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IDENTIFICATION OF KEY REGULATORS IN CELLULAR METABOLIC HOMEOSTASIS

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ABSTRACT

Cellular metabolic homeostasis is a highly regulated phenomenon that is necessary to maintain homeostasis of energy, cell integrity, and survival in different physiological conditions. Malfunction of metabolic pathways has been closely linked to the development of various disorders such as cancer, diabetes and metabolic syndromes. The current work will determine the main molecular regulators that can keep the cellular metabolism at homeostasis and learn about the coordinated actions of these molecules in response to the stress.

A mix of biochemical and molecular methods was used, which consisted of metabolic tests, ATP quantification, glucose uptake, and reactive oxygen species (ROS) tests, as well as genomic and proteomic expression data of genes and proteins via RT-qPCR and Western blotting assays. The key metabolic regulators, such as AMP-activated protein kinase (AMPK), mechanistic target of rapamycin (mTOR), sirtuin 1 (SIRT1), and peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 4), were analyzed.

The findings showed that metabolic stress causes a large discrepancy in cellular energy status, which is manifested by a decrease in ATP levels, changes in glucose metabolism, and oxidative stress. This imbalance was linked to the activation of AMPK signaling and repression of mTOR-mediated growth pathways, in addition to the regulation of SIRT1 and PGC-1A expression, showing a complex regulatory mechanism that re-established metabolic balance. The long-term stress levels led to the transition of adaptive metabolic regulation to the apoptotic signaling and the importance of setting a critical threshold beyond which the homeostasis becomes impossible. On the whole, the results indicate that the cellular metabolic stability is controlled by a network of interconnected regulatory signals and key signaling molecules. Knowledge of these regulatory systems has valuable information on the pathogenesis of illnesses and has provided other potential therapeutic control points in metabolic disorders.

KEYWORDS: Metabolic homeostasis, AMPK, mTOR, SIRT1, PGC-1, cellular metabolism, energy, oxidative stress, apoptosis.

1. INTRODUCTION

Cellular metabolic homeostasis is the dynamic balance of biochemical processes that produce energy, break down nutrients and help cellular activities. This balance plays a vital role in ensuring cell survival, growth and adapting to environmental changes. Disruption of the metabolic balance may result in metabolic instability and it is closely linked to cancer, diabetes, obesity and neurodegenerative diseases (West and Marnett, 2006).

Complex molecular signaling networks control energy balance and cellular survival. Adenosine triphosphate (ATP) is the most important energy currency in the cell and its synthesis and degradation must be very well regulated in response to cellular needs. Activating adaptive responses entails processes like metabolic reprogramming, enzyme regulation, and tune gene expression in response to changing stressors like nutrient deprivation or oxidative stress in order to recover energy homeostasis (West and Marnett, 2006).

AMP-activated protein kinase (AMPK) is one of the key regulators of metabolic homeostasis, the central cellular sensor of energy, which is triggered in low-energy states. AMPK activates ATP-producing pathways and inhibits anabolic energy-consuming mechanisms. Conversely, the mechanistic target of rapamycin (mTOR) stimulates cell proliferation, protein synthesis and nutrient consumption in the presence of ample energy supply. The AMPK-mTOR interaction creates an important regulatory axis that dictates cell metabolic outcome.

The recent molecular and cellular profiling technology has greatly enhanced the knowledge on metabolic regulation. The heterogeneous cellular responses to metabolic stress and drug exposure have been demonstrated with high-resolution methods like single-cell analysis and molecular characterization (Bendall et al., 2011). Research on stem cell biology and signaling regulation has also underscored the significance of checkpoint signaling pathways in ensuring cell stability and functional integrity (Kim et al., 2013; Rauch et al., 2011).

Moreover, microbial and environmental influences also play an important role in metabolic homeostasis. Shifts in the composition of gut microbiota have been linked with metabolic disorders and variations in energy-regulating gene expression (Demirci et al., 2022; Noh and Lee, 2020). Probiotics like Bifidobacterium are beneficial in maintaining metabolic homeostasis, as well as physiological stability in the host (Leser and Baker, 2023; Turroni et al., 2011). It has also been shown that biological systems have the capacity to adapt to extreme environments and maintain metabolism and gene expression in response to stress (Rego et al., 2019).

