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Biochemical and Histological Characterization of the Modulatory Effect of Ampk1/2 on Renal Function in Diabetic Rats

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ABSTRACT

Diabetic nephropathy is one of the major microvascular complications of diabetes mellitus. Inactivation of the AMPK1/2 pathway, a key player in energy metabolism, is thought to contribute to the glomerular and tubular dysfunction observed in diabetes. This study aims to characterize the biochemical, histological, and molecular effects of AMPK1/2 modulation on renal function in an experimental diabetes model. Twenty-four male Wistar rats were divided into four groups: controls, controls + dorsomorphin, diabetic rats, and diabetic rats + dorsomorphin. Diabetes was induced by alloxan (150 mg/kg), and AMPK was inhibited by dorsomorphin (25 mg/kg). Biochemical parameters (blood glucose, creatinine, urea, insulin, adiponectin) were measured, AMPK1/2 gene expression was assessed by RT-qPCR, and renal lesions were analyzed histologically. Alloxan induced severe hyperglycemia and altered renal parameters. AMPK1/2 inhibition worsened interstitial fibrosis and glomerulosclerosis. Conversely, untreated diabetic rats showed AMPK1/2 overexpression and improved renal integrity. Correlations showed a negative association between blood glucose and AMPK expression ($r = -0.74$; $p < 0.001$). Modulation of the AMPK1/2 pathway significantly influences the progression of diabetic nephropathy. Its activation appears to confer renal protection against oxidative stress and inflammation, suggesting a promising therapeutic target for the prevention of diabetic kidney damage.

Keywords

AMPK1/2, Experimental diabetes, Diabetic nephropathy, Renal function, Wistar rat.

Introduction

The prevalence of diabetes mellitus, particularly type 2, is increasing significantly overall, leading to a growing burden of microvascular complications, notably diabetic nephropathy (DN), which remains a leading cause of chronic kidney disease and end-stage renal disease. In this context, understanding and modulating the molecular pathways involved in DN progression is of major therapeutic interest [1,2].

Among these pathways, AMP-activated protein kinase (AMPK) plays a central role as a cellular energy sensor, capable of integrating metabolic signals (AMP/ATP ratio, oxidative stress,

hypoxia) and regulating key processes such as autophagy, lipid and carbohydrate metabolism, and the stress response (Essays in Biochemistry, AMPK special issue). In diabetic kidneys, several studies have documented a reduction in AMPK activity, associated with the stimulation of pro-fibrotic, inflammatory, and oxidative stress pathways [3,4].

AMPK dysfunction could thus constitute a key element in the pathophysiology of ND: it would promote decreased autophagy, increased accumulation of extracellular matrix, activation of the mTOR pathway, and ultimately progression towards glomerulosclerosis and tubulointerstitial fibrosis [5,6]. A recent study, for example, shows that in diabetic mice, the absence of the γ^2 subunit of AMPK eliminates female protection against nephropathy, highlighting the importance of this pathway for kidney function in the context of diabetes [7].

Furthermore, pharmacological or metabolic activation of AMPK appears as a potential strategy to limit diabetic kidney damage. For example, an AMPK activator, O304, has been shown to protect against cellular senescence and renal fibrosis in a renal aging model by restoring energy metabolism, promoting autophagy, and preserving mitochondrial homeostasis [8]. Similarly, AMPK/SP1 activation is involved in the protection of podocytes under diabetic conditions [9].

On a broader metabolic scale, a growing body of research indicates that mitochondrial metabolic reprogramming plays a central role in diabetic nephropathy, and that the AMPK pathway is involved as a key regulator [10,11]. Furthermore, approaches combining antidiabetic drugs such as Metformin and activators of the AMPK/SIRT1 pathway also show protective effects on the kidney in diabetic patients [12,13].

Despite these advances, several questions remain: When is AMPK activation most beneficial? What are the precise links between AMPK, autophagy, fibrosis, and renal inflammation in diabetes? And finally, what is the functional impact of AMPK modulation on biochemical, histological, and molecular parameters in an experimental model of diabetes (rats or mice)?

