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REVIEW

Low serum amylase and obesity, diabetes and metabolic syndrome: A novel interpretation

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Abstract

For the last decade, low serum amylase (hypoamylasemia) has been reported in certain common cardiometabolic conditions such as obesity, diabetes (regardless of type), and metabolic syndrome, all of which appear to have a common etiology of insufficient insulin action due to insulin resistance and/or diminished insulin secretion. Some clinical studies have shown that salivary amylase may be preferentially decreased in obese individuals, whereas others have revealed that pancreatic amylase may be preferentially decreased in diabetic subjects with insulin dependence. Despite this accumulated evidence, the clinical relevance of serum, salivary, and pancreatic amylase and the underlying mechanisms have not been fully elucidated. In recent years, copy number variations (CNVs) in the salivary amylase gene (*AMY1*), which range more broadly than the pancreatic amylase gene (*AMY2A* and *AMY2B*), have been shown to be well correlated with salivary and serum amylase levels. In addition, low CNV of *AMY1*, indicating low salivary amylase, was associated with insulin resistance, obesity, low taste perception/satiety, and postprandial hyperglycemia through impaired insulin secretion at early cephalic phase. In most populations, insulin-dependent diabetes is less prevalent (minor contribution) compared with insulin-independent diabetes, and obesity is highly prevalent compared with low body weight. Therefore, obesity as a condition that elicits cardiometabolic diseases relating to insulin resistance (major contribution) may be a common determinant for low serum amylase in a general population. In this review, the novel interpretation of low serum, salivary, and pancreas amylase is discussed in terms of major contributions of obesity, diabetes, and metabolic syndrome.

Key words: Serum amylase; Salivary; Pancreas; Diabetes; Metabolic syndrome; Obesity; *AMY1*; *AMY2*; Insulin resistance

Core tip: Low serum amylase was believed to occur in uncommon conditions such as type 1 diabetes, advanced chronic pancreatitis, and cystic fibrosis.

However, in the last decade, low serum amylase has been observed in more common conditions related with insulin resistance than was previously believed. In this review, a novel interpretation for low serum, salivary, and pancreatic amylase is discussed, particularly in terms of the cardiometabolic conditions of obesity, diabetes, and metabolic syndrome.

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INTRODUCTION

Traditionally, the level of serum amylase has been commonly measured to determine the presence of acute pancreatitis and biliary tract disease in primary clinical settings[1-3]. In contrast, most physicians seldom measure it to determine the degree of advanced chronic pancreatitis, which eventually results in secondary diabetes concomitant with weight loss, lipid diarrhea, and malnutrition[4-6]. The former condition predisposes to higher serum amylase (but not necessarily), whereas the latter condition lowers serum amylase. While some cancers, such as lung, ovarian, and colon cancer and myeloma, often produce amylase and increase serum amylase[7-9], such increased serum amylase may be exceptional in a general population. By contrast, low serum amylase has been empirically known in diabetic patients particularly with insulin-dependent diabetes (primarily type 1 diabetes), although the clinical relevance and precise underlying mechanism are not fully understood[10-15].

In earlier clinical studies, conflicting results were reported regarding the level of serum amylase in diabetic patients, possibly because a precise measurement for serum amylase had not then been established. In recent decades, however, the measurement of serum and its isoforms has been stably performed in clinical laboratories with several methods including electrophoresis, inhibitor method and antibody method[16]. Because serum amylase generally consists of salivary and pancreatic amylase at almost equal proportion, *i.e.*, 1:1[17], abnormal levels of both or one of the two isoforms affect the level of total serum amylase.

Low serum amylase was believed to occur in uncommon conditions such as type 1 diabetes, advanced chronic pancreatitis, and cystic fibrosis[1-3,10-15,18,19] (minor contribution) (Table 1). However, in the past decade, low serum amylase has been observed in more common conditions (major contribution) than was previously believed. In this review, a novel interpretation for low serum, salivary, and pancreatic amylase is discussed, particularly in terms of the cardiometabolic conditions of obesity, diabetes, and metabolic syndrome (MetS).

Table 1 Traditional and novel interpretations for low serum amylase

	Ref.
Traditional (minor contributions)	
Type 1 diabetes (juvenile diabetes)	[1,2,10-15]
Advanced chronic pancreatitis	[3-6]
Type 2 diabetes with insulin dependence	[14]
Cystic fibrosis	[18,19]
Novel (major contributions)	
Obesity	[35-38]
Insulin resistance (high HOMA-R)	[44]
Metabolic Syndrome	[38,41,42]
Type 2 diabetes with insufficient insulin action	[38,42]
Diabetic ketoacidosis	[13,23]
Non-alcoholic fatty liver disease	[40,43]
Smoking	[38,68-70]
Heavy alcohol drinking	[46,63]
Low CNVs of *AMY1*	[54,60,61]

HOMA-R: Homeostasis model of insulin resistance; CNV: Copy number variations; *AMY1*: Salivary amylase gene.

TRADITIONAL INTERPRETATION

Specific etiologies related to low serum amylase (minor determinant for low serum amylase)

In the general population, the cause of acute pancreatitis is mainly high alcohol intake and/or high serum triglycerides, both of which injure the pancreas[1-6]. Consequently, transient and acute increases (from several to ten times beyond the upper normal level) in serum amylase concentration may occur as a result of destruction of acinar cells in the pancreas. However, repeated acute pancreatitis eventually results in exhausted acinar cells and restricted flow of enzymes from pancreas parenchyma into the circulation[4-6], in turn leading to low serum amylase due to low pancreatic amylase. Secondary diabetes also develops because of the destruction of β-cells in the course of chronic pancreatitis. In addition, low serum amylase has been observed in patients with cystic fibrosis concomitant with pancreatic insufficiency[18,19].

Around 20% of patients with diabetic ketoacidosis develop hyperamylasemia[20-22]. In this author's opinion, however, because diabetic ketoacidosis is accompanied by severely insufficient insulin action, theoretically serum amylase should be decreased in such conditions, at least before insulin therapy is initiated.

Consistently with this, Yokoyama *et al*[13] and Somogyi *et al*[23] found that serum amylase activity was reduced at onset of the disease before treatment with insulin. The author of the current review and collaborators, have frequently observed low serum amylase in diabetic ketoacidosis before treatment with insulin in clinical settings (unpublished data). The unexpectedly high serum amylase in previous

studies[20-22] may be explained by the fact that diabetic ketoacidosis involves numerous etiologies that can contribute to high serum amylase: Acute pancreatitis (mild to moderate grades)[22] including hypertriglyceridemic pancreatitis[24], renal dysfunction, and dehydration, all of which increase serum amylase.

Meanwhile, low serum amylase has been empirically observed in clinical settings in patients with type 1 diabetes, type 2 diabetes with insulin dependence, or advanced overt pancreatitis[10-15]. The action of insulin is critical for the production of pancreatic amylase[25,26]. A common etiology in these conditions may be depleted secretion of insulin from the pancreas. However, patients with these specific conditions are minor populations compared with diabetic patients with insulin independence.

The clinical relevance of salivary amylase has been focused on diseases of the salivary glands, sympathetic nerve system, and oral health. Under physiological conditions, secretion and activation of salivary amylase, *i.e.,* hyperamylasemia, is reportedly stimulated by psychosocial stress[27,28]. Many α-amylase inhibitors, which are often extracted from plants, have also been intensively investigated in terms of carbohydrate digestion and diabetic treatment[29,30]. Unfortunately, however, few clinical studies have reported low salivary amylase.

Dentists and investigators who work with, or are interested in, oral care have addressed the etiology of low salivary amylase[31,32]. Although insulin may also exert its action on the production of salivary amylase in the salivary glands[33,34], physicians have paid little attention to the issue. Therefore, the clinical relevance of low salivary amylase has not been elucidated, particularly in cardiometabolic conditions.

NOVEL INTERPRETATION

Relationship with cardiometabolic conditions of obesity and obesity-related disease (major determinants for low serum amylase)

An early animal study by Schneeman *et al*[35] showed that pancreatic amylase activity was reduced in obese rats but remained elevated in lean rats. An early clinical study of healthy young men aged 19 to 22 years by Kondo *et al*[36] showed that serum pancreatic amylase and trypsin, but not lipase, were reduced in obese subjects (n = 85) compared with lean subjects (n = 75). The reduction in serum pancreatic amylase was significantly improved by a weight loss program over 6 mo, and remained improved for a further 10 mo. No such trend was observed in pancreatic trypsin. To the best of this author's knowledge, these are the first studies to show an inverse relationship between obesity and serum pancreatic amylase. However, in the clinical study[36], the sample size was relatively small and relevant confounding factors including smoking, alcohol intake, exercise, and kidney function were not adjusted for in the analysis. Another small clinical study in children (n = 58) showed that obese boys (n = 29) presented a significantly lower salivary amylase concentration than control boys[37]. Except for these early studies[35-37], no clinical studies have investigated the relationship between serum amylase and obesity and obesity-related conditions.

In our previous cross-sectional (n = 2425) and longitudinal (n = 571) studies during the last decade[38], low serum (total) amylase (⩽ 57 IU/L) was significantly associated with MetS, diabetes (mostly type 2 diabetes), and remained significant even after adjustment for relevant confounding factors including age, sex, smoking, alcohol drinking, and regular exercise, pharmacotherapies, and kidney function assessed by estimated glomerular filtration rate (eGFR). In this study, body mass index (BMI) was the factor most associated with serum amylase[38]. Furthermore, low serum amylase was associated with non-alcoholic fatty liver disease (NAFLD), a hepatic manifestation of MetS and insulin resistance[39], in asymptomatic adults independently of relevant confounding factors[40]. The results of these epidemiological studies[38,40] were subsequently confirmed in other large Asian populations[41-43]. Furthermore, in our previous study of asymptomatic subjects not being treated for diabetes[44], a homeostasis model assessment of insulin resistance, plasma insulin levels at fasting and at 60 min in the 75 g oral glucose tolerance test were significantly associated with low serum amylase (< 60 IU/L) after adjustment for relevant confounding factors including BMI, although the sample size was small (n = 54).

These results suggest that low serum amylase is observed in not only rare conditions of insulin depletion (minor contribution) but also in common cardiometabolic conditions such as MetS, type 2 diabetes, or NAFLD (major contribution). Obesity, as a condition associated with various cardiometabolic diseases concomitant with insulin resistance, may be a major determinant for low serum amylase in the general population (a novel interpretation). A clinical study in hospitalized patients by Curd *et al*[45] showed that hypoamylasemia was associated with cystic fibrosis, hypertriglyceridemia and use of the antibiotic gentamicin, besides diabetes mellitus. Although cystic fibrosis and use of gentamicin may be uncommon, hypertriglyceridemia is rather common.

Williams *et al*[46] mentioned in an early review article that insulin is necessary for normal acinar function and that endogenous insulin potentiates zymogen release. However, exogenous insulin supplementation can improve low serum amylase in type 1 diabetes[13]. Schneeman *et al*[35] proposed in an animal study that insulin resistance may prevent the potentiating effect of insulin on amylase synthesis, leading to lower amylase levels. Early clinical studies have also shown that serum pancreatic amylase was closely related to C-peptide concentration and pancreatic β-cell function[13,14]. One

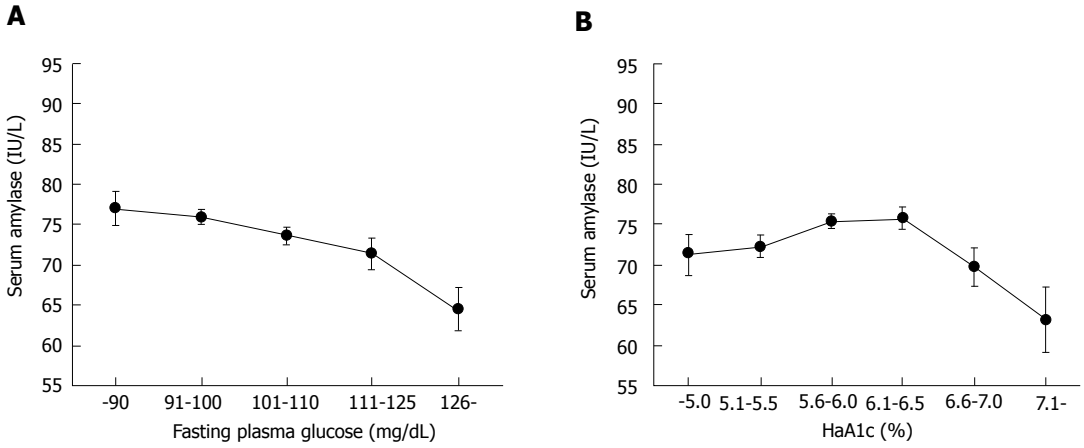

Figure 1 Relationship between serum amylase and fasting plasma glucose (A) and glycosylated hemoglobin (national glycohemoglobin standardization program) (B). Fasting plasma glucose of 90 mg/dL corresponds to HbA1c of 5.0% NGSP, as determined by linear regression analysis between FPG and HbA1c[47]. FPG: Fasting plasma glucose; NGSP: National glycohemoglobin standardization program; HbA1c: Glycosylated hemoglobin.

would therefore expect serum amylase to be reduced in obese and diabetic subjects.