Nonetheless, with these advances, the bulk of current research is centered on individual metabolic regulators or individual pathways, which restricts the general appreciation of how various signaling networks respond to one another to ensure the stability of metabolism. There is still a major gap on the way to defining the integrated regulatory network that governs cellular metabolic homeostasis in normal and stressful situations (Freedman et al., 2004; Pritchard and Donnelly, 2001). Thus, the current research is expected to explore the relationships among key metabolic regulators in an integrated context. The study combines biochemical analysis with gene and protein expression profiling to uncover the concerted actions of major regulators like AMPK, mTOR, SIRT1, and PGC-1 in ensuring metabolic homeostasis. The study also discusses the response of these pathways to metabolic stress and their role in the adaptive or dysfunctional response of cells and offers information on possible therapeutic actions to metabolic and chronic disorders.

2. RELATED WORK

Cellular metabolic homeostasis has become of great significance in the field of molecular biology because of its contribution to the development of diseases and cellular adaptation. The initial literature on genetic variation and population structure has stressed the need to detect good biological signals in complex data. Freedman et al. (2004) showed how stratification of a population can have a profound effect on genetic association studies, whereas Pritchard and Donnelly (2001) provided statistical models of structured population. In the same way, Lee et al. (2010) suggested spectral graph methods to infer genetic ancestry and to interpret molecular data. Most of the current methods of analysing metabolic and signalling pathways rely on the groundwork of these studies.

Recently, the advancements in methods of molecular and cellular profiling have made possible the high-resolution study of the metabolic regulatory processes. Bendall et al. (2011) also showed how the single-cell mass cytometry could be used to analyze different cellular responses in complicated biological systems, emphasizing the role of single-cell methods in the understanding of heterogeneous metabolic processes. The research of stem cells has also played a vital role in comprehending metabolic control. Kim et al. (2013) detected variations in proliferation and functional properties of mesenchymal stem cell populations whereas Rauch et al. (2011) focused on the importance of maintaining cellular differentiation and stability in cases of stress through signaling modulation.

Microbial and environmental research further reveals the importance of physiological and ecological conditions in ensuring homeostasis of metabolism. Demirci et al. (2022) reported a correlation between the composition of the gut microbiota and metabolic gene expression in diabetes patients, pointing to the significance of microbial systems in regulating metabolism. Positive microbes like Bifidobacterium have also been widely researched in terms of their role to metabolic balance and host health (Leser et al., 2023; Turroni et al., 2011). Further on, Noh and Lee (2020) noted that the changes in microbial populations in pathological conditions have the potential to disturb both cellular and systemic metabolic stability. Other studies by Rego et al. (2019) also emphasized the outstanding metabolic and genetic plasticity of microbial ensembles across harsh environmental changes.

Reactive intermediates and oxidative signaling pathways have also been identified as key modulators of metabolic regulation. West and Marnett (2006) showed the role played by endogenous reactive intermediates in signaling pathways associated with cell survival versus cell death and the close linkage between oxidative stress, signaling control and metabolic homeostasis.

Although there has been a significant advance in the study of metabolism regulation, the current literature is based on the predominance of isolated signaling pathways or biological systems. It is still unclear how several signaling networks interact with each other to maintain metabolic homeostasis even in physiological and stressful situations. The current paper suffers this limitation as it combines biochemical, molecular and signaling studies to determine key regulators and their integrated functions in preserving cellular metabolic homeostasis.

3. MATERIALS AND METHODS

3.1 Cell Model and Sample Selection

The current research has been achieved on the basis of the existing mammalian cell lines to explore the important regulators of the cellular metabolic homeostasis under regulated experimental conditions. The choice of human hepatocellular carcinoma cells (HepG2), as well as human cervical cancer cells (HeLa), was based on the well-characterized metabolic activity and environmental stress responses. The standard conditions of 37 C and 5% CO 2 were used to culture cells in Dulbecco's Modified Eagle Medium (DMEM) and 10% fetal bovine serum and 1% antibiotic solution.

Cells were categorized into control and treated group. A normal glucose condition was enforced on the control group although the treatment groups were exposed to metabolic stress conditions which comprised of glucose deprivation,

high-glucose exposure, and oxidative stress caused by hydrogen peroxide (H 2 O 2). These conditions were aimed at mimicking metabolic imbalance and cellular stress conditions.