This study falls within this framework. Using an alloxan-induced diabetic rat model, we sought to comprehensively characterize the modulatory effect of the AMPK1/2 pathway on renal function by combining biochemical measurements (blood glucose, creatinine, urea, adiponectin, etc.), an assessment of AMPK1/2 gene expression, and a histological examination of renal lesions. The objective is to better understand how AMPK modulation influences the development of diabetic nephropathy, and to open up therapeutic perspectives for the prevention of kidney damage in diabetes.

Materials and Methods

Experimental Animals

The study was conducted on twenty-four (24) male Wistar rats (Rattus norvegicus), aged 13 to 17 weeks and weighing between 150 and 345 g, obtained from the breeding center of Marien Ngouabi University (Brazzaville, Congo). The animals were kept in standard cages under controlled conditions (temperature: 22 ± 2°C; light/dark cycle: 12 h/12 h; humidity: 50–60%), with ad libitum access to water and a balanced diet (standardized pellets, U.M.N.). A 12-hour fast was observed before any experimental manipulation. The entire protocol was approved by the Ethics Committee of Marien Ngouabi University (ref. UMNG/CBES/2024/015) in accordance with international guidelines for the use of laboratory animals (National Research Council, 2011; Care and Use of Laboratory Animals, 8th ed.) [14].

Induction of diabetes and treatment protocol

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg, Sigma-Aldrich®) dissolved in ice-cold sterile saline (0.9% NaCl).

Control rats received an equivalent volume of saline. Dorsomorphine (Compound C, 25 mg/kg; Sigma-Aldrich®), a selective AMPK inhibitor, was administered intraperitoneally to groups 2 and 4 to inhibit AMPK1/2 activity [15]. To avoid acute post-injection hypoglycemia, a 5% glucose solution was administered ad libitum for the first 24 hours following injection. The rats were randomly divided into four groups (n = 6):

Table 1: Distribution of groups and treatment received.

Group	Treatment
G1	Untreated Controls (saline solution)
G2	Controls + Dorsomorphine
G3	Diabetics (Alloxan 150 mg/kg)
G4	Diabetics + Dorsomorphine

Biological monitoring and diabetes criteria

Capillary blood glucose was measured using a portable glucometer (OnCall Plus II, ACON®) on days 1, 3, 5, 7, and 14. Animals with a fasting blood glucose level greater than 280 mg/dL (≈ 15.5 mmol/L) on day 3 post-injection were considered diabetic [16]. Body weight was recorded with a precision electronic scale (Kern®, Germany).

Sample collection and preservation

After 14 days, the rats were anesthetized by inhalation of diethyl ether. Blood was collected by orbital sinus puncture, centrifuged at 3000 rpm for 10 min, and the serum was stored at −80°C for biochemical analyses. The kidneys and livers were removed immediately after cervical dislocation; the kidneys were fixed in 10% formalin, and the liver tissues were frozen at −80°C for molecular analyses.

Biochemical and hormonal analyses

Creatinine, urea, and blood glucose levels were measured by spectrophotometry (Cyanstart®, Cypress Diagnostics). Serum insulin and adiponectin were determined by ELISA (SunLong Biotech Co., LTD kits, lots SL0373Ra and SL0032Ra). The intra- and inter-assay coefficients of variation were <10% and <12%, respectively [17].

Histological analyses

Renal tissues were fixed in 10% formalin, dehydrated in a series of ethanols, embedded in paraffin, and then sectioned at 5 µm. The sections were stained with hematoxylin and eosin (H&E). Morphological observations were performed using a Leica® DM500 optical microscope, with semi-quantitative evaluation of glomerular, tubular, and interstitial lesions according to the method described by Glassock et al. [18].

