

openheart Autophagy-induced degradation of Notch1, achieved through intermittent fasting, may promote beta cell neogenesis: implications for reversal of type 2 diabetes

James J DiNicolantonio,¹ Mark McCarty²

To cite: DiNicolantonio JJ, McCarty M. Autophagy-induced degradation of Notch1, achieved through intermittent fasting, may promote beta cell neogenesis: implications for reversal of type 2 diabetes. *Open Heart* 2019;**6**:e001028. doi:10.1136/openhrt-2019-001028

Accepted 6 May 2019

INTERMITTENT FASTING BOOSTS NEUROGENIN-3 (NGN3) EXPRESSION AND BETA CELL NEOGENESIS

Several recent studies have found that repeated episodes of fasting (or of a low-carb/low-protein diet mimicking the metabolic impact of fasting), of 1–4 days of duration, interspersed with ad libitum food consumption, can induce neogenesis of pancreatic beta cells in several mouse models of diabetes (db/db, high-fat feeding and streptozotocin induced).^{1–3} This phenomenon is associated with increased expression of Ngn3 in islet cells. Ngn3 is a transcription factor expressed transiently in developing pancreatic islets in utero that is required for the further development of both alpha and beta cells.^{4,5} The increase in islet beta cells induced by intermittent fasting is accompanied by a corresponding increase in islet insulin content and a marked improvement in glycaemic control. This benefit of intermittent fasting is abrogated if autophagy is concurrently suppressed, suggesting that fasting-induced autophagy is a key mediator of the subsequent beta cell neogenesis.² It has also been noted that fasting-induced reductions in mTORC1 and protein kinase A (PKA) activity in islet cells are mediators of this phenomenon.¹ With respect to autophagy, it is notable that intermittent feeding of a leucine deprived, which would be expected to induce autophagy by episodic inhibition of mTORC1 activity, has likewise been shown to increase Ngn3 expression and beta cell mass in db/db mice.⁶

These observations are of the greatest interest, inasmuch as type 1 diabetes reflects near-complete inflammatory destruction of islet beta cells, and the later stages of severe type 2 diabetes are characterised by a marked decrease in islet beta cells, reflecting their

accelerated loss by apoptosis. If it proves feasible to replicate these observations in human diabetics, this strategy might arguably lend itself the *cure* of diabetes. In regard to type 2 diabetes, this disorder can sometimes be fully reversed in its early stages if the factors that impelled its onset are corrected, that is, if a diet and exercise regimen that supports insulin sensitivity is implemented and appropriate weight loss is achieved. Such a resolution is less common, however, in cases of long-standing diabetes, likely owing in large part to a marked decline in beta cell mass.^{7,8} Therefore, feasible measures that stimulate islet beta cell neogenesis might make it more feasible to reverse severe longstanding type 2 diabetes. With respect to type 1 diabetes, production of new beta cells might enable its resolution if the autoimmune attacks that destroyed the original complement of beta cells can somehow be quelled.

NOTCH1 SIGNALLING OPPOSES NGN3 EXPRESSION IN ISLET CELLS

How does intermittent fasting induce beta cell neogenesis associated with Ngn3 expression? There is reason to suspect that autophagy-mediated suppression of Notch1 signalling plays an important role in this regard. Ligand-stimulated cleavage of the transmembrane Notch1 protein by gamma-secretase generates an intracellular protein, Notch intracellular domain (NICD), which translocates to the nucleus to bind the CBF1, Suppressor of Hairless, Lag-1 (CSL) transcription factor and its coactivator Mastermind; this complex can then promote the transcription of a range of Notch target genes, notably those of the Hes family.⁹ One of these genes codes for Hes1, a basic helix–loop–helix transcription



© Author(s) (or their employer(s)) 2019. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

¹Mid America Heart Institute, Kansas, Kansas, USA

²Catalytic Longevity, Encinitas, California, USA

Correspondence to

Dr James J DiNicolantonio; jdinicol@gmail.com

factor that functions to repress transcription of the gene coding for the Ngn3 protein.^{10–12} Notch1 signalling is constitutively active in islet cells; Dll4 on neighbouring cells serves as the activating ligand.^{13 14} This signalling increases in mice that are obese, or in response to glucotoxicity.¹⁵ In non-obese diabetic (NOD) mice, a model for inflammation-induced diabetes, treatment with an antibody to Dll4 that blocks Notch1 signalling was found to increase islet mRNA expression of Ngn3 by 600-fold, while enhancing the production and proliferation of beta cells and increasing insulin production.¹³ Moreover, culturing rat pancreatic acinar cells with an external domain of the Notch1 protein that inhibits Notch1 activation, triggers a burst of Ngn3 expression and induces their differentiation into immature islet cells, about 30% of which are insulin-producing beta cells.¹⁶ Hence, it appears the episodic suppression of islet Notch1 signalling could be expected to boost Ngn3 expression and beta cell neogenesis.

MACROAUTOPHAGY MARKEDLY BOOSTS NOTCH1 DEGRADATION

A peculiarity of the Notch1 protein is that macroautophagy markedly decreases its expression by enhancing its lysosomal degradation—an effect that has been demonstrated in a number of cell types.^{17–21} This effect is accompanied by a corresponding decrease in cellular NCID levels and in expression of Notch1 targets such as Hes1. This effect does *not* reflect non-specific incorporation of Notch1 into endosomes that subsequently fuse with autophagosomes; rather, for some reason, Notch1 is incorporated into autophagosome precursor vesicles, positive for ATG16L1, that subsequently participate in autophagosome formation.¹⁷

Autophagic downregulation of Notch1 activity in uncommitted islet beta cells could therefore be expected to disinhibit transcription of the Ngn3 gene. The fact that increased protein expression of Ngn3 subsequently arises presumably reflects the fact that transcription factors capable of promoting transcription of this gene are active in these cells, at least temporarily; whether autophagy might play any role in stimulating their activity currently remains unclear. In any case, suppression of Notch1 activity seems likely to contribute importantly to renewed expression of Ngn3 in islet cells following intermittent fasting. Potentially, the refeeding periods following episodic fasts might support Ngn3's ability to reconstitute functional beta cells by enabling proliferation and suppressing apoptosis in these cells. It is important to note that Ngn3 is expressed only transiently—for several days—during islet development; it nonetheless has a long-lasting impact by triggering sustained production of other transcription factors that induce the characteristic behaviour of beta cells.⁵ Presumably, these factors then provide feedback inhibition of Ngn3 expression. These considerations explain why a transitory downregulation of Notch1 signalling and upregulation of Ngn3

expression induced by intermittent fasting could be expected to have a sustained impact on islet function.

With respect to the roles of decreased mTORC1 and PKA activities in promoting Ngn3 induction, it is well known that mTORC1 suppresses macroautophagy; it does so by conferring a phosphorylation on ULK1 that prevents its activation.^{22 23} Moreover, in at least some types of cells, mTORC1 increases Notch1 synthesis at the transcriptional level by upregulating STAT3 activity.²⁴ PKA likewise suppresses autophagy in yeast, although its impact on autophagy in mammalian cells is less clear.²⁵ However, a recent report indicates that cAMP/exchange protein directly activated by cAMP (EPAC) signalling inhibits autophagy in beta cells.²⁶ Also, PKA can upregulate Notch1 signalling by enhancing the activity of gamma secretase, the protease that is required for generation of NCID. It does so, at least in part, by increasing the expression of an essential component of the gamma secretase complex, PEN-2.²⁷ PKA boosts transcription of the gene coding for PEN-3 via activating phosphorylation of cyclic AMP-response element-binding protein (CREB), which binds to the *PEN-2* promoter.^{27 28} Arguably, this might represent an effect independent of autophagy whereby fasting decreases Notch1 activity.

Fasting and dietary strategies mimicking the metabolic impact of fasting are not the only ways to boost autophagy. AMP(adenosine 5' monophosphate)-activated kinase (AMPK) promotes autophagy by inhibiting mTORC1 via phosphorylations of TSC2 and Raptor and also by phosphorylating Ulk.^{22 29} In this regard, the AMPK-activating drug metformin has been shown to oppose Notch1 signalling in various contexts.^{24 30 31} Moreover, administration of metformin to pregnant mice has been shown to amplify the prenatal increase of Ngn3 in the pancreatic islets of the fetuses and to boost the beta cell fraction of the newborn pups.³² Metformin, and possibly also the phytochemical AMPK activator berberine, might thus have potential as adjuvants to intermittent fasting for promoting beta cell neogenesis.

SHIELDING BETA CELLS FROM GLUCOLIPOTOXICITY

If it proves clinically feasible to boost islet beta cell mass in individuals with type 2 diabetes with intermittent fasting strategies, the clinical utility of this approach would evidently be blunted if ongoing glucolipotoxicity rapidly renders these new cells dysfunctional and promotes their apoptosis. Hence, dietary, drug or nutritional strategies that alleviate the adverse impact of elevated glucose/ free fatty acid (FFAs) on beta cell function and that moderate episodic rises in glucose and FFAs by improving the insulin sensitivity of adipocytes, hepatocytes and skeletal muscle could importantly complement measures promoting beta cell neogenesis.

