

Molecular mechanism for metformin – A New Hypothesis

Abstract

Metformin, the anti- hyperglycaemic drug, though in use since 1957 eluded as to its mechanism of action till date. There is some truth but not the whole truth, in even the much favoured mechanisms of AMP stimulated protein kinase (AMPK) stimulation and inhibition of complex 1 of Electron Transport Chain {ETC} , as there are objections , unresolved ,as yet.. Subsequent innovative mechanisms, like gut- mediated responses- involving glucagon-like peptide {GLP 1} and sodium-glucose transporter protein { SGLT 1} or signalling pathways involving transcription factors like mammalian target of rapamycin (mTOR C2) , sirtuin 1 (SIRT 1) etc , and the recently proposed and; brain-gut- liver axis fared no better. The obvious truth to be accepted is that, probably no single mechanism can explain all the observed phenomena . An attempt is made to rope in all mechanisms into one, invoking the glucagon signalling pathway, by a non- AMPK ,non- Complex1 inhibitory mechanism by the proposed hypothesis. To this extent new concepts like gate control concept and Warburg- like effect in diabetes mellitus type2 (DM2) are proposed . It is conceptualised that deranged glycolysis is at root cause of the disturbed energy metabolism in DM 2 and the answer to restore the same lies in reversal of the factors that lead to derailed glycolysis, Besides, a brief recapitulation of what is known is attempted, with emphasis on the bottle necks of each of these mechanisms .

Key Words :

AMPK (AMP stimulated kinase) , ETC (Electron Transport Chain) , Glucagon signalling pathway, GLP 1 (Glucagon- like polypeptide 1), C AMP (Cyclic amp).

Introduction

Metformin, though in use since 1957, its mechanism of action in diabetes mellitus type 2 (DM2) , remained elusive. Traditionally, its effect is explained by inhibition of increased gluconeogenesis (GN), insulin sensitisation and increased peripheral utilisation. Stimulation of AMP- stimulated Kinase (AMPK)and inhibition of complex 1 of electron transport chain (ETC) mechanisms are on the fore front Recently Gut mediated mechanisms were discovered A novel concept of Gut-Brain- liver axis on the anvil, but the role of metformin's is yet to be elucidated on its basis. The target organs for hypoglycaemic actions are Liver, skeletal muscle and gut. discussed presently existing mechanism of actions of metformin and their limitations . The role of the target organs of metformin action ie. Liver, skeletal muscle and gut are reviewed.

Recapitulation of known mechanisms

AMPK :-

AMPK has a wider role in metabolic regulation .This includes fatty acid oxidation, muscle glucose uptake [1],expression of cyclic adenosine monophosphate (C AMP) -stimulated gluconeogenic genes

such as phosphoenolpyruvate kinase (PEPCK) and glucose 6 phosphatase (G 6 Pase) [2], and glucose-stimulated genes associated with hepatic lipogenesis, including fatty acid synthase (FAS), Spot-14 (S14), and I-type pyruvate kinase (PK) [3]. Chronic activation of AMPK may also induce the expression of muscle hexokinase and glucose transporters Glut4, Hexokinase and glycogen .[4] . Metformin by reversing these effects of AMPK, controls GN.

AMPK mediates a decrease in sterol regulatory element protein 1(SREBP-1) mRNA and protein expression. Known target genes for SREBP-1, which include fatty acid synthase (FAS and S14, are also down regulated in liver, further contributing to metformin's effects to modulate circulating lipids and to reduce hepatic lipid synthesis and fatty liver. increased SREBP-1 is postulated as a central mediator of insulin resistance in DM2 and related metabolic disorders .

AMPK probably also plays a role in increased peripheral insulin sensitivity, as metformin administration increases AMPK activity in skeletal muscle. Metformin also translocates glucose transporter 4 (GLUT 4)to the cell periphery.

Lysosomal pathway:- metformin is suggested to act through lysosomal pathway[5]

Three effects, have been implicated as major contributors to glucose-lowering efficacy.

