury together with glucose intolerance, insulin resistance, and dyslipidemia from 6 months post-surgery.[9,10] Conversely, others have reported a beneficial effect of UNx in rats from 13 weeks post-surgery, with a lower basal glycemia and an increase insulin sensitivity despite a mild decrease in renal function.[15] Moreover, a recent study in mice showed no differences in glucose tolerance or insulin sensitivity even 4 months post-UNx and reported an unexpected opposite effect on liver and skeletal muscle in obese mice, whereby UNx protected HFD-fed mice from hepatic insulin resistance and steatosis but led to skeletal muscle insulin resistance.[16] Despite all these studies, the metabolic consequences of UNx are still unclear and systemic insulin signaling in insulin target tissues (liver, muscle, and adipose tissue) post-UNx remain to be investigated. Therefore, it remains critical to investigate the effect of UNx on glucose homeostasis and systemic insulin signaling and gain more understanding on the crosstalk between the kidney and insulin target tissues.

FGF21 serum concentrations have been shown to be elevated in kidney donors post-UNx suggesting renal excretion as a major route for FGF21 elimination and making this endocrine/paracrine factor an attractive candidate to play a role in the crosstalk between the kidney and insulin target tissues.[17] Recently, FGF21 has emerged as an important metabolic regulator having a favorable effect on glucose and lipid metabolism.[18-21] FGF21 is mainly produced by the liver and initiates its action by activating a unique dual receptor complex consisting of a co-receptor βklotho and the tyrosine kinase FGF receptor 1 (FGFR1).[21] Transgenic mice overexpressing FGF21 or pharmacological administration of FGF21 in mice induces Akt phosphorylation in metabolic organs, improves insulin sensitivity, and protects against HFD-induced obesity, insulin resistance, and liver steatosis.[22] The crosstalk between FGF21 and insulin sensitivity is further supported by recent multiple reports demonstrating a synergy between FGF21 and insulin through a mutual sensitization to synergistically increase Akt signaling and regulate glucose and lipid metabolism in cell culture, animals, and humans.[20,23-25] Conversely, although FGF21 performs several beneficial functions, its serum level is known to be paradoxically elevated in several metabolic disorders, such as obesity, insulin resistance, and liver steatosis suggesting a potential FGF21-resistant state.[26-28] In support of this hypothesis, mRNA expression and activity of FGFR1 have been shown to be reduced in liver and white adipose tissue (WAT) in both obese and diabetic mice.

In the present study, we first addressed the impact of UNx on whole-body glucose homeostasis and systemic insulin signaling in mice. To gain more understanding on the crosstalk between the kidney and insulin target tissues, we also explored the possible implication of the class II PI3K-C2β isoform for several reasons. First, PI3K-C2β gene expression has been found upregulated in kidneys of diabetic nephropathy patients.[29] Second, this lipid kinase plays an important role in insulin signaling and it has been reported that its inactivation increased insulin sensitivity and protects against HFD-induced liver steatosis in mice.[30] Altogether, this suggested a potential role of PI3K-C2β in the crosstalk between the kidney and insulin target tissues to regulate glucose metabolism in UNx context. To assess the role of PI3K-C2β after UNx, we performed UNx in PI3K-C2β kinase-dead knock-in mice[30] (further referred to as $C2\beta^{D1212A/D1212A}$ mice) as a model to mimic the impact of a pharmacological PI3K-C2β inhibitor. Because of the high probability of developing obesity with age after kidney donation, we investigated both lean and obese mice and used HFD mice as an obesity mouse model.

2 | METHODS

2.1 | Mice

All animal experiments were conducted in accordance with the United Kingdom Home Office Animals 1986 Scientific Procedures. All experiments were performed on 7-week-old male C57BL/6J mice. Mice were kept on standard chow diet (20% protein, 75% carbohydrate, 5% fat) on a 12-h light–dark cycle, with free access to water in individually ventilated cages. For HFD experiments, mice were maintained on diet 824053 from Special Diet Services Inc. (20% protein, 35% carbohydrate, and 45% fat) for 16 weeks. Mice underwent UNx or sham operation at 7 weeks of age. Left nephrectomy was performed following an incision on the left dorsolateral paralumbar region. Sham-operated control mice underwent identical procedure except for kidney removal. The mice were monitored until they recovered from anesthesia. After a week, UNx mice were given high-fat diet for 16 weeks. PI3K-C2β kinase-dead knock-in mice ($C2\beta^{D1212A/D1212A}$ mice) have been reported earlier.[30]

2.2 | Metabolic analysis

Oral glucose tolerance test and insulin tolerance test were performed at 13 and 15 weeks post-UNx, respectively. The procedure was described in supplemental material. In vivo insulin stimulation was performed as already described.[30] Serum levels of insulin, IGF1, leptin, triglyceride, cholesterol, adiponectin, and FGF-21 were measured by ELISA and colorimetric kit (Alpha diagnostic for insulin, Thermofisher for IGF-1, Millipore for leptin and adiponectin; Cayman Chemical Company for Triglyceride and cholesterol; R&D System for FGF-21). Triglyceride levels in liver tissue were determined by ELISA (Abcam).

2.3 | Western blot analysis

Procedure has been described earlier.[30] All antibodies were against mouse proteins as follows: pAkt-S473, total Akt, FAS, FGFR1 (Cell Signaling Technology) all used at 1:1000 dilution, G6Pase (Santa Cruz) used at 1:500 dilution, and α-tubulin (Sigma) used at 1:5000 dilution.