As the insulin resistance associated with metabolic syndrome and visceral obesity worsens, beta cells initially compensate by multiplying and by secreting increased amounts of insulin, maintaining glycaemia at reasonably

normal levels. However, this adaptation often ultimately fails, as episodic exposure to elevated levels of glucose and of free fatty acids (especially saturated fatty acids) drives a dysdifferentiation of beta cells—a phenomenon known as glucolipotoxicity.^{33–34} Owing in large part to decreased expression and nuclear localisation of the PDX-1 transcription factor, as well as decreased expression of the MafA transcription factor, synthesis of both GLUT2 and glucokinase declines, leading to a disruption of glucose-stimulated insulin secretion (GSIS).^{35–36} In healthy beta cells, the increase in glycaemia following a meal evokes a corresponding increase in beta cell uptake and oxidation of glucose, associated with an increase in beta cell ATP levels that depolarises the cell by inhibiting ATP-sensitive potassium channels; this depolarisation in turn triggers calcium influx via voltage-sensitive channels, leading to exocytosis of insulin-loaded granules.³⁷ As contrasted to hexokinase, glucokinase has a lower affinity for glucose ($K_m=10$ mM) and is not feedback inhibited by its product glucose-6-phosphate, so glucose oxidation and ATP production in beta cells rise as plasma glucose rises throughout the physiological range; hence, glucokinase functions as the beta cell ‘glucosensor’.³⁸ In beta cells subjected to glucotoxicity, reduced expression of both GLUT2 and glucokinase blunts the increase in glucose oxidation following a meal, such that postprandial insulin secretion fails to rise appropriately. This phenomenon is further exacerbated by a reduction in beta cell insulin synthesis, reflecting key roles for nuclear PDX-1 and MafA in promoting transcription of the insulin gene.³⁶ The resultant failure of beta cells to secrete adequate amounts of insulin in response to postprandial elevations of glucose in turn leads to more sustained elevations of plasma glucose and FFAs that further exacerbate glucolipotoxicity and beta cell dysdifferentiation in a vicious cycle. Moreover, over the course of time, this situation worsens as glucolipotoxicity drives an apoptotic loss of beta cell mass.³⁴

OXIDANT PRODUCTION DRIVES GLUCOLIPOTOXICITY; cAMP AND cGMP COUNTERACT IT

Fortunately, however, we have gained considerable insight into how glucolipotoxicity causes beta cell dysfunction. In particular, increased oxidant production by NOX2-dependent NADPH oxidase complexes has been shown to be a key mediator in this regard.^{39–45} Glucose exposure stimulates the activity of this complex by promoting activation of Rac1, likely via upstream activation of Vav2 and the tyrosine kinase Yes.^{46–47} While Rac1 activity is required for the cytoskeletal remodelling that enables second-phase release of insulin secretory granules, it also promotes assembly of active NADPH oxidase complexes. Additionally, FFA interaction with the GPR40 activates phospholipase C and protein kinase C; while this boosts GSIS, it also promotes assembly of NADPH oxidase in a manner complementary to Rac1.^{48–49} While transient moderate activation of beta cell NADPH oxidase

postprandially appears to aid GSIS, the sustained high activation of this complex associated with glucolipotoxicity adversely affects beta cell differentiation and function.⁵⁰ The hydrogen peroxide stemming from increased NADPH oxidase activity boosts activation of stress-activated MAP kinases, notably JNK, by reversibly inhibiting MAP kinase phosphatase activities.^{40–51} JNK, primarily by conferring serine phosphorylations on insulin receptor substrate-2, impairs autocrine insulin signalling as well as insulin-like growth factor-1 (IGF-I) signalling.⁵² This in turn blunts Akt activation, leading to increased nuclear localisation of the FOXO1 transcription factor, which suppresses transcription of the PDX-1 gene.^{53–55} Moreover, activated JNK promotes export of PDX-1 from the nucleus, further amplifying the loss of PDX-1 activity.^{56–58} Glucotoxicity-triggered excessive oxidant production in beta cells also reduces transcription of the gene coding for MafA by boosting expression of c-Jun.^{59–60} Hence, the oxidant stress triggered by glucolipotoxicity suppresses the function of both PDX-1 and MafA, crucial for effective beta cell function, and sustained activation of JNK also drives the increased beta cell apoptosis that leads to loss of beta cell mass in progressing diabetes.⁵¹

Whereas sustained oxidative stress can disrupt beta cell differentiation, measures that boost beta cell production of cAMP and of cGMP can aid proper beta cell differentiation and function. The trophic effects of GLP-1 and of glucose-dependent insulinotropic peptide (GIP) on beta cell function are mediated primarily by stimulation of cAMP synthesis.⁶¹ Acting via both PKA and the EPAC guanine nucleoside exchange factor, cAMP acutely amplifies GSIS.^{62–67} Moreover, by conferring an activating phosphorylation on the CREB transcription factor, cAMP increases the expression of IRS-2, thereby aiding insulin-mediated and IGF-I-mediated activation of Akt, which is of key importance to PDX-1 expression; CREB also promotes expression of various antiapoptotic proteins.^{68–70} Drugs that either boost GLP-1 production (acarbose), sustain its activity by inhibiting its proteolytic degradation by DPP4 (sitagliptin) or that directly mimic its activity (exenatide) are known to aid glycaemic control in type 2 diabetes by boosting cAMP production in beta cells.^{71–73} Another of the consequence of beta cell glucolipotoxicity is downregulated expression of the receptors for both GLP-1 and GIP.⁷⁴

In regard to cGMP, the elevations of intracellular free calcium triggered by glucose uptake promote its production in beta cells via activation of nitric oxide synthase. Like cAMP, cGMP acts acutely to potentiate GSIS⁷⁵, and for reasons that remain rather obscure, cGMP, acting specifically via PKG-I α , stimulates PI3K in beta cells, thereby boosting Akt activity and complementing the impact of insulin/IGF-I in this regard.⁶¹ cGMP also potentiates the efficacy of agents that elevate cAMP; the chief phosphodiesterase in beta cells targeting cAMP, PDE3B, is inhibitable by cGMP.⁷⁵ It seems likely that the oxidative stress associated with glucolipotoxicity could impede cGMP production in beta cells by inducing uncoupling of nitric

oxide synthase, such as effect would also be expected to increase beta cell oxidant load.

NUTRACEUTICALS AND DRUGS HAVE POTENTIAL FOR OPPOSING GLUCOLIPOTOXICITY

Measures that can downregulate activation of NADPH oxidase in beta cells have evident potential for stemming glucolipotoxicity. In this regard, the unconjugated bilirubin generated intracellularly by heme oxygenase activity has been shown to inhibit Nox2-dependent NADPH oxidase activity. (Heme oxygenase cleaves heme to produce biliverdin, which is rapidly reduced to bilirubin by biliverdin reductase.) In epidemiological studies, increased plasma levels of bilirubin are associated with decreased risk for type 2 diabetes.^{76–78} Since bilirubin is too insoluble for oral administration, Ikeda and colleagues⁷⁹ administered biliverdin orally to db/db mice, and demonstrated that this aided preservation of beta-cell function and PDX-1 expression, while quelling oxidant stress. While the current high cost of biliverdin renders use of this agent as a nutraceutical unfeasible, cyanobacteria such as spirulina make substantial amounts of the biliverdin metabolite phycocyanobilin (PhyCB), which they employ as a collector of light energy. Fortunately, PhyCB, like biliverdin, is a substrate for biliverdin reductase and shares the ability of biliverdin/bilirubin to inhibit NADPH oxidase complexes.^{80–81} Furthermore, whether administered in free or protein-bound form, orally administered PhyCB has marked antioxidant activity, likely explaining many of the health-protective effects of oral spirulina in rodent and clinical studies.^{80–82–84} Hence, it has been proposed that oral administration of spirulina or of PhyCB-enriched spirulina extracts may have potential for protecting beta cells from glucolipotoxicity.⁸⁵ PhyCB may also have potential for preventing diabetic complications, independent of its influence on glucose control; in this regard, increased plasma bilirubin predicts lower risk for complications in diabetics.^{86–87}

Beta cell expression of enzymes and peptides that clear hydrogen peroxide or that reverse oxidant-mediated modifications of cysteine residues can be amplified with phase 2-inducing nutraceuticals, such as lipoic acid or ferulic acid.⁸⁸ Indeed, studies in rodents and in cell cultures have found that these agents can favourably influence the function of beta cells exposed to high-glucose or pro-oxidant toxins.^{89–92}