- ❖ Control of hepatic glucose production
- ❖ Increased skeletal myocyte glucose uptake,
- ❖ Metformin also decreases hepatic lipids in obese mice . There by reducing insulin resistance.
 - It is now appreciated, however, that many of the effects of metformin are AMPK-independent.
 - The drug effect on AMPK is not necessary , as it is also found in primary hepatocytes. [6]
 - The effects of metformin on hepatic glucose output are preserved in AMPK-deficient mice. [7]
 - Furthermore, metformin antigen-induced T cell proliferation independently of AMPK .[8]
 - metformin-induced suppression of glucose production is more pronounced in AMPK α 1 α 2-null hepatocytes compared with control cells
 - The ' energy crunch' produced by inhibition of complex 1 of ECT stimulates AMPK , relegating it to a second position to inhibition of complex 1 .
 - It is presently held that AMP can itself might be involved in metformin action by allosterically inhibiting genes of glucogenic enzymes like FBPase .
 - It was suggested that metformin might act through LKB1-AMPK signalling pathway. But since the drug did not influence the phosphorylation of AMPK by LKB1 in cell free assay, It is felt that metformin might not activate directly AMPK or LKB1.
 - Howley et have shown that AMP activation by metformin abolished in cell lines stably expressing AMPK complexes containing AMP insensitive gamma. w mutant, demonstrating that increased cytosolic AMP induced triggers the activation of the kinase by the drug. [9]
 - The biggest draw back of AMPK being the point of regulation by metformin is that whatever advantage is gained by reducing GN is offset by stimulation of B-Oxidation

83 Inhibition of complex 1 of ETC :

84 Metformin accumulates in mitochondrial matrix in good concentration because of its positive
85 charge. [9] metformin acts as an inhibitor of complex I of the electron transport chain(ETC) This

observation was made in both perfused livers and isolated hepatocytes from rodents but later extended to other tissues, including skeletal muscle , endothelial cells, pancreatic beta cells and **nuerons**.

- The possible ways metformin cause the complex 1 are
- Production of reactive oxygen species (ROS). [10]
- Inhibition of glucose 3 phosphate dehydrogenase (G3PDH) . [11]
- Uncoupling of proton pump. [12]
- However, the notion that metformin acts directly on mitochondria to inhibit complex I is controversial
- Recent work on the sensitivity of cancer cells to the direct actions of metformin further highlighted the controversy surrounding the mode of action of metformin. These studies demonstrate that cancer cells that are deficient in mitochondrial functions (rho0 cells) are sensitive to the action of metformin. [13]
- and that cancer cells harbouring complex I mutations are more sensitive to the action of metformin compared with cancer cells without these mutations [14]
- The ROS induced inhibition of complex 1 is not seen products of citric acid cycle pass through complex 2 of ETC. This is precisely what happens in DM2 , in the sense that, the products of B-Oxidation pass through complex 2 which will render metformin ineffective.
- It is also inconceivable that inhibition of complex 1 is enough to cause energy crunch as , infarct more energy (ATP) is produced by B- oxidation which by passing through complex 2 escape the inhibition at complex 1 is connected to disulfide bonds formation of insulin receptor, which if metformin inhibits , it makes endogenous insulin ineffective , hence counterproductive to metformin action
- NAD is essential to start glycolysis and it is regenerated from G3PDH. If the later were to be inhibited by metformin again it is counterproductive to its own action!
- Uncoupling of coupled reactions of energy production is also not a valid reason as coupling reaction occurs between electrons pumped out and protons pumped into the mitochondrial matrix from complex 3 and 4 of ETC .
- Metformin probably exerts some non- mitochondrial action as it is effective in erythrocytes, a cell that lacks mitochondria, by modulating membrane fluidity.[15]

Gut mediated mechanisms of metformin

- A) metformin increases glucose uptake and utilisation in the human intestine. The role of sodium-glucose transport protein 1 (SGLT 1) and glucose transporter 2 (GLUT2) are suggested. [16]
- B) Stimulation of GLP 1
Metformin could also act indirectly to stimulate GLP-1 secretion, via alterations in the bile acid pool. Metformin inhibits the farnesoid X receptor (FXR) via an AMPK-mediated mechanism, resulting in reduced sensing and ideal absorption of bile acids. The increase in the bile **acid** pool may then stimulate TGR5 bile acid receptors on the L cell causing an increase in glucagon like peptide 1 (GLP-1) secretion via mitochondrial oxidative phosphorylation and calcium influx
- 2) Metformin treatment has been shown to increase the glucagon-like peptide 1 (GLP-1) concentration in both mouse and human studies . GLP-1 is secreted.
Metformin could potentially increase the GLP-1 concentration by increasing its secretion from L cells, and/or by reducing its breakdown by **diphosphopeptidyl** 4 (DPP4) [17]
- 3) Microbes :-

132 It has been postulated that an increase in bacteria producing the short-chain fatty acids
133 butyrate and propionate may improve glycaemia .] Butyrate and propionate increase
134 intestinal gluconeogenesis. In rodents, increased intestinal gluconeogenesis results in a
135 reduction in hepatic gluconeogenesis, appetite and weight, leading to improved glucose
136 homeostasis. [18]