Molecular analyses

Total RNA was extracted from the liver using the Total RNA Purification Kit (Norgen Biotek Corp., Canada). Amplification was performed by RT-qPCR (One-Step Luna Universal Kit, New England Biolabs®) with primers specific for AMPKα1, AMPKα2, and GAPDH as the reference gene (Table 1). The amplification conditions were: reverse transcription at 55°C (10 s); activation at 95°C (60 s); and 40 cycles (95°C 10 s, 60°C 30 s).

Relative quantification was calculated using the 2ΔCt method of Livak and Schmittgen [19].

Table 2: Primers used for RT-qPCR.

Gene	Primer direction (5'–3')	Primer antisense (5'–3')
AMPKα1	GAC AGC CGA GAA GCA GAA AC	AGG ATG CCT GAA AAG CTT GA
AMPKα2	GAC GGG TTG AAG AGA TGG AA	CCT GCA TAC AAT CTG CCT GA
GAPDH	GTC CAC TGG CGT GTT CAC CA	GTG GCA GTG ATG GCA TGG AC

Statistical analyses

Data were expressed as mean ± standard deviation (M ± SD). Intergroup comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test with a significance level set at $p < 0.05$. Statistical analysis was performed using GraphPad Prism version 05 (GraphPad Software, USA), and images were analyzed using J software.

Results

Changes in body weight and blood glucose

Analysis of this curve shows that mean body weight decreased significantly in diabetic rats (DG: −18.6%; DD: −15.2%) compared to controls (CG: +1.7%; CD: −0.8%) at day 14 (two-way ANOVA, $p = 0.001$). Dorsomorphine did not cause a significant change in controls ($p = 0.61$) but slightly limited weight loss in diabetic rats ($p = 0.047$). These results suggest a partial modulating effect of AMPK on energy metabolism and tissue catabolism.

Data are expressed as mean ± SEM. Statistical analysis.
We observed that mean blood glucose increased from 92 ± 6 mg/dL to 410 ± 25 mg/dL in diabetic rats (DG) and to 398 ± 30 mg/dL in diabetic rats (DD) by day 3 post-alloxane ($p < 0.0001$). Controls (CG and CD) remained normoglycemic (mean = 101 ± 8 mg/dL, $p > 0.05$). Dorsomorphine did not restore normoglycemia, suggesting a worsening of metabolic dysregulation under AMPK inhibition.

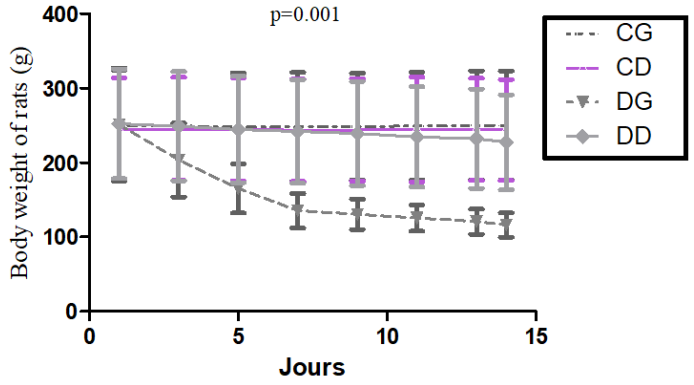

Figure 1A: Changes in body weight.

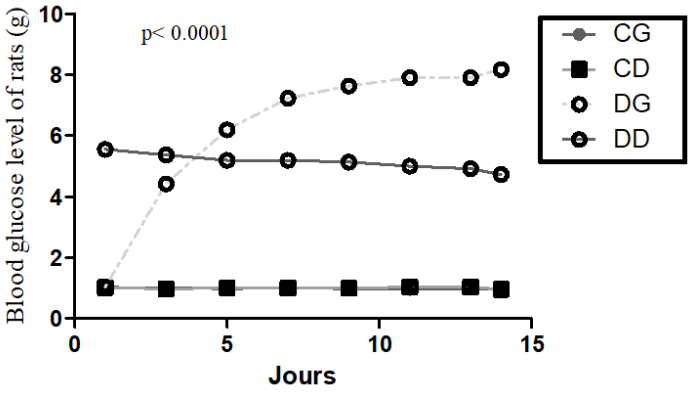

Figure 1B: Evolution of fasting blood glucose.