Regarding the relationship between diabetes and serum amylase, it is noteworthy that serum amylase levels are not linearly correlated with HbA1c values in the general population including healthy individuals and diabetic patients, although fasting plasma glucose was negatively and linearly correlated with serum amylase[47]. In this study, serum amylase showed an inverse U-shaped relationship with HbA1c categories (Figure 1). Unexpectedly, serum amylase level was highest in subjects with HbA1c of 5.6%-6.5%. We experienced a similar result in an entirely different Japanese population (unpublished data). These findings may be consistent with the results of an early study by Dandona *et al*[14], which showed no significant correlation between HbA1c and pancreatic amylase activity. The discrepancy between HbA1c and fasting plasma glucose may be owing to the presence of postprandial hyperglycemia, *i.e.,* impaired glucose tolerance, a common finding in obese individuals[48,49], primarily related to HbA1c only. Additionally, hyperinsulinemia induced by insulin resistance for the maintenance of euglycemia, a common finding in early type 2 diabetes, may increase pancreatic amylase production.

After progression to overt diabetes with HbA1c over 6.5%, insulin resistance is not managed by hyperinsulinemia and insulin secretion begins to decline, resulting in a corresponding decrease in pancreatic and/or salivary amylase. Therfore, a linear relationship between HbA1c and serum amylase was observed in the data when only overt diabetic patients were studied[13,47].

Taken together, these results suggest that the relationship between serum amylase and diabetes and obesity is an exocrine-endocrine interrelationship, which in turn may contribute to the feedback system in energy homeostasis.

SALIVARY AMYLASE

While vertebrate animals express amylase in the pancreas, its expression in the salivary gland is limited to some primates and other herbivores and omnivores[50]. Carnivores (domesticated dogs and cats) do not have salivary amylase, whereas many herbivores (including goats, cows, horse, koala, rabbit, and elephant) do. Conversely, most omnivores, including humans, have considerable amounts of salivary amylase. Salivary amylase is higher in humans compared with many other animals including ape species, suggesting a dietary shift in the direction of high starch content during evolution[50,51].

Salivary amylase can affect an individual's oral sensory properties, in turn altering the threshold of satiety and appetite. While amylase expression particularly in salivary glands may be roughly determined by genetic regulation, high amylase levels can be induced by carbohydrate-rich diets passed on over generations. This hypothesis warrants further study.

Postprandial plasma glucose concentrations after ingestion of a 50 g starch solution were significantly higher in healthy nonobese adults with low salivary amylase than in those with high salivary amylase[52]. High salivary amylase activity is associated with a rapid insulin response accompanied by a swift reduction in blood glucose levels following starch ingestion. A plausible explanation is the response of insulin secretion at the early cephalic phase.

Genetic regulation

Genetic regulation is likely to play a key role in the primary determination of salivary amylase[53,54]. In newborns the predominant amylase isozymes seen in the urine are of salivary origin and later both salivary and pancreas, which increases during development. *AMY1* is expressed as early as 18 wk of gestation and salivary amylase gradually increases during

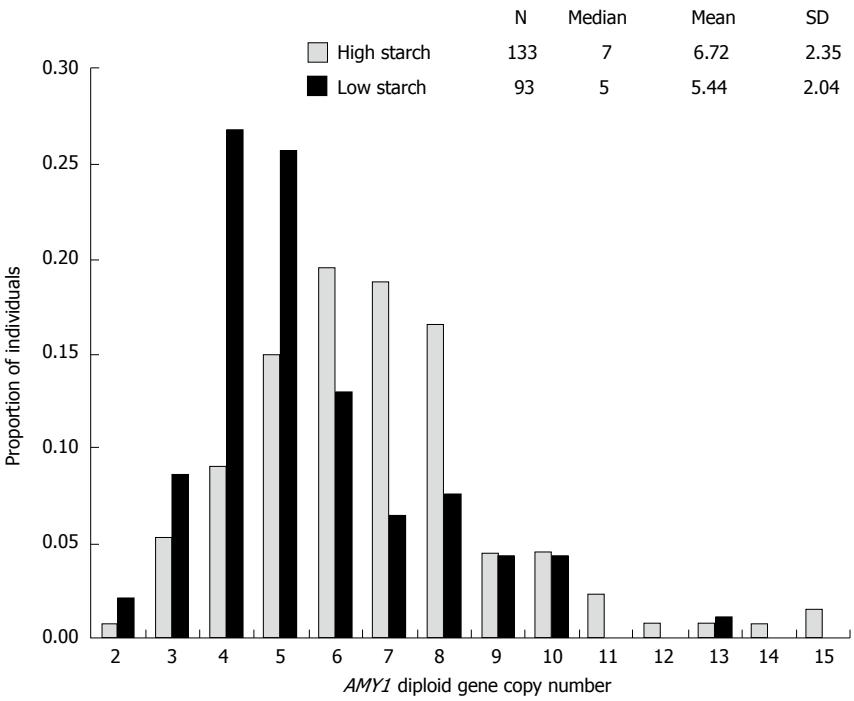

Figure 2 Copy number variations of the salivary amylase gene (*AMY1*) in relation to dietary starch[58].

development, as the total amylase activity approaches adult values.

In recent years, several studies have reported that serum and salivary amylase was significantly correlated with copy number variations (CNVs) of salivary amylase gene (*AMY1*). Moreover, CNV of *AMY1* was inversely associated with BMI, insulin resistance, and glucose tolerance[52,55-57]. CNVs seem to be higher in humans, particularly in American Europeans and Japanese, who relied on a starch-rich diet in the remote past[58] (Figure 2). According to Falchi *et al*[55], CNV of *AMY1* had a stronger association with BMI than polymorphisms in *FTO*, although conflicting results exist[59]. These findings suggest a genetic link between carbohydrate metabolism and obesity, possibly also involving gut microbiota[60].

A clinical study in Mexico suggests putative benefits of a high number of *AMY1* copies (and related production of salivary amylase) on obesity and energy metabolism in children[61]. Furthermore, a clinical study in Finland showed that low CNV of *AMY1* was associated with early-onset female obesity[62]. In this context, it is possible that individualized carbohydrate diets according to CNV of *AMY1* may help prevent obesity and type 2 diabetes.

Compared with *AMY1*, the relation of *AMY2* with cardiometabolic conditions is equivocal. In humans, the variation of CNVs of *AMY1* was wider than those of *AMY2*[57,59]. Furthermore, CNV of *AMY1* was independent of those of *AMY2*, which showed no association with BMI[59]. Meanwhile, in domesticated dogs for instance, pancreatic amylase not salivary amylase likely contributes to total amylase[50,63]. However, even in such animals, the variation of CNV of *AMY2B* was estimated to explain only 14.8% of the variance in amylase activity, indicating that additional factors may explain the majority of the variation[63].

OTHER CONDITIONS AFFECTING AMYLASE LEVELS

Alcohol intake, smoking, exercise, stress, and other factors

Other common conditions besides obesity-related conditions have been reported (Table 1). Alcohol consumption may affect the serum amylase level independently of BMI[47,64,65]. This may occur *via* damage of pancreatic tissue, *i.e.,* chronic pancreatitis, and reduced salivary amylase[66]. However, the underlying mechanism may be complicated because the effect of alcohol on glucose homeostasis can differ according to the quantity consumed[67], age, and lifestyle[68]. Low serum amylase was also observed in smokers compared with nonsmokers[69-71]. In contrast, high serum amylase has been observed in individuals who exercise regularly[72] and in high-performance long distance runners[73]. Because both smoking and fitness have a substantial impact on insulin action, these results may be explained from the view of insulin sensitivity[74]. Furthermore, low serum pancreatic enzyme levels predict mortality and are associated with malnutrition-inflammation[75], although the underlying mechanism remains unknown.

A recent study by Shimizu *et al*[76] showed that circulating pancreatic amylase was higher in female subjects with O blood type than those with A blood

type (lower pancreatic amylase in A blood type). Serum total amylase was also higher (but not statistically significant) in O blood type in both sexes. Coincidentally, we confirmed a similar finding of lower serum amylase in A blood type relative to O blood type in a general Japanese adult population (n = 1185), although no ABO blood types were associated with hyperglycemia (HbA1c $\geqslant$ 5.7% and/or pharmacotherapy for diabetes)[77]. Meanwhile, some clinical studies[78,79] have shown that people with the O blood type had a lower risk of developing type 2 diabetes compared with other blood types. Therefore, it is possible that unknown and unquantified factors including CNV of *AMY1* and prevalence of Rhesus factor in the study may also contribute to the relationship among ABO blood types, serum amylase, and impaired glucose metabolism. Together with the putative lower prevalence of pancreatic cancers in individuals with O blood type[80-82], this indicates a possible relationship between ABO blood type, which is under strict genetic regulation, and susceptibility to pancreatic disease.

Eating disorders

Intriguingly, elevated serum amylase has been sporadically observed in a series of eating disorders. Anorexia nervosa (AN) and bulimia nervosa (BN) are two major eating disorders with a complex relationship with abnormal physical conditions such as severe weight loss, binge eating, frequent vomiting or endocrine disorders[83,84]. Several studies have shown that serum amylase levels were significantly elevated in patients with BN[85-87]. It is likely that vomiting, rather than binge behavior, increases amylase in BN patients[87]. Frequent vomiting may also be associated with enlarged submandibular and parotid glands. It was also confirmed that hyperamylasemia in patients with AN or BN was caused by increased salivary-type amylase activity[85]. Consequently, these results suggest that the direct effect on elevated serum amylase may be primarily enlarged salivary glands. Conversely, it is also possible that concomitant low body weight or depleted energy storage, common findings in AN, cause the increased serum amylase. This needs to be studied further.

CAUTION IN INTERPRETING SERUM AMYLASE

Psychosocial stress contributes to elevated salivary amylase even in a healthy population[27,28], which likely leads to elevated total serum amylase. However, whether psychosocial stress has a long-term effect on serum amylase has not been confirmed in clinical studies. Investigators should pay attention to the mental and physical conditions in their patients when measuring salivary and serum amylase levels. Kidney function is also a crucial modifier that affects the clearance of circulating amylase in the blood[38,88]. Serum amylase is expected to be elevated in patients with renal dysfunction, and is a permanent phenomenon. Marked serum amylase elevation is observed in patients with only chronic kidney disease (CKD)[89]. While renal dysfunction should be kept in mind when high amylase levels are detected, the conditions of low serum amylase can be hidden and thus overlooked in patients with CKD and renal dysfunction, because lower amylase can be converted to normal amylase as a result of diminished clearance. GFR, for instance by the use of inulin, is not measured in usual clinical settings, so eGFR should be at least considered as a relevant confounding factor in the analysis of serum amylase. Nevertheless, it is unknown whether hyperfiltration, which is often observed in early diabetes, lowers serum amylase.

