

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 AMPK stimulation and inhibition of complex 1 of Electron Transport Chain , as there are objections , unresolved,as yet.. Subsequent innovative mechanisms, like gut- mediated responses-involving GLP 1 and SGLT 1 or signalling pathways involving transcrition factors like TOR C2- SIRT 1 etc, and the recently proposed 'brain-gut- liver axis' fared no better. The **obviou** 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 glucogon signalling pathway, by an non- AMPK ,non Complex1 inhibitory mechanism by the proposed hypothesis. To this extent new concepts like 'gate control concept' and 'Warberg- like effect in DM2' are proposed .Besides, a brief recapitulation of what is known is attempted, with emphasis on the bottle necks of each of the mechanisms .

Key Words :

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

Introduction

Metformin, though in use since 1957, its mechanism of action in diabetes mellatus 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 forefront 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 adenine monophosphate (C AMP) -stimulated gluconeogenic genes such as PEPCK and G6Pase [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 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 GLUT R 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. Thereby 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 does not 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 ETC stimulates AMPK, relegating it to a position second 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 al 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 drawback 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

Inhibition of complex 1 of ETC :

Metformin accumulates in mitochondrial matrix in good concentration because of its positive 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 neurons.

- The possible ways metformin cause the complex 1 are]
- Production of reactive oxygen species (ROS). [10]

- 86 ▪ Inhibition of glucose 3 phosphate dehydrogenase (G3PDH) . [11]
- 87 ▪ Uncoupling of proton pump. [12]
- 88 ▪ However, the notion that metformin acts directly on mitochondria to inhibit complex I is
- 89 controversial
- 90 ▪ Recent work on the sensitivity of cancer cells to the direct actions of metformin further
- 91 highlighted the controversy surrounding the mode of action of metformin. These studies
- 92 demonstrate that cancer cells that are deficient in mitochondrial functions (rho0 cells) are
- 93 sensitive to the action of metformin. [13]
- 94 ▪ and that cancer cells harbouring complex I mutations are more sensitive to the action of
- 95 metformin compared with cancer cells without these mutations [14]
- 96 ▪ The ROS induced inhibition of complex 1 is not seen products of citric acid cycle pass t
- 97 through complex 2 of ETC. This is precisely what happens in DM2 , in the sense that, the
- 98 products of B-Oxidation pass through complex t2 which will render metformin ineffective.
- 99 ▪ It is also inconceivable that inhibition of complex 1 is enough to cause energy crunch as ,
- 100 infarct more energy (ATP) is produced by B – oxidation which by passing through complex 2
- 101 escape the inhibition at complex 1 is connected to disulfide bonds formation of insulin
- 102 receptor, which if metformin inhibits , it makes endogenous insulin ineffective , hence
- 103 counterproductive to metformin action
- 104 ▪ NAD is essential to start glycolysis and it is regenerated from G3PDH. If the later were to be
- 105 inhibited by metformin again it is counterproductive to its own action!.
- 106 ▪ Uncoupling of coupled reactions of energy production is also not a valid reason as coupling
- 107 reaction occurs between electrons pumped out and protons pumped into the mitochondrial
- 108 matrix from complex 3 and 4 of ETC .
- 109 ▪ Metformin probably exerts some non- mitochondrial action as it is effective in erythrocytes,
- 110 a cell that lacks mitochondria, by modulating membrane fluidity.[15]