137 **Glucagon signalling.**

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139 1) Miller et al found that metformin antagonises effects of glucagon on adenylate cyclase,
140 attenuating glucagon-dependent increases in cAMP levels and concomitant activation
141 of PKA signalling pathway. This is discussed more elaborately under the new hypothesis to
142 be proposed shortly. if post-glucagon receptor modification of glucagon signalling, as
143 reported by Miller et al for metformin treatment in rodents , can be shown to be an
144 important effector of metformin therapy in humans, the allosteric inhibitor P-site on
145 adenylate cyclase provides an alternative approach to blocking the receptor itself, and this
146 offers an intriguing novel mechanism for inhibiting glucagon action in the liver.

147 The role of glucagon signalling system is considered more elaborately under the proposed
148 hypothesis.

149 **ROLE OF LIVER**

150 Liver is the primary target of metformin as GN occurs here. It acts byA)

151 **A) controlling nutrient supply to GN.**

152 Takashima et al. have proposed a role for Krüppel-like factor 15 (KLF15) in the metformin-
153 induced inhibition of genes coding for both gluconeogenic and amino acid catabolic
154 enzymes, these later being potentially implicated in the regulation of gluconeogenesis
155 through the control of gluconeogenic substrate availability .[19]

156 **B) By reduction of energy needed for GN.**

157 1) Since the rate of hepatic glucose production is closely linked to hepatic energy
158 metabolism (6 ATP equivalents are required per molecule of glucose synthesised),
159 disruption of the main energy supply in hepatocytes (mitochondrial oxidative
160 phosphorylation) through inhibition of the respiratory-chain complex 1 would have a
161 profound effect on the flux through gluconeogenesis .

162 2)In addition, as AMP tends to rise whenever ATP falls, this could cause

163 A) an acute inhibition of gluconeogenesis by metformin via allosteric regulation of key enzymes in
164 this pathway, such as fructose-1,6-bisphosphatase . [20]

165 .B) allosterically inhibit cAMP–PKA signalling through suppression of adenylate cyclase,)

166 3) Activates AMPK.

167 **The role of OCT 1 and CCK 2** – are more elaborately discussed under the proposed hypothesis

168 **Gut- Brain – Liver axis[21]** brain–Gut -liver axis has recently been proposed to detect blood lipids to
169 inhibit It is demonstrated that direct administration of lipids into the upper intestine increased
170 upper intestinal long-chain fatty acyl-coenzyme A (LCFA-CoA) levels and suppressed glucose
171 production. Sub diaphragmatic vagotomy interrupts the neural connection between the gut and the
172 brain, and blocks the ability of upper intestinal lipids to inhibit glucose production. Finally, hepatic
173 vagotomy negated the inhibitory effects of upper intestinal lipids on glucose production. These

findings indicate that upper intestinal lipids activate an intestine–brain–liver neural axis to inhibit glucose production, and thereby reveal a previously unappreciated pathway that regulates glucose homeostasis. There is no indication that metformin acts through this mechanism as yet.

Role of skeletal muscle[22]

Metformin increases transport of glucose into skeletal muscles through insulin dependent and independent mechanisms.

Insulin sensitivity:-[23]

Metformin causes insulin sensitising effect by direct stimulation of insulin receptor. AMPK probably also plays a role in increased peripheral insulin sensitivity, as metformin administration increases AMPK activity in skeletal muscle. AMPK is known to cause GLUT4 deployment to the plasma membrane, resulting in insulin-independent glucose uptake.

A prelude to the proposed hypothesis :