Data are expressed as mean ± SEM. Statistical analysis: Two-way ANOVA followed.

Variations in biochemical parameters

To assess renal function, serum creatinine and urea concentrations were measured in the different experimental groups. A significant increase in creatinine was observed in diabetic rats (DG: 1.65 ± 0.12 mg/dL; DD: 1.83 ± 0.15 mg/dL) compared to controls (CG: 1.05 ± 0.08 mg/dL; $p = 0.02$), corresponding to a 56% increase. Similarly, serum urea was markedly increased (DG: 72.4 ± 6.3 mg/dL; DD: 78.2 ± 5.8 mg/dL vs. CG: 42.6 ± 4.1 mg/dL; $p = 0.002$). The more pronounced increase observed under dorsomorphine (DD) highlights an aggravating effect of AMPK inhibition on renal function.

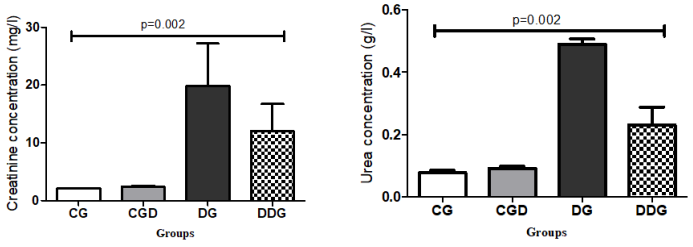

Figure 2: Serum creatinine and urea concentrations.

Data are expressed as mean ± SEM. Statistical analysis: One-way ANOVA followed by Tukey's post-hoc test. AMPK inhibition accentuates the renal functional impairment observed in experimental diabetes.

Hormonal profile: insulin and adiponectin

Serum insulin concentration was significantly reduced in diabetic rats compared to controls. Mean levels decreased from 11.8 ± 1.3 µIU/mL in controls (CG) to 3.2 ± 0.9 µIU/mL (DG) and 2.9 ± 0.7 µIU/mL (DD), representing a reduction of nearly 75% (p = 0.0005). No significant difference was observed between the control groups treated with or not treated with dorsomorphine (p > 0.05). These results confirm the destruction of pancreatic β-cells induced by alloxan and show that AMPK inhibition does not attenuate this insulin deficiency.déficit.

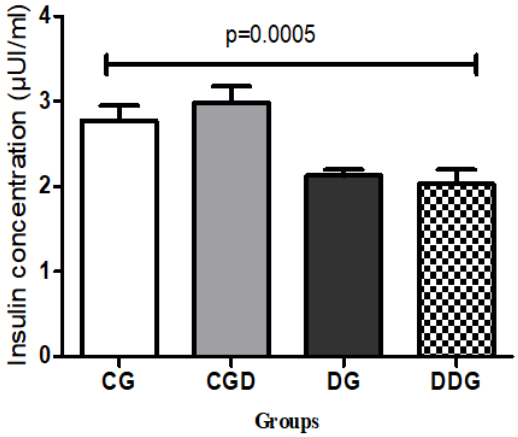

Figure 3A: Serum insulin concentration.

Data are expressed as mean ± SEM. Statistical analysis: One-way ANOVA (p = 0.0005).

Serum adiponectin levels increased in untreated diabetic rats (DG: 14.6 ± 1.2 µg/mL) compared to controls (CG: 10.3 ± 0.9 µg/mL; p = 0.048). A non-significant increase was noted in the dorsomorphine-treated diabetic group (DD: 15.4 ± 1.4 µg/mL; p = 0.06). These results suggest adaptive activation of energy metabolism via adiponectin in response to insulin resistance.à l'insuline.

Data are expressed as mean ± SEM. Statistical analysis: One-way ANOVA. Data are expressed as mean ± SEM. (p=0.06).

