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Review

Metformin and calorie restriction modulate gene-environment interaction to prevent premature senescence

***Dr. Oriaifo Stephen E[1] Dr. Nicholas Oriaifo[2] Prof. Omogbai E.K.I [3]**

[1] Department of pharmacology and therapeutics, aau, Ekpoma
[2] Department of obstetrics and gynaecology, Isth, Irrua
[3] Department of pharmacology and toxicology, Uniben, Benin-City
*Corresponding author's email: stephen.oriaifo@yahoo.com

Abstract

A catalogue of evidence shows that the cardiometabolic disorders of diabetes mellitus and hypertension are related to other age-related diseases which shorten life. Data also mount that they are linked to environmental factors inducing alterations in genetic and epigenetic mechanisms, for example, telomerase expression. Calorie restriction and the biguanide metformin have been acquitted as plausible agents for extending health span and lifespan for they suppress age-related disorders. Their mode of action appear to converge on AMPK, micro RNAs and purinergic mechanisms and, through these, modulation of matrix metalloproteinases and its down-stream target, glycogen synthase kinase-3 β. A retrospective report also shows that metformin and calorie restriction more effectively suppress deterioration in cardiac function compared to other hypoglycaemic agents such as insulin and sulphonylureas in a Nigerian population. More research is needed to discover agents that may possess the pharmacologic actions of metformin, to further elucidate the mechanisms of action of metformin and calorie restriction and whether and how their beneficial effects hold fort in all races and in all climes.

Keywords: Metformin, Calorie restriction, Environment, Diabetes mellitus, Hypertension, Health span

OVERVIEW

The rising burden of diabetes mellitus and hypertension

Type 2 diabetes mellitus and hypertension have emerged as major medical and public health issues worldwide (Kings and Rewers, 1991). Type 2 diabetes mellitus is increasing in epidemic proportions globally. According to WHO, the prevalence of diabetes mellitus in adults worldwide was estimated to be 4.0% in 1995 and is predicted to rise to 5.4% by the year 2025, an estimate that might have already been exceeded. In 2010, the estimate of presenting diabetes was 6.4% of the global adult population, that is, 285 million (Bruce, 2014).

Diabetes and hypertension reduce quality of life

Additionally, hypertension affects one billion people worldwide and it is estimated that by 2025, up to 1.56 billion adults worldwide will be hypertensive.Diabetes and hypertension exert a significant burden resulting in increased morbidity and mortality (Moller, 2001), decreased life expectancy and reduced quality of life. For example, according to WHO, the life expectancy of Nigerians fell from 51-56 years in 2000 to 47.56 years in 2011 due to the epidemic of the metabolic or insulin resistance syndrome (Mohan et al, 2013; Awosan et al, 2013; Udenze et al, 2013). Metabolic syndrome is a

cluster of cardiometabolic risk factors which include insulin resistance, prediabetes, type 2 diabetes mellitus, central obesity, dyslipidaemia, hypertension, atherosclerotic cardiovascular disease and microalbuminuria (WHO, 1999; Alberti et al, 2005).

Diabetes mellitus disrupts homeostasis and increase risk of age-related diseases

Age-related diseases include cognitive impairment occasioned or exacerbated by diabetes; stroke and neurodegenerative diseases; glaucoma- and macular degeneration-induced falls secondary to diabetes and hypertension; immune dysfunction, atherosclerosis, coronary heart disease, cancer, prostate enlargement, osteoporosis-induced fractures, all resulting in frailty and faulty physiological response to stressors; and constipation/incontinence. Diabetes mellitus may be at the epicentre in the causation of most of these illnesses which decrease healthspan and lifespan because it particularly disruptsthe 'fixity' of the *milieu interieur* or internal environment which according to Claude Bernard is the condition (or pre-requisite) of a free, independent life (Holmes, 1986; Gross, 1998) and, *pari passu*, the balance in the functions of the nervous, immune and cardiovascular systems. While the possible mediators of this association, such as polymorphism in the matrix metalloproteinase-9 (MMP9) gene (Rybakowski, 2009) and microRNA-regulated pathways (Hebert, 2009) are being unravelled, metformin and calorie restriction, at present, may be in the forefront in the prevention of these age-related illnesses (Blagosklonny, 2009).

The influence of environmental factors

Environmental factors (Liu et al, 2008), particularly diet and sedentary lifestyles, have major roles in diabetes risk by accelerating prevalence of early onset pre-diabetes and type 2 diabetes mellitus. The common forms of diabetes mellitus present multifactorial aetiologies with involvement of intricate interactions of genetic, epigenetic and environmental attributes (Chukwuma, 2014).