One of the key effects of phase 2 inducers is to upregulate the enzyme rate limiting for glutathione synthesis, γ -glutamyl cysteine ligase.^{93–94} In addition to acting as a prominent intracellular scavenging antioxidant, glutathione functions to promote catabolism of hydrogen peroxide and to reverse its oxidising effects on proteins, thereby opposing many proinflammatory effects of oxidative stress.^{95–97} Since cysteine availability is also rate limiting for glutathione production, supplementation with NAC is a clinically effective strategy for boosting

tissue glutathione levels, particularly in the elderly in whom glutathione levels tend to be depressed.^{98–100} Hence, it is not surprising that feeding NAC helps to prevent or slow the deterioration of glucose tolerance and loss of effective beta cell function in Zucker diabetic fatty rats and db/db mice.^{101–102} However, part of this benefit might be mediated by increased islet production of hydrogen sulfide (H_2S), for which cysteine is also the key precursor.¹⁰³ H_2S , although it can downregulate GSIS in healthy islets, exerts an antiglucotoxic effect on beta cells, apparently by suppressing the expression or interfering with the function of thioredoxin-interacting protein (Txnip); the latter is known to play a key mediating role in glucotoxicity.^{104–108}

Txnip expression is elevated in diabetic beta cells and promotes glucolipotoxicity by opposing the antioxidant effects of thioredoxin.¹⁰⁷ This elevated expression reflects, at least in part, activation of the transcription factor carbohydrate response element-binding protein (ChREBP) by increased beta cell glucose metabolism; ChREBP binds to the promoter of the Txnip gene and boosts its transcription.¹⁰⁹ However, the transcriptional activity of ChREBP can be opposed by AMPK, the key target of the antidiabetic agents metformin and berberine.^{110–111} Hence, in addition to quelling excessive hepatic glucose output, metformin and berberine can aid diabetic control by opposing beta cell Txnip activity and hence countering glucotoxicity.^{112–113}

As noted above, acarbose, DPP4 inhibitors and GLP-1 receptor agonists, which function to increase GLP-1 signalling in beta cells, can be employed to boost beta cell cAMP levels. Exenatide therapy, as opposed to insulin therapy, has a more favourable impact on beta cell function over 3 years of follow-up.^{114–115} Recent evidence indicates that oral arginine can potentiate meal-evoked GLP-1 secretion in mice and humans; whether citrulline shares this property remains to be determined.^{114–116} Slowly digested (lente) carbohydrate and certain probiotics may also promote increased GLP-1 secretion.⁷¹

With respect to cGMP, supraphysiological concentrations of the B vitamin biotin (roughly two orders of magnitude above the physiological range) are capable of directly activating soluble guanylate cyclase.^{117–118} Since biotin's maximal impact in this regard is moderate (2–3 fold increase over basal activity, whereas nitric oxide can increase its activity dose dependently up to a hundred fold), high-dose biotin tends to be well tolerated; indeed, intakes of 100 mg daily or more are feasible in children with biotin-responsive genetic disorders. Systemic activation of guanylate cyclase with high-dose oral biotin has been demonstrated in spontaneously hypertensive rats without evident toxicity.¹¹⁹ These considerations likely explain why high-dose biotin has shown trophic effects on beta cells in vitro and in rodents.^{120–125} Moreover, it has been reported to aid glycaemic control both in animal models of diabetes and in diabetic patients, although favourable impacts on hepatocyte function also contribute in this regard.^{126–128}

The oxidative stress associated with glucolipotoxicity in beta cells might be expected to uncouple nitric oxide synthase, impairing endogenous production of nitric oxide (NO)/cGMP and increasing oxidant load. Nonetheless, it appears that this possibility has received little research attention. However, uncoupling of nitric oxide synthase has been demonstrated in the islets of healthy ageing rats; one would expect this phenomenon to be accentuated in individuals with diabetes.¹²⁹ Supplementation with arginine or citrulline can counteract the NO synthase uncoupling induced by asymmetric dimethylarginine (ADMA); in young rats that are the offspring of diabetic mothers, arginine supplementation boosts subnormal NO production in their islets as well as Akt phosphorylation and PDX-1 expression.^{130 131} In prospective epidemiology, increases in plasma arginine or in arginine/ADMA ratio predict lower risk for type 2 diabetes.¹³² Like PhyCB, supplemental citrulline, which raises plasma and tissue levels of arginine more effectively than arginine supplementation does, may have potential for prevention of diabetic complications.⁸⁶ Whether high-dose folate might reverse NO synthase uncoupling induced by peroxynitrite in beta cells has not yet been studied.^{133 134}

Supplemental zinc modestly improves glycaemic control in type 2 diabetes, as confirmed by meta-analysis of placebo-controlled studies; average reduction of HbA1c was 0.54%.^{135 136} Although zinc has the potential to upregulate insulin signalling via inhibition of protein tyrosine phosphatase-1B, recent research in fat-fed insulin-resistant mice suggests that a potentiation of beta cell GSIS is primarily responsible for the favourable impact of zinc on diabetic glycaemic control.¹³⁷ Notably, this effect is also seen in healthy chow-fed mice, so it does not appear to reflect opposition to glucolipotoxicity. A possible explanation for zinc's upregulatory impact on GSIS is at hand. Insulin granules contain also ATP and zinc, which are released into the extracellular space when insulin is secreted. ATP, via stimulation of P2X receptors—most notably P2X(3) in human islets—induces an influx of cations that depolarises the beta cell membrane, thereby inducing further influx of calcium via voltage-sensitive channels.¹³⁸ Although zinc does not directly activate P2X receptors, it potentiates their response to ATP.^{139 140} Hence, when beta cells are relatively high in zinc, this positive feedback mechanism amplifying GSIS should be more meaningful. While there is no reason at present to suspect that zinc counteracts the adverse effect of hyperglycaemia on beta cell differentiation, zinc might be indirectly beneficial in this regard by modestly aiding glycaemic control.

PLANT-BASED DIETS FOR DIABETES PREVENTION AND THERAPY

As noted, glucolipotoxicity can also be minimised by measures that improve tissue insulin sensitivity, decrease hepatic glucose output and lessen postprandial

elevations of glucose and FFAs. Standard therapies such as metformin and pioglitazone evidently have utility in this regard.

The muscle and hepatic insulin resistance associated with increased visceral adiposity results, at least large part, from exposure of muscle fibres and hepatocytes to FFAs in excess of metabolic need. Saturated fatty acids, in particular, are prone to give rise to proinflammatory mediators such as diacylglycerol and ceramide and can also trigger inflammatory signalling by interacting with fetuin A to stimulate Toll-like receptor 4. This signalling compromises insulin responsiveness by conferring inhibitory phosphorylations on IRS-1 and other mediators of the insulin signal.

This excessive FFA exposure stems largely from improper function of hypertrophied, insulin-resistance adipocytes. Properly functioning adipocytes are crucially important to metabolic health. After a fat-rich meal, they efficiently store chylomicron triglycerides; then, during fasting metabolism, when glucose and insulin levels are relatively low, they release FFA in response to metabolic need. That is how adipocytes function in women with gynoid obesity who maintain good insulin sensitivity. However, the hypertrophied insulin-resistant adipocytes associated with metabolic syndrome, while they cleave chylomicron triglycerides effectively following a fatty meal, fail to store the derived FFAs efficiently, resulting in excessive flux of FFAs into liver and muscle when glucose and insulin levels are high and FFA oxidative is suppressed. Conversely, when the body's tissues need FFA as metabolic fuel, these dysfunctional adipocytes fail to adequately upregulate FFA release in response to elevated catecholamines and low insulin, which is why they remain hypertrophied.

These considerations help to explain the utility of a plant-based diet, low in saturated fat, for preventing or reversing tissue insulin resistance. Plant-based diets are usually characterised by a low ratio of saturated to unsaturated fat, and if they exclude the few types of oil high in saturates (eg, palm oil and coconut oil), postprandial overexposure of tissues to saturated fatty acids will be minimised. Moreover, the fraction of saturated fatty acids in stored in adipocytes will be relatively low, so that tissue exposure to saturated FFAs throughout the day will be moderated.