The factors that lead to the blocking of glycolysis in DM2, should be reversed if glycolysis is to be re-established. The increased gluconeogenesis which is secondary to the disturbed glycolysis automatically gets corrected, once the glycolysis is re-established. A drug like metformin, to be effective has to restore glycolysis. So it is imperative that both of the factors that block and restore glycolysis should be surmised to see as to how metformin works. A gate control concept in DM2 is proposed, where shutting and opening these gates would close and open the pathway of glycolysis. In all, 4 gates and 2 sub-gates are envisaged of which 3 main gates remain closed i.e. Phosphofructokinase, pyruvate kinase and pyruvate dehydrogenase. (PFK, PK and PDH) and one gate (PEPCK) and two sub-gates (glycogen synthase and glucose 6 phosphate dehydrogenase (G-6PDH)), remain opened in untreated DM2. The events that lead to closure/opening of these gates are traced, leading to stopping/starting of glycolysis in DM2 untreated, and treated with metformin, respectively. The new hypothesis, shows as to how Metformin opens these closed gates to restore the glycolysis and controls the hyperglycaemia in DM2. Metformin by mechanisms other than AMPK stimulation and inhibition of Complex 1 of ETC. are explored. Thus the hypothesis is centred around the **glucagon** signalling pathway. The factors involved in mechanism of anti—hyperglycaemic action of Metformin are re-interpreted in terms of invoking the glucagon signalling pathway. The basis of the proposed hypothesis is that, even under physiological conditions, there is interplay between the two regulatory hormones of energy metabolism, the insulin and glucagon, one prevailing over the other in the prandial and interprandial states respectively. In DM2, due to relative insufficiency of functionally active insulin (inspired by prevailing hyperinsulinemia), the metabolism becomes glucagon oriented. Inhibiting glucagon is as good as increasing endogenous insulin, in restoring glycolysis. Metformin not being a secretagogue, is likely that it works by down-regulating glucagon. The pathway is modulated by metformin through control of enzymes, both glucogenic and glycolytic through allosteric, covalent, and hormonal regulators or through expression / down-regulation of their corresponding genes, and a host of transcription factors - the respective elements are summarised in table 1 and table 2. The spectrum of Glucagon actions and GLP stimulation are depicted in table 3. Glucagon signalling pathway is shown in Box - 1.

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Table 1

Hormones	--	Enzymes
Glucagon		glycolytic
GLP 1		PFK
G.H		PK
FGF 21		PDH
Glucocorticoids		Glucogenic
		F1,6BPase
		F2,6BPase
		PEPCK

Table 2

Gene transcription Factors	Transporters
TOR C2	OCT 1
PGC alfa	SGLT 1
HNF alfa	GLUTs
SHP	(2,4)
Others	
ERR alfa	
NR4As	
ROR alfa	
TR4	

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Table 3		

Spectrum of actions		
Glucagon		GLP 1

A) By phospho-rylation		A) Reduced glucogon level
Inhibition		
PFK		b) insulino-
PK		trophic
PDH		effect
F2,6BP		
B) gene stimulation		C) increased glucose utilisation
FBPase		by entero-
PEPCK		Cytes.

286 BOX NO 1

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288 **Glucagon Signalling pathway:**

289 glucagon binds to G8 protein - coupled- receptor causing stimulation of Adenylyl cyclase which
290 produces C- AMP . C AMP Stimulates PKA that phosphorylates proteins- the target enzymes. The
291 phosphorylation of target proteins by protein kinase A is reversed by the action of protein
292 phosphatase .

293 Inactive form of protein kinase A consists of two regulatory (R) and two catalytic (C) subunits.
294 Binding of cAMP to the regulatory subunits induces a conformational change that leads to
295 dissociation of the catalytic units which are translocated into the nucleus. and phosphorylates the
296 transcription factor CREB (CRE-binding protein), leading to expression of C AMP-inducible genes.

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298 **Synthesis and degradation of C AMP:**

299 Cyclic AMP is synthesised from ATP by adenylyl cyclase and degraded to AMP by C

300 - AMPphosphodiesterase

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302 **The proposed hypothesis**

303 **The gate 1- PFK/ FBPase**

304 The crucial step whether Glycolysis or GN should proceed is decided by the bifunctional protein,
305 PFK/ FBPase . It is the first committed step towards glycolysis, by PFK while FBPase is decisive in
306 regulating GN pathway. Since both are reciprocally related only one process at a time occurs to
307 prevent a futile cycle. This reciprocal control is achieved as follows

308 FBPase and C AMP :-

309 In human, over expression of FBPase I among other gluconeogenic enzymes was reported in type 2
310 diabetic patients, [24]

311 FBPase I mRNA is rapidly induced by cAMP within 24 h, while insulin suppresses its expressions
312 seen in primary rat hepatocytes, [25]

313 The presence of putative CRE in the promoter of rat FBPase I gene is consistent with the ability of
314 C AMP to induce expression of FBPase 1 mRNA in rat hepatocytes although fold-response to cAMP in
315 the hepatocytes transfected with FBPase I promoter-reporter construct is somewhat low.

316 Dual role of F2, 6 bisphosphate:-

317 F,6 BP is Stimulated by PFK and inhibited by FBPase [26]

318 After ingestion of a meal, fructose-2,6-bisphosphate is elevated advancing a speedy glycolysis
319 reaction by stimulating PFK.. Before meal, the concentration of fructose-2,6- bisphosphate is
320 decreased, resulting in up regulation of FBPase and facilitation of gluconeogenesis. [27]

321 Glucagon:-

322 In untreated DM2, the PFK, is inhibited by the Glucagon by phosphorylation through PKA and at the
323 same time, FBPase and PEPCK genes regulating GN are over expressed.