Genetic and epigenetic implication

DNA methylation and histone modifications are two of the chromatin remodelling processes which help in the integration of environmental signals for optimal genomic output (Liu et al, 2008). Aberrations in chromatin remodelling are associated with both genetically and environmentally-related diseases such as cancer and type 2 diabetes mellitus. DNA methylation of certain genes has been found to be important in type 2 diabetes mellitus (Carless et al, 2013); to mediate persistent epileptiform activity *in vitro* and *in vivo* (Machnes et al, 2013); and also implicated in bipolar disorder (Ikegame et al, 2013). Obesity caused by high-fat diet increases DNA methylation at the leptin promoter in rat adipocytes (Kalliman and Parrizas, 2011). Diabetes mellitus and a hyperactive mTOR signalling increase generation of ROS which may cause genetic mutation by damaging DNA and mitochondria (Potter et al, 2010; Robertson et al, 2004; Zhang, H (Sfrbm.org). CR (Olivo-Marston et al, 2014) and metformin (Paulito et al, 2013) modulate microRNAs (MiR-122, MiR-33 and MiR-200) involved in cholesterol metabolism and cancer growth.

Telomere and ageing

Attrition in telomere length may be the main determinant of human ageing and in this regard diabetes mellitus has a significant role to play in causing telomere attrition and decreasing lifespan and healthspan. White blood cells telomere length is shorter in males with type 2 diabetes mellitus and microalbuminuria (Tentolouris et al, 2007). High-glucose-induced DNA degradation, mitochondrial oxidative degradation and protein glycation resulting in the formation of advanced glycation end-products (AGEs) augment telomere attrition and this can be prevented by metformin and calorie restriction (CR). The molecular mechanisms involved in the premature senescence associated with hyperglycaemia include oxidative stress, decreased mitochondrial and nuclear DNA repair capacity, and protein glycation through the Maillard's reaction and Amadori re-arrangement resulting in the formation of advanced glycosylation end products such as the dicarbonyl methylglyoxal (Del Nogal-Avila et al, 2013). Plasma methylglyoxal levels, a major precursor of advanced glycation end-products, are increased in hypertension and diabetes mellitus and is related to upregulation of the renin-angiotensin-aldostrone (RAA) system (Dhar et al, 2013) which may enhance telomere attrition. Diabetes mellitus is a disease characterised by accelerated chemical ageing of long-lived tissue proteins (Dyer et al, 1993). These molecular mechanisms lead to the telomeres gradually decreasing in length (telomere attrition) culminating in cellular senescence (Von Zglinicki, 2001; Mayer et al, 2006).

Calorie restriction

Calorie restriction (CR) is the moderate reduction of about 20-40% in calorie intake compared with a*d libitum* feeding without compromising the basic nutritional needs (Blagosklonny, 2009; Canto and Auwerx, 2011). It is the most consistent intervention increasing lifespan. It protects against the deterioration in biological function and reducing the risk factor for diabetes-associated cardiovascular disease and cancer.

AMPK is a key sensor and effector of the molecular effects of calorie restriction and metformin

The serine/threonine kinase, 5'-adenosine monophosphate activated protein kinase (AMPK) is the key energy sensor with the ability to transcriptionally reprogram the cell and metabolically adapt to external cues. It is activated upon an increase in AMP/ATP ratio. AMPK acts as important mediator of the beneficial effects of CR (Canto and Auwerx, 2011) and of metformin, leptin, adiponectin, adenosine, adenine nucleotides and ghrelin that regulate energy expenditure and food intake (Towler and Hardie, 2007). Adenosine nucleotide biosynthesis and AMPK regulate lifespan and mediate the longevity benefit of calorie restriction (Stenesen et al, 2013).

Insulin sensitivity and AMPK signalling decrease with ageing

The responsiveness of AMPK signalling and insulin sensitivity decline with ageing (Salminen and Kaamiranta, 2012; Escriva et al, 2007).This increases oxidative stress and reduces autophagic clearance. There is then activation of innate immunity defence, triggering low-grade inflammation and metabolic disorders.