Some pharmacotherapies, particularly against diabetes, for instance dipeptidyl peptidase-4 inhibitors and glucagon-like peptide 1 receptor agonists, reportedly increase serum amylase. Several investigators[90-92] have recommended caution when starting incretin therapy to avoid pancreatitis. However, theoretically, a mild increase in serum amylase levels within the normal range or from low to normal levels can represent a potential beneficial effect of incretin therapy on glucose homeostasis especially in individuals with appreciable weight loss. Physicians may withdraw incretin therapy in patients with a slight increase in serum amylase of 10-20 IU/mL, for example, interpreting this as a sign of pancreatitis. Increase in serum amylase, particularly from low to normal levels, may reflect improved glucose homeostasis rather than acute pancreatitis. However, it remains pivotal to accumulate further data to investigate the clinical relevance of increases in pancreatic enzymes during incretin therapy.

For several decades, some clinical studies have revealed that α-amylase inhibitors, most of which are extracted from plants (clinically unavailable), can improve postprandial hyperglycemia[93] and obesity[94,95]. It is unclear whether α-amylase inhibitors would be truly effective for preventing diabetes and obesity if clinically available. Furthermore, while acarbose, an α-glucosidase inhibitor that also inhibits α-amylase, is available, such agents may be ineffective in obese subjects who have already low serum amylase.

CONCLUSION

Collectively, low serum amylase may reflect a manifestation of insufficient insulin action regardless of cause including insufficient pancreatic insulin secretion and/or systemic insulin resistance. Unfortunately, the cut-off point for low serum amylase has not been defined, primarily because of the lack of a concept for low serum amylase and the differences in assay methods. Unlike minor contributions, major contributions for low serum

amylase include common cardiometabolic conditions such as obesity, MetS, and type 2 diabetes, which are all increasing in incidence worldwide. Although genetic regulation may have a substantial impact on primary salivary amylase, whether epigenetic background and individual diet can alter salivary amylase and thus affect serum amylase is unclear, and requires further investigation.

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MINIREVIEWS

Metformin revisited: Does this regulator of AMP-activated protein kinase secondarily affect bone metabolism and prevent diabetic osteopathy?

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Abstract

Patients with long-term type 1 and type 2 diabetes mellitus (DM) can develop skeletal complications or "diabetic osteopathy". These include osteopenia, osteoporosis and an increased incidence of low-stress fractures. In this context, it is important to evaluate whether current anti-diabetic treatments can secondarily affect bone metabolism. Adenosine monophosphate-activated protein kinase (AMPK) modulates multiple metabolic pathways and acts as a sensor of the cellular energy status; recent evidence suggests a critical role for AMPK in bone homeostasis. In addition, AMPK activation is believed to mediate most clinical effects of the insulin-sensitizer metformin. Over the past decade, several research groups have investigated the effects of metformin on bone, providing a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone. However, two caveats should be kept in mind when considering metformin treatment for a patient with type 2 DM at risk for diabetic osteopathy. In the first place, metformin should probably not be considered an anti-osteoporotic drug; it is an insulin sensitizer with proven macrovascular benefits that can secondarily improve bone metabolism in the context of DM. Secondly, we are still awaiting the results of randomized placebo-controlled studies in humans that evaluate the effects of metformin on bone metabolism as a primary endpoint.

Key words: Diabetes mellitus; Osteoporosis; Bone fractures; Metformin; AMP-activated kinase

Core tip: Patients with long-term type 1 and type 2 diabetes mellitus (DM) can develop skeletal complications. These include osteopenia, osteoporosis and increased incidence of low-stress fractures. In this context, it is important to evaluate whether current anti-diabetic treatments can secondarily affect bone metabolism. Over the past decade, several research

groups have investigated the effects of metformin on bone, providing a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone. This could be particularly relevant when considering treatment options for DM in the context of diabetic osteopathy.

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INTRODUCTION

Diabetes mellitus (DM) is a highly prevalent global disease associated with long-term microvascular and macrovascular complications. Over the past 30 years, an increasing body of experimental and clinical evidence has reported the association of type 1 and type 2 DM with osteopenia, osteoporosis and an increased incidence of low-stress fractures, in what has been called diabetic osteopathy[1]. Many adult patients with type 1 DM show mild osteopenia, with a decrease in bone mineral density (BMD) of around 10%[2] that would be expected to double the risk of non-vertebral fragility fractures[3]. However, the incidence of low-stress fractures in type 1 DM is 7-12 times that of age-matched non-diabetic individuals[4,5]. On the other hand, patients with type 2 DM tend to have normal or even moderately elevated BMD. Although this would be expected to reduce their incidence of osteoporotic fractures, they actually show a 2-fold increase in hip, extremity and vertebral fractures[3-7]. Taken together, these clinical observations are considered to be evidence for a significant decrease in bone quality of patients with both types of DM[8] that would explain their increase in low-stress fractures.

Several mechanisms have been proposed to explain diabetic osteopathy, such as disturbed glucose metabolism, tissue (bone) and systemic low-grade inflammation, changes in the secretory pattern of growth factors and/or cytokines, increased oxidative stress and excess accumulation of advanced glycation end products (AGEs). In particular, excess accumulation of AGEs in bone extracellular matrix (ECM) occurs as a function of aging and duration of Diabetes, and has been found to impair the mechanical properties of bone[9]. Poorly compensated DM elevates circulating reactive oxygen species (ROS), glucose and/or carbonyl stress, which can induce excess AGEs formation on bone ECM, reducing bone strength and post-yield properties. Additionally, collagen-AGEs interact with the receptor for AGEs (RAGE) expressed by osteoblasts and osteoclasts, inhibiting their functionality and decreasing bone turnover. This induces an even greater accumulation of AGEs in bone that contributes to diabetic osteopathy, and can increase fracture risk[1].

Treatment of patients with DM can include either an absolute requirement for exogenous insulin (type 1 DM), or relative requirement of glucose-lowering medication such as insulin and/or oral drugs (type 2 DM). Oral glucose-lowering agents fall into different classes that include (but are not limited to) insulin secretagogues, insulin sensitizers, incretin-based treatments and inhibitors of renal proximal tubule glucose reabsorption. Each class operates through distinct biological pathways and has certain advantages as well as disadvantages. Recently, several commonly prescribed oral medications for type 2 DM have been found to secondarily affect bone metabolism, in some cases modifying the incidence of fragility fractures[10]. Depending on their specific skeletal effects these drugs could either help to prevent diabetic osteopathy, or contribute to worsen this complication of DM.

Members of the thiazolidinediones (TZD) family of insulin-sensitizers such as rosiglitazone have been shown to be detrimental for bone health. In rodents, TZD increase the adipocytic commitment of mesenchymal stem cells (MSC) while decreasing their osteogenic potential, *via* a decrease in the Runx2/PPARγ ratio. This increases bone marrow adiposity and promotes bone loss[11]. In the ADOPT clinical trial[12], results showed a higher risk of fracture in diabetic women, but not men, on rosiglitazone monotherapy.

Post-prandial incretin secretion is believed to play a physiological role linking nutrient ingestion to suppression of bone resorption and stimulation of bone formation. Thus, incretin-based treatments would be expected to show anabolic effects on bone, as has been suggested in a recent meta-analysis[13]. However, this may not be so in certain cases such as the DPP4 inhibitor saxagliptin, which has been found to impair MSC osteogenic potential and bone micro-architecture in rodent models[14].

Inhibitors of the sodium glucose cotransporter 2 such as dapagliflozin and canagliflozin, that decrease plasma glucose and body weight by impairing proximal tubule glucose reabsorption, have recently been associated with alterations in mineral metabolism and with an increase in bone fractures. This undesirable effect is probably due to the fact that these drugs can induce hyperphosphataemia and an increase in fibroblast growth factor-23 and para-thyroid hormone levels[15].

Metformin is an insulin-sensitizing biguanide that was developed several decades ago; however, it is still the most widely used oral anti-diabetic medication, particularly since the United Kingdom Prospective Diabetes Study demonstrated its efficacy for reducing macrovascular complications in obese type 2 DM patients[16]. Although the exact mechanism of action for metformin is still incompletely understood, in various tissues and organs it improves glucose metabolism *via* activation of the ubiquitously expressed AMP-activated protein kinase (AMPK)[17,18]. AMPK subunit expression and activation is tissue-specific, with the α1 subunit

accounting for most of bone AMPK[19]. Over the last 10 years, pre-clinical and clinical evidence has accumulated pointing to an anabolic effect of metformin on bone, in part due to AMPK activation.

AMPK-A KEY ENERGY SENSOR

AMPK is a Ser/Thr protein kinase that modulates multiple metabolic pathways and acts as a sensor of the cellular energy status[20]. AMPK is a heterotrimer of three subunits. The α subunit holds the catalytic domain with a Ser/Thr kinase domain (KD). It contains a Thr172 residue, critical for the kinase activity. AMPK-α also contains a regulatory domain, which interacts with the KD of the unphosphorylated inactive form. The β subunit contains a carbohydrate-binding module and acts as a scaffold for the assembly of α and γ subunits. It also defines the subcellular localization of AMPK as well as its substrate specificity. The γ subunit contains four cystathionine-β-synthase (CBS) domains, which bind adenine nucleotides. Expression of all three subunits is required for AMPK activity. The mechanism of activation first requires the reversible phosphorylation of the α subunit; then after AMP binding to the CBS domain of γ subunit, an allosteric stimulatory effect occurs. Several upstream kinases (AMPKK) are involved in the phosphorylation of AMPK, such as liver kinase B1 (LKB1) and Ca^{2+}/calmodulin-dependent protein kinase kinase beta (CaMKKβ). Activation of AMPK can occur by two mechanisms: One is mediated by an increase of AMP/ATP ratio (for instance during exercise), so this mechanism coordinates anabolic and catabolic pathways to equilibrate nutrient supply with energy expenditure[21]. Several compounds can activate AMPK by this mechanism: 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), H_2O_2, MAPK inhibitors, TZD, leptin, adiponectin and α-lipoic acid[22]. In the second mechanism, AMPK activation can be independent of AMP/ATP ratio and involves alternative AMPK regulation. Agents such as peroxynitrite, metformin, estradiol, low glucose levels and several membrane receptor agonists can induce AMPK activation by this way.

It has recently been demonstrated that AMPK can participate in the control of whole-body energy homeostasis by integrating signals from diverse cellular environments[23]. AMPK participates in several physiological events, such as survival, growth and development. This kinase could also be implicated in pathological conditions such as type 2 DM, insulin resistance, cardiovascular disease and cancer. For instance, AMPK can control epigenetic processes in certain cells to avoid reproductive defects in their subsequent generations[24]; or it can suppress proinflammatory-signalling pathways in adipocytes[25]. It has recently been reported that in patients with insulin resistance, AMPK is depressed in adipose tissue; an effect that is associated with oxidative stress, increased expression of inflammatory cytokines and decreased expression of genes regulating oxidative phosphorylation. On the contrary, AMPK activation increases mitochondrial biogenesis[26]. AMPK can also inhibit the proliferation of muscle stem cells, and induce the differentiation of endothelial cell progenitors[21].

ROLE OF AMPK IN CELL METABOLISM

AMPK functions as an intracellular sensor regulating energy balance in different cell types, and thus can regulate diverse metabolic pathways (Table 1).

In the liver, AMPK acts as a "metabolic master switch"[27]; its activation inhibits energy-consuming pathways and stimulates ATP-producing catabolic pathways. For instance, after AMPK activation in a fasting state, fatty acid synthesis is inhibited while mitochondrial oxidative phosphorylation is stimulated. These effects occur by a reduction in malonyl-CoA content that is mediated by inhibition of Acetyl-CoA carboxylase. In addition, malonyl CoA decarboxylase is activated, thus further increasing fatty acid oxidation. Activation of AMPK also suppresses glucose production (gluconeogenesis), as has been demonstrated in metformin-treated primary hepatocyte cultures[17]. This has been confirmed in mice with a liver-selective deletion of the *AMPKα2* gene, which exhibit hyperglycaemia and glucose intolerance in the fasting state[27].