324 c amp -Xu 5P(Xylulose .5 phosphate) control of F2, 6 BP/PFK. 2

325 Both have reciprocal control under physiological conditions -on PFK, the Xu5P stimulating (through
326 F2,6 BP) and C amp inhibiting PFK(by phosphorylating).In uncontrolled DM2, it was shown that
327 HMP shunt is opened due to the stimulatory effect on G-6PD (sub gate B), (which remains inhibited
328 under physiological conditions) by the increased G -6 P flux, consequent to block at PFK level .Xu 5P
329 is an intermediary product in HMP path way. The intermediary, Xu 5P stimulates 2PA which
330 produces F2;6 BP, the powerful stimulator of PFK. In DM2 Glucagon, through C AMP- inhibits PFK by
331 phosphorylation and inhibition of F2, 6 BP, stimulatory action of Xu 5P is overcome by CAMP.[28]
332 Metformin inhibits CAMP[29] and the first gate is closed.

333 Modulate the activity of glucagon through adenylyl calyces [30] lowers the glucagon level through
334 stimulation of GLP 1 and facilitates the F2,6BP, which is the most powerful stimulator of FBS. This
335 opens the first closed gate.6 the glycolytic flux to pass uninterrupted up-to PEP where the
336 second gate in the form of PK decides the fate of the glycolytic flux in reciprocal relation to the dose
337 third gate, the PEPCK.

338 **Gate 2:- The pyruvate Kinase (PK) :-**

339 PK in normal physiological conditions, converts PEP to pyruvate. PK in DM2 is inhibited and hence
340 the glycolytic flux from PEP is routed through the GN pathway. Reciprocally the PEPCK is stimulated
341 directing the flux from OA also into the GN pathway. The end product, G3PDH of HMP shunt
342 (hexose monophosphate shunt) is directed also into the GN Pathway which accounts for the
343 increased GN seen in DM2

344 PK is inhibited by

345 1)Glucagon through adenylyl cyclase- c amp -PKA signalling pathway inhibits PK by phosphorylation.

2) It is also inhibited by FBS (which is a allosteric stimulator /positive feed back) being itself inhibited by glucagon. [31]

3) Increased ATP inhibits PK by negative feedback.

Metformin causes a significant decrease in the cellular ATP concentration, which is a known allosteric inhibitor of this enzyme, This could explain the stimulating pyruvate-kinase activity and thus inhibiting gluconeogenesis.

4) Metformin decreases gluconeogenesis by enhancing the pyruvate kinase flux in isolated rat hepatocytes.[32]

5) ChREB which is stimulated by Xu5P through 2PA also inhibits L type Pyruvate kinase C AMP by phosphorylating and dephosphorylating ChREBP regulates PK . [33]

6) The reciprocal inhibition of PK by gluconeogenic enzyme genes ie. PEPCK and FBPase, which are over expressed in DM2.

7) guanidine-containing drugs induce anti-hyperglycaemic effects by displacing calcium from proteins such as pyruvate kinase.[34]

8) direct targeting of metal ions —which takes the form of an unusual electron delocalised planar ring structure, where square planar geometry replaces more conventional tetragonal geometry .

10) AMP mediated:

AMP attaching to adenylyl cyclase inhibits C Amp.

a) Increased cellular AMP has been proposed to explain the inhibition of glucagon-induced increase in cAMP and activation of PKA.

b) It was also demonstrated that metformin elicited a decrease in ATP and a concomitant rise in AMP levels, and that there was a tight correlation between the magnitude of ATP reduction and inhibition of glucose.

c) metformin increases the concentration of cytosolic adenosine monophosphate (AMP) (as opposed to a change in total AMP or total AMP/adenosine triphosphate).

through the glycolytic cycle is more important determinant in inhibiting the increased GN in DM2.

d) As AMP tends to rise whenever ATP falls, this could cause an acute inhibition of gluconeogenesis .

.The two isoforms of PK- PK M1 and M2 :

The isoenzyme PK M1 promotes glycolysis by converting PEP to pyruvate. Where as PK M2 promotes the GN at the expense of glycolysis. In tumour cells- PKM2 suspends glycolysis and favours GN in tumour cells- the Warburg effect.