The actions of calorie restriction and metformin converge

The actions of CR and the synthetic biguanide metformin appear to converge. Both increase mitochondrial biogenesis crucial for a healthy cellular and whole-body ageing (Martin-Montalvo et al, 2013; Canto andAuwerx, 2011); reduce MMP-2 activity and retards age-associated aortic restructuring in rats (Wang et al, 2006); down-regulate increased susceptibility of aging kidney to ischaemic injury by inhibiting MMP-7 gene (Chen et al, 2007), counter the age-related decline in ischaemic tolerance (Peart et al, 2012) and antagonise MMP-2-mediated augmentation of GSK-3 beta kinase activity which may contribute to cardiac injury resulting from enhanced oxidative stress (Kandasamy and Schulz, 2009). GSK-3 beta also mediates high-glucose-induced ubiquitination and proteasome degradation of insulin receptor substrate I (Leng et al, 2010) and its levels strongly correlates with increased gamma-secretase activity in the brain (Ho et al, 2004). Thus both metformin and calorie restriction enhance insulin signalling and may be beneficial in the prevention of Alzheimer's disease due to diet-induced amyloidosis (Ho et al, 2004)

Metformin has calorie restriction-mimetic effectsto attenuate augmented telomere attrition occasioned by high-glucose induction

Metformin has calorie restriction-mimetic effects (Martin-Montalvo et al, 2013), suppresses memory of hyperglycaemia stress (Zheng et al, 2012) via Sirtuin I (silent information regulator) activation and prevents progression of impaired fasting plasma glucose (Oriaifo et al, 2013). Metformin stands to potentiate the effect of calorie restriction in the management of diabetes mellitus where the activity of FOXO3a is reduced. Calorie restriction with weight loss increases insulin sensitivity by activating FOXO3a, a key regulator of insulin and IGF-I signalling (Qin et al, 2006). Independently, calorie restriction induces SIRT I activation. Metformin also suppresses hepatic gluconeogenesis and modulates hyperglycaemia-induced endothelial senescence and apoptosis through induction of SIRT I (Caton et al, 2010; Arunachalam et al, 2014).

Thus, metformin and calorie restriction, alone or in combination, attenuate telomere erosion associated with ageing (Vera et al, 2013), and may synergise with telomerase in promoting longevity (Anisimov et al, 2005; Everitt and Le Couteur, 2007; Wang et al, 2015). The calorie restriction regimen practised in our clinics to improve cardiometabolic health include reducing calorie intake by reductions in carbohydrate, meat, oil, salt and sugar in-take (C.MOSS) and increase of in-take of fruits and vegetables, legumes, unsaturated oils such as soya bean oil, water, decrease in periods of non-activity by increasing exercise and leading a regulated life free of alcohol and drugs (FLOWER). A tilt of the ratio of C.MOSS/FLOWER towards C.MOSS or a decrease in the FLOWER items of diet may increase cardiometabolic risk. 'Moss' literally means stagnation and relative exaggeration of MOSS items in foods, especially from obesogenic centres, poses increased risk for stagnation (clog) in the circulation. Metformin has been shown to recover pancreatic β-cell cell dysfunction and death due to endoplasmic reticulum stress (Jung et al, 2012; Cheang et al, 2014) which may be exacerbated by ageing (Naidoo et al, 2014). Although the adaptations to calorie restriction (CR) may involve reductions in post-priandal GLP-I concentrations, CR + exercise have additive beneficial effects on glucoregulation and insulin sensitivity (Weiss et al, 2015). Metformin's upregulation of GLP-I, the incretin of major importance, which enhances pancreatic beta-cell neogenesis like GLP-I (Verspohl, 2012; DeFronzo et al, 2014) and which does not cause weight gain unlike insulin, the sulphonylureas or thiazolinediones, makes metformin + CR + exercise training, at present, pivotal or optimal in preventing progression of prediabetes to type 2 diabetes. The regimen of low-dose metformin (500 mg daily) and calorie restriction has been found to be beneficial in one cohort of patients with impaired fasting plasma glucose.

Metformin and CR restore leptin sensitivity and increase a diponectin levels occasioned by insulin resistance

There is hypoadiponectinaemia in insulin resistance

which is corrected by chronic metformin therapy and by food restriction (Adamia et al, 2007; Escriva et al, 2007). The levels of adiponectin decreases with obesity or food addiction (Barry et al, 2009) while that of leptin increases (Putz et al, 2004) and these aberrations increase cardiovascular risk (Im et al, 2006; Koh et al, 2008) and hepatic fibrosis (Tsochatzis et al, 2006).Hyperleptinaemia impairs insulin signalling (Perez et al, 2004) and predicts acute cardiovascular events by exerting actions that are potentially atherogenic, thrombotic and angiogenic. It stimulates production of pro-inflammatory cytokines and increase reactive oxygen species (ROS) and sympathetic activity, an independent predictor of diabetes mellitus (Koh et al, 2008). The increased plasma levels of leptin and C-reactive protein (CRP) in obesity increase cardiovascular risk and CRP may bind leptin extra-cellularly thus impairing its activity and creating insulin resistance (Hribal et al, 2014). Metformin decreases CRP levels (Oriaifo et al, 2013) andboth metformin and CR restore leptin sensitivity in rodents with high-fat-induced insulin resistance (Kim et al, 2006; Wilsley and Scarpace, 2004; Escriva et al, 2007).