2.4 | Histology

Procedure has been described earlier.[30]

2.5 | RNA extraction, cDNA synthesis, and quantitative RT PCR

20 mg of liver and 80 mg of WAT tissue were used for RNA extraction using the Qiagen RNeasy® Fibrous Tissue Mini Kit (Qiagen) for the liver and Qiagen RNeasy® Lipid Tissue Mini Kit (Qiagen) for the WAT. 500 ng of extracted RNA was used for the reverse transcription using a superscript first-strand synthesis kit (RT2 First Strand Kit (Qiagen). Real-time PCRs were performed using SYBR Green master mix (Qiagen) using 5 ng of cDNA. See the primers used in supplemental material.

2.6 | Statistical analysis

All data are shown as mean ± SEM. Data sets were compared for statistical significance using a two-tailed Student's *t* test. All statistical analyses were generated using Excel software and statistical significance indicated as $^{*}p < .05$, $^{**}p \leq .01$, and $^{***}p \leq .001$.

3 | RESULTS

3.1 | Combination of UNx and PI3 K-C2β inactivation decreases HFD-induced obesity

We first investigated the impact of UNx and PI3K-C2β inactivation on body weight under both NCD and HFD. Under NCD, body weight gain was not affected either by UNx or/and PI3K-C2β inactivation (Figure 1A). However under HFD, while WT UNx-operated and $C2\beta^{D1212A/D1212A}$ mice showed a similar body weight gain compared to WT sham-operated mice, $C2\beta^{D1212A/D1212A}$ UNx-operated mice showed a synergistic reduction in body weight compared to WT UNx-operated or $C2\beta^{D1212A/D1212A}$ mice ($p < .05$ and $p < .01$, respectively; AUC graph). Thus, $C2\beta^{D1212A/D1212A}$ UNx-operated mice had a 17% reduction in body weight compared to WT sham-operated mice (Figure 1B; $p < .001$; AUC graph).

This decrease in body weight was not due to a decrease in food intake between WT and $C2\beta^{D1212A/D1212A}$ mice under any condition tested (Figure S1). Moreover, we have not observed an effect of UNx on PI3K-C2β mRNA or protein expression (Figure S2).

3.2 | UNx and PI3K-C2β inactivation synergistically protect against HFD-induced insulin resistance

While CKD results in multiple metabolic derangements, the metabolic impact of UNx remains largely unclear. Therefore, we next studied the effect of UNx on glucose metabolism and insulin sensitivity. Under both NCD and HFD, fasting blood glucose, insulin, and IGF-1 levels were not affected either by UNx or/and PI3K-C2β inactivation (Figure 2A,B; Figure S3). Surprisingly, in both WT lean and obese mice, UNx significantly improved glucose tolerance (Figure 2C,D; $p < .05$; AUC graph) and insulin sensitivity (Figure 2E,F; $p < .01$; AUC graph),

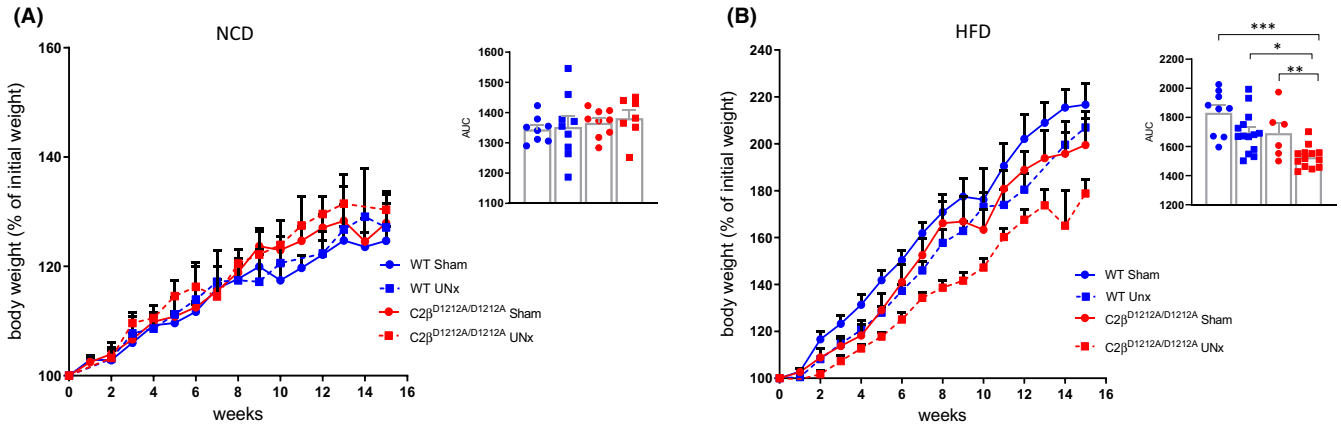

FIGURE 1 Combination of UNx and PI3K-C2β inactivation decreases HFD-induced obesity. (A) Changes in body weight of UNx and sham-operated mice fed a NCD or (B) HFD throughout the study. Body weight is expressed as a percentage of the basal starting at week 0 when UNx was performed. Area under the curve (AUC) is shown. For all experiments shown, ≥6 mice/genotype/condition were used. Data represent mean ± SEM. $^{*}p < .05$, $^{**}p \leq .01$, $^{***}p \leq .001$

highlighting a beneficial effect of UNx on glucose metabolism and insulin sensitivity. Interestingly, PI3K-C2β inactivation, which has been previously shown to increase glucose tolerance and insulin sensitivity, did not further improve UNx-induced positive impact on glucose tolerance under both NCD and HFD, and insulin sensitivity under NCD (Figure 2C–E) but accentuated the UNx-induced positive impact on insulin sensitivity under HFD only (Figure 2F; $p < .01$; AUC graph).