Indeed, clinical studies show that a low-fat plant-based diet, in ad libitum amounts, favourably influences insulin sensitivity and, in obese subjects, improves beta cell function; in the long term, such a diet also tends to promote appropriate weight loss.^{141–143} Such a diet appears to be superior to conventional diabetes diets with respect to glycaemic control, weight loss and modulation of cardiovascular risk factors.^{144–146} Choosing foods relatively low in glycaemic index (owing to structural intactness and/or high content of amylose, soluble fibre or resistant starch) has been shown to amplify the weight loss—and, consequently, the improvement in glycaemic control—achieved with such diets.¹⁴⁷ An epidemiological study in

Loma Linda found that long-term vegans were at substantially lower risk for diabetes than omnivores, independent of their body mass index (BMI).¹⁴⁸ Whereas vegans were about only 40% as likely to become diabetic as omnivores after adjustment for BMI, this may represent overadjustment, as vegans tend to have lower BMIs owing to their diets; when BMI was not corrected for, risk for diabetes found to be 25% that of omnivores.¹⁴⁸

Plant-based diets also tend to be relatively low certain essential amino acids, and this triggers hepatic signalling that promotes leanness, insulin sensitivity and proper beta cell function.¹⁴⁹ A moderate degree of essential amino acid restriction, by activating the GCN2 kinase, promotes increased hepatic production of fibroblast growth factor 21 (FGF21), which in turn stimulates increased production of adiponectin by adipocytes.^{149 150} The latter acts on both skeletal muscle and the liver to promote insulin sensitivity and FFA oxidation and also opposes hepatic steatosis.^{151–155} Moreover, FGF21 acts directly on beta cells to boost Akt activity and improves GSIS in diabetic mice.^{156 157} In conjunction the low saturated/unsaturated ratio of dietary fat, these phenomena likely explain the markedly favourable impact of plant-based diets on risk for diabetes and diabetes control, and FGF21 may be a mediator of the characteristic relative leanness of vegans; FGF21 acts to counterweight gain in rodent models of obesity.¹⁵⁸

As is well known, aerobic exercise training can aid weight control and can promote insulin sensitivity in the muscle groups that are exercised. A single exercise session, via AMPK activation, enhances insulin sensitivity of the exercised muscles for up to 48 hours; exercise training induces a more durable improvement in this regard that may reflect, in part, increased mitochondrial biogenesis.^{159–161}

NUTRACEUTICAL SUPPORT OF INSULIN SENSITIVITY

Certain nutraceuticals also have potential for improving tissue insulin sensitivity in the context of metabolic syndrome. Glycine can act on adipocytes to improve their insulin sensitivity and boost their production of adiponectin.^{162 163} There is reason to suspect that the bilirubin-mimetic PhyCB may exert comparable effects by downregulating activation of NADPH oxidase in hypertrophied adipocytes.¹⁶⁴ The muscle insulin resistance evoked by fatty diets in rodents has recently been reported to be mediated by NOX2-dependent NADPH oxidase activity, so PhyCB might also act directly on muscle fibres to promote insulin sensitivity.¹⁶⁵ In the elderly, in whom tissue levels of carnitine tend to be diminished, supplemental carnitine may boost mitochondrial biogenesis in muscle fibres, aiding their capacity to oxidise FFAs and thereby ward off insulin resistance¹⁶⁶, and berberine, via activation of AMPK, can be expected to mimic metformin's ability to quell excessive hepatic glucose output and protect beta cells from glucolipotoxicity.

Arguably, the goal in diabetes reversal therapy should be to transition individuals with diabetes to a state in which glycaemic control can be normalised without concurrent drug therapy, entailing compliance with a diabetes-preventive diet and exercise programme, and possibly employment of certain nutraceuticals that aid prevention/control of diabetes and reduce risk for other important pathologies.

BREAKING THE VICIOUS CYCLE OF GLUCOLIPOTOXICITY WITH PROLONGED FASTING

Whereas intermittent fasting protocols may prove useful for boosting beta cell mass in individuals with diabetes, a prolonged fast, by restoring normoglycaemia for the duration of the fast, can be expected to improve beta cell function by alleviation of glucolipotoxicity.¹⁶⁷ Thus, when protein-sparing fasts or very-low-calorie diets have been employed to achieve weight loss in individuals with diabetes, the resulting improvement in beta cell function has been found to be more substantial than that attributable to achieved weight loss alone.^{168–174} If patients are then transitioned to a diabetes-preventive diet, while receiving nutraceuticals and drugs that shield beta cells from glucotoxicity, it might be feasible to conserve the improvements in beta cell function observed during the fast, effectively reversing diabetes. This strategy would be less likely to succeed in patients with long-term diabetes whose beta cell mass is substantially diminished. In such patients, previous use of intermittent fasting protocols to boost beta cell mass—presuming that this strategy proves workable in humans—might greatly improve chances for successful reversal of diabetes.

TOWARDS A PRACTICAL STRATEGY FOR REVERSING TYPE 2 DIABETES

In light of the recent revelation that intermittent fasting can induce beta cell neogenesis in rodents, it seems likely that repeated bouts of induced autophagy, by episodically suppressing Notch1 signalling and thereby promoting temporary expression of Ngn3, can drive the generation of new beta cells in the pancreas. Presumably that this finding can be replicated clinically, the way may then be open to reversing type 2 diabetes in patients who have enough discipline and commitment to adopt a lifestyle that would have prevented diabetes in the first place.

As a first step, patients should adopt and practise a diabetes-preventive lifestyle, preferably, a plant-based diet avoiding oils rich in saturated fats, consisting primarily of whole foods, complemented with a regular exercise programme that is practical for the patient on a continuing basis. This should achieve some initial weight loss, while improving insulin sensitivity, and diminishing the fraction of saturated fats in the body's fat stores. In some patients whose diabetes is of recent origin, this in itself may prove sufficient for diabetes reversal over the course of time.

In those who fail to respond optimally to lifestyle modification alone, an intermittent fasting (or fasting mimetic) protocol can be implemented to boost islet beta cell mass. When the transition back to a health-protective diet is made, nutraceutical and drug measures can be implemented that shield the nascent and pre-existing beta cells from the impact of glucolipotoxicity, so that they retain good functional capacity. These measures should be designed to inhibit islet oxidative stress (spirulina or PhyCB-enriched spirulina extracts, a phase 2 inducer such as lipoic or ferulic acid, NAC, metformin or berberine) and support production of both cAMP and cGMP in beta cells (sitagliptin, high-dose biotin and citrulline). Zinc supplementation might also be employed, as this potentiates GSIS.

If these measures fail to achieve diabetes reversal, a modified fast of several weeks' duration could be implemented—while continuing the beta cell-shielding measures—to expose beta cells to a prolonged period of normoglycaemia, during which they should achieve a more normal functional capacity, with restored expression of GLUT2, glucokinase and insulin. If this improvement is sufficient, it may then be possible to achieve normal glycaemic control after transition back to a diabetes-preventive diet and lifestyle.

The ultimate goal should be to achieve normal control of glycaemia without drugs, maintained by compliance with a diabetes-preventive diet and lifestyle, and the use of nutraceutical that help to stave off return of diabetes and provide protection from other important pathologies.

Contributors All authors contributed to the final manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests JJD is author of *The Salt Fix*, *Superfuel* and *The Longevity Solution*. MM is an owner of a nutraceutical company and coinventor and co-owner of a US patent covering nutraceutical uses of phycocyanobilin oligopeptides derived from cyanobacteria such as spirulina.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; internally peer reviewed.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