Metformin and CR Decrease Age-Related Increased Risk of Cardiomyo-Vasculopathies.

The cardiovascular diseases of hypertension, coronary heart disease, peripheral artery disease, atherosclerosis and stroke increase risk for premature senescence (Blagosklonny, 2009; Nicolli and Partridge, 2012) and are accelerated by diabetes.

Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus (Fadini et al, 2005). Metformin modulates hyperglycaemia-induced endothelial senescence and apoptosis through SIRT I (Arunachalam et al, 2014) and increases endothelial progenitor cells (EPCs) in diabetic mellitus (Liao et al, 2010). It may also activate cardiac progenitor cells. Metformin and CR possess anti-hypertensive (Bhalla et al, 1996) and sympathoinhibitory effects.

Through their activation of AMPK and inhibition of glycogen synthase kinase 3 beta (GSK-3 beta), metformin and CR stand in good stead as a therapeutic agent for atherosclerosis (Motoshima et al, 2006; McAlpine et al, 2012); and could also help in thromboembolism through suppression of matrix metalloproteinase-9 (MMP-9) and -2 (Morizane et al, 2011). In fructose-induced insulin resistant rats, Lu et al (2013) showed that metformin prevented neo-intima formation and enhanced methacholine - induced relaxation whilst decreasing phenylephrine-induced vasoconstriction. It was also shown to decrease vascular smooth muscle cell (VSMC) proliferation, migration and inflammation.

Metformin and CR arecardioprotectives

Metformin limits cardiac infarct size and remodelling (El-Messaoudi et al, 2011); and AMPK activation by metformin or CR is a preventive therapeutic target in the transition from cardiac injury to heart failure (Beauloye et al, 2011; Fu et al, 2011). K (ATP) activation by metformin coupled with its inhibition of MMP-9 could attenuate left ventricular dilatation in the infarcted heart (Gao et al, 2009; Creemers et al, 2001). Metformin through the Silent Information Regulator (SIRT I) regulates Protein Kinase B (Akt) implicated in modulating cardiac hypertrophy and ageing (Pillai et al, 2014).

Diabetes causes bone marrow autonomic neuropathy and impairs stem cell mobilisation (Albiero et al, 2014) which may be modulated by metformin and CR. Restoration of cardiac progenitor cells after myocardial infarction is by self-proliferation and selective homing of bone marrow-derived stem cells (Mouquet et al, 2005). Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells (Urbich et al, 2006).

Proviral Insertion Site for Moloney Murine Leukaemia Virus-I (Pim-I), a critical participant in Akt-mediated cardioprotection (Sussman, 2009), is a close homologous gene to Pim-2 (van der Lugt et al, 1995) which is known to be upregulated by metformin (Leclerc et al, 2013). Metformin's and CR induction of the anti-oxidant hemeoxygenase-I (HO-I) or heat-shock protein-32 (Liu et al, 2011) could off-set effects of polymorphisms in the detoxification enzymes, quinoneoxido-reductase (NQOI), glutathione-S-transferase theta (GSTT) and glutathione-S-transferaseMμ (GSTM) genes which are associated with coronary heart disease (CHD) (Martin et al, 2009) in patients with type 2 diabetes mellitus.

Cognitive decline and Neurodegeneration

Cognitive decline and neurodegeneration are frequently age-related disorders (Blagosklonny, 2009) in tandem with the fact that obesity and diabetes are associated with cognitive impairment and early neurodegeneration (Kim et al, 2011)

Brain insulin resistance is an early and common feature of Alzheimer's disease (Talbot et al, 2012) and is associated with IGF-I resistance, insulin receptor substrate-I (IRS-I) dysregulation and cognitive decline. Alzheimer's disease shows many age-related pathophysiological features of type 2 diabetes mellitus which include insulin resistance, disrupted glucose metabolism in non-neuronal tissues, peripheral oxidative and inflammatory stress, amyloid aggregation, neural atrophy and cognitive decline. Advanced Maillard's reaction end-products are associated with Alzheimer's disease and ageing pathology (Smith et al, 1994; Dyer et

al, 1993). Alzheimer's disease may now be termed type 3 diabetes mellitus (de la Monte, 2012; Yanev et al, 2013; Chaldakov et al, 2014;Chaldakov et al, 2009; Manev, 2009). Additionally, hypoxia-dependent amyloidogenesis, a sequelae of hypertension, could lead to Alzheimer's disease, creating the cerebrovascular-Alzheimer's disease spectrum that may be responsive to some anti-hypertensives that have neuroprotective effects (Jin et al, 2014; Valenzuela et al, 2012; Alexander et al, 2011; Wang et al, 2007).