Table 1: Experimental Conditions

Group	Condition	Glucose Level	Treatment	Treatment
Control	Normal	5 mM	None	None
Low Glucose	Stress	1 mM	None	None
High Glucose	Stress	25 mM	None	None
Oxidative Stress	Stress	5 mM	H_2O_2	H_2O_2

3.2 Experimental Design

To assess a time-varying behavior of metabolic regulation, cells were subjected to different time-periods of stress which were early (6 hours) and chronic (24 hours) exposures. The glucose variation (low glucose: 1 mM; high glucose: 25 mM) and oxidative stress were induced with the H_2O_2 at optimized concentrations. After the treatment, cells were harvested at the required time points to undergo biochemical and molecular analyses. Such design allowed evaluating both the adaptive responses in the short term and the long-term outcomes of the metabolic stress imposed on the cellular regulatory mechanisms.

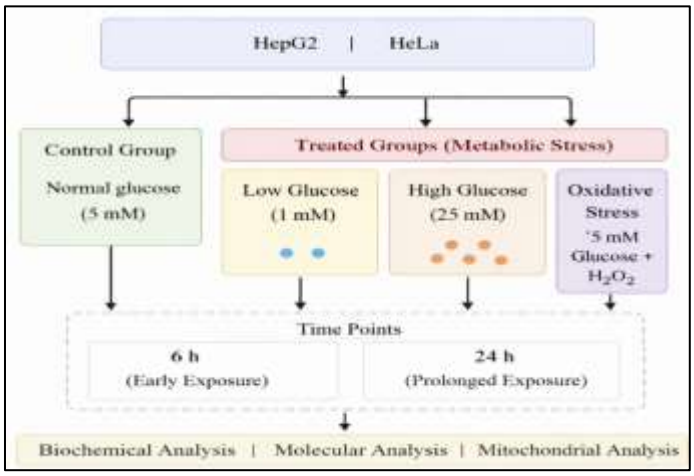

Fig 1: Experimental Design Diagram

3.3 Biochemical Analysis

Multiple biochemical assays were used in measuring cellular metabolic status. Luminescence based ATP assay kit was taken on intracellular ATP levels, which gives an approximation of the cellular energy status. A fluorescent glucose analog was used to measure glucose uptake, a measure of metabolic activity and utilization of nutrients in cells.

Lactate production was quantified as a parameter of glycolytic flux by a colorimetric lactate assays. Fluorescence intensity measured at excitation/emission wavelengths of 485/ 530 nm was used to measure the level of reactive oxygen species (ROS) with the use of DCFH-DA fluorescent probe. An elevation of ROS was taken as the sign of metabolic and oxidative stress in the cells.

3.4 Molecular Analysis

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to analyze gene expression. TRIzol reagent was used to extract total RNA and a reverse transcription kit was used to synthesize cDNA. Quantitative PCR was done with SYBR Green chemistry and relative levels of expression were determined by the 2 -ΔΔCt method where GAPDH was the internal control. Major metabolic regulators were examined such as AMPK, mTOR, SIRT1 and PGC-1α. Western blot analysis was used to determine the level of protein expression. Proteins in the total protein were extracted on lysis buffer, filtrated on Bradford assay, separated on SDS-PAGE and transferred to PVDF membranes. Primary antibodies of AMPK, mTOR, SIRT1, and PGC-1ala were then added to membranes with secondary antibodies added subsequently. Chemiluminescence was used to visualise protein bands. The JC-1 dye test was used to evaluate the mitochondrial membrane potential ($\Delta \psi m$) in mitochondrial functioning. Reduction in the ratio of red/green fluorescence was a measure of the metabolic stress conditions which depolarized and dysfunctioned the mitochondria.

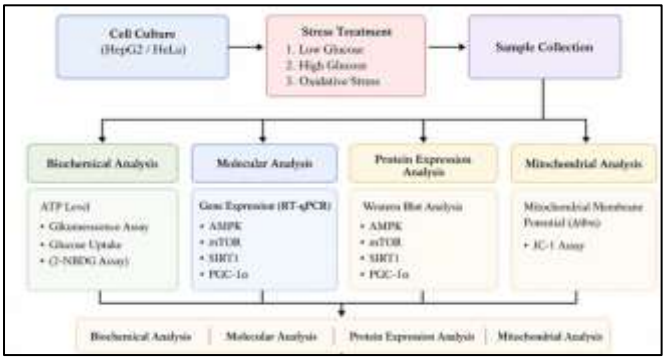

Fig 2: Biochemical & Molecular Workflow

3.5 Statistical Analysis

Wholesomely, all the experiments were done thrice and results were given in mean and standard deviation (SD). One-way analysis of variance (ANOVA) with a subsequent pairwise comparison by Student t-test was taken as the statistical analysis. The p-value of less than 0.05 was deemed as statistically significant. Analysis of the data and visualization was done in Graphpad Prism and R Software.