In skeletal muscle, AMPK regulates energy expenditure during exercise in order to optimize and enhance energy production. It participates in the transition from more glycolytic fibres to more oxidative fibres, following exercise training[28]. A role for AMPK in the myocytic uptake and oxidation of fatty acids has also been postulated. In addition, during exercise an increase in ATP turnover is accompanied by enhanced glucose uptake, in turn associated with an increase in myocyte plasma membrane GLUT4 expression[21]. Thus, use of metformin in patients with type 2 DM will increase their AMPK-induced glucose uptake and disposal. AMPK activation has also been postulated to induce skeletal muscle regeneration, by regulating its post-injury inflammatory response[29]. Postnatal skeletal muscle regeneration involves stem cell reprogramming that induces their proliferation, differentiation and/or self-renovation, and activation of the AMPK pathway is believed to regulate these processes[21]. Due to the regulatory effects of AMPK on integrated metabolism, activation of AMPK is considered a therapeutic target for hyperglycaemic states. For instance metformin, an anti-diabetic drug that is widely used for treatment of patients with type 2 DM, suppresses hepatic glucose production and decreases plasma glucose levels *via* activation of AMPK pathways.

The precise effects of AMPK on bone metabolism are incompletely known; however, recent evidence supports an active role for this kinase in bone physiology[30]. Several reports have demonstrated that AMPK modulates bone cell differentiation and function. In AMPKα-deleted animals, a reduction was found in

Table 1 Role of AMP-activated protein kinase activation on cell metabolism in different organs

Organ	Effect	Mechanism of action	Ref.
Liver	Inhibition of anabolic pathways	Inhibition of fatty acid synthesis	[27]
		Inhibition of gluconeogenesis	
	Stimulation of ATP synthesis	Stimulation of Mitochondrial oxidative phosphorylation	[27]
Skeletal muscle	Regulation of energy expenditure during exercise	Favours the transition from glycolitic to oxidative skeletal muscle fibers	[28]
		Regulation of myocitic uptake and oxidation of fatty acids	[21]
		Enhanced glucose uptake *via* an increase in GLUT4 expression	[21]
	Increase in skeletal muscle regeneration	Regulation of post-injury inflammatory response	[29]
		Stem cell reprogramming: Induction of proliferation, differentiation and self-renewal	[21]
Bone	Increase in osteoblastogenesis	Increases MSC differentiation towards the osteoblastic lineage favouring Runx2 expression	[30,37]
		Decreases PPARγ expression diminishing MSC differentiation towards the adipocytic phenotype	[36,37]
	Decrease in osteoclastogenesis	Negative regulation of RANKL expression by osteoblasts	[35]

MSC: Mesenchymal stem cells; ATP: Adenosine triphosphate; GLUT4: Glucose transporter type 4; RANKL: Receptor activator for nuclear factor κB ligand.

trabecular bone mass[31]. Single α1 or α2 knockout (KO) mice are viable, but the double KO is embryonically lethal[27]. In addition, histomorphometric analysis revealed that AMPKα1 KO mice show an elevated rate of bone remodelling *in vivo*, associated with increased osteoclastogenesis *in vitro*.

In vitro experiments have demonstrated that AMPK activation enhances osteogenesis[30,32] while compound C (an AMPK inhibitor) reduces osteoblastic mineralization[30]. In other experiments AICAR (an activator of AMPK) was found to stimulate alkaline phosphatase activity (ALP) and mineral nodule formation by rat calvaria-derived cells, while compound C suppressed these effects[33]. A decrease in AMPK activity has been reported during osteoblastic differentiation; this could be associated with the high-energy requirements of maturing osteoprogenitor cells[34]. Although studies of AMPK action on bone resorption have led to conflicting results, it appears that AMPK is a negative regulator for RANKL and can thus decrease osteoclast-mediated bone resorption[35]. The AMPK pathway may also be involved in regulating the fate of bone marrow stromal cells (MSC) toward the osteoblastic or adipocytic lineage by reciprocally regulating the expression of Runx2 and PPARγ[30]. AMPK has been shown on one hand to induce phosphorylation of β-catenin, suppress PPARγ expression and thus reduce adipogenesis[36]; while on the other hand it increases Runx2 expression and thus osteoblastic differentiation of MSC[37]. This evidence suggests a critical role for AMPK in bone homeostasis.

MOLECULAR MECHANISMS OF METFORMIN ACTION

Metformin has been widely used in the United States since 1995 as an oral anti-diabetic treatment for type 2 DM[22]. Even though its precise mechanism of action is not completely known, metformin is known to activate AMPK[17]; however, AMPK-independent pathways have also been postulated[38].

Metformin can be incorporated into cells by a facilitated mechanism that is mediated by different isoforms of the organic cation transporter. Metformin induces mild and specific inhibition of the mitochondrial respiratory chain complex in hepatocytes and other tissues[39]; it can also inhibit the mitochondrial production of ROS. Inhibition of mitochondrial activity induces a decrease in the cell energy status, which in turn triggers depletion of ATP and a diminished ATP/AMP ratio. This effect then induces phosphorylation and activation of AMPK *via* LKB1. Metformin also induces an acute inhibition of gluconeogenesis: this can be explained by the decrease in ATP/AMP ratio, which inhibits key enzymes of the gluconeogenic pathway such as fructose-1,6-bisphosphatase. New evidence suggests that this metformin-induced inhibition of glucose production could also be mediated by a down-regulation of gluconeogenic genes *via* a transcription-independent process. It has been suggested that reduction in energy status, but not AMPK activation, is critical for metformin inhibition of hepatic glucose production[38].

In addition to its effects in the liver, metformin can also affect other organs *via* multiple molecular mechanisms. One important action of metformin is its reduction of endothelial activation and of athero-genesis[16]. Metformin decreases intracellular ROS production in endothelial cells by inhibiting both NADPH oxidation and the respiratory chain complex[40], and this effect appears to be independent of AMPK activation.

METFORMIN MEETS BONE

Over the past decade, several research groups have investigated the effects of metformin on bone. The results of these studies are discussed in detail below. They have provided a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone, which could be particularly relevant when considering treatment

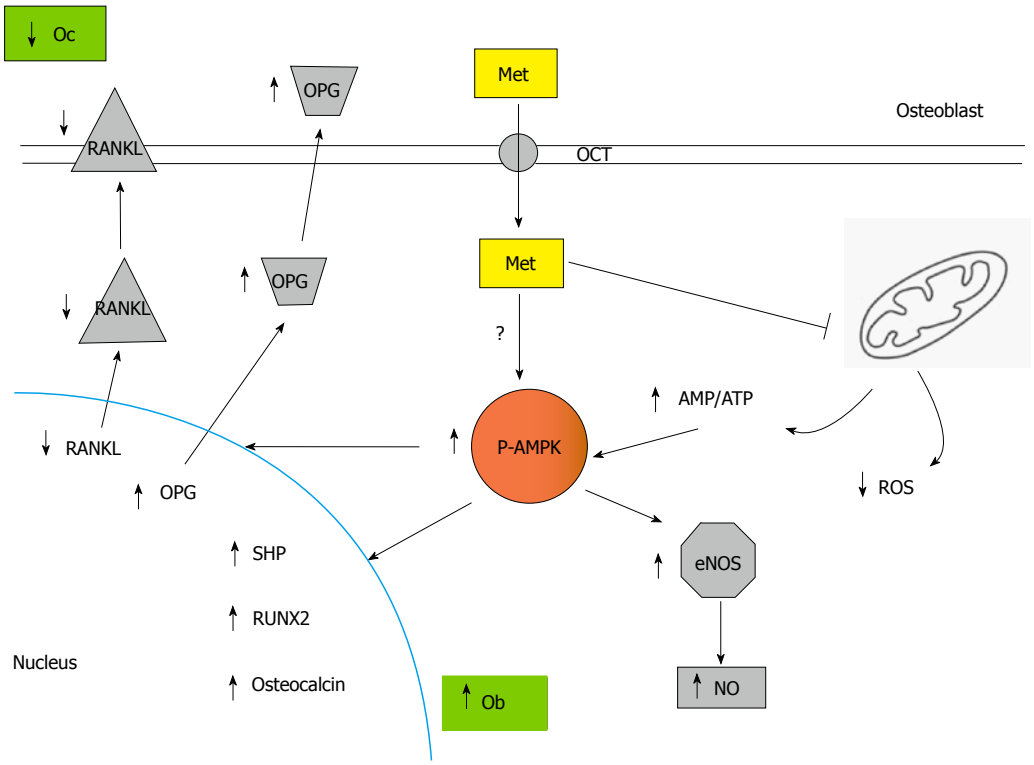

Figure 1 Metformin actions *via* osteoblasts are pro-osteogenic and anti-resorptive. Metformin is incorporated into osteoblasts, where it inhibits intracellular ROS production and induces AMPK phosphorylation/activation. This increases eNOS activity and NO production, promoting osteoblast proliferation. In addition, activated AMPK up regulates osteoblastic differentiation and mineralization *via* expression of Runx2 and SHP, while decreasing osteoclastic recruitment and bone-resorbing activity through a reduction in osteoblastic RANKL/OPG ratio. ROS: Reactive oxygen species; AMPK: AMP-activated protein kinase; OPG: Osteoprotegerin; RANKL: Reduced receptor activator of nuclear factor-κB ligand; SHP: Small heterodimer partner; RUNX2: Runt-related transcription factor 2; AMP: Adenosine monophosphate; ATP: Adenosine triphosphate; Met: Metformin.

options for DM in the context of diabetic osteopathy.

In vitro effects of metformin on bone cells

Metformin has been found to modulate the physiology of osteoblasts (Figure 1) and osteoclasts, as well as influencing the phenotypic progression of bone MSC.

Cortizo *et al*[41] were the first to describe an *in vitro* effect of metformin on bone-derived cells, showing that it dose-dependently increased osteoblastic proliferation, differentiation and mineralization. This effect was mediated by activation of extracellular-regulated kinases and by induction of NO synthases. Several researchers have corroborated these results[19,42-44], additionally showing that the osteogenic *in vitro* action of metformin on osteoblasts is dependent on activation of the AMPK signalling pathway and subsequent bone morphogenetic protein-2 production. In an interesting mechanistic study, Jang *et al*[45] found that metformin increased the osteoblastic transcription of small heterodimer partner (SHP) and osteocalcin genes, an effect that was inhibited either by a dominant negative form of AMPK or by compound C. They also found that metformin-induced *SHP* gene expression was mediated by upstream stimulatory factor-1 (USF-1), that AMPK activation increased the expression of Runx2 and that SHP interacts physically and forms a complex with Runx2 on the osteocalcin gene promoter in osteoblastic cells. Thus, metformin appears to enhance osteoblast differentiation through the transactivation of Runx2 *via* the AMPK/USF-1/SHP regulatory cascade[45]. In another study, Mai *et al*[46] found that metformin dose-dependently stimulated osteoprotegerin (OPG) and reduced receptor activator of nuclear factor-κB ligand (RANKL) mRNA and protein expression by cultured osteoblastic cells, a potentially anti-osteoclastogenic effect that they were able to block by inhibition of AMPK[46].