T- state of PK stabilised by PEPCK and F 1,6 BP promotes glycolysis. R state of Pk (high substrate affinity state) is stabilised by PEPCK and F1,6 BP stabilise it promoting glycolysis. The R state (low affinity substrate state) stabilised by ATP and alanine, promotes GN than glycolysis. By interpolation the above states which are interchangeable might be due to the effect of alternate splicing of PKM gene and expression of the M1 and M2 iso -enzymes. In other words, the splicing can be induced by glucogenic enzymes which are subject to regulation by glucagon signalling and other regulators of PK.

PK M2 - mTOR-HIF -1 alfa cascade: PKM2 is a crucial glycolytic enzyme in the oncogenic mTOR-induced Warburg effect, in which hypoxia inducible factor-1 α (HIF-1 α) and c-Myc-hnRNP cascades are the transducers of mTOR regulation of PKM2. Notably, the reduction in Under hypoxic conditions, the PKM2 gene interacts directly with HIF-1 α , which activates the hypoxia response element that is required for HIF-1 α binding u

Warburg -like effect in DM2-Is proposed by this author by drawing a similarity between both malignancy and DM 2 as regards to circumstances and mechanism responsible for both. The Warburg effect occurs by preference of malignant cells for glucose to ATP by oxidative phosphorylation. This occurs in the hypoxic circumstances in which cancer cells grow. In DM 2 also due to inhibition of PDH (blocking access to citric acid cycle- ECT of

acetyl CoA) anaerobic glycolysis promotes a hypoxic state . Also the **glycolysis** is suspended in preference to GN- So the circumstances (hypoxia) are common to both even though the purpose is different.

The Warburg effect is induced by mTOR on M2.m TOR is elevated in DM2 also making M2 stimulation a possibility. In fact some studies noted increased M2 in DM2.

C AMP by up **regulating** and Metformin by inhibiting C AMP down regulates m and mTOR thus inhibiting the GN and promoting glycolysis.

Gate -3 PPEPCK

- 1))Over-**expression** of PEPCCK is sufficient to cause A DM2 like state in mice. [35]
- 2) Knock out studies of pck 1 gene reduced hyperglycaemia and insulin resistance in db/ db mice . G METFORMIN ACTION :-
- 3) Down regulation of PEPCCK genes by metformin results in shutting off of GN pathway resulting in- **reduced** flux through G6Pase.[36]
- 4) suppression of hepatic glucose production by metformin in insulin-resistant high-fat fed rats is dependent on an inhibition of the substrate flux through G6Pase. [37]
- 5) KLF 15 expression IS high in fasting and diabetic mice which caused increased expression of glucogenic enzymes like PEPCCK and G 6 phosphatase. metformin down regulated these enzymes and reduced hepatic gluconeogenesis[38]
- 6) PGC-1ALFA:-(Peroxisome Proliferator-Activated Receptor Gamma co- activator 1-alpha.) metformin selectively affects hepatic PGC-1 α -mediated gene regulation and prevents activation of gluconeogenesis.
- Metformin inhibits PGC 1 ALFA by acetylating it and reducing GN. [39]
- 7) TOR C2

TORC2, the transducer of regulated CREB protein 2 is a cAMP responsive co- activator that, in concert with LKB1 and AMPK, controls glucose homeostasis in the liver.

In the nucleus, TORC 2 phosphorylates to FORM TORC 2- CERB- CBP complex which causes induction of PGC alfa, a co-activator for Pck 1 gene. Glucagon dephosphylates the CREB controlled TORC 2.

Once activated PGC1 ALFA forms complex with Foxo and hnf alf4 which is a glucogenic genes stimulation including Pck 1

8)) SIRT 1

- a) deacetylates and inhibits TORC2 (which suppresses the GN gene expression.
- b) SIRT I dephosphorylates PGC alfa 1 there by permitting the formation of PGC 1 ALFA- FOXO 4- Hnf1 complex which stimulates GN gene Expression. GCN 1 (general control nonderepressible homolog) 1
- C) SIRT 1 also deacetylates Foxo 4 alfa, causing nuclear transduction and Induction of GN gene)[40]

Through SIRT 1 and GCN 1 stimulation , metformin controls DM

β -adrenergic activation of the cAMP/PKA pathway rapidly increases SIRT1 activity in a NAD+-independent fashion.[41]

Gate 4. - The PDH

When the gate is open, glycolytic products enter citric acid cycle..When it is closed, the glycolytic flux is directed to aerobic glycolytic pathway leading to GN.. The energy metabolism is switched over Beta- Oxidation of fats.