Data normality and homogeneity of variance were checked before statistical tests to ascertain the robustness and reliability of the results. Where needed, post hoc tests, such as the multiple comparison test of Tukey, were used to determine significant differences between multiple groups. Correlation analysis was also conducted to assess the relationship between metabolic parameters (ATP levels and glucose uptake and ROS production) and expression levels of the key regulatory genes. This integrated statistical methodology allowed correct analysis of experimental evidence and supported the validity of the identified changes of the molecules and metabolism in the circumstances of various stresses.

Table 2: Analysis Methods And Parameters

Analysis	Method	Tool/Kit	Purpose
ATP Measurement	Luminescence assay	ATP Kit	Energy status
Glucose Uptake	Fluorescent assay	2-NBDG	Metabolic activity
Lactate	Colorimetric assay	Lactate Kit	Glycolysis
ROS	DCFH-DA assay	Fluorescence reader	Oxidative stress
Gene Expression	RT-qPCR	SYBR Green	Regulatory genes
Protein Analysis	Western blot	PVDF membrane	Protein levels
Mitochondrial Function	JC-1 assay	Fluorescence	$\Delta\psi m$

4. RESULTS AND DISCUSSION

4.1 Alteration of Metabolic Markers

The stress conditions associated with metabolic stress levels led to great changes in important cellular metabolic indicators, which implies disturbance of the metabolic homeostasis. There was significant ATP drop in the intracellular space of the control cells (7.2 + 0.6 ug/g protein) to the treated cells (3.9 + 0.5 ug/g protein) which attests to lack of cellular energy production. Conversely, there was significant increase in the levels of reactive oxygen species (ROS) with the levels increasing to 126.8 ± 8.7 AU in the case of stress conditions compared to 48.5 ± 5.2 AU in control cells ($p < 0.001$) which is an indication of the increase in the oxidative stress.

The analysis of glucose uptake also showed a two-fold reaction to the duration of stress. In early exposures, there was an approximate 1.6-fold increment in glucose uptake appearing as a compensatory mechanism in metabolism. But, when subjected to a long term stress period, the uptake of glucose reduced considerably to 0.7-fold that at control conditions, meaning metabolic fatigue and poor utilization of nutrients. These findings altogether indicate that metabolic stress interrupts the energy balance in cells, externalizes oxidative stress, and disturbs metabolic activity.

Table 3: Key Metabolic and Regulatory Changes

Parameter	Control	Treated	Trend
ATP	7.2 ± 0.6	3.9 ± 0.5	↓

ROS	48.5 ± 5.2	126.8 ± 8.7	↑
Glucose Uptake	1.0	1.6 → 0.7	↑ then ↓
AMPK	1.0	2.8	↑
mTOR	1.0	0.52	↓
Akt	1.0	1.7 → 0.65	↑ then ↓
p53	1.0	3.9	↑
CASPASE-3	1.0	2.7	↑
BCL-2	1.0	0.48	↓

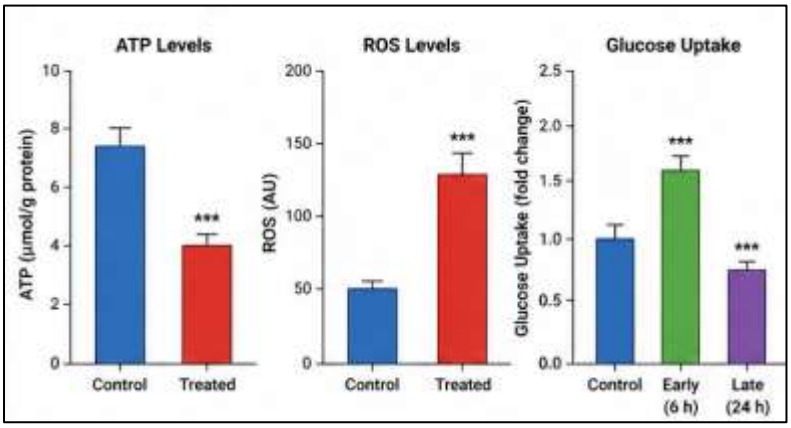

Fig 3: Metabolic markers (ATP, ROS)