Molecular expression of AMPK1/2

Molecular expression of AMPK1

RT-qPCR analysis showed significant overexpression of the AMPK1 gene in untreated diabetic rats (DR: 2.1 times the value in controls; p = 0.0019). Dorsomorphine administration reduced this expression by 37% in controls (CRD vs. CR) and by 42% in diabetic rats (DRD vs. DR). This reduction confirms the specific inhibitory effect of dorsomorphine on AMPK1 transcriptional activity.

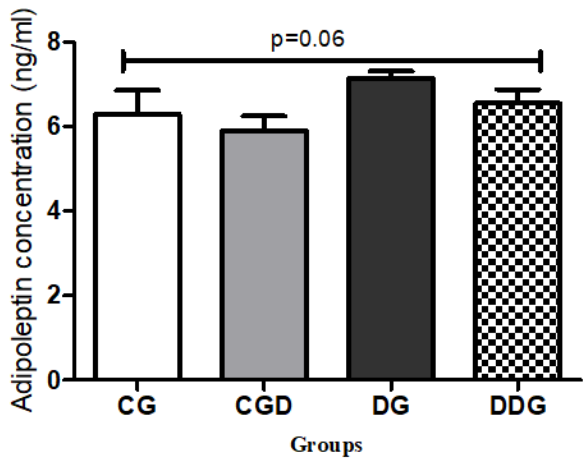

Figure 3B: Serum adiponectin concentration.

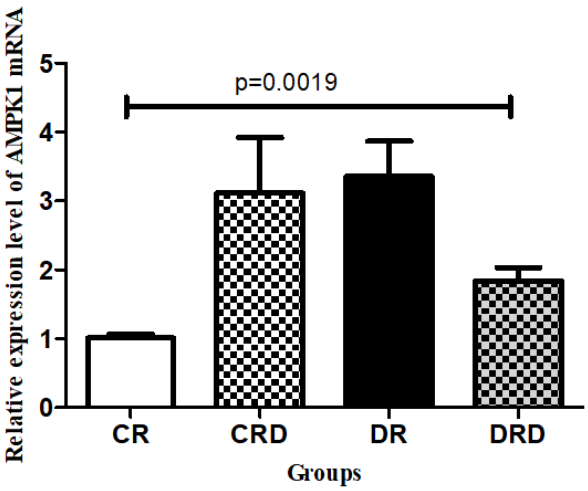

Figure 4: Relative AMPK1 expression measured by RT-qPCR in the different experimental groups.

CR (control rats), CRD (control rats + dorsomorphine), DR (diabetic rats), DRD (diabetic rats + dorsomorphine). Data are expressed as mean ± SEM. Statistical analysis: ANOVA followed by the Kruskal-Wallis post-test, p = 0.0019

Molecular expression of AMPK2

AMPK2 gene expression is significantly increased in untreated diabetic rats (DR: +3.9 times vs CR; p = 0.0004). Under dorsomorphine, this expression decreases by 60% in controls (CRD vs CR) and by 68% in diabetic rats (DRD vs DR). These variations demonstrate adaptive regulation of AMPK2 in response to metabolic stress and its high sensitivity to pharmacological inhibition.

CR (control rats), CRD (control rats + dorsomorphine), DR (diabetic rats), DRD (diabetic rats + dorsomorphine). Data are expressed as mean ± SEM. Statistical analysis: ANOVA followed by multiple comparisons test, p = 0,0004