CR and metformin possess anti-hypertensive effects

CR (Dolinsky et al, 2010; Young et al, 1978) and metformin (Muntzel et al, 1999) possess anti-hypertensive effects. Together with their neuroprotective effects and actions against insulin resistance, their deployment in diabetes mellitus and co-occurring hypertension treatment may be indispensable.

Both CR and metformin display anti-depressant effects

Chronic metformin administration display anti-depressant effects which is enhanced by CR and by the recognised anti-hypertensive, valsartan (Oriaifo and Omogbai, To Be Published). Both metformin through AMPK activation and CR may enhance serotonin, orexin, endorphin and neurotrophic signalling (Zhang et al, 2015; Lim et al, 2010; Tsuneki et al, 2013; Ou et al, 2006; Satoh et al, 2010) for their anti-depressant-like effects which reinforce the will for life amongst diabetics.

CR and metformin show benefit in Alzheimer's disease

The energy sensor, AMPK, when activated by insulin sensitizers such as metformin + exenatide (Li, 2007) or calorie restriction (Ho et al, 2004) reduce oxidative stress, improves mitochondrial dysfunction, improves glucose uptake in Alzheimer's disease (AD) and slows down the main amyloidogenic protein, Aβ, accumulation extracellularly and intracellularly or tau hyper-phosphorylation intracellularly (Kim et al, 2011) to reduce the memory impairment in the disease (Kim et al, 2013).Whitmer (2013) has reported that metformin cuts AD-linked dementia rates more than insulin, thiazolidinediones or sulphonylureas. Moreover, the initial report by Chen et al (2009) has also been straightened by recent investigations by Hettich et al (2014) who showed that the biguanide, metformin, reduces β-site amyloid precursor protein cleaving enzyme-I(BACEI) levels making it of value in treating or preventing AD. Metformin decreases neuronal insulin resistance and is associated with neurogenesis (Wang et al, 2012). It also protects against vascular dementia. Metformin, not rosiglitazone, attenuates the increasing plasma levels of the new cardiovascular/bipolar disorder I marker, fibulin-I (Skov et al, 2014; Greenwood et al, 2012).

Network analysis of neurodegenerative diseases highlights a role of Toll-like receptor signalling (Nguyen et al, 2014) which metformin and CR suppress acutely and chronically (Soraya et al, 2014; Sun et al, 2007). Reduced cell proliferation and neuroblast differentiation in the dentate gyrus of lipotoxic mice are ameliorated by metformin (Yoo et al, 2011) which promotes neurogenesis and spatial memory formation (Potts and Lim, 2012; Aravi et al, 2008; Zhang et al, 2011).

Molecular effects of metformin in neurogenesis

Metformin's and CR's upregulation of BDNF upregulates neurogenesis by enhancing the activities of neurotrophic factors such as basic fibroblast growth factor-2 (Lamba et al, 2008). Metformin activates an atypical protein kinase C-(PKC)-cAMP response element binding protein (CBP) pathway to promote neurogenesis (Wang et al, 2012). It also promotes epidermal growth factor-induced proliferation and migration of human fetal neural stem/progenitor cells with the PIK3/Akt pathway (Zhang et al, 2011; Menendez and Vazquez-Martin, 2012)). Metformin also enhances BDNF-neuropeptide Y (NPY) signalling which decreases excitability and neuro-apoptosis (Silva et al, 2007).

CR and metformin attenuate inflammatory cytokines and are beneficial in epilepsy

CR and K(ATP) channel openers increase seizure threshold (Greene et al, 2001; Ghasemi et al, 2010) while metformin has been demonstrated to reduce epileptogenesis (Zhao et al, 2014) and experimentally-induced epilepsy in our laboratory. Epilepsy and bipolar disorder have overlapping aetiopathogenic factors (Kanner et al, 2014).Pathological behaviours, such as epilepsy, hypersexuality, hyperlocomotion and aggression, may be mediated by dopamine D3/2 receptors (Kelly et al, 2012), signalling through GSK-3 beta (Li and Gao, 2011) in a hyperdopaminergic state.