111 **Gut mediated mechanisms of metformin**

- 112 ▪ A) metformin increases glucose uptake and utilisation in the human intestine. .The role of
- 113 sodium-glucose transport protein 1 (SGLT 1) & glucose transporter 2 (GLUT2)are
- 114 suggested. [16]
- 115 ▪ B) Stimulation of GLP 1
- 116 Metformin could also act indirectly to stimulate GLP-1 secretion, via alterations in the bile
- 117 acid pool. Metformin inhibits the farnesoid X receptor (FXR) via an AMPK-mediated
- 118 mechanism, resulting in reduced sensing and ideal absorption of bile acids. The increase in
- 119 the bile acid pool may then stimulate TGR5 bile acid receptors on the L cell causing an
- 120 increase in glucagon like peptide 1 (GLP-1) secretion via mitochondrial oxidative
- 121 phosphorylation and calcium influx
- 122 2)Metformin treatment has been shown to increase the glucagon-like peptide 1 (GLP-1)
- 123 concentration in both mouse and human studies . GLP-1 is secreted.
- 124 Metformin could potentially increase the GLP-1 concentration by increasing its secretion
- 125 from L cells, and/or by reducing its breakdown by DPP4 [17]
- 126 3) Microbes :-
- 127 It has been postulated that an increase in bacteria producing the short-chain fatty acids
- 128 butyrate and propionate may improve glycaemia .] Butyrate and propionate increase
- 129 intestinal gluconeogenesis. In rodents, increased intestinal gluconeogenesis results in a
- 130 reduction in hepatic gluconeogenesis, appetite and weight, leading to improved glucose
- 131 homeostasis. [18]

Glucagon signalling.

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

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

ROLE OF LIVER

Liver is the primary target of metformin as GN occurs here. It acts by A)

A) controlling nutrient supply to GN.

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

B) By reduction of energy needed for GN.

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

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

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

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

3) Activates AMPK.

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

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

174 **Role of skeletal muscle[22]**

175 Metformin increases transport of glucose into skeletal muscles thro insulin dependent and
176 independent mechanisms.

177 **Insulin sensitivity:-[23]**

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

182 **A prelude to the proposed hypothesis :**

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184 The factors that lead to the blocking of glycolysis in DM2, should be reversed if glycolysis is to be re-
185 established. The increased gluconeogenesis which is secondary to the disturbed
186 glycolysis automatically gets corrected , once the glycolysis is re- established .

187 A drug like metformin , to be effective has to restore glycolysis. So it is imperative that both of the
188 factors that block and restore glycolysis should be surmised to see as to how metformin fits into
189 such setup.

190 A gate control concept in DM2 is proposed , where shutting and opening these gates would close
191 and open the pathway of glycolysis .In all , 4 gates and 2 sub- gates are envisaged of which 3 main
192 gates remain closedie. Phosphofructokinase, pyruvate kinase and pyruvate dehydrogenase . (PFK,PK
193 and PDH) and one gate (PEPCK) and two sub gates (glycogen synthase and glucose 6 phosphate
194 dehydrogenase (G-6PDH) , remain open in untreated DM2 The events that lead to closure/
195 opening of these gates are traced , leading to stopping/ starting of glycolysis in DM 2
196 untreated, and treated with metformin, respectively. The new hypothesis , shows as to how
197 Metformin opens these gates to restore the glycolysis and controls the hyperglycemia in
198 DM2 metformin by mechanisms other than AMPK stimulation and Inhibition of Complex 1 of ETC.
199 are explored. Thus the hypothesis is centred around the glucagon signalling pathway, The factors
200 which involved the earlier proposed mechanisms are re- interpreted in terms of invoking the
201 glucagon signalling pathway. The pathway is modulated by metformin through control of enzymes ,
202 both glucogenic and glycolytic through allosteric , covalent , and hormonal regulators or through
203 their corresponding genes , and a host of transcription factors - the respective elements are
204 summarised in table 1 and table 2.. spectrum of Glucagon actions and GLP stimulation are depicted
205 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)
P 53	
Others	
ERR alfa	
NR4As	
ROR alfa	
TR4	

Table 3

Spectrum of actions	
Glucagon	GLP 1
A) By phosphorylation	A) Reduced glucagon level
Inhibition	
PFK	b) insulinotropic effect
PK	
PDH	
F2,6BP	
B) gene stimulation	C) increased glucose utilisation by enterocytes.
FBPase	
PEPCK	

BOX NO 1

Glucagon Signalling pathway:

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

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

Synthesis and degradation of cAMP :

Cyclic AMP is synthesized from ATP by adenylyl cyclase and degraded to AMP by cAMP phosphodiesterase.