3.3 | UNx and PI3K-C2β inactivation synergistically protect against HFD-induced liver steatosis

With accumulating evidence demonstrating a link between CKD and hepatic steatosis and dyslipidemia,[31-33] we investigated the impact of UNx on lipid metabolism and the development of liver steatosis. Therefore, we analyzed liver histology and lipid profile in mice after UNx and PI3K-C2β inactivation. When fed NCD, liver weight was not affected either by UNx or/and PI3K-C2β inactivation (Figure 3A). However, under conditions of obesity, UNx induced a 40% reduction in liver weight compared to sham-operated mice (Figure 3A; $p < .001$). Interestingly, liver from C2β$^{D1212A/D1212A}$ mice showed a similar weight reduction under both sham and UNx conditions (Figure 3A; $p < .001$).

Histological examination of liver sections revealed no apparent differences in all groups under NCD (Figure 3B). However, in conditions of obesity, both WT UNx-operated and C2β$^{D1212A/D1212A}$ mice showed a 45% reduction in liver steatosis compared to sham-operated mice (as assessed by the presence of vacuoles) (Figure 3B; $p < .01$). Interestingly, C2β$^{D1212A/D1212A}$ UNx-operated mice were further protected against HFD-induced liver steatosis compared to WT UNx-operated and C2β$^{D1212A/D1212A}$ mice (Figure 3B; $p < .01$) and were almost completely protected against HFD-induced liver steatosis compared to control group, with a 80% reduction of hepatic lipid accumulation (Figure 3B; $p < .001$). This was correlated with a significant reduction in levels of triglycerides in the liver of C2β$^{D1212A/D1212A}$ UNx-operated mice compared to WT sham and Unx-operated mice (Figure 3C; $p < .01$ and $p < .05$, respectively). However, serum triglyceride levels did not differ between all groups in both NCD and HFD (Figure S4A). Only serum cholesterol levels were found to be decreased in C2β$^{D1212A/D1212A}$ UNx-operated mice compared to WT sham and UNx-operated animals (Figure S4B; $p < .05$). Adipokine levels such as leptin and adiponectin implicated in protecting from hepatic steatosis and lipid accumulation were unchanged in all conditions (Figure S4C,D). Correlating with the previous data, under HFD, while hepatic fatty acid synthase (FAS) and glucose 6-phosphatase (G6Pase) expression were similar in both UNx-operated and C2β$^{D1212A/D1212A}$ mice