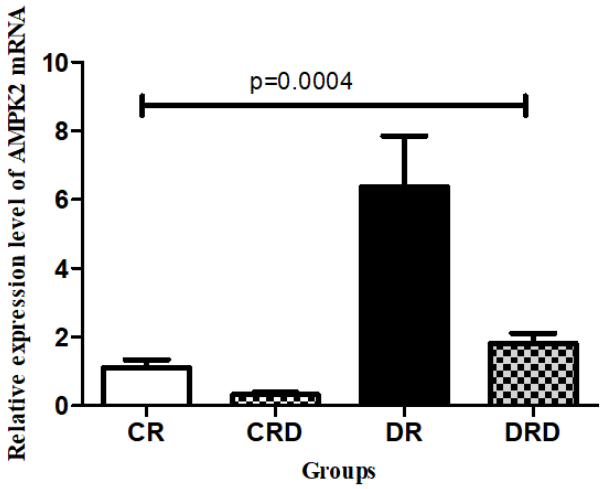

Figure 5: Relative AMPK2 expression measured by RT-qPCR in the different experimental groups.

Renal histological analysis

Microscopic examination shows that control kidneys exhibit normal architecture, with intact glomeruli and a non-fibrotic interstitium. In diabetic rats, glomerular hypertrophy, tubular vacuolization, and marked interstitial fibrosis are observed (+65% fibrotic surface area; $p < 0.01$). These alterations are more severe under dorsomorphine, indicating a worsening of glomerulosclerosis and cortical disorganization in cases of AMPK inhibition.

Biochemical and molecular correlation

Pearson correlation analysis showed a negative association between blood glucose and AMPK1/2 expression ($r = -0.74$; $p < 0.001$), as well as between creatinine and adiponectin ($r = -0.61$; $p = 0.004$). A significant positive correlation was observed between AMPK1 and AMPK2 ($r = +0.82$; $p < 0.0001$). These results highlight a strong link between metabolic stress, renal dysfunction, and AMPK activity.

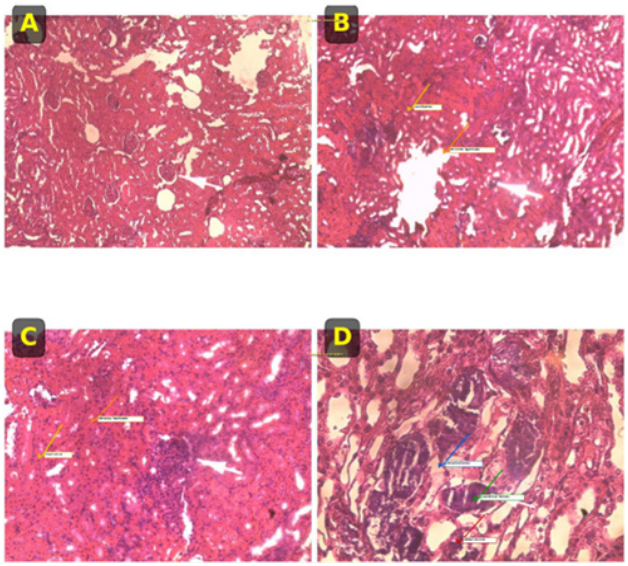

Figure 6: Histological image after microscopic examination.

Typical histological observation of diabetic glomerulosclerosis aggravated by dorsomorphine.

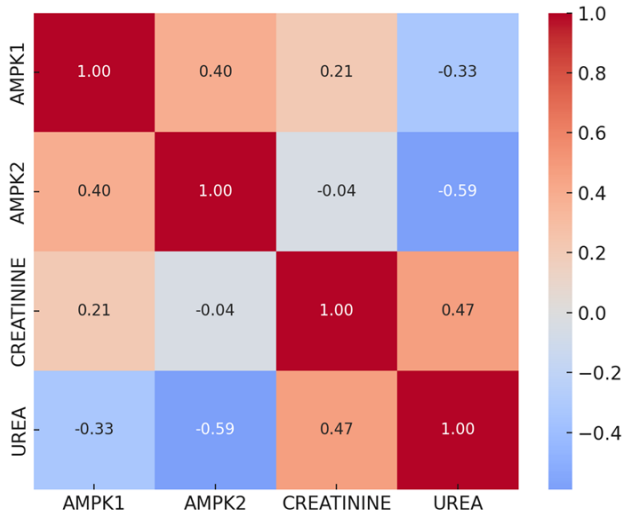

Figure 7: Correlation matrix between biochemical and molecular parameters.