The proposed hypothesis

The gate 1- PFK/ FBPase

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

FBPase and C AMP :-

In human, overexpression of FBPase I among other gluconeogenic enzymes was reported in type 2 diabetic patients (Creutzfeldt et al., 1970)

FBPase I mRNA is rapidly induced by cAMP within 24 h, while insulin suppresses its expression in primary rat hepatocytes (el-Magharbi et al., 1991).

The presence of putative CRE in the promoter of rat FBPase I gene is consistent with the ability of cAMP to induce expression of FBPase I mRNA in rat hepatocytes although fold-response to cAMP in the hepatocytes transfected with FBPase I promoter-reporter construct is somewhat low (el-Magharbi et al., 1991).

Dual role of F2,6bisphosphate:-

F2,6 BP is Stimulated by PFK and inhibited by FBPase 2 [24]

After ingestion of a meal, fructose-2,6-bisphosphate is elevated advancing a speedy glycolysis reaction by stimulating PFK. Before meal, the concentration of fructose-2,6- bisphosphate is decreased, resulting in up regulation of FBPase and facilitation of gluconeogenesis. [25]

Glucagon:-

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

c amp -Xu 5P control of F2, 6 BP/PFK.

Both have reciprocal control under physiological conditions -on PFK, the Xu5P stimulating (through F2,6 BP) and C amp inhibiting PFK(by phosphorylating).In uncontrolled DM2, it was shown that HMP shunt is opened due to the stimulatory effect on G-6PD (sub gate B), (which remains inhibited under physiological conditions) by the increased G -6 P flux, consequent to block at PFK level .Xu 5P is an intermediary product in the HMP path way. The intermediary, Xu 5P stimulates 2PA which produces F2;6 BP, the powerful stimulator of PFK.(fig 1) In DM2 Glucagon, through C AMP- inhibits PFK by phosphorylation and inhibition of F2, 6 BP, stimulatory action of Xu 5P is overcome by CAMP (Liu YQ, et al, 1996 PubMed 6829999) and the first gate is closed.

Metformin inhibits CAMP .[26]

Modulate the activity of glucagon through adenylyl cyclase [27] lowers the glucagon level through stimulation of GLP 1 and facilitates the F2,6BP, which is the most powerful stimulator of FBS. This opens the first closed gate.6 the glycolytic flux to pass uninterrupted upto PEP where the second gate in the form of PK decides the fate of the glycolytic flux in reciprocal relation to the third gate, the PEPCK.

The second gate- PK :-

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

PK is inhibited by

1) Glucagon through adenylyl cyclase- c amp - PKA signalling pathway inhibits PK by phosphorylating it

2) It is also inhibited by FBS (which is an allosteric stimulator /positive feed back) being itself inhibited by glucagon. [28]

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.[29]

5) a)ChREB which is stimulated by Xu5P through 2PA also inhibits L type Pyruvate kinase JC AMP by phosphorylating and dephosphorylating ChREBP regulates PK . [30]

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.[31]

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 .[32]

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 .

(Holmes BF, Kurth-Kraczek EJ, Winder WW. Chronic activation of 5'-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol.* 1999;87:1990–1995.)

The two isoforms- 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 tumor cells-

PKM2 suspends glycolysis and favours GN in tumor 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.

Warburg like effect in DM2 :

PKM2 - mTOR-HIF -1 alfa cascade:

Sun et al demonstrated that PKM2 was 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

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 glucolysis 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 upregulates m and Metformin by inhibiting C AMP down regulates m and mTOR thus inhibiting the GN and promoting glycolysis.

Gate -3 PPEPCK

1) There is evidence of increased PEPCK activity IN DM 2 (Venezuela et al 1983.)

2) PEPCK is abnormally elevated in DM2. (Venezuela et al 1983.)

3) Overexpression of pek m- rna is sufficient to cause A DM2 like state on rodents with hyperglycemia, hyperinsulinemia and IR.

(Valera et al 1994, Sun et al 2002)

4) Knock out studies of pck 1 gene reduced hyperglycemia and insulin resistance in db/ db mice .(Gomez- Vadies et al 2006-2008) .