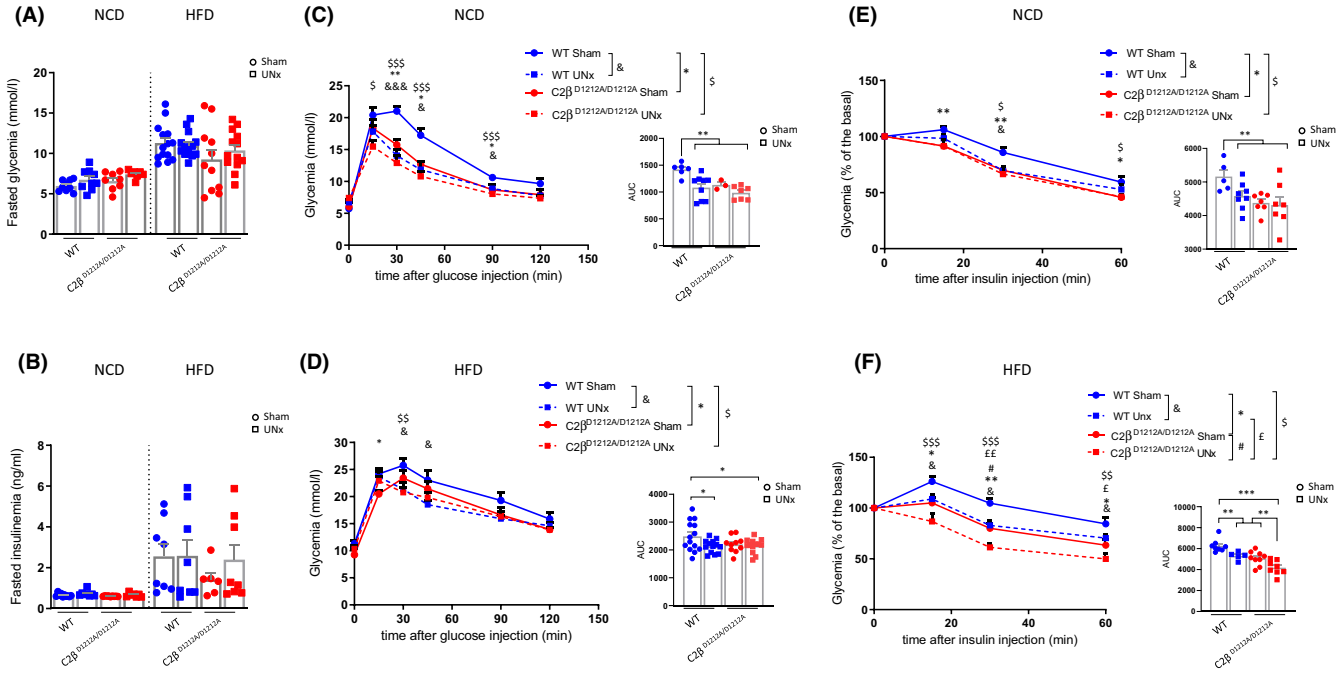

FIGURE 2 UNx and PI3K-C2β inactivation synergistically protect against HFD-induced insulin resistance. (A) Serum glucose levels and (B) serum insulin levels under fasted conditions measured in both NCD (left panel) and HFD (right panel) conditions after overnight starvation. (C) Glucose tolerance test after orally administration of 2 g/kg of glucose in mice after overnight starvation under NCD and (D) HFD. AUC is shown. (E) Insulin tolerance test after intraperitoneal injection of 0.75 U/kg of insulin in mice after overnight starvation under NCD and (F) HFD. Glucose levels are expressed relative to the levels in mice of the same genotype before injection of insulin. AUC is shown. For all experiments shown, 4–14 mice/genotype/condition were used. Data represent mean ± SEM. $^{*}p < .05$, $^{**}p \leq .01$, $^{***}p \leq .001$. $^{\&}p$ value WT sham vs WT UNx; $^{*}p$ value WT sham vs C2β$^{D1212A/D1212A}$ sham; $^{\$}p$ value WT sham vs C2β$^{D1212A/D1212A}$ sham WT UNx; $^{\#}p$ value C2β$^{D1212A/D1212A}$ sham vs C2β$^{D1212A/D1212A}$ UNx; $^{£}p$ value WT UNx vs C2β$^{D1212A/D1212A}$ UNx

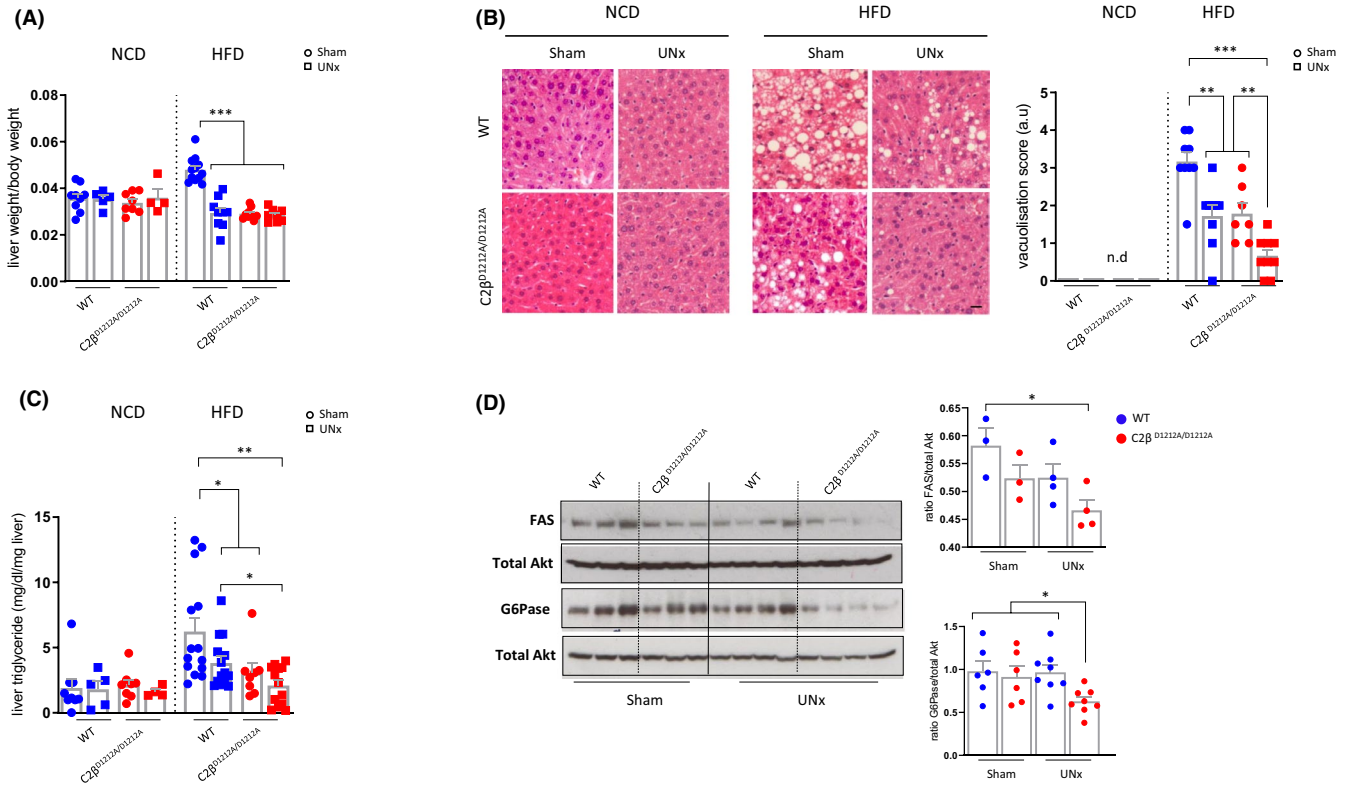

FIGURE 3 UNx and PI3K-C2β inactivation synergistically protect against HFD-induced liver steatosis. (A) Liver weight/body weight ratio of the indicated genotypes under both NCD (left) and HFD (right). (B) Liver histology. Hematoxylin and eosin staining of liver sections of mice post-UNx. Quantification of vacuolization of 7–9 livers/genotype/condition is shown on the right. a.u., arbitrary units. n.d., not detected. Scale bar, 20 μm. (C) Liver triglyceride levels of 4–14 livers/genotype/conditions is shown. (D) Liver homogenates isolated from overnight starved mice fed a HFD were analyzed by SDS-PAGE and immunoblotting using the indicated antibodies. Each lane represents an individual mouse; 3–8 mice/genotype/condition were used. Data represent mean ± SEM. *$p < .05$, **$p \leq .01$, ***$p \leq .001$

