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Bariatric surgery-mediated weight loss and its metabolic consequences for type-2 diabetes

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Abstract

The worldwide epidemic of obesity and its medical complications are being dealt with a combination of life style changes (*e.g.*, healthier diet and exercise), medications and a variety of surgical interventions. The Roux-en Y gastric bypass (RYGB) and laparoscopic adjustable gastric banding (LAGB) are two of the most common weight loss surgeries for morbid obesity-associated metabolic syndrome and insulin resistance. A vast majority of patients that undergo RYGB and LAGB are known to experience marked weight loss and attenuation of diabetes. A number of recent studies have indicated that the rates of remission in glycemic control and insulin sensitivity are significantly greater in patients that have undergone RYGB. A plausible hypothesis to explain this observation is that the gastric bypass surgery as opposed to the gastric banding procedure impinges on glucose homeostasis by a weight loss-independent mechanism. In a recent paper, Bradley *et al* have experimentally explored this hypothesis. The authors compared several clinical and laboratory parameters of insulin sensitivity and β-cell function in cohorts of RYGB and LAGB patients before and after they lost approximately 20% of their body mass. After weight loss, both groups of patients underwent similar changes in their intra-abdominal and total adipose tissue volume, hepatic triglyceride and circulating leptin levels. The RYGB patients who lost 20% body mass, manifested higher postprandial output of glucose, insulin and glucagon-like peptide-1; these laboratory parameters remained unchanged in LABG patients. Irrespective of the observed differences in transient responses of RYGB and LAGB patients to mixed meal, the overall glycemic control as judged by glucose tolerance, multi-organ insulin sensitivity and β-cell function were nearly identical in the two groups. Both RYGB and LAGB patient cohorts also experienced similar changes in the expression of a number of pro- and anti-inflammatory markers. Based on these analyses, Bradley *et al* concluded that similar restoration of insulin sensitivity and b-cell function in non-diabetic obese patients that have undergone RYGB and LAGB were directly due to marked weight loss. These data have important implications for the risk/benefit analysis of weight loss therapy by bariatric procedures.

Key words: Bariatric surgery; Roux-en Y gastric bypass; Laparoscopic adjustable gastric banding; Weight loss; Type-2 diabetes

Core tip: This report demonstrates that the positive effects of Roux-en Y gastric bypass and laparoscopic adjustable gastric banding are mainly caused by weight loss. Quantitatively similar losses of intra-abdominal and total adipose were seen in both groups of patients who also experienced improved glucose tolerance, multi-organ insulin sensitivity and cell function. Weight loss was associated with positive changes in a number of pro- and anti-inflammatory markers, regardless of the type of gastric surgery. In light of these findings the risk/benefit ratio of weight loss therapy by bariatric procedures with varying degrees of invasiveness, post-surgical complications and cost need to be re-evaluated.

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COMMENTARY ON HOT TOPICS

Obesity-associated type-2 diabetes mellitus (T2DM) and metabolic syndrome, and the cardiovascular consequences of chronic obesity are steadily emerging as key global healthcare challenges of the 21st century (World Health Organization Global Infobase: data on overweight and obesity mean body mass index, healthy diets and physical inactivity; www.who.int/mediacenter/). Surgical procedures such as gastric banding, gastric bypass and biliopancreatic diversion/duodenal switch have proven to be highly effective therapies for weight loss in morbidly obese individuals[1-4]. Regardless of whether weight loss is achieved by a combination of diet and/or exercise, or by surgery, such interventions, invariably, lead to improved metabolic profiles and amelioration of diabetes.

A comprehensive review and meta-analysis of 621 studies involving different types of bariatric surgeries revealed that a vast majority of patients that underwent weight loss following these procedures also experienced improvement in the clinical and laboratory manifestations of their diabetes[5]. Furthermore, it was noted that the improved glucose homeostasis and weight loss were progressively more significant with laparoscopic adjustable gastric banding (LAGB), gastroplasty, Roux-en-Y gastric bypass (RYGB) and bilio-pancreatic diversion/duodenal switch procedures[5]. Whether variable metabolic outcome of different types of bariatric procedures is caused by weight loss alone or involves other factors remains controversial. Two common bariatric surgeries used for weight loss therapy are RYGB that diverts the ingested food from passage through the upper gastrointestinal (GI) tract[6] and the laparoscopic adjustable gastric banding (LAPG) technique that reduces the size of the stomach[7]. The meta-analytical observations of Buchwald *et al*[5] and a number of other experimental findings have led some investigators to question the exclusive cause and effect relationship between weight loss and diabetes in patients undergoing bariatric surgeries[8,9]. In light of these data, it has been posited that bariatric interventions impinge on the mechanisms of glucose homeostasis that may be independent of weight loss[8,10]. However, unequivocal experimental data that support this tantalizing hypothesis are currently missing. In the December 2012 issue of the *Journal of Clinical Investigation*, Bradley *et al*[11] have described a set of experiments that were specifically aimed at testing this hypothesis. Based on these data authors concluded that upper GI tract diversion by RYGB improved insulin sensitivity and β-cell function by a weight loss-dependent mechanism.