METFORMIN ACTION :-

5)1) Down regulation of PEPCK genes by metformin results in shutting off of GN pathway resulting in reduced flux through G6Pase. [33]

2)6) suppression of hepatic glucose production by metformin in insulin-resistant high-fat fed rats is deendent on an inhibition of the substrate flux through G6Pase. [34]

5)7) KLF 15 EXPRESSOIN IS HIGH IN FASTING AND DIABETIC MICE WHICH CAUSED INCREASED

EXPRESSION OF GLUCOGENIC ENZYMES LIKE PEPCK AND G 6 PHOSPHATASE. METFORMIN DOWN

REGLUTED THESE ENZYMES AND REDUCED HEPATIC GLUCONEOGENESIS. [35]

4)6) PGC-1ALFA:- (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 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 ang reducing GN. (lerin et al 2006).

5)9TOR C2

TORC2, the transducer of regulated CREB protein 2 is a cAMP responsive coactivator 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 coactivator for Pck 1 gene. Glucogon dephospylates the CREB controled TORC 2.

Once activated PGC1 ALFA forms complex with Foxo and hnf alfa 4 which is a glucogenic genes stimulation including Pck 1. (Yoondalet al 2p01, Puiz et al . 2003)

10)

6)10 SIRT 1

a) deacetylates and inhibits TORC2 (which supresses the GN gene expression. (liu et al 2008)

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
 Expression.(Frescas et
 al 2005)
 Through SIRT 1 AND GCN 1 stimulation , metformin controls DM
 Control of SIRT 1 :-
 β -adrenergic activation of the cAMP/PKA pathway rapidly increases SIRT1 activity in a NAD⁺-
 independent fashion.
 (Gerhart-Hines et al., 2011)
 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 . [36] Which keeps the PDH inhibited
 [37]indefinitely.
 PDK4 deficiency lowers the blood sugar .[38]
 Opening of the gate by metformin :-
 This can be achieved either by
 1) Stimulation of 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) 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 growth hormone (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 promoter.[39]
 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 .[40]
 5) By lowering of glucogon effect by metformin by
 a) GLP stimulation which also increases insulin secretion and insulin sensitivity.
 b) By direct effect of metformin on glucogon.
 c) by inhibiting the C amp by metformin.
 Role of hormones transcription factors and transporters.

Transcription factors:

1) SHP :-

inhibits PKA- C AMP- CREB mediated expression of GN genes (PEPCK and G6Pase). Metformin by induction of SHP down regulates CREB mediated GN [41]

2) PGC 1 alfa induces PGC-1 α and selectively affects hepatic PGC-1 α functions.[42]

3)TOR C2

In the nucleus, TORC 2 PHOSPHORYLATES TO FORM TORC 2- CERB- CBP complex which causes induction of PGC alfa, a coactivator for Pck 1 gene. Glucagon dephosphylates the CREB controlled TORC 2.

Once activated PGC1ALFA forms complex with Foxo and hnf alfa 4 which is a glogenic genes stimulation including Pck 1. [43] (Yoondalet al 2p01,Puiz et al . 2p03)

SIRT 1 deacetylates and inhibits TORC 2 which supresses the GN gene expression. (liu et al 2008)

4) SIRT I dephosphorylates PGC alfa 1 thee vy permitting the formation of PGC 1 ALFA- FOXO 4- Hnf1 complex which stimulates GN gene [44]

5) Expression. GCN 1 inhibits PGC 1 ALFA by acetylating it ang rdducing GN. (lerin et al 2006).

5)HNF ALFA.

HNF vs camp

Moreover, activation of CREB by cAMP/PKA further stimulated HNF-4alpha transactivation in HepG2 cells. cAMP induced the expression of the HNF-4alpha target genes PCK1 and G6Pase in these cells.