compared to control animals, we observed a significant downregulation of FAS and glucose G6Pase expression in C2β$^{D1212A/D1212A}$ UNx-operated mice, showing that Unx and PI3K-C2β inactivation combination induced a significant decrease in hepatic lipogenesis and gluconeogenesis (Figure 3D; $p < .05$).

3.4 | Combination of UNx and PI3K-C2β inactivation increases FGF21 responsiveness

We hypothesized that FGF21 could be an interesting candidate as it has been shown to be elevated in kidney donors and has recently emerged as a beneficial factor to increase insulin sensitivity.[17-21] Therefore, we measured serum FGF21 levels after UNx and PI3K-C2β inactivation. As previously observed in humans, we observed an increase in fasted serum FGF21 levels in UNx-operated animals compared to sham controls in both NCD and HFD conditions (Figure 4A; $p < .05$ under NCD and $p < .01$ under HFD). Next, we investigated whether FGF21 serum levels were also affected in C2β$^{D1212A/D1212A}$ mice, which are as insulin sensitive as UNx-operated mice and surprisingly, unlike UNx-operated mice, C2β$^{D1212A/D1212A}$ animals showed decreased serum FGF21 levels under HFD and more significantly under NCD condition, suggesting a potentially enhanced FGF21 sensitivity upon PI3K-C2β inactivation (Figure 4A; $p < .01$). Interestingly, under HFD, serum FGF21 levels in C2β$^{D1212A/D1212A}$ UNx-operated mice were similar to Unx-operated animals and remained higher compared to sham-operated mice (Figure 4A; $p < .01$). Because the elevation in circulating FGF21 levels can be explained not only by its decreased renal clearance but also by enhanced FGF21 expression and secretion; therefore, we assessed the impact of UNx and PI3K-C2β inactivation on FGF21 mRNA expression in the liver, which is the primary organ for production of circulating FGF21. Correlated with serum FGF21 levels, hepatic FGF21 mRNA expression was markedly higher in UNx-operated mice and lower in C2β$^{D1212A/D1212A}$ mice compare to WT sham mice (Figure 4B; $p < .05$), suggesting an opposite impact of UNx and PI3K-C2β inactivation on FGF21 responsiveness. However, hepatic FGF21 expression of C2β$^{D1212A/D1212A}$ UNx-operated mice was lower compared to UNx-operated animals and was comparable to sham-operated mice (Figure 4B; $p < .05$).

Recently, extensive crosstalk between FGF21 and insulin sensitivity has been reported, demonstrating mutual sensitization between these growth factors to regulate glucose and lipid metabolism.[20,23,24] These observation led us to speculate that UNx and PI3K-C2β inactivation induced insulin sensitization that could increase FGF21