Animal models support a potential role of pathogenic mechanisms of mood disorders in the development of epileptic seizures and epileptogenesis. A common mechanism may be their anti-inflammatory and MMP-9 inhibitory effects important in the aetiopathogenesis of epilepsy and bipolar disorder. MMP-9 which signals through GSK-3 beta decreases seizure threshold (Wilcynski et al, 2008). Importantly, chronic epilepsy may increase cardiometabolic risk (Katsiki et al, 2014) related to the pharmacoresistance in epilepsy. Inflammatory

cytokines in infection, inflammation, stress and neurodegeneration couple hyperexcitability and seizures (Vezzani et al, 2011) and both metformin and CR decrease chemoattractant cytokine signalling in adipose tissue of obese animals (Wasinski et al, 2013; Yung et al, 2007) and IL-I betahyperresponsiveness in aged animals (Rutkute et al, 2007; Hattori et al, 2006; Liu et al, 2011).

Preliminary results from our laboratory reveal that metformin and CR attenuate some facets of bipolar disorder such as aggression and hypersexuality partly through upregulation of central serotonergic signalling (Schweiger et al, 1989; Aravi et al, 2008; Marson and McKenna, 1992).

Improvement of functional recovery following stroke

Chronic metformin treatment may improve functional recovery following stroke, evident from limited observational studies in a small cohort. Metformin is associated with increased angiogenesis and neurogenesis following experimental stroke (Jin et al, 2014) partly through increasing neurotrophic support by upregulation of brain-derived neurotrophic factor (Paintlia et al, 2013), ATP P2X7 (Neary and Kang, 2006) purinergic signalling and induction of autophagy (Dong et al, 2013). It also inhibits class II histone deacetylase (Mihaylova et al, 2011) and mTOR; and dampens hyperglutamatergic signalling to prevent excitotoxicity (Kim et al, 2013; Shen et al, 2014). CR prevents stroke and improves functional outcome after stroke through increasing neurotrophic support and dampening inflammatory pathways as does metformin (Manzanero et al, 2011; Arumugam et al, 2010).

Metformin and CR enhance immune function via AMPK

The dysregulated immune function especially in aged and high-fat-fed animals are normalised by metformin and CR. Metformin increases extra-cellular ATP concentration and the ATP P2YI purinergic G-protein coupled receptor may be central in autocrine stimulation of the human pancreatic β-cells (Tengholm, 2014). It also increases levels of adenosine which increases phagocytosis of *S. aureus* by endothelial cells via increase in cAMP levels (Ryan et al, 1969).

ATP, which stimulates human macrophages to kill intracellular virulent *Mycobacterium tuberculosis* via calcium-dependent phagosome-lysosome fusion, acts as a competitive antagonist of NMDA receptors at low glutamate concentrations and a positive allosteric modulator at high glutamate concentrations engendered by infections (Ortinau et al, 2003; Kloda et al, 2004). Thus, in this setting the ATP P2X7 purinergi receptor may lead to decrease in synaptic strength and neurodegeneration.

Activation of AMPK by metformin enhances neutrophil chemotaxis and bacterial killing (Park et al, 2013). This means that the biguanide, metformin, may facilitate bacterial eradication in sepsis.

There is a functional role of leptin in enhancing Th I (cell-mediated) lymphocyte functions and there is an impaired T cell immunity in mice deficient in leptin or its receptor (Martin-Romero et al, 2000; Lord et al, 1998). Thus leptin resistance in obesity and diabetes impairs T cell-based immunity, and this can be rescued by metformin and calorie restriction which improve leptin sensitivity. Leptin sensitivity helps T cells upregulate glucose uptake and metabolism (Saucillo et al, 2014). Metformin's activation of AMPK increases Treg (Regulatory T cells) differentiation *in vitro* and *in vivo*which inhibit elements of the metabolic syndrome (Feuerer et al, 2009). These Treg cells which are CD4(+) Foxp3(+) regulatory cells are reduced in insulin resistance. Metformin is also reported to be important for memory CD8(+) T-cell differentiation (Araki and Ahmed, 2013), controlling T-cell metabolism and determining the effector versus memory fate of CD8(+) T-cells (Finlay and Cantrell, 2011)

AMPK is a likely component of the intrinsic innate immune response against RNA viruses and may provide a target for broadly anti-viral therapeutics (Moser, 2011).

In the same vein, CR increases CD4(+) T-cell/CD8(+) T-cell ratio and decreases monocyte recruitment to adipose tissue in order to attenuate expression of inflammatory cytokines (Wasinski et al, 2013).