4.2 Activation of Energy Sensor Pathways (AMPK)

Metabolically stress elicited a strong response in the AMP-activated protein kinase (AMPK) pathway, which indicates cellular efforts to replenish the energy homeostasis. RT-qPCR showed that the expression of AMPK gene increased by 2.8 folds in cells treated over controls ($p < 0.001$). On the same note, the activation at both transcriptional and post-translational levels was confirmed by Western blot analysis which revealed that the levels of phosphorylated AMPK protein increased at an approximate of 2.5 times.

This stimulation demonstrates that AMPK is a central energy sensor when there is stress present, and stimulates catabolic pathways to produce ATP at the expense of inhibiting energy-consuming activities. The high activity of AMPK indicates the adaptive mechanism of the cell to maintain homeostasis of metabolism in the conditions of energy deficiency.

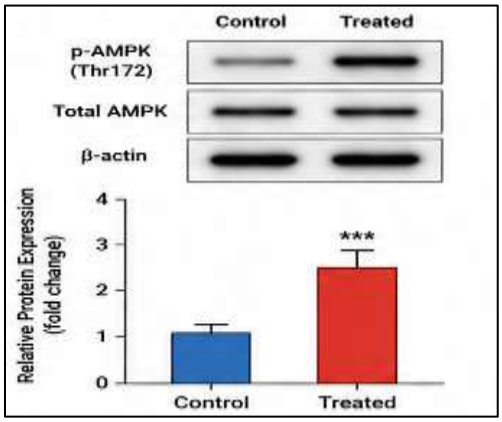

Fig 4: AMPK Activation

4.3 Modulation of mTOR Signaling

Compared with AMPK activation, the mechanistic target of rapamycin (mTOR) signaling pathway was greatly repressed in metabolic stress conditions. Analysis of gene expression revealed that the expression of mTOR was reduced to 0.52-fold relative to control ($p < 0.001$) and protein analysis revealed that the phosphorylated levels of the mTOR had also been reduced.

This inhibition of mTOR signaling indicates inhibition of anabolic events like protein synthesis and cell growth and is compatible with an energetically saving cellular phenotype. The negative correlation between the activation of the

AMPK and the inhibition of mTOR is an indicator of a significant regulatory axis that regulates the metabolic balance. The findings indicate metabolic stress rearranges cellular priorities in a way that cells prioritize survival over growth.

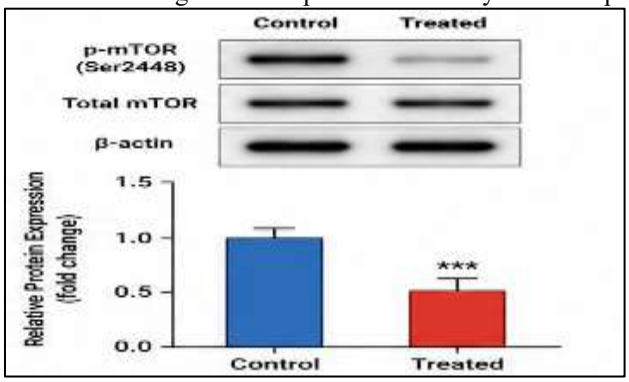

Fig 5: mTOR Suppression

4.4 Dysregulation of PI3K/Akt Pathway

Metabolic stress condition resulted in biphasic PI3K/Akt signaling pathway response. Phosphorylation of Akt rose to a level of about 1.7 times after early exposure (6 hours) which indicates activation of the survival signaling pathways. Nevertheless, when exposed over a long period (24 hours), Akt activity decreased significantly to 0.65-fold of control levels although PI3K expression remained relatively stable.

Such a tendency implies that cells initially respond to metabolic stress by turning on survival signals, but prolonged stress causes signaling pathway breakdown and cellular death. The loss of association of PI3K and Akt signaling is another indication of downstream signaling disturbance, which causes metabolic dysfunction.