The PDC complex is inactivated by PDKs and activated by PDPs.

.The PDC is usually active during the fed-state in most tissues, where it suppresses pyruvate dehydrogenase kinase (PDK)-induced phosphorylation

Pdk4 levels are elevated in fasting and diabetic individuals . [42] Which keeps the PDH inhibited [43]indefinitely. PDK4 deficiency lowers the blood sugar .[44]

Opening of the gate by metformin :-

This can be achieved either by

1) **Stimulation of PDH :-**

.The PDC is inhibited in DM2 allosterically. By increased levels of acetyl CoA/ CoA and NADH/NAD and ATP/ADP.

ADP, AMP and COA are allosteric stimulators. The FBP which is by now activated , stimulates the PDH

2) **By increasing the glycolytic flux through PDH ,**

When inhibition of PK is lifted .Its stimulatory effect of increased flux through PDH overcomes the allosteric PDH inhibition.

3) **By inhibiting PDK 4 :-**

The role of growth hormone (GH):-

GH stimulates PDK4 expression in the liver of wild-type mice during fasting by activating the janus kinase/signal transducer and activator of transcription (STAT5) pathway and increasing gluconeogenesis. Metformin inhibits GH-induced PDK4 expression via the AMP-activated protein kinase/small heterodimer partner-dependent pathway that inhibits the combination of STAT5 to the PDK4 [46]

4) **SHP :-**

inhibits PKA- C AMP- CREB mediated expression of GN genes (PEPCK and G6Pase). Metformin by induction of SHP down regulates CREB mediated GN .[47]

5) **By lowering of glucagon effect by metformin by**

a) GLP stimulation which also increases insulin secretion and insulin sensitivity.

b) By direct effect of metformin on glucagon.

c) by inhibiting the C amp by metformin.

Role of hormones transcription factors and transporters. Transcription factors

1)) **SHP (Small heterodimer partner) :-**

Metformin Inhibits Hepatic Gluconeogenesis Through AMP-Activated Protein Kinase–Dependent.Regulation of the Orphan Nuclear Receptor SHP. [48]

2) **PGC 1 alfa(Peroxisome proliferator-activated receptor coreceptor) :**

Metformin induces PGC-1 α and selectively affects hepatic PGC 1 alfa mediated gene regulation and prevents activation of GN.. Metformin inhibits PGC alfa by acetylating it and reduces GN. [49]

3) **CRTC2**, initially called **TORC2**, is a transcriptional **coactivator** for the transcription ate responsive binding protein **CREB** and a central regulator of **gluconeogenic gene expression** in response to **cAMP**.

In the nucleus, TORC 2 phosphorylates lo form TORC 2- CERB- CBP complex which causes induction of PGC alfa, a co-activator for Pck 1 gene. Glucagon dephosphylates the CREB controlled **TOR C2**.

Once activated PGC 1 alfa forms complex with Foxo and hnf alfa 4 which is a glucogenic genes stimulation including Pck 1. [50]

4) **HNF ALFA (hepatocyte nuclear**Cyclic nucleotide regulation of Na⁺/glucose cotransporter (SGLT1) mRNA stability. Interaction of a nucleocytoplasmic protein with a regulatory domain in the ;-

483 untranslated region critical for stabilisation. [51]

484 Recent **factor** :

485 Activation of CREB by cAMP/PKA further stimulated HNF-4 α transactivation in HepG2 cells.
486 cAMP induced the expression of the HNF-4 α target genes PCK1 and G6Pase in these cells. In
487 conclusion, our results suggest that the level of PGC-1 α determines whether the cAMP/PKA-
488 pathway overall stimulates or inhibits HNF-4 α transcriptional activation.[50]

489 5) **KLF. (kruppel like factor)**

490 KLF15 is increased by fasting and decreased by feeding and insulin via PI3K signalling. KLF15 was
491 increased by glucocorticoid signalling and was also increased by inhibition of PI3K. Insulin and its
492 counteracting hormones regulate the hepatic expression of KLF1h5. Forced expression of KLF15 in
493 cultured hepatocytes increased both the expression and the promoter activity of the gene
494 for phosphoenolpyruvate carboxykinase (PEPCK).[52]

495 **6) sirtuin 1**

496 deacetylates and inhibits TORC 2 which surpasses the GN gene expression. (liu et al2008)) SIRT I
497 dephosphorylates PGC alfa 1 thee by permitting the formation of PGC 1 ALFA- FOXO 4- Hnf1
498 complex which stimulates GN gene. C AMP/PKA pathway activates Beta oxidation-of fats by an NAD
499 independent mechanism. [53.] Metformin by inhibiting CAMP down regulates Beta oxidation .