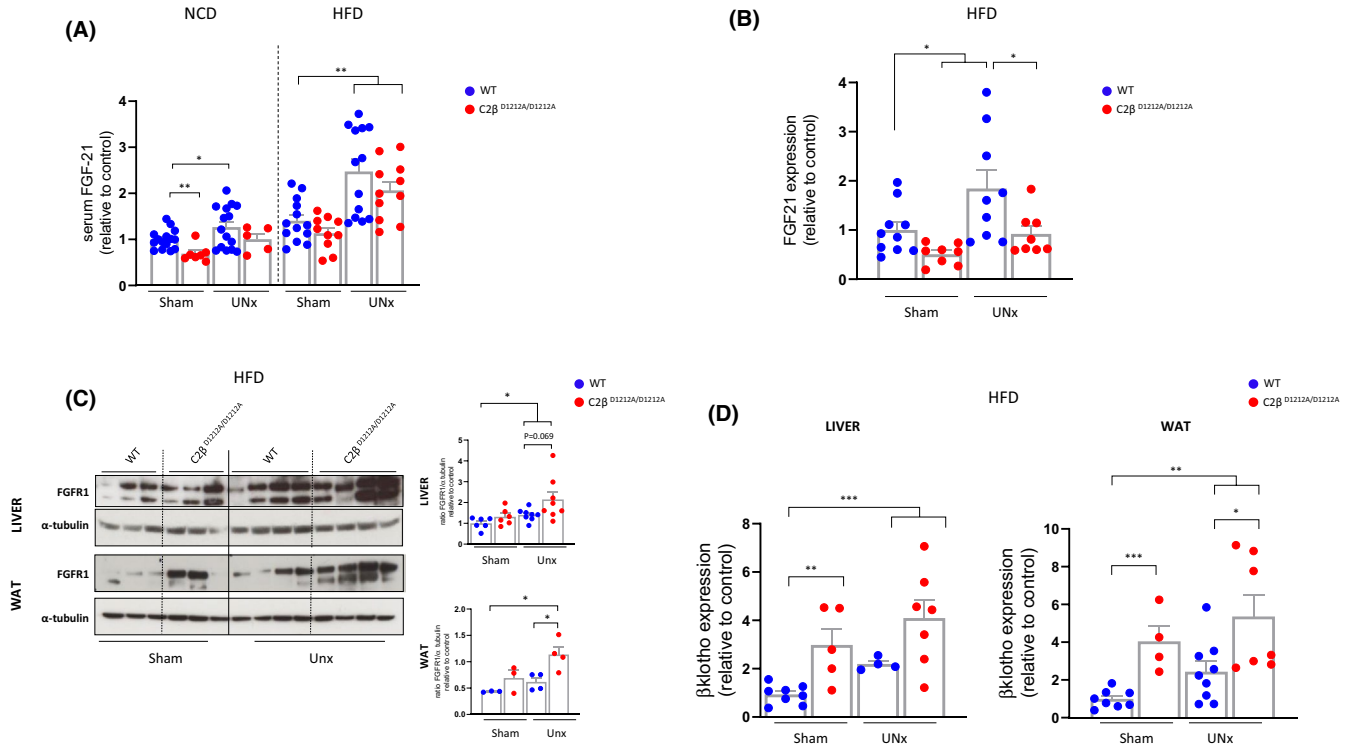

FIGURE 4 Combination of UNx and PI3K-C2β inactivation increases FGF21 responsiveness. (A) Serum FGF21 levels under fasted conditions measured in both NCD and HFD conditions after overnight starvation; 5–15 mice/genotype/condition were used. (B) Liver FGF21 mRNA expression. mRNA was extracted from overnight starved mice fed a HFD and was analyzed by QPCR; 8–10 mice/genotype/condition were used. (C) Liver and WAT homogenates isolated from overnight starved mice fed a HFD were analyzed by SDS-PAGE and immunoblotting using the indicated antibodies. Each lane represents an individual mouse; 3–8 mice/genotype/condition were used. (D) Liver and WAT β klotho mRNA expression. mRNA was extracted from overnight starved mice fed a HFD and was analyzed by QPCR; 4–9 mice/genotype/condition were used. Data represent mean ± SEM. $^{*}p < .05$, $^{**}p \leq .01$, $^{***}p \leq .001$

responsiveness.[20,23-25] We therefore investigated the impact of UNx and PI3K-C2β inactivation on expression of FGFR1 and its co receptor βklotho in the liver and WAT which are two major targets of FGF21 (Figure 4C,D). We showed that FGFR1 protein expression was slightly increased in either WT UNx-operated or C2β$^{D1212A/D1212A}$ animals and further increased in C2β$^{D1212A/D1212A}$ UNx-operated group compared to WT UNx-operated animals in both liver and WAT (Figure 4C; $p = .068$ in liver and $p < .05$ in WAT). Similar to FGFR1, we found that βKlotho mRNA expression was also upregulated in UNx-operated or C2β$^{D1212A/D1212A}$ animals which was further increased in C2β$^{D1212A/D1212A}$ UNx-operated mice compared to WT UNx-operated liver and WAT (Figure 4D; $p < .001$ in liver and $p < .05$ in WAT).

3.5 | UNx and PI3K-C2β inactivation synergistically enhance insulin-stimulated Akt signaling in metabolic tissues

Several reports have demonstrated a synergy between insulin and FGF21 to regulate glucose and lipid metabolism via a synergistic increase in Akt signaling.[20,23-25] To investigate whether this also occurs upon combined UNx and PI3K-C2β inactivation, we examined the impact of UNx and PI3K-C2β inactivation on Akt signaling pathway in metabolic tissues (liver, muscle, and WAT) with Akt phosphorylation being a readout for insulin sensitivity. Compared to sham-operated mice, *in vivo* insulin stimulation of UNx-operated mice led to enhanced Akt phosphorylation in the liver and muscle under NCD (Figure 5A–C; $p < .05$) and in the liver under HFD conditions (Figure 5D; $p < .05$), correlating with the improved insulin sensitivity observed in UNx mice in vivo. When UNx was combined with PI3K-C2β inactivation, the inhibition of which has been reported to result in enhanced insulin-stimulated Akt signaling, we observed a remarkable synergistic enhancement of insulin-induced Akt phosphorylation in the muscle of lean mice and more significantly in the liver and muscle of obese mice compared to groups with Unx or PI3K-C2β inactivation alone (Figure 5; $p < .05$ in liver and $p < .01$ in muscle).

4 | DISCUSSION

Living kidney donation for transplantation is currently the optimal treatment for the ever-increasing number of patients suffering from end-stage renal disease. Therefore, it is imperative to increase our knowledge on the short- and long-term risks involved