Bradley *et al*[11] recruited two groups of obese subjects, ten in each cohort, that were insulin-resistant, as judged by homeostasis model assessment of insulin resistance (HOMA-IR) values of > 2.5. The choice of insulin-resistant obese patients that were not diabetic was made specifically with a goal to minimize the confounding variables of baseline glycemic control, glucose toxicity and interference with medications used to treat diabetes. The patient cohorts underwent RYGB or LAGB surgeries and were allowed to reach the target weight loss of 20% at 22 ± 7 and 16 ± 2 wk, respectively. The clinical and laboratory measurements that included body composition, insulin sensitivity and metabolic response to mixed meal were carried out in both groups of patients, before and after weight loss. These analyses revealed that total fat mass, intra-abdominal adipose tissue volume, intrahepatic triglyceride content and plasma leptin concentration were altered similarly in RYGB and LAGB subjects after weigh loss. Both cohorts of patients also elicited similar beneficial changes in the steady state levels of their plasma glucose, C-peptide, adiponectin and C-reactive protein.

Bradley *et al*[11] noted that following approximately 20% weight loss, the HOMA-IR scores decreased by more than 2-fold in both LAGB and RYGB patients who also displayed similar reductions in total insulin secretion rates (ISR) and total β-cell sensitivity. However, the kinetics of plasma glucose concentration after a mixed-meal was significantly different in RYGB and LAGB cohorts after target weight loss. Thus, postprandial rate of appearance (Ra) of glucose in RYGB patients increased from 70% ± 19% to 92% ± 2%, before and after weight loss, respectively; the RYGB patients also showed a higher peak in the rise of plasma insulin, C-peptide, and a marked increase in glucagon-like peptide-1 (GLP-1) after a mixed meal. The higher values of glucose in RYGB patients after weight loss likely reflected a more rapid emptying of their meal into small intestine; a higher dynamic ISR in these patients reflected a rapid rise in circulating glucose combined with increased plasma GLP-1. This explanation of greater dynamic ISR in RYGB patients is reasonable since postprandial rates of endogenous glucose production (EGP) were similar before and after weight loss in LAGB patients. In contrast, following a mixed meal, RYGB subjects elicited a faster and almost complete suppression of EGP that also rapidly returned to baseline. These differences in kinetics of EGP underscore a critical role of hepatic gluconeogenesis in preventing postprandial hypoglycemia in RYGB patients. The observed differences Ra to mixed meal notwithstanding, the area under the curve measurements of plasma insulin and C-peptide values, decreased to a similar extent in LAGB and RYGB patients after undergoing weight loss; both groups of patients also exhibited a near doubling of their disposition index (DI). It should be noted that the plasma concentration of glucagon did not change in either group of patients.

The authors observed that the whole body rise in insulin sensitivity, as judged by a 25% decline in insulin secretion in response to oral glucose challenge, occurred in

both LAGB and RYGB patients. However, as assessed by DI, there was a 2-fold enhancement of insulin sensitivity. Thus, β-cell function, assessed as total meal-induced insulin secretion in relationship to DI increased by about 75% in both LAGB and RYGB patients. Based on these data strongly suggested that weight loss, regardless of whether it occurred as a result of upper GI tract diversion or gastric banding could restore β-cell function, insulin sensitivity and oral glucose tolerance in non-diabetic patients.

Since a 5%-10% weight loss was shown to be insufficient to alter insulin sensitivity of skeletal muscle in previous studies[8,12-14], Bradley *et al*[11] speculated that for skeletal muscle to become more insulin sensitive a more marked weight loss is needed. The data in the current study support this notion as judged by nearly 2-fold improvement in skeletal muscle insulin sensitivity after 20% weight loss in both LAGB and RYGB patients. It should be pointed out however, that weight loss in either group of patients did not change the intramyocellular content of diacylglycerol or ceramide; these two lipids have been associated with skeletal muscle insulin resistance in rodents[15,16].