Since one of the proposed mechanisms for diabetic osteopathy is hyperglycaemia-mediated accumulation of AGEs on bone collagen, and AGEs can decrease osteoblastic maturation and survival *via* binding to their receptor RAGE, Schurman *et al*[47] investigated whether this process could be modulated *in vitro* by metformin. They found that metformin was able to prevent the increase in apoptosis, caspase 3 activity, inhibition of ALP and alterations in intracellular oxidative stress induced by AGEs in osteoblastic cells, *via* a metformin-dependent down regulation in the osteoblastic expression of RAGE[47]. In another study, Zhen *et al*[33] evaluated whether metformin could prevent the anti-proliferative effect of high-glucose exposure on primary osteoblasts in culture. They found that incubation with metformin decreased the high-glucose-induced intracellular ROS production and apoptosis, and that it additionally induced an osteogenic effect on osteoblasts that was mediated by an increase in *Runx2* and *IGF-1*

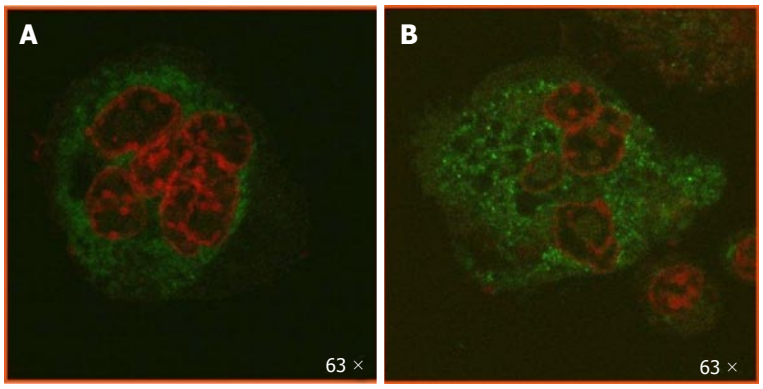

Figure 2 Metformin induces an increase and redistribution of activated AMP-activated protein kinase in multinucleated osteoclasts. UMR106 osteoblasts and Raw 264.7 macrophages were co-cultured for 7 d, in the absence (A) or presence (B) of 500 mol/L metformin. Cells were fixed, permeabilized and incubated with an anti-phosphorylated AMPK antibody, followed by a FITC-conjugated secondary antibody (green). Nuclei were counterstained with propidium iodide (red). Cells were visualized with a Leica TSC SP5 AOBS confocal microscope. Metformin induced an increase in activated AMPK with a punctillate and predominantly cytoplasmic distribution. AMPK: AMP-activated protein kinase.

gene expression[33]. These results have been recently confirmed by other investigators[48].

Three different studies have investigated the *in vitro* effects of metformin on stromal cells isolated from bone marrow (MSC). In the first report, Gao *et al*[49] found that metformin increased Runx2 and decreased PPARγ expression, and consequently stimulated ALP and mineralization while inhibiting the intracellular accumulation of lipid droplets. These results suggest that metformin could influence the reciprocal relationship between osteoblastic and adipogenic differentiation of MSC, tipping the balance towards osteogenesis. Molinuevo *et al*[37] further demonstrated that metformin could induce an *in vitro* dose-dependent increase in MSC osteogenic potential (ALP, type 1 collagen secretion and mineralization). They also found that metformin dose-dependently prevents rosiglitazone-induced intracellular lipid accumulation by MSC[37]. In another study, Sedlinsky *et al*[50] demonstrated that the *in vitro* osteogenic effect of metformin on MSC is AMPK-dependent, and that it can be completely blocked by the AMPK inhibitor compound C.

Metformin has also been found to modulate *in vitro* osteoclast recruitment, differentiation and bone-resorbing activity in some[35,46,51] but not all[52] published reports. OPG and RANKL are predominantly secreted by osteoblasts and play critical roles in osteoclast physiology. As stated above, *in vitro* experiments have shown that metformin increases OPG and reduces RANKL mRNA and protein expression by osteoblasts, which is potentially anti-osteoclastogenic. Additionally, when a macrophage cell line was incubated with the supernatant of osteoblasts treated with metformin, this reduced the formation of tartrate resistant acid phosphatase (TRAP)-positive multi-nucleated osteoclasts[46]. In another interesting *in vitro* study, AMPK was found to be expressed by bone marrow pre-osteoclasts and as such is a regulatory target for osteoclast differentiation and resorptive activity. Pharmacological inhibition of pre-osteoclastic AMPK with compound C increased the RANKL-induced formation of TRAP-positive multinucleated cells and their resorptive activity on dentine discs, *via* downstream activation of p38, JNK, NF-κB, Akt, CREB, c-Fos, and NFATc1. On the contrary, metformin dose-dependently suppressed formation of TRAP-positive multinucleated cells and dentine resorption[35]. In unpublished results using indirect immunofluorescence, we have found a metformin-induced increase and sub-cellular redistribution in phosphorylated (activated) AMPK of multinucleated osteoclasts obtained from osteoblast-macrophage co-cultures (Figure 2), which could be mediating the effects of metformin in this cell type.

All in all, these *in vitro* results point to a global bone-anabolic effect of metformin: Tipping the phenotypic balance of bone MSC towards osteoblastogenesis, increasing the bone-forming capacity of osteoblasts, and decreasing the recruitment and bone-resorbing activity of osteoclasts (Figure 3). These findings are further supported by *in vivo* and *ex vivo* (pre-clinical) as well as clinical evidence, pointing to an osteogenic action of metformin in the context of DM.

In vivo and ex vivo effects of metformin on bone metabolism: Animal models

Most studies using animal models (but not all) have shown beneficial actions of metformin on bone metabolism and on bone lesion repair (Figure 4).

Molinuevo *et al*[37] demonstrated an *ex vivo* osteogenic effect of metformin: *i.e.*, that bone MSC obtained from rats after a 2-wk treatment with oral metformin, exhibit increased osteogenic potential (Runx2 expression, ALP activity, type 1 collagen production, osteocalcin synthesis, and mineral nodule deposition) *vs* MSC obtained from non-treated animals. In addition, these metformin-induced effects were found to be secondary to AMPK activation. In this study, metformin treatment also stimulated the repair of a minimal parietal lesion *in vivo*, both in diabetic and non-diabetic rats.

As stated above, rosiglitazone is a TZD that induces

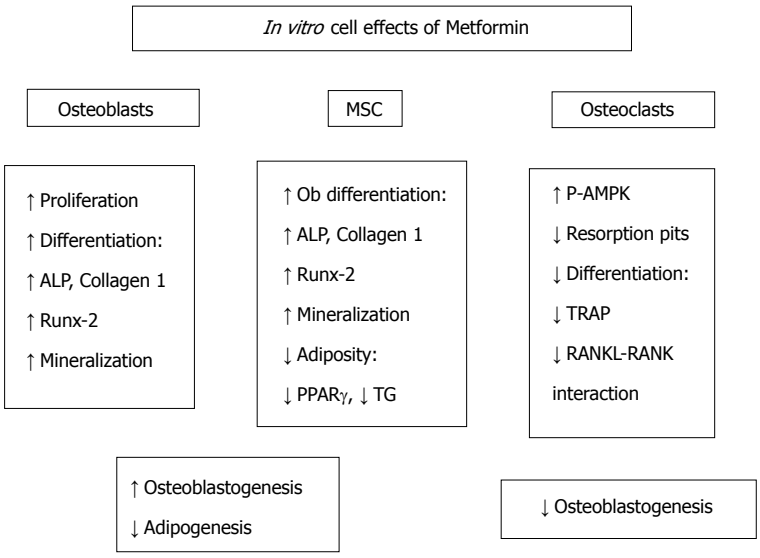

Figure 3 Effects of metformin on bone-derived cells. The results of several in vitro studies show that metformin modulates the phenotypic balance of bone marrow stromal cells (MSC) away from adipogenesis and towards osteoblastogenesis. In addition, metformin increases *in vitro* the bone-forming capacity of osteoblasts, while decreasing the recruitment and bone-resorbing activity of osteoclasts. ALP: Alkaline phosphatase; TG: Triglycerides; TRAP: Tartrate-resistant acid phosphatase; RANK: Receptor activator for nuclear factor κB; RANKL: RANK ligand.

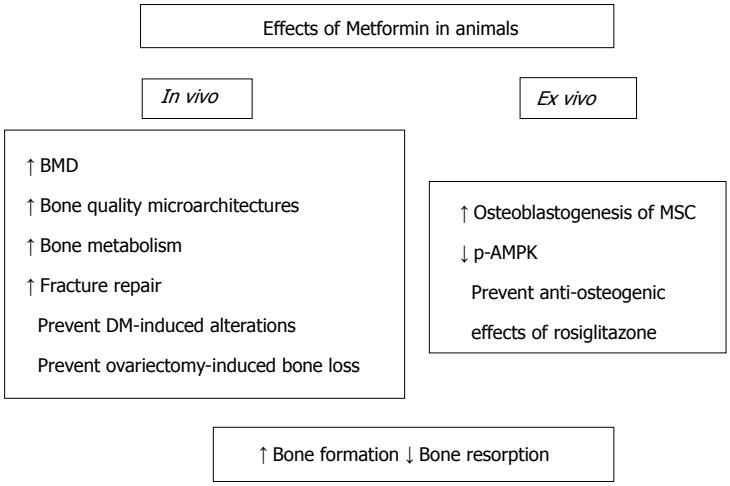

Figure 4 Actions of metformin on bone metabolism - animal studies. Orally administered metformin promotes the osteogenic potential of bone MSC, increases the quality of bone tissue (improving its micro-architecture and mineral density) and facilitates the repair of bone lesions. In addition, metformin may prevent experimental diabetic osteopathy as well as ovariectomy-induced bone loss. MSC: Marrow stromal cells; BMD: Bone mineral density; p-AMPK: Phosphorylated AMP-activated kinase.

deleterious effects on osteoblast differentiation[53] and on osteocyte survival[54], diverting MSC differentiation toward the adipocyte lineage. In view of the opposite effect that has been demonstrated for metformin on these cell types, Sedlinsky *et al*[50] investigated the effect of a 2-wk metformin/rosiglitazone combined oral treatment of rats on long-bone metaphyseal microarchitecture, minimal parietal lesion repair and MSC osteogenic potential. Compared to untreated controls, rosiglitazone monotherapy decreased femoral metaphysis trabecular area, osteoblastic and osteocytic density, and TRAP activity associated with epiphyseal growth plates. In addition, it greatly diminished bone repair. It also decreased the *ex vivo* osteogenic potential of MSC, inducing an increase in PPARγ and a decrease in Runx2 expression, as well as a decrease in phosphorylated (active) AMPK. Metformin/rosiglitazone co-treatment prevented all the *in vivo* (bone repair and diaphyseal microarchitecture) and *ex vivo* anti-osteogenic effects of rosiglitazone monotherapy, with a reversion back to control levels of PPARγ, Runx2 and AMPK phosphorylation in MSC[50].

In another study, the skeletal (femoral) effects of rosiglitazone were compared to those of metformin in insulin-resistant female C57BL6J ob/ob mice. The metformin-treated group showed higher BMD, higher trabecular bone volume/total bone volume, higher osteoid width and mineral apposition, lower trabecular spacing and lower bone marrow adiposity, when compared with the rosiglitazone-treated group[55].

Gao *et al*[56] studied the *in vivo* effect of oral metformin on bone mass in ovariectomized rats. They found that metformin dose-dependently reverted ovariectomy-induced bone loss, showing an improvement in BMD measured by DEXA, and in bone microarchitecture measured both by micro CT and by bone histology. By real-time PCR of MSC, they found a metformin-dependent increase in Runx2 and Lrp5 (co-receptor for Wnt) expression, both of which are involved in osteoblastic proliferation and differentiation[56].

In another report, Jeyabalan *et al*[57] studied either ovariectomized C57BL/6 mice or young Wistar rats to evaluate the effect of oral metformin on bone metabolism and fracture repair, respectively. In both models, metformin did not modify bone microarchitecture or cellular activity *in vivo* as evaluated by micro-CT and bone histomorphometry. In addition, metformin had no significant effect on the repair of a midshaft femoral fracture in Wistar rats[57].

Mai *et al*[46] further investigated the effects on bone of an oral metformin treatment in ovariectomized (OVX) adult rats. They found that metformin treatment of OVX animals significantly increased total body BMD, enhanced bone mineral content and decreased trabecular separation; supporting the concept that metformin can prevent OVX-induced bone loss. The authors also found that metformin reverted the OVX-associated increase in TRAP-positive osteoclasts of proximal tibiae resorption pits. Metformin treatment also increased serum OPG, and decreased RANKL expression by MSC, in OVX rats. Further *in vitro* experiments showed that these effects were regulated by AMPK and by its upstream activator CaMKK.