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501 **Other Transcription factors in relation to regulation of GN :**

502 A) ERR Alfa:- a downstream glucagon modulator of GN pathway.

503 B)NR 4As

504 C) ROR alfa

505 D)TR4

506 It is beyond the scope of present article to elaborate on these transcription factors.

507 **Hormones vs metformin.**

508 1) **Glucagon:** Is inhibited by metformin acting through C AMP as seen already. Further it's level
509 is lowered by the metformin stimulation of GLP 1 , the later also producing its antagonistic
510 hormone ,the insulin from pancreatic B -cells.

511 2) **Adiponectin** vs C AMP

512 The circulating concentration of adiponectin is decreased in obesity and Type 2 diabetes .
513 insulin and β -agonists act directly at the adipocytes in opposing fashions to regulate the
514 production of adiponectin and leptin, and that a PI3K-PDE3B-cAMP pathwayformin
515 mediates the effects of insulin to restore β -agonist/cAMP-suppressed secretion and
516 expression of these two adipokines. [54]

517 3)**FGF 21 (fibrocyte growth factor)**

518 In humans, plasma levels of FGF21 are elevated in obese subjects and patients with type 2
519 diabetes,

520 Glucagon Stimulates Hepatic FGF21 Secretion through a PKA- and EPAC-Dependent Post-
521 transcriptional Mechanism .[55]Metformin down regulating the glucagon signalling.

522 4) **GLP 1:**

523 The role of GLP 1 IS summarised IN TABLE 2.

524 Cyclic AMP triggers glucagon-like peptide-1 secretion from the GI entero- endocrines cell line

525 5) GH :

526 The growth hormone (GH):- stimulates PDK4 expression in the liver of wild-type mice
527 during fasting by activating the janus kinase/signal transducer and activator of transcription
528 (STAT5) pathway and increasing gluconeogenesis. Metformin inhibits GH-induced

529 PDK4 expression via the AMP-activated protein kinase/small heterodimer partner-dependent
530 pathway that inhibits the combination of STAT5 to the PDK4 promoter.[57]

531 1) OCT 1 (**organic cation transporter**)

532 Metformin exerts major effects on glucose and lipid metabolism in the liver, and OCT1 has been well
533 established as the primary hepatic transporter for the drug. It functions as a repressor of Cdx-2, a
534 proglucagon gene activator.

535 elevation of cAMP leads to enhanced phosphorylation and nuclear exclusion of Oct-1 and reduced
536 interactions between Oct-1 or nuclear co-repressors and the Cdx-2 gene promoter.

537 POU homeodomain protein Oct-1 functions as a sensor for cyclic AMP

538 2) SGLT 1:

539 Cyclic nucleotide regulation of Na⁺/glucose cotransporter (SGLT1) mRNA stability. Interaction of a
540 nucleocytoplasmic protein with a regulatory domain in the 5'-untranslated region critical for
541 stabilisation. [56]

542 Recent studies of the mouse intestine in vitro demonstrated that Na⁺/glucose cotransport is
543 increased two- to eightfold within minutes by the application of forskolin, an agent that increases
544 intracellular cyclic AMP levels . [58]

545 2) cAMP-dependent stabilisation of the SGLT1 message was correlated with the protein
546 phosphorylation-dependent binding of cytoplasmic proteins to a uridine-rich sequence (URE) in the 5'-
547 untranslated region

548 Conclusion:

549 The glucogenic enzymes and their corresponding genes, the hormones, and the transcription
550 factors that tend to produce hyperglycaemia in DM2, are shown to be controlled by metformin
551 through inhibition of the glucagon- C AMP-PKA pathway. The basic objections to AMPK mechanism
552 of metformin action is that, if Metformin were to act by stimulating it, any benefit of controlling GN
553 is offset by stimulation of Beta oxidation of fats, which brings the matter back to square one. The
554 other mechanism, inhibition of complex 1, is also believed to be insufficient to produce a low
555 energy state that could explain either the stimulation of AMPK or cutting down the fuel needed for
556 GN. While the above two mechanisms are centred around correcting the GN, an off-shoot of
557 blocked glycolysis in DM 2, ie. the GN, the proposed hypothesis aims at correction of the basic
558 defect of blocked glycolysis which automatically corrects the secondary effects like the GN. Hence
559 the proposed new hypothesis, basing on the glucagon signalling pathway, assumes significance as a
560 break-away from earlier approaches.

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