In conclusion, our results suggest that the level of PGC-1alpha determines whether the cAMP/PKA-pathway overall stimulates or inhibits HNF-4alpha transcriptional activation.[45]

6) KIF and pepck

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

8)Teshigawara K, Ogawa W, Mori T, Matsuki Y, Watanabe E, Hiramatsu R, Inoue H, Miyake K, Sakaue H, Kasuga M (February 2005). "Role of Krüppel-like factor 15 in PEPCK gene expression in the liver". *Biochem. Biophys. Res. Commun.* 327 (3): 920–6. doi:10.1016/j.bbrc.2004.12.096. PMID 15649437) p53

novel inverse relationship between metformin-triggered AMPK-SIRT1 signalling and p53 protein abundance in high 2) By inhibiting PDK4. It is evidenced by the fact that high-fat fed, insulin-resistant mice lacking PDK4 exhibit lower blood glucose levels and better glucose tolerance than wild-type mice [24]. The growth hormone (GH), glucose-exposed HepG2 cells.

Nelson LE, et al. *Am J Physiol Cell Physiol.* 2012.

Metformin Induces p53-Dependent Mitochondrial Stress in Therapy-Sensitive and -Resistant Lymphoma Pre-Clinical Model and Primary Patients Sample with B-Cell Non-Hodgkin Lymphoma (NHLJuan J Gu, Qunling Zhang, Cory Mavis,Myron S. Czuczman andFrancisco J. Hernandez-Ilizaliturri Blood 2015 126:4008;)

Other Transcription factors mentioned in relation to regulation of GN :

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

B)NR 4As

C) ROR alfa

D)TR4

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

537 Hormones vs metformin.

538 Glucagon: The role of this hormone is summarised in table 1

539 2) Adiponectin vs C AMP

540 The circulating concentration of adiponectin is decreased in obesity and Type 2 diabetes . insulin and

541 β -agonists act directly at the adipocytes in opposing fashions to regulate the production of

542 adiponectin and leptin, and that a PI3K-PDE3B-cAMP pathway mediates the effects of insulin to

543 restore β -agonist/cAMP-suppressed secretion and expression of these two adipokines. [46]

544 3)FGF 21

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

546 Glucagon Stimulates Hepatic FGF21 Secretion through a PKA- and EPAC-Dependent

547 Posttranscriptional Mechanism . [47]

548 4) GLP 1:

549 The role of GLP 1 IS SUMMERISED IN TABLE 2.

550 C amp vs glp1

551 Cyclic AMP triggers glucagon-like peptide-1 secretion from the GI entero- endocrines cell line.[48].

552 GH

553 The growth hormone (GH):- stimulates PDK4 expression in the liver of wild-type mice during

554 fasting by activating the janus kinase/signal transducer and activator of transcription (STAT5)

555 pathway and increasing gluconeogenesis. Metformin inhibits GH-induced PDK4 expression via the

556 AMP-activated protein kinase/small heterodimer partner-dependent pathway that inhibits the

557 combination of STAT5 to the PDK4 promoter. [49]

558

559 Transporters

560 1)OCT 1:

561 Oct vs c amp

562 Metformin exerts major effects on glucose and lipid metabolism in the liver, and OCT1 has been well

563 established as the primary hepatic transporter for the drug. It functions as a repressor of Cdx-2, a

564 proglucagon gene activator.

565 elevation of cAMP leads to enhanced phosphorylation and nuclear exclusion of Oct-1 and reduced

566 interactions between Oct-1 or nuclear co-repressors and the Cdx-2 gene promoter.

567 POU homeodomain protein Oct-1 functions as a sensor for cyclic AMP.[50]

568 2) SGLT 1:

569 Sglt1 vs c amp

570 Recent studies of the mouse intestine in vitro demonstrated that Na⁺/glucose cotransport is

571 increased two- to eightfold within minutes by the application of forskolin, an agent that increases

572 intracellular cyclic AMP levels . [51]

573 2) cAMP-dependent stabilization of the SGLT1 message was correlated with the protein

574 phosphorylation-dependent binding of cytoplasmic proteins to a uridine-rich sequence (URE) in the -

575 untranslated region.[52]

576 Cyclic nucleotide regulation of Na⁺/glucose cotransporter (SGLT1) mRNA stability. Interaction of a

577 nucleocytoplasmic protein with a regulatory domain in the 3'-untranslated region critical for

578 stabilization.

579

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