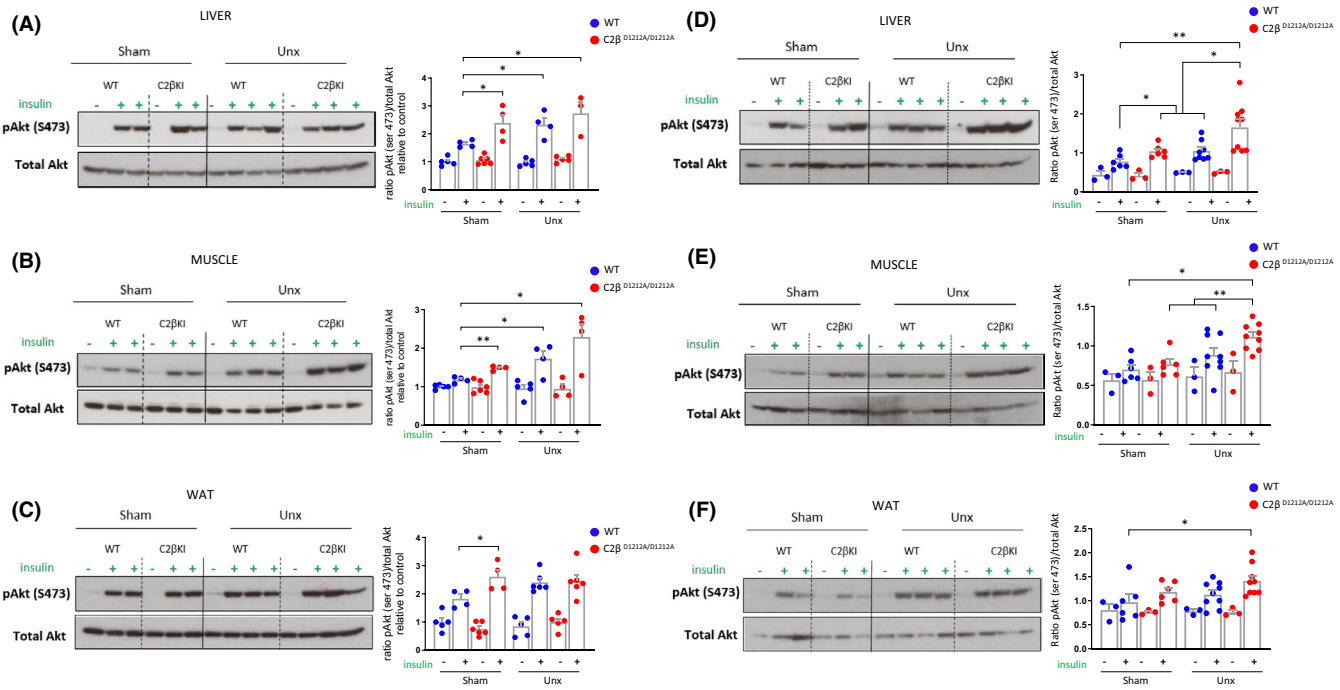

FIGURE 5 UNx and PI3K-C2β inactivation synergistically enhance insulin-stimulated Akt signaling in metabolic tissues. (A–C) Tissue homogenates: liver, muscle, and WAT, isolated from overnight starved mice fed a NCD or (D–F) HFD, 30 min after intraperitoneal injection of 0.75UI/kg insulin or PBS, were analyzed by SDS-PAGE and immunoblotting using the indicated antibodies. Each lane represents an individual mouse. Quantification of the signals in tissues is shown on the right. WAT, white adipose tissue. For all experiments shown, 3–9 mice/genotype/condition were used. Data represent mean ± SEM. $*p < .05$, $**p \leq .01$, $***p \leq .001$

in kidney donation. Most CKD patients, with bilateral renal dysfunction, develop insulin resistance, type 2 diabetes, and liver steatosis leading to cardiovascular disease and increased mortality.[1-3] While CKD patients develop metabolic disorders, the metabolic impact of kidney donation through UNx remains unclear. Despite numerous experimental and clinical studies documenting the metabolic consequences of UNx so far, conflicting data have been published.[6-13] Therefore, it remains critical to investigate the effect of UNx on glucose homeostasis, systemic insulin signaling in insulin target tissues, and gain more understanding on the crosstalk between the kidney and insulin target tissues. In the present study, we investigated the impact of UNx on whole-body glucose homeostasis together with a systemic insulin signaling analysis in both lean and obese mice using UNx mouse model. UNx in rodents is an ideal model to study the metabolic effects of reduced renal function and mimic the clinical situation of kidney donation.[6,34,35]

Surprisingly, our data showed an improved glucose tolerance and insulin sensitivity in UNx animals fed on both NCD and HFD with a decrease in HFD-induced liver steatosis. This correlated with a significant increase in serum FGF21 and *in vivo* insulin-stimulated Akt signaling in metabolic tissues (particularly in liver and muscle). Remarkably, the combination of UNx and PI3K-C2β inactivation protected against HFD-induced obesity and further potentiated the metabolic improvement observed in WT UNx mice. Moreover, combined UNx and PI3K-C2β inactivation induced a synergistic inhibition of hepatic lipogenesis and gluconeogenesis with the decrease in fatty acid synthase and glucose 6-phosphatase expression. This metabolic improvement correlated with a synergistic increase in metabolic tissues of (1) insulin-stimulated Akt signaling and (2) FGFR1 and β Klotho expression, suggesting an increased sensitization to FGF21. This study highlights a beneficial effect of UNx alone and more effectively in combination with PI3K-C2β inhibition to protect kidney donors against obesity, insulin resistance, and liver steatosis through a mutual insulin and FGF21 sensitization.

Our findings are in contrast to some of previous experimental studies on mice and rats and clinical reports from living kidney donors showing that UNx correlates with insulin resistance and diabetes.[4,7,9,10] One of the reasons for such discrepancies could be the length of the starvation period prior to metabolic studies. Indeed, inconsistencies with respect to fasting duration have been previously reported to alter insulin responsiveness.[36-38] While most of experimental studies showing that UNx leads to glucose intolerance or has no impact on insulin sensitivity have been performed after a short starvation, the only metabolic study showing that UNx induced an insulin sensitivity in rats was performed after an overnight starvation. Our experiments are in line with the latest study as we have performed all our studies after an overnight starvation. Indeed, our data unambiguously showed that UNx results in better insulin sensitivity and increase insulin-stimulated Akt signaling both in lean (liver and muscle) and obese mice (liver). Thus, it appeared that the starvation period prior metabolic studies is an important parameter to take into consideration. In addition, other parameters

have to be considered such as glucose and insulin dose and route of administration, age, strain, sex of the animals, and duration of post-UNx analyzed. Regarding clinical studies, parameters such as the cohort size, health condition, and diet of kidney donors should also be considered.