REFERENCES

- Cheng C-W, Villani V, Buono R, *et al*. Fasting-Mimicking diet promotes Ngn3-Driven β -cell regeneration to reverse diabetes. *Cell* 2017;168:775–88.
- Liu H, Javaheri A, Godar RJ, *et al*. Intermittent fasting preserves beta-cell mass in obesity-induced diabetes via the autophagy-lysosomal pathway. *Autophagy* 2017;13:1952–68.
- Wei S, Han R, Zhao J, *et al*. Intermittent administration of a fasting-mimicking diet intervenes in diabetes progression, restores β cells and reconstructs gut microbiota in mice. *Nutr Metab* 2018;15.
- Rukstalis JM, Habener JF. Neurogenin3: a master regulator of pancreatic islet differentiation and regeneration. *Islets* 2009;1:177–84.
- Zhu Y, Liu Q, Zhou Z, *et al*. Pdx1, neurogenin-3, and MafA: critical transcription regulators for beta cell development and regeneration. *Stem Cell Res Ther* 2017;8.
- Wei S, Zhao J, Wang S, *et al*. Intermittent administration of a leucine-deprived diet is able to intervene in type 2 diabetes in db/db mice. *Heliyon* 2018;4:e00830.
- Kaneto H, Matsuoka T-aki, Kimura T, *et al*. Appropriate therapy for type 2 diabetes mellitus in view of pancreatic β -cell glucose toxicity: "the earlier, the better". *J Diabetes* 2016;8:183–9.
- Kimura T, Obata A, Shimoda M, *et al*. Protective effects of the SGLT2 inhibitor luseogliflozin on pancreatic β -cells in db/db mice: The earlier and longer, the better. *Diabetes Obes Metab* 2018;20:2442–57.
- Hori K, Sen A, Artavanis-Tsakonas S. Notch signaling at a glance. *J Cell Sci* 2013;126:2135–40.
- Sumazaki R, Shiojiri N, Isoyama S, *et al*. Conversion of biliary system to pancreatic tissue in Hes1-deficient mice. *Nat Genet* 2004;36:83–7.
- Salama-Cohen P, Arevalo M-A, Grantyn R, *et al*. Notch and NGF/p75NTR control dendrite morphology and the balance of excitatory/inhibitory synaptic input to hippocampal neurons through neurogenin 3. *J Neurochem* 2006;97:1269–78.
- Fukuda A, Kawaguchi Y, Furuyama K. Ectopic pancreas formation in Hes1-knockout mice reveals plasticity of endodermal progenitors of the gut, bile duct, and pancreas. *J Clin Invest* 2006;116:1484–93.
- Billiard F, Karaliota S, Wang B, *et al*. Delta-like Ligand-4-Notch signaling inhibition regulates pancreatic islet function and insulin secretion. *Cell Rep* 2018;22:895–904.
- Mirtschink P, Chavakis T. The missed Notch to bring down diabetes. *Trends Endocrinol Metab* 2018;29:448–50.
- Bartolome A, Zhu C, Sussel L, *et al*. Notch signaling dynamically regulates adult β cell proliferation and maturity. *J Clin Invest* 2019;129:268–80.
- Baeyens L, Bonn S, Bos T, *et al*. Notch signaling as gatekeeper of rat Acinar-to- β -Cell conversion in vitro. *Gastroenterology* 2009;136:1750–60.
- Wu X, Fleming A, Ricketts T, *et al*. Autophagy regulates notch degradation and modulates stem cell development and neurogenesis. *Nat Commun* 2016;7.
- Li BO, Duan P, Li C, *et al*. Role of autophagy on bone marrow mesenchymal stem-cell proliferation and differentiation into neurons. *Mol Med Rep* 2016;13:1413–9.
- Zeng J, Jing Y, Shi R, *et al*. Autophagy regulates biliary differentiation of hepatic progenitor cells through Notch1 signaling pathway. *Cell Cycle* 2016;15:1602–10.
- Ahn J-S, Ann E-J, Kim M-Y, *et al*. Autophagy negatively regulates tumor cell proliferation through phosphorylation dependent degradation of the Notch1 intracellular domain. *Oncotarget* 2016;7:79047–63.
- Choi HK, Yuan H, Fang F, *et al*. *Tsc1* Regulates the Balance Between Osteoblast and Adipocyte Differentiation Through Autophagy/Notch1/ β -Catenin Cascade. *J Bone Miner Res* 2018;33:2021–34.
- Kim J, Kundu M, Viollet B, *et al*. AMPK and mTOR regulate autophagy through direct phosphorylation of ULK1. *Nat Cell Biol* 2011;13:132–41.
- Castets P, Rügge MA. mTORC1 determines autophagy through ULK1 regulation in skeletal muscle. *Autophagy* 2013;9:1435–7.
- Li H, Lee J, He C, *et al*. Suppression of the mTORC1/STAT3/Notch1 pathway by activated AMPK prevents hepatic insulin resistance induced by excess amino acids. *Am J Physiol Endocrinol Metab* 2014;306:E197–E209.
- Stephan JS, Yeh Y-Y, Ramachandran V, *et al*. The TOR and PKA signaling pathways independently target the Atg1/Atg13 protein kinase complex to control autophagy. *Proc Natl Acad Sci U S A* 2009;106:17049–54.
- Chu KY, O'Reilly L, Mellett N, *et al*. Oleate disrupts cAMP signaling, contributing to potent stimulation of pancreatic beta-cell autophagy. *J Biol Chem* 2018.
- Angulo-Rojo C, Manning-Cela R, Aguirre A, *et al*. Involvement of the Notch pathway in terminal astrocytic differentiation: role of PKA. *ASN Neuro* 2013;5:e00130.
- Wang R, Zhang Y-w, Sun P, *et al*. Transcriptional Regulation of PEN-2, a Key Component of the -Secretase Complex, by CREB. *Mol Cell Biol* 2006;26:1347–54.
- Gwinn DM, Shackelford DB, Egan DF, *et al*. AMPK phosphorylation of raptor mediates a metabolic checkpoint. *Mol Cell* 2008;30:214–26.
- Yang B, Huang C-Z, Yu T, *et al*. Metformin depresses overactivated Notch1/Hes1 signaling in colorectal cancer patients with type 2 diabetes mellitus. *Anticancer Drugs* 2017;28:531–9.
- Yang Y, XH W. Study on the influence of metformin on castration-resistant prostate cancer PC-3 cell line biological behavior by its inhibition on PLCepsilon gene-mediated Notch1/Hes and