In a rat model of partially insulin-deficient nicotinamide/streptozotocin-induced DM, tibia histomorphometry showed a diabetes-induced decrease in trabecular bone volume, osteocyte density, growth plate height and osteoclast (TRAP positive) activity in the primary spongiosa, as well as an increase in bone marrow adiposity. MSC from diabetic animals showed a decrease in their osteoblastic potential, an increase in adipocytic commitment, a reduction in their Runx2/PPARγ ratio and an increased expression of the AGEs receptor RAGE. A 2-wk oral treatment with metformin prevented all these Diabetes-induced alterations in bone micro-architecture and MSC osteogenic potential, and also induced a down-regulation of RAGE expression by MSC[58].

Clinical evidences of metformin effects on bone

There are few published clinical studies reporting the skeletal effects of metformin. In addition, randomized placebo-controlled studies in humans that evaluate the effects of metformin on bone metabolism as a primary end point are so far unavailable. The results of published clinical reports are summarized in Table 2.

Several epidemiological studies have reported the effects of diabetes and antidiabetic agents on bone fracture risk. In 2005, Vestergaard *et al*[59] published a Danish population-based study evaluating risk of fractures and its relationship with T1DM and T2DM and anti-diabetic agents. They found that both T1DM and T2DM patients had a significant increase in bone fracture risk, and that the use of metformin was associated with a significantly decreased risk for fracture at any site.

Melton *et al*[6] conducted another population-based study in Rochester, United States, to evaluate fracture risk factors in T2DM patients. They found that patients had an increased risk of hip fracture after 10 years of DM, and that use of biguanides such as metformin was protective even after adjusting for other risk factors (HR, 0.7; 95%CI: 0.6-0.96).

Monami *et al*[60] conducted a case-control study, nested within a retrospective cohort, comparing 83 case subjects with a history of bone fractures and 249 control subjects, in all cases exposed to insulin, insulin secretagogues or metformin treatment for the past 10 years, in order to assess the risk for bone fractures associated with exposure to insulin or different oral hypoglycaemic agents. This study was unable to demonstrate a reduction in bone fractures associated with metformin treatment, but showed an increased rate of fractures in patients on insulin treatment, probably related to worse diabetes control or to hypoglycaemic episodes. Nevertheless, the authors acknowledged that the lack of a statistically significant fracture reduction associated with metformin treatment was probably related to an insufficient sample size.

The ADOPT study (A Diabetes Outcome Progression Trial), that compared the glycaemic effects of rosiglitazone, metformin and glyburide, showed that among the adverse effects of rosiglitazone was an increased risk of fracture in women. At the same time they showed that metformin had a lower risk of fracture, both in women and men, for every skeletal site assessed[12]. In an add-on report to the ADOPT study, C-telopeptide levels (CTX, a bone resorption marker) were found to be reduced by metformin treatment and increased in rosiglitazone-treated patients, suggesting that changes in bone resorption may be partly responsible for the differences in fracture risk observed for both treatments[61].

A randomized, parallel group, double-blind, multicentre study comparing the efficacy and safety of rosiglitazone/metformin co-treatment (RSG/MET) *vs* metformin monotherapy (MET) was conducted in order to assess glycaemic control and BMD after 80 wk of treatment in drug-naïve T2DM patients. Although the RSG/MET combination was superior to MET in achieving significant reductions in glycated haemoglobin and fasting plasma glucose, RSG/MET was associated with a significantly lower BMD in comparison with MET at week 80 in the hip and lumbar spine[62].

In another study, Hegazy[63] evaluated the possible anti-osteoporotic effect of metformin *vs* sitagliptin in 40 post-menopausal diabetic women. They were randomly divided into two groups, one receiving 500 mg

Table 2 Clinical evidences of metformin effects on bone

Study design	Study population	*n*	Outcome	Ref.
Case control study	All subjects with bone fracture in Denmark (year 2000), *vs* 3-fold controls	124655 fracture patients 373962 control	Fracture risk = 0.81 (95%CI: 0.70-0.93)[1] (for metformin)	[59]
Cohort Study	Rochester residents first meeting Diabetes glycaemic criteria (1970-1994)	1964 diabetic patients	Fracture risk = 0.7 (95%CI: 0.6-0.96)[2] (for metformin)	[6]
Case control study	A study nested within a cohort of 1945 diabetic Tuscany outpatients (1998-2004)	83 fracture patients 249 control	Fracture risk = 0.60 (95%CI: 0.34-1.08)[3] (for metformin)	[60]
Double-blind, randomized, controlled clinical trial	Recently diagnosed, drug-naïve patients with type 2 diabetes, treated for a median of 4 yr with rosiglitazone, metformin, or glyburide	Rosiglitazone: *n* = 1456; Metformin: *n* = 1454; Glyburide: *n* = 1441	N° Fractures (%): Rosiglitazone 60 (9.30) Metformin 30 (5.08)[4] Glyburide 21 (3.47)[4]	[12]
Double-blind, randomized, controlled clinical trial	Recently diagnosed, drug-naïve patients with type 2 diabetes, treated for a median of 4 yr with RSG, MET, or GLY	Paired baseline and 12-mo stored serum samples from 1605 patients	In women, CTX increased by 6.1% with RSG, decreased by 1.3% with MET (P = 0.03) In men, CTX was unchanged on RSG (-1.0%) and fell with MET -12.7% (P = 0.001)	[61]
Randomized, parallel group, double-blind, multicentre study	Drug naïve, male and female patients who had an established clinical diagnosis of type 2 diabetes mellitus	688 patients equally randomized to RSG/MET or MET	BMD at week 80: Lumbar = (-2.2) (95%CI: -3.5, -0.9) Total hip = (-1.5) (95%CI: -2.3, -0.7)[5]	[62]
Prospective randomized study with active comparator study	Forty postmenopausal diabetic women recruited from Tanta University Hospitals	20 patients on metformin and 20 on sitagliptin, for 12 wk	BMD was unchanged in both groups at week 12 Bone turnover markers remained unchanged from baseline in MET	[63]
Prospective randomized double-blind, double-dummy with active comparator	Men with uncomplicated type 2 diabetes mellitus, aged 45-65 yr	71 men were randomized to PIO once daily or MET twice daily	Sclerostin levels at week 24 increased by 11% in PIO-treated patients and decreased by 1.8% in MET-treated patients (P = 0.018)	[64]

[1]Relative risk of any fracture interpreted as OR with 95%CI for several variables in the population of Denmark (National Health Registry, year 2000); [2]Multivariate HR for the development of any new fracture among 1964 Rochester, MN, United States residents after recognition of diabetes mellitus in 1970-1994; [3]Exposure for at least 36 mo to hypoglycemic treatments in case subjects and matched control subjects, interpreted as OR with 95%CI; [4]$P < 0.01$ for comparison of fracture risk in women with rosiglitazone (unadjusted, contingency χ^2 test); [5]Percentage of change in BMD at week 80, comparing RSG/MET *vs* MET. RSG: Rosiglitazone; MET: Metformi; GLY: Glyburide; PIO: Pioglitazone; BMD: Bone mineral density; OR: Odds ratio; HR: Hazard ratios.

metformin twice a day, and the other 100 mg sitagliptin once a day, for 12 wk. In the metformin-treated group, serum ALP and urinary D-piridinoline (DPD) were not significantly different from baseline; conversely in the sitagliptin group, serum ALP and urinary DPD decreased significantly after 12 wk, although BMD was unchanged in both groups.

The effects of pioglitazone and metformin on circulating sclerostin (an osteocyte-derived osteoblast proliferation inhibitor), and biochemical markers of bone turnover were studied in 71 men with T2DM. This group as a whole showed higher serum sclerostin levels than healthy controls. Sclerostin levels were further increased in the sub-set of patients that were treated with pioglitazone, who also showed an increase in serum CTX. On the contrary, metformin-treated patients *vs* healthy controls showed significantly lower sclerostin levels and unchanged CTX levels[64]. Although sclerostin is a well-established inhibitor of bone formation, recent evidence indicates that it can also promote osteoclastogenesis by stimulating RANKL produced by osteocytes[65], suggesting that pioglitazone could increase bone resorption while decreasing bone formation, and the opposite would occur with metformin.

Finally, metformin was tested for bone-defect healing purposes in a clinical study, adding this biguanide to platelet-rich fibrin in order to treat intrabony defects in patients with chronic periodontitis. The study was designed to evaluate the efficacy of platelet-rich fibrin, 1% metformin gel, or platelet-rich fibrin plus 1% metformin gel, in all cases with open flap debridement, for treatment of intrabony defects in 120 patients with chronic periodontitis. The group treated with platelet-rich fibrin plus 1% metformin gel showed the greatest improvements in clinical parameters, with an increase in percentage radiographic defect depth reduction when compared to metformin alone, platelet-rich fibrin alone or open flap debridement alone[66].

CONCLUSION AND PERSPECTIVES

Patients with long-term T1 DM and T2 DM can develop skeletal complications or "diabetic osteopathy". These include osteopenia, osteoporosis and an increased incidence of low-stress fractures. In this context, it is important to evaluate whether current anti-diabetic treatments can secondarily affect bone metabolism. Over the past 10 years, many investigators have studied the effects of metformin on bone, providing a considerable body of pre-clinical (*in vitro*, *ex vivo* and *in vivo*) as well as clinical evidence for an anabolic action of metformin on bone. However three reports (one *in vitro*, one *in vivo*, one clinical) have been unable to link

metformin treatment with bone anabolic processes, underscoring the differences that exist between experimental models in pre-clinical studies, and the low statistical potency inherent in clinical reports that include a relatively small number of patients. In this sense, two caveats should be kept in mind when considering metformin treatment for a patient with T2DM at risk for diabetic osteopathy. In the first place, metformin should probably not be considered an anti-osteoporotic drug; it is an insulin sensitizer with proven macrovascular benefits that can secondarily improve bone metabolism in the context of DM. Secondly, we are still awaiting the results of randomized placebo-controlled studies in humans that evaluate the effects of metformin on bone metabolism as a primary endpoint.

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MINIREVIEWS

Infantile onset diabetes mellitus in developing countries - India

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Abstract

Infantile onset diabetes mellitus (IODM) is an uncommon metabolic disorder in children. Infants with onset of diabetes mellitus (DM) at age less than one year are likely to have transient or permanent neonatal DM or rarely type 1 diabetes. Diabetes with onset below 6 mo is a heterogeneous disease caused by single gene mutations. Literature on IODM is scanty in India. Nearly 83% of IODM cases present with diabetic keto acidosis at the onset. Missed diagnosis was common in infants with diabetes (67%). Potassium channel mutation with sulphonylurea responsiveness is the common type in the non-syndromic IODM and Wolcott Rallison syndrome is the common type in syndromic diabetes. Developmental delay and seizures were the associated co-morbid states. Genetic diagnosis has made a phenomenal change in the management of IODM. Switching from subcutaneous insulin to oral hypoglycemic drugs is a major clinical breakthrough in the management of certain types of monogenic diabetes. Mortality in neonatal diabetes is 32.5% during follow-up from Indian studies. This article is a review of neonatal diabetes and available literature on IODM from India.

Key words: Infants; Diabetes mellitus; Monogenic diabetes; Co-morbid state; Mortality

Core tip: The clinical presentation of infantile onset diabetes mellitus (IODM) as syndromic and non-syndromic forms from South India is discussed in this article. Associated co-morbid states, mortality pattern, difficulty in the management and need for genetic evaluation among this group of infants are also discussed. Identification of this form of monogenic diabetes by clinical evaluation and appropriate genetic evaluation to identify the subtypes helps in the management of these infants to improve the overall morbidity and mortality. Reported mortality in IODM is high from South India.