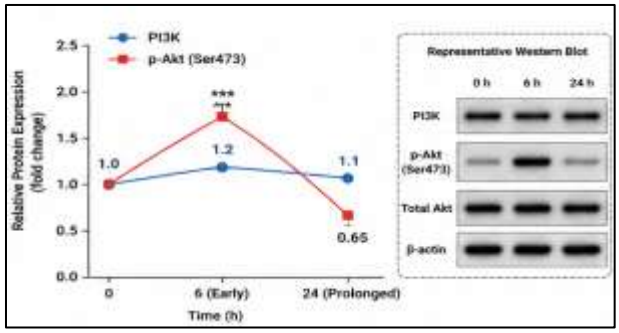

Fig 6: Dysregulation of PI3K/Akt Pathway

4.5 Mitochondrial Dysfunction

Metabolic stress caused major disturbances in the functioning of mitochondria as demonstrated by a reduction in the mitochondrial membrane potential. The results of JC-1 assay indicated that the ratio of red/green fluorescence reduced by about 5.6 in the control cells to 2.2 in the treated cells, which is about 60 percent drop.

This decrease is a sign of depolarization of the mitochondria, one of the characteristics of a metabolic failure and an initial apoptotic process. The ATP production and oxidative vulnerability are implied by the loss of mitochondrial integrity. The present results prove that metabolic stress can influence not only the cytoplasmic signaling pathway but also destabilize the core energy-producing organelles, thereby causing cellular energy breakdown.

4.6 Activation of Metabolic Apoptosis

The induction of apoptosis-related signaling pathways due to metabolic stress triggered signaling involves a change in the adaptive response to programmed cell death. The analysis of gene expression showed that the p53 level increased 3.9-fold, with the levels of CASPASE-3 (2.7-fold) and the anti-apoptotic BCL-2 protein (0.48-fold) being up and downregulated, respectively, in comparison with control cells ($p < 0.001$).

These alterations point to the engagement in the intrinsic apoptotic cascade that is caused by mitochondrial dysfunction and metabolic instability. The enhanced levels of pro-apoptotic indicators and the inhibition of survival indicators prove that metabolic stress with prolonged duration overwhelms cellular resistance, and endorses the occurrence of apoptosis.

4.7 Discussion

The current paper proves the idea that metabolic stress impairs cellular homeostasis by causing major changes in energy status, oxidative status and signaling pathways. The reported reduction in ATP levels, augmentation in the generation of ROS and fluctuation in glucose uptake confirm the existence of metabolic imbalance. AMPK activation and mTOR repression point to a reduction of anabolic processes that consume energy and smaller energy-conserving

reactions in response to stress, reflecting a cellular adaptation to the stress. Besides, biphasic nature of PI3K/Akt pathway indicates that cells initially respond by turning on survival mechanisms but cannot maintain them in the stressful conditions that persist over time.

Moreover, the dysfunction of mitochondria is crucial in the switch of metabolic adaptation to apoptosis. Programmed cell death pathways are activated as the mitochondrial membrane potential (Δ) and the expression of pro-apoptotic proteins like p53 and CASPASE-3 and the drop in BCL-2 are increased. These results highlight the concerted action of metabolic and signaling networks to regulate cell fate, and long-term metabolic stress can ultimately suppress adaptive mechanisms and cause apoptosis.

5. CONCLUSION

The paper has achieved success in determining the major molecular regulators that play a role in ensuring levels of cellular metabolic homeostasis and their collective action during situations of metabolic stress. The results show that metabolic imbalance is defined by low ATP levels, high levels of oxidative stress and changes in nutrient consumption all of which interfere with cellular stability. The core regulators AMPK, mTOR and PI3K/Akt are important in setting cellular responses, whereby their activation and inhibition respectively are involved in energy conservation and dysregulation of PI3K/Akt signaling, respectively, in the loss of survival capacity. In addition, the paper establishes that a long period of metabolic stress causes mitochondrial dysfunction and opening of apoptotic pathways, which are characterized by elevated levels of p53 and CASPASE-3 and reduced levels of BCL-2. These findings indicate that there is a shift in adaptive metabolic responses to programmed cell death when cellular stress reaches a critical level.

On the whole, this study provides a solid framework of metabolic imbalance-signaling pathway dysregulation and apoptosis. These key regulators are identified and this information can be used to help understand the molecular origin of metabolic disorders and may offer a possible target in therapeutic intervention. The prospective research combining multi-omics studies and in vivo experiments will also contribute to the knowledge of metabolic homeostasis and its impact on the development of diseases.

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