- androgen receptor signaling pathway. *Eur Rev Med Pharmacol Sci* 2017;21:1918–23.
32. Gregg B, Elghazi L, Alejandro EU, *et al*. Exposure of mouse embryonic pancreas to metformin enhances the number of pancreatic progenitors. *Diabetologia* 2014;57:2566–75.
 33. Alejandro EU, Gregg B, Blandino-Rosano M, *et al*. Natural history of β -cell adaptation and failure in type 2 diabetes. *Mol Aspects Med* 2015;42:19–41.
 34. Poitout V, Amyot J, Semache M, *et al*. Glucolipotoxicity of the pancreatic beta cell. *Biochim Biophys Acta* 2010;1801:289–98.
 35. Kaneto H, Matsuoka T-aki. Role of pancreatic transcription factors in maintenance of mature β -cell function. *Int J Mol Sci* 2015;16:6281–97.
 36. ———, Miyatsuka T, Kawamori D, *et al*. PDX-1 and MafA Play a Crucial Role in Pancreatic β -Cell Differentiation and Maintenance of Mature β -Cell Function. *Endocr J* 2008;55:235–52.
 37. Matschinsky FM, Collins HW. Essential biochemical design features of the fuel-sensing system in pancreatic β -cells. *Chem Biol* 1997;4:249–57.
 38. Matschinsky F, Liang Y, Kesavan P, *et al*. Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J Clin Invest* 1993;92:2092–8.
 39. Yuan H, Lu Y, Huang X, *et al*. Suppression of NADPH oxidase 2 substantially restores glucose-induced dysfunction of pancreatic NIT-1 cells. *FEBS J* 2010;277:5061–71.
 40. ———, Zhang X, Huang X, *et al*. NADPH oxidase 2-derived reactive oxygen species mediate FFAs-Induced dysfunction and apoptosis of β -cells via JNK, p38 MAPK and p53 pathways. *PLoS One* 2010;5:e15726.
 41. Mohammed AM, Kowluru A. Activation of apocynin-sensitive NADPH oxidase (Nox2) activity in INS-1 832/13 cells under glucotoxic conditions. *Islets* 2013;5:129–31.
 42. Kowluru A, Kowluru RA. Phagocyte-like NADPH oxidase [Nox2] in cellular dysfunction in models of glucolipotoxicity and diabetes. *Biochem Pharmacol* 2014;88:275–83.
 43. Sato Y, Fujimoto S, Mukai E, *et al*. Palmitate induces reactive oxygen species production and β -cell dysfunction by activating nicotinamide adenine dinucleotide phosphate oxidase through Src signaling. *J Diabetes Investig* 2014;5:19–26.
 44. Sidarala V, Veluthakal R, Syeda K, *et al*. Phagocyte-like NADPH oxidase (Nox2) promotes activation of p38MAPK in pancreatic β -cells under glucotoxic conditions: evidence for a requisite role of Ras-related C3 botulinum toxin substrate 1 (Rac1). *Biochem Pharmacol* 2015;95:301–10.
 45. Koulajian K, Desai T, Liu GC, *et al*. NADPH oxidase inhibition prevents beta cell dysfunction induced by prolonged elevation of oleate in rodents. *Diabetologia* 2013;56:1078–87.
 46. Veluthakal R, Tunduguru R, Arora DK, *et al*. Vav2, a guanine nucleotide exchange factor for Rac1, regulates glucose-stimulated insulin secretion in pancreatic beta cells. *Diabetologia* 2015;58:2573–81.
 47. Yoder SM, Dineen SL, Wang Z, *et al*. Yes, a Src family kinase, is a proximal glucose-specific activator of cell division cycle control protein 42 (Cdc42) in pancreatic islet β cells. *J Biol Chem* 2014;289:11476–87.
 48. Fujiwara K, Maekawa F, Yada T. Oleic acid interacts with GPR40 to induce Ca²⁺ signaling in rat islet β -cells: mediation by PLC and L-type Ca²⁺ channel and link to insulin release. *Am J Physiol Endocrinol Metab* 2005;289:E670–E677.
 49. Yamada H, Yoshida M, Ito K, *et al*. Potentiation of glucose-stimulated insulin secretion by the GPR40–PLC–TRPC pathway in pancreatic β -cells. *Sci Rep* 2016;6.
 50. Kowluru A. Friendly, and not so friendly, roles of Rac1 in islet β -cell function: lessons learnt from pharmacological and molecular biological approaches. *Biochem Pharmacol* 2011;81:965–75.
 51. Hou N, Torii S, Saito N, *et al*. Reactive oxygen species-mediated pancreatic β -cell death is regulated by interactions between stress-activated protein kinases, p38 and c-Jun N-terminal kinase, and mitogen-activated protein kinase phosphatases. *Endocrinology* 2008;149:1654–65.
 52. Solinas G, Naugler W, Galimi F, *et al*. Saturated fatty acids inhibit induction of insulin gene transcription by JNK-mediated phosphorylation of insulin-receptor substrates. *Proc Natl Acad Sci U S A* 2006;103:16454–9.
 53. Kitamura T, Nakae J, Kitamura Y, *et al*. The forkhead transcription factor Foxo1 links insulin signaling to Pdx1 regulation of pancreatic β cell growth. *J Clin Invest* 2002;110:1839–47.
 54. ———. The role of FOXO1 in β -cell failure and type 2 diabetes mellitus. *Nat Rev Endocrinol* 2013;9:615–23.
 55. Wong JC, Vo V, Gorjala P, *et al*. Pancreatic- β -cell survival and proliferation are promoted by protein kinase G type I α and downstream regulation of Akt/FoxO1. *Diab Vasc Dis Res* 2017;14:434–49.
 56. Kawamori D, Kajimoto Y, Kaneto H, *et al*. Oxidative stress induces nucleo-cytoplasmic translocation of pancreatic transcription factor PDX-1 through activation of c-Jun NH2-terminal kinase. *Diabetes* 2003;52:2896–904.
 57. Kaneto H, Kawamori D, Matsuoka T-aki, *et al*. Oxidative Stress and Pancreatic β -Cell Dysfunction. *Am J Ther* 2005;12:529–33.
 58. Kawamori D, Kaneto H, Nakatani Y, *et al*. The forkhead transcription factor Foxo1 bridges the JNK pathway and the transcription factor PDX-1 through its intracellular translocation. *J Biol Chem* 2006;281:1091–8.
 59. Harmon JS, Stein R, Robertson RP. Oxidative stress-mediated, post-translational loss of MafA protein as a contributing mechanism to loss of insulin gene expression in glucotoxic beta cells. *J Biol Chem* 2005;280:11107–13.
 60. Matsuoka T-a, Kaneto H, Miyatsuka T, *et al*. Regulation of MafA Expression in Pancreatic β -Cells in db/db Mice With Diabetes. *Diabetes* 2010;59:1709–20.
 61. Furman B, Ong WK, Pyne NJ. Cyclic AMP signaling in pancreatic islets. *Adv Exp Med Biol* 2010;654:281–304.
 62. Seino S, Takahashi H, Fujimoto W, *et al*. Roles of cAMP signalling in insulin granule exocytosis. *Diabetes Obes Metab* 2009;11(Suppl 4):180–8.
 63. Chepurny OG, Kelley GG, Dzura I, *et al*. PKA-dependent potentiation of glucose-stimulated insulin secretion by Epac activator 8-pCPT-2'-O-Me-cAMP-AM in human islets of Langerhans. *Am J Physiol Endocrinol Metab* 2010;298:E622–E633.
 64. Kai AKL, Lam AKM, Chen Y, *et al*. Exchange protein activated by cAMP 1 (*Epac1*)-deficient mice develop β -cell dysfunction and metabolic syndrome. *FASEB J* 2013;27:4122–35.
 65. Yosida M, Dezaki K, Uchida K, *et al*. Involvement of cAMP/EPAC/TRPM2 activation in glucose- and incretin-induced insulin secretion. *Diabetes* 2014;63:3394–403.
 66. Kang G, Chepurny OG, Malester B, *et al*. cAMP sensor Epac as a determinant of ATP-sensitive potassium channel activity in human pancreatic β cells and rat INS-1 cells. *J Physiol* 2006;573:595–609.
 67. Leech CA, Chepurny OG, Holz GG. Epac2-dependent Rap1 activation and the control of islet insulin secretion by glucagon-like peptide-1. *Vitam Horm* 2010;84:279–302.
 68. Dalle S, Quoyer J, Varin E, *et al*. Roles and regulation of the transcription factor CREB in pancreatic β -cells. *Curr Mol Pharmacol* 2011;4:187–95.
 69. Liu B, Barbosa-Sampaio H, Jones PM, *et al*. The CaMK4/CREB/IRS-2 cascade stimulates proliferation and inhibits apoptosis of β -cells. *PLoS One* 2012;7:e45711.
 70. Pugazhenth S, Qin L, Bouchard R. Dipeptidyl peptidase-4 inhibition in diabetic rats leads to activation of the transcription factor CREB in β -cells. *Eur J Pharmacol* 2015;755:42–9.
 71. McCarty MF, DiNicolantonio JJ. Acarbose, lente carbohydrate, and prebiotics promote metabolic health and longevity by stimulating intestinal production of GLP-1. *Open Heart* 2015;2:e000205.
 72. Janardhan S, Sastry G. Dipeptidyl peptidase IV inhibitors: a new paradigm in type 2 diabetes treatment. *Curr Drug Targets* 2014;15:600–21.
 73. Htike ZZ, Zaccardi F, Papamargaritis D, *et al*. Efficacy and safety of glucagon-like peptide-1 receptor agonists in type 2 diabetes: a systematic review and mixed-treatment comparison analysis. *Diabetes Obes Metab* 2017;19:524–36.
 74. Xu G, Kaneto H, Laybutt DR, *et al*. Downregulation of GLP-1 and GIP receptor expression by hyperglycemia: possible contribution to impaired incretin effects in diabetes. *Diabetes* 2007;56:1551–8.
 75. Undank S, Kaiser J, Sikimic J, *et al*. Atrial natriuretic peptide affects stimulus-secretion coupling of pancreatic β -cells. *Diabetes* 2017;66:2840–8.
 76. Nano J, Muka T, Cepeda M, *et al*. Association of circulating total bilirubin with the metabolic syndrome and type 2 diabetes: a systematic review and meta-analysis of observational evidence. *Diabetes Metab* 2016;42:389–97.
 77. Abbasi A, Deetman PE, Corpeleijn E, *et al*. Bilirubin as a potential causal factor in type 2 diabetes risk: a Mendelian randomization study. *Diabetes* 2015;64:1459–69.
 78. Jung CH, Lee MJ, Kang YM, *et al*. Higher serum bilirubin level as a protective factor for the development of diabetes in healthy Korean men: a 4year retrospective longitudinal study. *Metabolism* 2014;63:87–93.
 79. Ikeda N, Inoguchi T, Sonoda N, *et al*. Biliverdin protects against the deterioration of glucose tolerance in db/db mice. *Diabetologia* 2011;54:2183–91.