The metabolic improvement observed in UNx mice cannot be explained by a decrease in insulin clearance given that insulin levels were unaffected by UNx. However, we cannot rule out that the metabolic improvement observed after UNx, can be due to a 50% reduction of renal gluconeogenesis caused by the 50% reduction of kidney mass. Overall, our findings suggest an inter-organ communication between the kidney and insulin target tissues through a paracrine/endocrine factor not properly excreted in Unx animals. Thus, we hypothesized that FGF21 could be an interesting candidate for several reasons. First, FGF21 has been shown to be elevated in kidney donors.[17] Second, FGF21 recently emerged as a beneficial factor in glucose and lipid metabolism.[18-21] Lastly, FGF21 has been shown to be rapidly induced by fasting and mediates critical aspects of the adaptive response to starvation.[39] Thus, differential FGF21 levels could explain the discrepancies between metabolic studies performed after different starvation periods. Because of all these reasons, the elevated serum FGF21 levels observed in UNx-operated animals could be responsible for the improvement of glucose metabolism and insulin sensitivity but more work is needed to confirm this hypothesis. Moreover, our study showed the elevated FGF21 serum concentrations observed post-UNx was not only due to a decrease in renal excretion as previously suggested but is also due to an increase in hepatic FGF21 expression. Because of the increase hepatic FGF21 expression together with its receptor components in UNx-operated animals, we cannot exclude a potential development of a FGF21-resistant state over time (similarly to hyperinsulinemia inducing insulin resistance).

To gain more understanding on the crosstalk between the kidney and insulin target tissues, we also explored the possible implication of the class II PI3K-C2β isoform for several reasons. First, PI3K-C2β gene expression has been found upregulated in kidneys of diabetic nephropathy patients.[29] Second, this lipid kinase plays an important role in insulin signaling and it has been reported that its inactivation increased insulin sensitivity in mice.[30] Altogether, this suggested a potential role of PI3K-C2β in the crosstalk between the kidney and insulin target tissues to regulate glucose metabolism in UNx context. One of the most remarkable findings of our study is the synergistic effect of the combination of UNx and PI3K-C2β inactivation to protect against HFD-induced obesity and further potentiates the metabolic improvement observed in UNx alone. Our data suggested that the combination of UNx and PI3K-C2β inactivation cooperatively plays a positive role on FGF21 pathway through independent mechanisms with Unx mainly increasing serum FGF21 levels and PI3K-C2β upregulating FGFR1 and βKlotho expression in liver and WAT leading to a synergistic upregulation of FGFR1 and βKlotho expression. This was accompanied with a synergistic enhancement of insulin-induced Akt phosphorylation in metabolic tissues.

We believe that our study has an important clinical relevance as it demonstrates a potential beneficial effect of living kidney donation to protect against metabolic disorders. However, this animal experimental study has certain limitations. First, UNx was performed on young (7-week old) mice, whereas live donors are typically older than 30 years. Despite undergoing UNx at young age, we believe that our study has a long-term relevance as most of the analyses were performed 16 weeks post-UNx, which is equivalent to 16 years post-donation in human.[40,41] Second, while live kidney donors are in general both men and women, our study only used male mice. Based on the recent National Institutes of Health (NIH) guide notice to consider sex as a biological variable, we can therefore not generalize our study to female mice.[42,43] Moreover, PI3K-C2β inhibitor is not available yet. However, there is an increased interest in the development of specific PI3K-C2β inhibitors with many pharmaceutical companies involved. Finally, our current study does not have any direct impact in the context of APOL1 high-risk variants. Indeed, APOL1 high-risk variants seen in patients of black ethnicity have been reported to confer risk of developing non diabetic kidney disease, hypertension, and higher rate of end-stage renal disease with no additive role in diabetic kidney disease (DKD) prevalence.[44,45] Nevertheless, the APOL1 risk variants relationship to DKD still remain somehow mysterious. Indeed, obesity that coexist with diabetes and induces glomerular injury can complicate this relationship. Therefore, it would be interesting to assess in further studies the impact of PI3K-C2β inactivation on APOL1 high-risk variant-related diseases particularly in obesity condition. Moreover, our data suggest that even overweight patients or those with pre-diabetes could be safely used as potential kidney donors without subjecting them to increased risk of insulin resistance, type 2 diabetes, and hepatic steatosis, increasing the number of potential kidney donors for transplantation. Furthermore, we identified PI3K-C2β as a new therapeutic target for improving and preserving the health of kidney donors, thus encouraging living kidney donation.

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DISCLOSURE

The authors of this manuscript have conflicts of interest to disclose as described by the *American Journal of Transplantation*. B.V. is a consultant for Karus Therapeutics (Oxford, UK), iOnctura (Geneva, Switzerland), and Venthera (Palo Alto, CA) and has received speaker fees from Gilead Sciences (Foster City, CA). The other authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

ORCID

Samira Alliouachene https://orcid.org/0000-0002-0998-8997

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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