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INTRODUCTION

Infantile onset diabetes mellitus (IODM) is a rare form of diabetes with onset at less than one year of age. Onset of diabetes in the first 6 mo of life is termed neonatal diabetes[1]. The majority of neonatal diabetes cases are monogenic. However, recent reports have suggested evaluation for monogenic diabetes among those with onset in later infancy (onset between 6 mo and 1 year), too[2]. The two phenotypes of neonatal diabetes include transient neonatal diabetes mellitus (TNDM) and permanent neonatal diabetes mellitus (PNDM). The reported incidence of neonatal diabetes varies from 1 in 200000 to 1 in 400000[3]. Infants with PNDM continue to require insulin for maintaining euglycemia. TNDM usually resolves by 18 mo of age. In a study from Chennai in India, the incidence of infantile onset diabetes was 7.9% among all diabetic children in a pediatric diabetic clinic[4]. Another hospital based retrospective study from South India revealed the incidence to be 7%[5]. A recent international cohort study used comprehensive genetic testing to identify causal mutations which were found in nearly 80% of samples in neonatal diabetes[6]. This indicates that genetic diagnosis results in a phenomenal change in the management of infantile onset diabetes.

Genetics of TNDM

Among the neonatal diabetes cases, 50%-60% of affected neonates are due to TNDM based on the Western literature[7,8]. However, in a study from Chennai in India, TNDM only accounted for 5% of all neonatal DM cases[4]. TNDM is commonly due to a developmental defect in the pancreatic beta cell function. The common genetic defect (60%-70%) is due to mutations on chromosome 6q24[3,9,10]. The cause seems to be a defect in maternal methylation, most often due to recessive mutations in the *ZPF57* (Zinc Finger Protein) gene[11]. There are three types of abnormalities leading to overexpression of the paternal allele at this locus: (1) a paternally inherited duplication; (2) paternal uniparental disomy (UPD); and (3) an epimutation resulting in a complete loss of methylation of the maternal allele on chromosome 6q24[12,13]. Mutations in *KCNJ11* (Potassium channel subfamily J member 11) and *ABCC8* (ATP binding cassette transporter subfamily C, member 8) leading to about 20%-25% of TNDM cases[13-16]. Rarely mutations in *HNF 1B*, insulin gene and solute carrier 2 family 2 gene (*SLC2A2*) can result in neonatal diabetes. TNDM remits permanently or may relapse later during adulthood. Thus, TNDM may be a permanent beta cell defect with variable expression during growth and development. The clinical presentation includes hyperglycemia, dehydration, and failure to thrive with or without ketoacidosis. The associated features in chromosome 6q24 mutations include macroglossia (35%), umbilical hernia (14%) or more rarely cardiac and brain developmental defects. Infants with TNDM resulting from a K_{ATP} channel mutation are often heavier than patients with chromosome 6q24 induced TNDM at birth, are diagnosed with diabetes later, remit later and relapse earlier[17]. Chromosome 6q24 induced TNDM should be treated with insulin. Relapse due to UPD chromosome 6 mutation or *ABCC8* mutation in puberty responds well to sulphonylurea therapy[18,19]. Relapsing diabetes due to 6q24 related diabetes has been successfully treated with dipeptidyl peptidase 4 inhibitor[20]. The majority (> 90%) of TNDM cases due to *ABCC* 8/*KCNJ11* mutation respond to sulphonylurea therapy[21]. Infants born with TNDM harbour a greater risk of developing type 2 diabetes later in the life[22]. There were only few studies, and most were case reports about TNDM in the Indian literature[23-29]. Rarely encountered is type 1 diabetes in infancy especially in the non-syndromic diabetes with onset in later part of infancy[30].

Genetics of PNDM

Children with PNDM have their onset in early infancy and continue to be hyperglycemic, which needs lifelong insulin therapy. The genetic defect involves mutation of genes involving the pancreatic B cell development, function, apoptosis and insulin molecule. Nearly 40% of the defects are in the genes regulating the K_{ATP} (potassium ATP) channel. As early as 1997 mutations leading to PNDM have been described. The first is pancreatic agenesis due to mutations in *IPF*/pancreatic and duodenal homeobox 1 (*PDX1*). Between 2004 and 2007 the mutations of *KCNJ11*, *ABCC8* and *INS* genes were identified for PNDM. *KCNJ11* and *ABCC8* mutations account for nearly 40%-50% of all PNDM cases[31]. PNDM can be nonsyndromic or syndromic (associated with other systemic features). The five genes in which mutations in nonsyndromic PNDM occur include *KCNJ11* (approximately 30% of NDM cases), *ABCC8* (approximately 19%), *INS* (approximately 20%), *GCK* (approximately 4%), and *PDX1* (< 1%)[32]. The mode of inheritance of PNDM is autosomal dominant for mutations in *KCNJ11*, autosomal dominant or autosomal recessive for mutations in *ABCC8* and *INS*, and autosomal recessive for mutations in *GCK* and *PDX1*.

K_{ATP} CHANNEL MUTATION

Glucose sensing and insulin release from beta cells are a complex process. Glucose enters through GLUT receptors and generates energy in the form of ATP. This increased ratio of ATP to ADP results in closing of the potassium channels and depolarization. This activates the calcium channels and influx of calcium into cells, leading to release of insulin by exocytosis. The potassium channel subunit Kir 6.2 (potassium inward receptor) and SUR (sulphonylurea receptor 1) are encoded by genes called *KCNJ11* and *ABCCC8*. Mutations in these two genes are common in PNDM. The majority of these children respond to sulphonylurea, which acts on the potassium channels,

keeps them open and prevents depolarization[33]. These channels are present in non-pancreatic tissues like the brain, heart and skeletal muscles and this explains the associated co-morbid states like developmental delay, muscle weakness and seizures in DEND syndrome [developmental delay, epilepsy, neonatal diabetes mellitus (NDM)][34,35]. A number of patients with K_{ATP} channel mutations present with a milder phenotype without epilepsy [termed intermediate DEND (iDEND] syndrome[36-38]. Infants or children with Kir mutation respond well to sulphonylurea therapy, which is preferred than insulin as sulphonylurea therapy is associated with improvement of other systemic features along with glycemic control[39]. Among the published data from South India, out of the 9 identified mutations, 7 were due to *KCNJ11* and *ABCC8* mutations and successful switch was done in these children following genetic reports except one child with *ABCC8* mutation induced hyperinsulinemic hypoglycemia[40].

INSULIN

Insulin gene defects lead to defective folding of insulin in the endoplasmic reticulum and this affects the insulin release. They may present as NDM or MODY[41]. These infants do not have any extra-pancreatic features. The heterozygous form presents during the first 6 mo of age and 50% have keto acidosis. The more severe form with homozygous mutation presents much earlier and have low birth weight. Management includes insulin, prevention of hyperglycemia through diet and use of insulin sensitizers like metformin. Sulphonylureas are not effective. Insulin gene mutations leading to neonatal diabetes have been described even in Indian infants[40,42,43].

GLUCOKINASE

Glucokinase (GCK) is the glucose sensor of the cell. It is essential for phosphorylation of the glucose molecule that enter the cells. Mutations lead to defective glycolytic activity, and thereby the cascade leading to insulin release is affected. Homozygous infants present with NDM. They need lifelong insulin therapy. Heterozygotes may present later as MODY 2 (maturity onset diabetes in young). A homozygous *GCK* mutation has been described in an infant from Chennai[4,43]. Although *GCK* mutations in neonates do not respond well to sulphonylurea, there are a few case reports suggesting a role for glibenclamide along with insulin in children with homozygous *GCK* mutations[44,45].

PDX1

The homozygous form presents as PNDM and has both pancreatic exocrine and endocrine dysfunctions. The heterozygous form present as MODY 4[46].

Syndromic causes of PNDM are due to mutations in *GATA6*, *PTF1A*, *FOXP3*, *GLIS3*, *RFX6*, *NEUROD1*, *NEUROG3*, *HNF1B*, *PAX6*, *SLC19A2* and *WFS1* genes.

MUTATIONS IN *GATA6* GENE

This is the most common cause for pancreatic agenesis. Extra-pancreatic features are common and include structural heart defects, biliary tract and gut anomalies, and other endocrine abnormalities. Inheritance is autosomal dominant, but in most reported cases the mutations have arisen *de novo*[47].

PANCREATIC TRANSCRIPTION FACTOR 1, A SUBUNIT

This factor is essential for pancreatic development and function as well as cerebellar development. Other than pancreatic hypoplasia, cerebellar hypoplasia, microcephaly and respiratory distress may also develop[48].

FOXP3 GENE

The dysfunction of the transcription factor FOXP3 may result in X-linked-IPEX syndrome (immune dysregulation, polyendocrinopathy and enteropathy). Although autoimmune diabetes is uncommon in infancy, most of the autoimmune children may be due to *FOXP3* mutations[49]. The IPEX syndrome gene on the X chromosome, which codes for a forkhead domain-containing protein known as "scurfin", is required for immune homeostasis. These children present with intractable diarrhea with villous atrophy, exfoliative dermatitis, autoimmune hypothyroidism, hemolytic anaemia and recurrent infections. They may test positive for islet cell auto-antibodies.

GLI SUBFAMILY OF KRUPPEL-LIKE ZINC FINGER PROTEIN-3

This transcription factor is involved in various processes in multiple tissues. These infants present with multi-system involvement like diabetes, congenital hypothyroidism, congenital glaucoma, renal cysts and dysmorphic facies[50].

Other causes for syndromic neonatal diabetes include the following: *MNX1* (motor neuron and pancreas homeobox I) mutations - neonatal diabetes with developmental delay, neurogenic bladder, sacral agenesis, and imperforate anus; *NKX2* (NK homeobox 2) mutations - neonatal diabetes with developmental delay, hypotonia, hearing impairment, cortical blindness and short stature; *RFX6* mutations - pancreatic hypoplasia, intestinal atresia, and gall bladder hypoplasia[51]. Pancreatic exocrine function is normal. Inheritance is autosomal recessive; *NEUROD1* mutations - cerebellar hypoplasia, sensorineural deafness, and visual impairment[52]. Pancreatic exocrine function is normal and the inheritance is autosomal recessive; *NEUROG3* mutations - congenital malabsoptive diarrhea and the exocrine

pancreatic function may be affected[53]. Inheritance is autosomal recessive; *HNF-1β* mutations - hypo plastic pancreas and renal abnormalities. The inheritance is autosomal recessive with incomplete penetrance; *PAX 6* mutations - central nervous system phenotype such as microcephaly and panhypopituitarism. The ocular phenotype includes aniridia, keratopathy, optic nerve defects, cataracts, microphthalmia and anophthalmia. Wolfram syndrome - DM with optic atrophy, diabetes insipidus and/or deafness, usually presents a little later in life although it can present in the neonatal period. Optic atrophy and diabetes may present in the first decade of life while diabetes insipidus and deafness present later in the 3rd or 4th decade[32]; *SLC19A2* (soluble carrier family 19, member 2; thiamine transporter) mutations. Recessive mutations lead to NDM, thiamine responsive megaloblastic anemia and deafness. They may have cardiac manifestations. This is also called Rogers syndrome and is inherited as an autosomal recessive disorder[54,55]; *SLC2A2* (soluble carrier family 2 member 2) mutations - Fanconi bickel syndrome. DM with hepatomegaly, glycosuria, proteinuria, hypophosphatemic rickets are the presenting features[56]. *EIF2AK*3 (eukaryotic translation initiation factor 2 alpha kinase 3) mutations - Wolcott-Rallison syndrome. Infants with *EIF2AK* mutations present with neonatal diabetes, liver failure, growth retardation, epiphyseal dysplasia, developmental delay, hypothyroidism and renal failure. Higher mortality has been reported among these children. This is more common in infants born of consanguinity. Mortality is predominantly due to liver cell failure in these children[4,40].

The course of PNDM varies by genotype. Pancreatic agenesis/hypoplasia caused by homozygous mutations in *PDX1* results in severe insulin deficiency and exocrine pancreatic insufficiency. The morbidity and mortality vary according to the co-morbid conditions of the infant. Rarely congenital hyperinsulinemia can present with neonatal diabetes with ABCC8 mutations and is refractory to insulin therapy as in congenital lipodystrophy[57].