80. McCarty MF. Clinical Potential of *Spirulina* as a Source of Phycocyanobilin. *J Med Food* 2007;10:566–70.
81. Zheng J, Inoguchi T, Sasaki S, et al. Phycocyanin and phycocyanobilin from *Spirulina platensis* protect against diabetic nephropathy by inhibiting oxidative stress. *Am J Physiol Regul Integr Comp Physiol* 2013;304:R110–R120.
82. Romay C, Gonzalez R, Ledon N, et al. Rimbau V. C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Curr Protein Pept Sci* 2003;4:207–16.
83. Liu Q, Huang Y, Zhang R, et al. Medical application of *Spirulina platensis* derived C-phycocyanin. *Evid Based Complement Alternat Med* 2016;2016.
84. Huang H, Liao D, Pu R, et al. Quantifying the effects of *Spirulina* supplementation on plasma lipid and glucose concentrations, body weight, and blood pressure. *Diabetes Metab Syndr Obes* 2018;11:729–42.
85. McCarty MF, Barroso-Aranda J, Contreras F. NADPH oxidase mediates glucolipotoxicity-induced beta cell dysfunction – clinical implications. *Med Hypotheses* 2010;74:596–600.
86. McCarty M. Supplementation with phycocyanobilin, citrulline, taurine, and Supranutritional doses of folic acid and Biotin – Potential for preventing or slowing the progression of diabetic complications. *Healthcare* 2017;5.
87. Zhu B, Wu X, Bi Y, et al. Effect of bilirubin concentration on the risk of diabetic complications: a meta-analysis of epidemiologic studies. *Sci Rep* 2017;7.
88. Dinkova-Kostova AT, Talalay P. Direct and indirect antioxidant properties of inducers of cytoprotective proteins. *Mol Nutr Food Res* 2008;52(Suppl 1):S128–S138.
89. Yang Y, Wang W, Liu Y, et al. α -lipoic acid inhibits high glucose-induced apoptosis in HIT-T15 cells. *Dev Growth Differ* 2012;54:557–65.
90. Koh G, Yang E-J, Kim M-K, et al. Alpha-lipoic acid treatment reverses 2-deoxy-D-ribose-induced oxidative damage and suppression of insulin expression in pancreatic beta-cells. *Biol Pharm Bull* 2013;36:1570–6.
91. Nobakht-Haghighi N, Rahimifard M, Baeeri M, et al. Regulation of aging and oxidative stress pathways in aged pancreatic islets using alpha-lipoic acid. *Mol Cell Biochem* 2018;449:267–76.
92. Roy S, Metya SK, Sannigrahi S, et al. Treatment with ferulic acid to rats with streptozotocin-induced diabetes: effects on oxidative stress, pro-inflammatory cytokines, and apoptosis in the pancreatic β cell. *Endocrine* 2013;44:369–79.
93. Wild AC, Moinova HR, Mulcahy RT. Regulation of γ -Glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. *J Biol Chem* 1999;274:33627–36.
94. Lee J-S, Surh Y-J. Nrf2 as a novel molecular target for chemoprevention. *Cancer Letters* 2005;224:171–84.
95. Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 2001;57:1825–35.
96. Dickinson DA, Forman HJ. Glutathione in defense and signaling: lessons from a small thiol. *Ann N Y Acad Sci* 2002;973:488–504.
97. Shelton MD, Chock PB, Miesel JJ. Glutaredoxin: Role in Reversible Protein S -Glutathionylation and Regulation of Redox Signal Transduction and Protein Translocation. *Antioxid Redox Signal* 2005;7:348–66.
98. Dodd S, Dean O, Copolov DL, et al. N -acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert Opin Biol Ther* 2008;8:1955–62.
99. Droge W, Kinscherf R, Hildebrandt W, et al. The deficit in low molecular weight thiols as a target for antiageing therapy. *Curr Drug Targets* 2006;7:1505–12.
100. Sekhar RV, McKay SV, Patel SG, et al. Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. *Diabetes Care* 2011;34:162–7.
101. Tanaka Y, Gleason CE, Tran POT, et al. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. *Proc Natl Acad Sci U S A* 1999;96:10857–62.
102. Kaneto H, Kajimoto Y, Miyagawa J, et al. Beneficial effects of antioxidants in diabetes: possible protection of pancreatic beta-cells against glucose toxicity. *Diabetes* 1999;48:2398–406.
103. DiNicolantonio JJ, O'Keefe JH, McCarty MF. Boosting endogenous production of vasoprotective hydrogen sulfide via supplementation with taurine and N-acetylcysteine: a novel way to promote cardiovascular health. *Open Heart* 2017;4:e000600.
104. Okamoto M, Ishizaki T, Kimura T. Protective effect of hydrogen sulfide on pancreatic beta-cells. *Nitric Oxide* 2015;46:32–6.
105. ———, Yamaoka M, Takei M, et al. Endogenous hydrogen sulfide protects pancreatic beta-cells from a high-fat diet-induced glucotoxicity and prevents the development of type 2 diabetes. *Biochem Biophys Res Commun* 2013;442:227–33.
106. Mao Z, Huang Y, Zhang Z, et al. Pharmacological levels of hydrogen sulfide inhibit oxidative cell injury through regulating the redox state of thioredoxin. *Free Radic Biol Med* 2019.
107. Shalev A. Lack of Txnip protects β -cells against glucotoxicity: Figure 1. *Biochem Soc Trans* 2008;36:963–5.
108. Rani S, Mehta JP, Barron N, et al. Decreasing TXNIP mRNA and protein levels in pancreatic MIN6 cells reduces reactive oxygen species and restores glucose regulated insulin secretion. *Cell Physiol Biochem* 2010;25:667–74.
109. Cha-Molstad H, Saxena G, Chen J, et al. Glucose-stimulated expression of Txnip is mediated by carbohydrate response element-binding protein, p300, and histone H4 acetylation in pancreatic beta cells. *J Biol Chem* 2009;284:16898–905.
110. Kawaguchi T, Osatomi K, Yamashita H, et al. Mechanism for fatty acid "sparing" effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. *J Biol Chem* 2002;277:3829–35.
111. Shaked M, Ketzinel-Gilad M, Cerasi E, et al. AMP-activated protein kinase (AMPK) mediates nutrient regulation of thioredoxin-interacting protein (TXNIP) in pancreatic beta-cells. *PLoS One* 2011;6:e28804.
112. Masini M, Anello M, Bugliani M, et al. Prevention by metformin of alterations induced by chronic exposure to high glucose in human islet beta cells is associated with preserved ATP/ADP ratio. *Diabetes Res Clin Pract* 2014;104:163–70.
113. Baidwan S, Chekuri A, Hynds DL, et al. Glucotoxicity promotes aberrant activation and mislocalization of Ras-related C3 botulinum toxin substrate 1 [Rac1] and metabolic dysfunction in pancreatic islet β -cells: reversal of such metabolic defects by metformin. *Apoptosis* 2017;22:1380–93.
114. Bunck MC, Corner A, Eliasson B, et al. Effects of Exenatide on Measures of β -Cell Function After 3 Years in Metformin-Treated Patients With Type 2 Diabetes. *Diabetes Care* 2011;34:2041–7.
115. van Raalte DH, Bunck MC, Smits MM, et al. Exenatide improves β -cell function up to 3 years of treatment in patients with type 2 diabetes: a randomised controlled trial. *Eur J Endocrinol* 2016;175:345–52.
116. Alamshah A, McGavigan AK, Spreckley E, et al. L-arginine promotes gut hormone release and reduces food intake in rodents. *Diabetes Obes Metab* 2016;18:508–18.
117. Vesely D. Biotin enhances guanylate cyclase activity. *Science* 1982;216:1329–30.
118. ———, Wormser H, bramson H. Biotin analogs activate guanylate cyclase. *Mol Cell Biochem* 1984;60:109–14.
119. Watanabe-Kamiyama M, Kamiyama S, Horuchi K, et al. Antihypertensive effect of biotin in stroke-prone spontaneously hypertensive rats. *Br J Nutr* 2008;99:756–63.
120. Borboni P, Magnattera R, Rabini RA, et al. Effect of biotin on glucokinase activity, mRNA expression and insulin release in cultured beta-cells. *Acta Diabetol* 1996;33:154–8.
121. Furukawa Y. Enhancement of glucose-induced insulin secretion and modification of glucose metabolism by biotin]. *Nihon Rinsho* 1999;57:2261–9.
122. Lazo de la Vega-Monroy ML, Larrieta E, German MS, et al. Effects of biotin supplementation in the diet on insulin secretion, islet gene expression, glucose homeostasis and beta-cell proportion. *J Nutr Biochem* 2013;24:169–77.
123. Tixi-Verdugo W, Contreras-Ramos J, Sicilia-Argumedo G, et al. Effects of biotin supplementation during the first week postweaning increases pancreatic islet area, beta-cell proportion, islets number, and beta-cell proliferation. *J Med Food* 2018;21:274–81.
124. Vilches-Flores A, Tovar AR, Marin-Hernandez A, et al. Biotin increases glucokinase expression via soluble guanylate cyclase/protein kinase G, adenosine triphosphate production and autocrine action of insulin in pancreatic rat islets. *J Nutr Biochem* 2010;21:606–12.
125. McCarty MF. cGMP may have trophic effects on beta cell function comparable to those of cAMP, implying a role for high-dose biotin in prevention/treatment of diabetes. *Med Hypotheses* 2006;66:323–8.
126. Maebashi M, Makino Y, Furukawa Y, et al. Therapeutic evaluation of the effect of biotin on hyperglycemia in patients with non-insulin dependent diabetes mellitus. *J Clin Biochem Nutr* 1993;14:211–8.
127. Zhang H, Osada K, Sone H, et al. Biotin administration improves the impaired glucose tolerance of streptozotocin-induced diabetic Wistar rats. *J Nutr Sci Vitaminol* 1997;43:271–80.
128. McCarty MF. In type 1 diabetics, high-dose biotin may compensate for low hepatic insulin exposure, promoting a more normal