The correlations demonstrate the inverse relationship between hyperglycemia and AMPK activation

Discussion

The aim of this study was to evaluate the impact of AMPK pathway inhibition on renal function in an experimental rat model of diabetes by analyzing biochemical, molecular, and histological parameters. Our results show that diabetes induction led to significant weight loss (-18.6% in diabetic rats), persistent hyperglycemia (410 ± 25 mg/dL), a marked increase in creatinine (+56%) and serum urea, and a drastic decrease in insulin (-75%). Concurrently, we observed a moderate increase in adiponectin and overexpression of the AMPKα1 (2.1x) and AMPKα2 (3.9x) isoforms in untreated diabetic rats. Histological analysis confirmed significant renal lesions, including glomerular hypertrophy, tubular vacuolization, and increased interstitial fibrosis (+65%). Pharmacological inhibition of AMPK by dorsomorphine exacerbated these alterations, with worsening glomerulosclerosis, a further increase in creatinine (1.83 ± 0.15 mg/dL), and a reduction in AMPKα1 and α2 expression of 42% and 68%, respectively. These data highlight the protective role of AMPK in diabetic nephropathy.

These results support the current understanding of the central role of AMPK as a cellular energy sensor. Its activation under metabolic stress aims to restore energy homeostasis [15]. The AMPKα1/α2 overexpression observed in our untreated diabetic rats likely reflects an adaptive response to hyperglycemic stress. Conversely, the increased functional and structural degradation under dorsomorphine confirms that this pathway is essential for limiting renal damage, consistent with its described role in preserving tubular and podocyte function [7,9].

Several pathophysiological mechanisms can explain this protective

action. AMPK limits oxidative stress and apoptosis, modulates autophagy via the AMPK/mTOR axis, and inhibits pro-fibrotic pathways (TGF-β, NF-κB) [8,20]. Our histological observations of exacerbated interstitial fibrosis under AMPK inhibition support this view. Furthermore, the negative correlation between blood glucose and AMPK expression (r = -0.74) suggests a close link between hyperglycemia and the suppression of this protective pathway. Moreover, the partial preservation of body weight in diabetic rats treated with dorsomorphine, although modest, could reflect a modulation of energy metabolism independent of blood glucose levels, warranting further investigation.

The elevation of renal markers (creatinine, urea) and their worsening under dorsomorphine confirm the attenuating role of AMPK in diabetic renal dysfunction. These data are consistent with the protective effects reported with metformin, an indirect activator of AMPK [21]. Furthermore, the strong positive correlation between AMPKα1 and AMPKα2 (r = +0.82) indicates a coordinated regulation of the two isoforms in response to metabolic stress, an aspect that is still poorly documented in the literature.

All of these experimental results reinforce the idea that AMPK represents a promising therapeutic target for diabetic nephropathy [22]. The evidence of its protective role, supported by our biochemical, molecular, and histological data, opens the way to pharmacological or nutritional activation strategies (berberine, resveratrol, etc.) aimed at preserving renal function in diabetic conditions [17,23].

However, certain limitations must be acknowledged. The study relies on an acute induction model using alloxan and global AMPK inhibition. While informative, gene expression assessment would benefit from being complemented by measurements of AMPK phosphorylation and its targets (p-ACC, p-ULK1) [18]. Similarly, the absence of proteinuria or albuminuria measurements limits glomerular function assessment. Finally, the beneficial effect of pharmacological AMPK activation remains to be demonstrated in this model.

Conclusion

This study provides strong experimental evidence for the protective role of AMPK in diabetic nephropathy. Inhibition of this pathway exacerbates the functional and structural degradation of the kidney, highlighting its essential contribution to the regulation of energy metabolism, the fight against oxidative stress, and the limitation of fibrosis. These results confirm AMPK as a promising therapeutic target whose activation could slow the progression of diabetic kidney complications and improve long-term renal prognosis.

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