CLINICAL PRESENTATION OF NDM

Clinical features of TNDM and PNDM are indistinguishable. NDM often presents with hyperglycemia incidentally identified during evaluation for intercurrent illness or may present with keto acidosis. Rarely candida infection of the genitalia can be a presenting feature. A higher incidence of consanguinity was encountered in the study of 12 children with infantile diabetes from Chennai, India. Initial presentation as diabetic keto acidosis (DKA) was encountered in 83% of the study group. Mortality at 1-year follow-up was 16.6%[5]. Another study by Varadarajan *et al*[4] from South India revealed 67.5% of infants with ketotic onset. The median age at diagnosis was 3.75 mo. Based on the study published from South India only 32% of infants were diagnosed to have diabetes or DKA at presentation. Missed diagnosis was common in infants with diabetes (67.5%). Infantile onset diabetes present with a history of polyuria, polydipsia, irritability, vomiting, seizures, breathlessness, poor feeding, white discharge from genitals, or sticky urine. Low birth weight and monogenic diabetes were more common in those infants with onset at less than 6 mo of age in comparison with those with onset beyond 6 mo. Sixty-three percent of infants with onset in the first 6 mo of life were of low birth weight. Eighty-five percent of infants with onset less than 6 mo were identified to be monogenic in comparison to 55% if the onset was more than 6 mo. This study revealed Wolcott Rallison to be most common type of monogenic diabetes[4]. This is similar to the study by Ganesh *et al*[5] where 50% of the study group had Wolcott Rallison syndrome. Among the non-syndromic type KCNJ11/8 was common and among the syndromic type Wolcott Rallison syndrome was common. Ten percent (4 out of 40) of infants had transient NDM with remission of hyperglycemia in the first few months and one of them relapsed at 9.7 years of age. Children with Wolcott Rallison syndrome had a higher mortality than any other group. Hepatic failure was the most common cause of death. Co-morbid states encountered in infants with diabetes include developmental delay, seizures, hepatic involvement, hypothyroidism, optic atrophy, hepatomegaly, short stature and rickets. The mean insulin requirement was 1.19 units/kg per day in those with onset less than 6 mo or 1.4 units/kg per day in those with onset more than 6 mo. Among those children with *KCNJ11* or *ABCC8* mutation the response to oral sulphonylurea is excellent and better metabolic control has been documented even during follow-up. Other than glibenclamide, glyburide has also been tried in children with *KCNJ11* or *ABCC8* mutation. Relapsing NDM in older children do respond well to sulphonylurea without need for insulin therapy. Despite the advances of diagnosis and management of IODM, day-to-day problems exist in the management in developing countries. One of the problems of concern is the assessment of glycemic control among infants who are on 2-3 hourly breast feeds or demand feeds. Difficulty in dispensing very low doses of insulin is a problem in developing countries. Delivering insulin less than 1 unit is difficult as there are no diluents available in developing countries. Dilution with normal saline have been used in such situations but the evidence for the same to be effective is lacking. Currently available insulin pens to dispense 0.5 units may be useful in such infants. The psychological trauma to the family members and lack of adequate support in the community through a structured diabetic care team makes day-to-day management of diabetes difficult in these infants. Frequent hospitalization, glycemic fluctuations, poor weight gain and co-morbid conditions were other problems reported in studies from South India[4]. Literature reveals continuous subcutaneous infusion of insulin as a useful intervention, although

affordability and cost are limiting factors in developing countries. Limited availability of genetic studies in India may cause delay in the diagnosis of monogenic diabetes. Reported mortality in infantile onset diabetes is very high in developing countries. The mortality at 1-year follow-up was 16%[5]. However, another study of 40 infants with diabetes from South India had revealed a mortality of 32.5% over a 12-year period[4]. Cerebral edema, sepsis, acute respiratory distress syndrome, disseminated intravascular coagulation, hypoglycemia, refractory cardiac failure, septic shock, renal failure and hepatic failure were the causes of mortality in infantile onset diabetes.

Comparing the literature about IODM in developed countries, the following needs to be emphasized. TNDM is reported to constitute 40%-60% of IODM cases in Western literature while it is less than 10% from developing countries. The infrequent sequencing for 6q mutations may be a possible explanation. Those with onset between 6 to 12 mo were commonly identified to have *INS* gene mutation in comparison to the *EIF2AK* mutation in developing countries, and a higher rate of consanguinity may be a contributory factor. Comprehensive genetic testing has identified genetic cause in more than 80% of IODM cases in developed countries, while it is much lower in developing countries. Nearly 60%-80% of IODM cases present with DKA in developing countries while no such data are available from developed countries. Developmental delay and neuropsychological dysfunction are common in children with IODM from developed countries while developmental delay and hepatosplenomegaly have been reported from developing countries. Insulin pumps have been used for insulin requiring mutations in developed countries while they have been managed with conventional injections in developing countries like India. Overall mortality in IODM was reported to be 33% in developing countries while no such data on mortality in IODM are available from developed countries.

DIAGNOSTIC APPROACH TO INFANTILE ONSET NEONATAL DIABETES

A low C peptide level and high HBA1c level are supporting lab parameters to confirm infantile onset diabetes from stress induced hyperglycemia in infants. Initial evaluation should include a search for syndromic features. Associated features like hypothyroidism, elevated liver enzymes, skeletal dysgenesis, pancreatic agenesis, enteropathy, developmental delay, anemia, umbilical hernia and macroglossia should be looked for (Table 1). All infants with onset of diabetes at less than one year of age need to undergo genetic evaluation at the earliest for monogenic diabetes. Older children of any age with infantile onset diabetes can undergo genetic work-up as therapy with sulphonylurea at a later age is still useful for good glycemic control and management

Table 1 Diagnostic clues for type of mutation in infantile onset diabetes mellitus

Associated features	Diagnostic possibility of mutation
Umbilical hernia, macroglossia	6q 24
Developmental delay	*KCNJ11, ABCC8, EIF2AK3*
Microcephaly	*PTF 1A*
Hypothyroidism	*EIF2AK3, GLIS 3, FOXP3*
Diarrhea, eczema	*IPEX*
Anemia	*EIF2AK3, SLC19A2*
Hepatomegaly with liver dysfunction	*EIF2AK3*
Cerebellar hypoplasia	*PTF1A, NEUROD 1*
Pancreatic hypoplasia	*RFX 6, HNF1B, PTF1A, GATA6*
Ocular manifestations	*PAX 6*
Rickets, round facies, mild hyperglycemia	*SLC2A2*
No syndromic features	*KCNJ11, ABCC8, INS*
Renal abnormalities	*GLIS3, HNF1B*
Hirsutism, failure to thrive	Insulin resistance syndromes

KCNJ11: Potassium channel subfamily J member 11; *ABCC8*: ATP binding cassette transporter subfamily C, member 8; *EIF2AK3*: Eukaryotic translation initiation factor 2 alpha kinase 3; *PTF*: Pancreatic transcription factor; *GLIS3*: GLI subfamily of Kruppel-like zinc finger protein-3; *SLC2A2*: Solute carrier 2 family 2 gene; *INS*: Insulin.

of other comorbid factors. However, long-term insulin therapy may reduce the available beta cell mass and they may need additional glucose reducing drugs. Hence, an earlier genetic confirmation or therapy with sulphonylurea is warranted. Comprehensive genetic screening has been found to be more useful for early diagnosis than the conventional genetic screening. Conventional genetic tests analyze few genes based on the clinical features. With improved sequencing methods simultaneous analysis of multiple genes is possible. The genetic result predicts the best diabetes treatment and development of associated features. Evaluation with auto-immune antibodies may be warranted in infants with onset of diabetes in late infancy as the chances of type 1 diabetes presenting in late infancy has been reported in the literature.

Management of IODM

Adequate hydration of infants with acute presentation in DKA is essential. Infants with DKA may need much more fluids than the management of older children. Associated predisposing factors like sepsis or bronchopneumonia need to be treated for early control of hyperglycemia. Infusions of short-acting insulin in DKA and subcutaneous doses of insulin are the therapy of choice until evaluation for monogenic diabetes. These infants and toddlers may be very sensitive to small doses of insulin and careful watch for hypoglycemia is a must. Avoiding hypoglycemia is essential in these infants as sequelae of hypoglycemia on the developing brain leads to increased morbidity. Short-acting and rapid-acting insulin may sometimes cause hypoglycemia that is difficult to control in infants. Intermediate-acting insulin is preferred to be given as once or twice a day therapy[4]. Initial insulin dose for stabilization may

range from 0.35 units/kg per day to 3 units/kg per day. Insulin pumps have been used successfully in developed countries[58,59], but the initial cost and subsequent maintenance are major issues in using insulin pumps in infants from developing countries like India. If genetic reports suggest mutations in *KCNJ11* or *ABCC8* mutations which are responsive to sulphonylurea, transfer to oral drugs should be undertaken. Earlier identification of sulphonylurea responsiveness (*KCNJ11* and *ABCC8* mutations) is essential as the insulin therapy will only achieve glycemic control. Other systemic manifestations like seizures, muscular involvement, and developmental delay do not respond to insulin therapy. Earlier institution of sulphonylurea is essential to prevent worsening of these co-morbid factors in IODM. Following genetic confirmation, insulin can be switched slowly by outpatient protocols or by rapid inpatient protocols[21,60-66]. The initial median dose for sulphonylurea used to treat patients with K_{ATP} channel mutations is 0.45 mg/kg per day (range, 0.05-1.75 mg/kg per day). The switch over from insulin therapy to sulphonylurea may take more than 6 wk as some infants need very high doses up to 2 mg/kg per day of sulphonylurea[4]. In developing countries like India awaiting genetic reports for sulphonylurea therapy may cause a delay in specific treatment for co-morbid conditions. Studies have been undertaken to assess the risk and benefit of sulphonylurea therapy prior to genetic confirmation. In view of the potential benefit in the neurodevelopmental outcome and glycemic control, one may consider empirical inpatient trial with sulphonylurea if the genetic results are likely to be delayed. However, further studies with a large number of infants are warranted to decide on empirical sulphonylurea therapy[67]. Age at initiation of sulphonylurea showed a linear correlation with the dose required at follow-up. Indeed, all patients required additional glucose lowering medications along with sulphonylurea, if drug therapy was started at the age of 13 years or older[68]. Short-term studies on sulphonylurea therapy in children have been found to be safe without major side effects. Diarrheal episodes and rarely discolouration of teeth have been reported with sulphonylurea. Diarrhea usually disappears with therapy. None of these side effects will affect the continuation of therapy[63,69]. It is imperative to confirm the genetic mutation to decide treatment, to assess prognosis and anticipate the long term-outcome. They need to be followed even after remission as relapse has been reported in early adolescence or adults. The relapse responds well to sulphonylurea therapy.

These children need to be followed for glycemic control, growth pattern, developmental delay, seizures, pancreatic exocrine function, hypothyroidism and other comorbid states depending on the type of mutation. Periodic monitoring for long-term complications is mandatory. Presently there are no guidelines for long-term follow-up for diabetes related complications in these children with IODM, as long-term follow-up studies are lacking. Evaluation of the parental diabetic status and the genetic mutations will help to plan counseling regarding the future conceptions in the family. A neonatal diabetes registry has been established in India where facilities are available to sequence *KCNJ11*, *ABCC8*, *insulin* genes and other syndromic mutations.

CONCLUSION

DKA is the most common presentation of IODM (67%-83%). Low birth weight is common in infants less than 6 mo of age at presentation. Sixty-seven point five percent of IODM cases had a missed diagnosis at presentation. Monogenic diabetes is the most common cause of infantile onset diabetes. *KCNJ11/ABCC8* and *EIF2AK* mutations are the commonly reported non-syndrome and syndromic types, respectively. TNDM was noted in 10% of all infantile onset diabetics in South India. Developmental delay and seizures are major co-morbid factors in IODM. Glycemic control assessment among breast-fed infants and dispensing very small doses of insulin are difficult in IODM in developing countries. Management is by once or twice a day injections of intermediate-acting subcutaneous insulin, and continuous subcutaneous insulin infusions if feasible. Potassium channel mutations *(KCNJ11* and *ABCC8*) are sulphonylurea responsive and infants may need higher doses up to 2 mg/kg per day. Reported mortality during follow-up of IODM is very high (33%) in India.

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