- expression of glycolytic and gluconeogenic enzymes and thereby Aiding glycaemic control. *Med Hypotheses* 2016;95:45–8.
129. Kassem M, Niazi ZR, Abbas M, *et al.* Senescence of pancreas in middle-aged rats with normal vascular function. *Ann Transplant* 2017;22:177–86.
 130. McCarty M. Asymmetric dimethylarginine is a well established mediating risk factor for cardiovascular morbidity and Mortality—Should patients with elevated levels be supplemented with citrulline? *Healthcare* 2016;4.
 131. Carvalho DS, Diniz MM, Haidar AA, *et al.* L-arginine supplementation improves insulin sensitivity and beta cell function in the offspring of diabetic rats through Akt and PDX-1 activation. *Eur J Pharmacol* 2016;791:780–7.
 132. Yu E, Ruiz-Canela M, Razquin C, *et al.* Changes in arginine are inversely associated with type 2 diabetes: a case-cohort study in the PREDIMED trial. *Diabetes Obes Metab* 2019;21:397–401.
 133. Gao L, Siu KL, Chalupsky K, *et al.* Role of uncoupled endothelial nitric oxide synthase in abdominal aortic aneurysm formation. *Hypertension* 2012;59:158–66.
 134. Chalupsky K, Kračun D, Kanchev I, *et al.* Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia-induced pulmonary hypertension by Recoupling endothelial nitric oxide synthase. *Antioxid Redox Signal* 2015;23:1076–91.
 135. Jayawardena R, Ranasinghe P, Galappathy P, *et al.* Effects of zinc supplementation on diabetes mellitus: a systematic review and meta-analysis. *Diabetol Metab Syndr* 2012;4.
 136. de Carvalho GB, Brandão-Lima PN, Maia CSC, *et al.* Zinc's role in the glycaemic control of patients with type 2 diabetes: a systematic review. *Biometals* 2017;30:151–62.
 137. Cooper-Capetini V, de Vasconcelos DAA, Martins AR, *et al.* Zinc supplementation improves glucose homeostasis in high fat-fed mice by enhancing pancreatic beta-cell function. *Nutrients* 2017;20:9.
 138. Jacques-Silva MC, Correa-Medina M, Cabrera O, *et al.* ATP-gated P2X3 receptors constitute a positive autocrine signal for insulin release in the human pancreatic cell. *Proc Natl Acad Sci U S A* 2010;107:6465–70.
 139. Schwiebert EM, Liang L, Cheng N-L, *et al.* Extracellular zinc and ATP-gated P2X receptor calcium entry channels: new zinc receptors as physiological sensors and therapeutic targets. *Purinergic Signal* 2005;1:299–310.
 140. Richards-Williams C, Contreras JL, Berecek KH, *et al.* Extracellular ATP and zinc are co-secreted with insulin and activate multiple P2X purinergic receptor channels expressed by islet beta-cells to potentiate insulin secretion. *Purinergic Signal* 2008;4:393–405.
 141. Kahleova H, Tura A, Hill M, *et al.* A plant-based dietary intervention improves beta-cell function and insulin resistance in overweight adults: a 16-Week randomized clinical trial. *Nutrients* 2018;10.
 142. Barnard ND, Scialli AR, Turner-McGrievy G, *et al.* The effects of a low-fat, plant-based dietary intervention on body weight, metabolism, and insulin sensitivity. *Am J Med* 2005;118:991–7.
 143. Kahleova H, Fleeman R, Hlozkova A, *et al.* A plant-based diet in overweight individuals in a 16-week randomized clinical trial: metabolic benefits of plant protein. *Nutr Diabetes* 2018;8.
 144. Barnard ND, Cohen J, Jenkins DJA, *et al.* A low-fat vegan diet and a conventional diabetes diet in the treatment of type 2 diabetes: a randomized, controlled, 74-wk clinical trial. *Am J Clin Nutr* 2009;89:1588S–96.
 145. Trapp C, Barnard N, Katcher H. a plant-based diet for type 2 diabetes: scientific support and practical strategies. *Diabetes Educ* 2010;36:33–48.
 146. Toumpanakis A, Turnbull T, Alba-Barba I. Effectiveness of plant-based diets in promoting well-being in the management of type 2 diabetes: a systematic review. *BMJ Open Diab Res Care* 2018;6:e000534.
 147. Turner-McGrievy GM, Jenkins DJA, Barnard ND, *et al.* Decreases in dietary glycemic index are related to weight loss among individuals following therapeutic diets for type 2 diabetes. *J Nutr* 2011;141:1469–74.
 148. Tonstad S, Stewart K, Oda K, *et al.* Vegetarian diets and incidence of diabetes in the Adventist health Study-2. *Nutr Metab Cardiovasc Dis* 2013;23:292–9.
 149. McCarty MF. GCN2 and FGF21 are likely mediators of the protection from cancer, autoimmunity, obesity, and diabetes afforded by vegan diets. *Med Hypotheses* 2014;83:365–71.
 150. Fontana L, Cummings NE, Arriola Apelo SI, *et al.* Decreased consumption of branched-chain amino acids improves metabolic health. *Cell Rep* 2016;16:520–30.
 151. Vu V, Liu Y, Sen S, *et al.* Delivery of adiponectin gene to skeletal muscle using ultrasound targeted microbubbles improves insulin sensitivity and whole body glucose homeostasis. *Am J Physiol Endocrinol Metab* 2013;304:E168–E175.
 152. Patel SA, Hoehn KL, Lawrence RT, *et al.* Overexpression of the adiponectin receptor AdipoR1 in rat skeletal muscle amplifies local insulin sensitivity. *Endocrinology* 2012;153:5231–46.
 153. Nawrocki AR, Rajala MW, Tomas E, *et al.* Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor γ agonists. *J Biol Chem* 2006;281:2654–60.
 154. Yamauchi T, Kamon J, Minokoshi Y, *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002;8:1288–95.
 155. Méndez-Sánchez N, Chavez-Tapia NC, Villa AR. Adiponectin as a protective factor in hepatic steatosis. *World J Gastroenterol* 2005;11:1737–41.
 156. Wente W, Efanov AM, Brenner M, *et al.* Fibroblast growth factor-21 improves pancreatic β -cell function and survival by activation of extracellular Signal-Regulated kinase 1/2 and Akt signaling pathways. *Diabetes* 2006;55:2470–8.
 157. WY S, Cheng Q, Xu A, *et al.* Loss of fibroblast growth factor 21 action induces insulin resistance, pancreatic islet hyperplasia and dysfunction in mice. *Cell Death Dis* 2015;6:e1707.
 158. Jimenez V, Jambrija C, Casana E, *et al.* Fgf21 gene therapy as treatment for obesity and insulin resistance. *EMBO Mol Med* 2018;10.
 159. Kjobsted R, Wojtaszewski JF, Treebak JT. Role of AMP-activated protein kinase for regulating post-exercise insulin sensitivity. *Exp Suppl* 2016;107:81–126.
 160. Hawley JA, Lessard SJ. Exercise training-induced improvements in insulin action. *Acta Physiologica* 2008;192:127–35.
 161. Hesselink MKC, Schrauwen-Hinderling V, Schrauwen P. Skeletal muscle mitochondria as a target to prevent or treat type 2 diabetes mellitus. *Nat Rev Endocrinol* 2016;12:633–45.
 162. Garcia-Macedo R, Sanchez-Muñoz F, Almanza-Perez JC, *et al.* Glycine increases mRNA adiponectin and diminishes pro-inflammatory adipokines expression in 3T3-L1 cells. *Eur J Pharmacol* 2008;587:317–21.
 163. Chen J, Ma X, Yang Y, *et al.* Glycine enhances expression of adiponectin and IL-10 in 3T3-L1 adipocytes without affecting adipogenesis and lipolysis. *Amino Acids* 2018;50:629–40.
 164. DiNicolantonio JJ, McCarty MF, O'Keefe JH. Antioxidant bilirubin works in multiple ways to reduce risk for obesity and its health complications. *Open Heart* 2018;5:e000914.
 165. Souto Padron de FA, salmon AB, Bruno F, Jimenez F, Martinez Hg, Halade GV, Ahuja SS, Clark Ra, DeFronzo Ra, Abboud He, El JA. Nox2 mediates skeletal muscle insulin resistance induced by a high fat diet. *J Biol Chem* 2015;290:13427–39.
 166. McCarty MF, DiNicolantonio JJ, O'Keefe JH. The ability of carnitine to act as a type 1 histone deacetylase inhibitor may explain the favorable impact of carnitine supplementation on mitochondrial biogenesis in the elderly. *Med Hypotheses* 2019. in submission.
 167. ————. Exenatide and biotin in conjunction with a protein-sparing fast for normalization of beta cell function in type 2 diabetics. *Med Hypotheses* 2007;69:928–32.
 168. Wing RR, Blair EH, Bononi P, *et al.* Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. *Diabetes Care* 1994;17:30–6.
 169. Henry RR, Gumbiner B. Benefits and limitations of very-low-calorie diet therapy in obese NIDDM. *Diabetes Care* 1991;14:802–23.
 170. Fukuda M, Tahara Y, Yamamoto Y, *et al.* Effects of very-low-calorie diet weight reduction on glucose tolerance, insulin secretion, and insulin resistance in obese non-insulin-dependent diabetics. *Diabetes Res Clin Pract* 1989;7:61–7.
 171. Weck M, Hanefeld M, Schollberg K. Effects of VLCD in obese NIDDM (non-insulin dependent diabetes) on glucose, insulin and C-peptide dynamics. *Int J Obes* 1989;13(Suppl 2):159–60.
 172. Bhatt AA, Choudhari PK, Mahajan RR, *et al.* Effect of a low-calorie diet on restoration of Normoglycemia in obese subjects with type 2 diabetes. *Indian J Endocrinol Metab* 2017;21:776–80.
 173. Jackness C, Karmally W, Febres G, *et al.* Very Low-Calorie diet mimics the early beneficial effect of Roux-en-Y gastric bypass on insulin sensitivity and β -cell function in type 2 diabetic patients. *Diabetes* 2013;62:3027–32.
 174. Malandrucko I, Pasqualetti P, Giordani I, *et al.* Very-low-calorie diet: a quick therapeutic tool to improve β cell function in morbidly obese patients with type 2 diabetes. *Am J Clin Nutr* 2012;95:609–13.