Dysregulated matrix metalloproteinase signalling in diabetes mellitus

The dysregulation of secretion of matrix metalloproteinases (MMPs) contribute to numerous disease processes, ranging from cancer primary tumor growth, invasion and metastasis to microbial infection, the mediation of tissue destruction in degenerative and inflammatory diseases; and thromboembolism (Gebbia et al, 2004; Rundhang, 2003;Baroncini et al, 2011). MMP- 2 inhibition may prevent platelet activation in thrombus formation (Momi et al, 2009). MMPs are now known to be at the intersection of the pathways regulating cardiometabolic diseases, neuropsychiatric diseases and cancer (Rybakowski, 2009).

The neuroadipokine, leptin, increases MMP-2, MMP-9 and tissue inhibitor of metalloproteinase (TIMP). It is also mitogenic for vascular endothelial cells and induces angiogenesis (Park et al, 2001). This role of leptin may be especially important in the setting of the metabolic syndrome when there is leptin resistance. Reactive oxygen species (ROS) (Nelson and Melendez, 2004);

nuclear factor-kappaβ (Chou et al, 2010); the nuclear transcription factor and cytokine activator which triggers inflammatory cascades, high mobility group box I (HMGB I) (Lee et al, 2015); interleukin-I beta (Liang et al, 2007) and Toll-like receptors (Paolillo et al, 2012) induce MMP expression.

Beneficial role of CR and metformin in MMP overexpression, cancer and gerosuppression

Calorie-restriction and metformin which restore leptin sensitivity inhibits the activity of MMPs in causing angiogenesis, proliferation and migration of human umbilical endothelial cells (Wilsley and Scarpace, 2004; Esfahanian et al, 2012; Soraya et al, 2012).Calorie restriction in rodents (Fontana et al, 2008); protein restriction in humans (Levine et al, 20140) and metformin down-regulate the insulin/IGF-I signalling pathway to exhibit gerosuppressant effects and inhibit metabolic syndrome-induced cancer growth (Sarfstein et al, 2013; Herranz et al, 2010). CR reduces IGF-I-dependent nuclear factor-kappaβactivation to decrease murine and human pancreatic tumor growth and nuclear factor-kappaβ activation (Harvey et al, 2014); and also reduces antigen load in the host from the gut microbiota to decrease metabolic syndrome-related diseases including cancer (Zhang et al, 2013). Both metformin and CR downregulate GSK-3 beta levels implicated in adipogenesis and hyperinsulinaemia. We have previously reported that metformin prevented the progression of prediabetes and CIN I cervical dysplasia in a Nigerian (Oriaifo et al, 2014).

CR and metformin reduce air-way inflammation

The obvious beneficial effects of CR and metformin in cardiac performance enhance pulmonary function. There is obstructive respiratory insufficiency with pCO2 retention in hypertensive patients with congestive failure and cardiac asthma (Cosby et al, 1957). CR (Johnson et al, 2007) and metformin (Park et al, 2012; Park et al, 2012; Calixto et al, 2013) reduce markers of oxidative stress in over-weight individuals with moderate bronchial asthma. Via AMPK activation, metformin suppressed eosinophilic inflammation, vascular permeability and peribronchial fibrosis by suppressing IL-5 and -13, HIF-alpha/VEGF-A pathway and TNF-alpha and NF-kappaβ mediated iNOS expression in lung tissue.

Beneficial action of metformin in preventing visual defect attributable to early glaucoma and macular degeneration

The gravity of diabetic retinopathy is highlighted by the finding that individuals with diabetes are 25 times more likely to become legally blind than non-diabetics (Azinge, 2013). Vascular endothelial growth factor is implicated in the aetiopathogenesis of neovascular glaucoma and cataracts due to diabetes and hypertension (Simha et al, 2013). Apart from effects in preventing progression of pre-diabetes, metformin and CR also inhibit vascular endothelial growth factor (Tadakawa et al, 2015; De Lorenzo et al, 2011) important in aetiopathology of diabetic retinopathy. These actions of metformin may be important in metformin's effect in preventing progression of early glaucoma and macular degeneration which we have observed in a small cohort.

Chronic use of metformin decreases constipation

Diabetes mellitus may be associated with autonomic neuropathy-induced constipation and spurious diarrhea (Spangeus et al, 1999). Acute use of metformin may be associated with gastrointestinal discomfort and diarrhea, but this abates with chronic use in most of our patients. Metformin, taken chronically plus the increase of the FLOWER items in diet improves gastrointestinal health and decreases constipation which may be of concern in the aged and diabetics. Slow-release metformin preparations such as Glucophage XR (500 mg and 750 mg tablets) may help in alleviating chronic constipation (www.google.com/patents/EP1865939A2).Individuals on CR may need to increase fruits and vegetables (FLOWER items) in diet in order to avoid risk of constipation.

Metformin improves osteoblast function and decreases osteoporosis

Falls and resultant fractures are age-relatedcomplaints. Diabetes mellitus is associated with bone loss (Molinuevo et al, 2009; Sedlinsky et al, 2011) who have shown in elaborate reports that bone remodelling is altered in the elderly and diabetic patients with consequent increased skeletal fragility and fracture risk. While rosiglitazone increases adipogenesis and osteoporosis, metformin increases the osteoblastic differentiation of bone marrow progenitor cells with resultant decrease in fracture risk and enhancement of bone healing in diabetic and non-diabetics. Their evidence is supported by Gao et al (2008) who showed that metformin markedly stimulated the deposition of mineralised nodules and blocked the formation of cytoplasmic lipid droplets. Moderate CR, with or without exercise, that preserves calcium intake does not significantly cause bone loss (Redman et al, 2008).

Metformin + calorie restriction promotes increased human healthspan over metformin alone

Our retrospective observational studies in patients have

Table I: Differential effect of metformin (M), metformin + calorie restriction(M+CR), sulphonylurea(S) and insulin(I) on cardiovascular morbidity

Age	Age at Commencement in years	No of Years on Treatment	No With Heart Failure	No Alive After 30Years
M n = 10	45 ± 3.0	30	1	10
M + CR n = 10	45 ± 5.0	30	-	10
S n = 10	45 ± 2.0	30	9	8
I n = 10	45 ± 3.0	30	8	6

The difference between M and M+CR was not significant (P > 0.5). The M group had 1 death, compared to the M+CR with no death. Compared to S and I groups (DMR), the M+CR group was most significant in reducing deaths due to cardiovascular causes.

shown that metformin + calorie restriction was more effective in maintaining euglycaemia, reducing body weight and promoting healthspan than metformin alone in pre-diabetics and type 2 diabetics. Further, in responsive patients, metformin was more effective than sulphonylureas or insulin in preventing all-cause mortality, especially deaths from microvascular and cardiovascular complications. This agrees with recent findings (DeFronzo, 2014; Nichols et al, 2001).

Animal experimentations have shown that metformin increases mean lifespan and healthspan in *Caenorhabditis elegans* and mice (Berstein, 2012; Sato et al, 2012).

In a retrospective comparison of the effects on metformin alone, metformin + calorie restriction, sulphonylurea and metformin on cardiovascular morbidity in patients between 1984 and 2014, metformin + calorie restriction was found to be most effective in prolonging health span (Table I).

Metformin, renal function and lactate levels

20-40% of patients with diabetes mellitus ultimately develop nephropathy and there is increased susceptibility of the aging kidney to ischemic injury. The genes implicated such as kidney injury molecule-I (Kim-I), MMP-7 and hypoxia-inducible factor-I alpha may be down regulated by metformin and CR that reduce oxygen consumption by renal tubular cells (Chen et al, 2007; Takiyama et al, 2011). Metformin exerts beneficial effects in obesity-induced renal injury by regulating systemic inflammation, insulin resistance and the renal AMPK/ACC pathway (Kim et al, 2013). However, concerns linger about the role of metformin in kidney function due to a probable risk of lactic acidosis. According to the European Association for the Study of Diabetes, metformin is safe unless the estimated glomerular filtration rate (eGFR) falls to below < 30 ml/min per I.73 m^2. This level has been revised upwards in a recent report (Warren et al, 2007) to 36-40 ml/min per I.73 m^2 which is equivalent to 1.7 mg/dL creatinine serum level. This is more than the previous cut-off point of I.4 mg/dL in women and I.5 mg/dL in men and which was based on the calculated ability of the kidneys to remove 3 g of metformin at steady-state levels within 24-48 hours. In fact, the ability to comfortably remove the drug extends up to creatinine levels of I.8-2.0 mg/dL (Lipska et al, 2011).

Metformin is primarily excreted by the kidneys unchanged, and so might accumulate in severe renal failure. The risk for lactic acidosis with metformin is 3 cases/100,000 patient years but this risk do not differ apparently in patients taking metformin versus other glucose-lowering drugs. Metformin levels are not linked to mortality in those who develop lactic acidosis (Lipska et al, 2011; McCormak et al, 2005) and hypoxia, haemodynamic compromise due to congestive cardiac failure may be more important.

CONCLUSION

Judging from their roles in infection, immune function, cancer prevention, cardiometabolic and neuropsychiatric health, the biguanide metformin in conjunction with calorie restriction seem licensed to prevent premature senescence and extend healthspan and lifespan. Metformin and CR through activation of AMPK may be key to the body's modulation of responses due to external environmental cues and regulation of homeostatic mechanisms of the internal environment.

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