Finally, Bradley *et al*[11] reported that weight loss following either RYGB or LAGB led to amelioration of pro-inflammatory factors putatively involved in aberrant regulation of metabolism in morbidly obese animals and man. They measured the steady state levels of mRNA encoding EMR1 and CD11B, cell surface markers of pro-inflammatory macrophages as well as the expression of pro-inflammatory cytokines [*e.g.*, colony-stimulating factor, interleukin-6 (IL-6), tumor necrosis factor-α, and leptin]. Weight loss led to reduced expression of markers of inflammation and a concomitant enhancement of expression of IL-10, an anti-inflammatory cytokine. It is noteworthy that concomitant up-regulation of pro-inflammatory and down-regulation of anti-inflammatory signals occurred to a similar extent in both RYGB and LAGB patients. These observations demonstrate that in addition to restoring insulin sensitivity and β-cell function, weight loss impinges on the pathways of inflammation known to exacerbate insulin resistance and T2DM.

In conclusion, the data of Bradley *et al*[11] indicate that marked weight loss in obese subjects is accompanied by changes in key parameters of postprandial glucose homeostasis, multi-organ insulin sensitivity, β-cell function and adipose tissue inflammation. The authors' assertion that manifestly different response of RYGB patients to a mixed meal, as judged by increased transient levels of plasma glucose, insulin and GLP-1 are unlikely to play a therapeutic role in alleviating insulin resistance is reasonable. Based on these data authors concluded that restoration of β-cell function and insulin sensitivity occurred primarily as a result of weight loss. Although this study employed non-diabetic obese patients who underwent RYGB- or LAGB-dependent weight loss, the insights of this excellent study are highly relevant to the causes and consequences of morbid obesity-associated T2DM and its attenuation by weight loss. The data of this study also have serious implications for the choices of bariatric procedures that differ in their pre- and post-surgical preparations and complications and cost.

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REVIEW

Gastrointestinal complications of diabetes mellitus

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Abstract

Diabetes mellitus affects virtually every organ system in the body and the degree of organ involvement depends on the duration and severity of the disease, and other co-morbidities. Gastrointestinal (GI) involvement can present with esophageal dysmotility, gastro-esophageal reflux disease (GERD), gastroparesis, enteropathy, non alcoholic fatty liver disease (NAFLD) and glycogenic hepatopathy. Severity of GERD is inversely related to glycemic control and management is with prokinetics and proton pump inhibitors. Diabetic gastroparesis manifests as early satiety, bloating, vomiting, abdominal pain and erratic glycemic control. Gastric emptying scintigraphy is considered the gold standard test for diagnosis. Management includes dietary modifications, maintaining euglycemia, prokinetics, endoscopic and surgical treatments. Diabetic enteropathy is also common and management involves glycemic control and symptomatic measures. NAFLD is considered a hepatic manifestation of metabolic syndrome and treatment is mainly lifestyle measures, with diabetes and dyslipidemia management when coexistent. Glycogenic hepatopathy is a manifestation of poorly controlled type 1 diabetes and is managed by prompt insulin treatment. Though GI complications of diabetes are relatively common, awareness about its manifestations and treatment options are low among physicians. Optimal management of GI complications is important for appropriate metabolic control of diabetes and improvement in quality of life of the patient. This review is an update on the GI complications of diabetes, their pathophysiology, diagnostic evaluation and management.

Key words: Gastrointestinal complications; Diabetes mellitus; Esophageal complications; Nonalcoholic fatty liver disease; Diabetic gastroparesis; Diabetic enteropathy; Glycogenic hepatopathy

Core tip: Although relatively common, gastrointestinal (GI) complications of diabetes mellitus are underrecognized by most physicians. Early identification and prompt management of GI complications are of paramount importance as they are associated with significant morbidity. Common GI complications are esophageal dysmotility, gastro-esophageal reflux disease, gastroparesis, enteropathy, non alcoholic fatty liver disease (NAFLD) and glycogenic hepatopathy. Damage to the myenteric neurons due to longstanding diabetes causes esophageal, gastric and enteric disease. NAFLD is a hepatic manifestation of metabolic syndrome and is commonly seen in type 2 diabetes while glycogenic hepatopathy is due to poor glycemic control in type 1 diabetes. Clinical manifestations, pathogenesis, diagnostic evaluation and management of GI complications of diabetes are discussed in this article.

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INTRODUCTION

The prevalence of diabetes mellitus has now reached epidemic proportions in both developed and developing countries, affecting more than 366 million people worldwide[1]. This number is likely to increase in the coming years as a result of an ageing global population, urbanization, rising prevalence of obesity and sedentary lifestyles. Diabetes affects virtually every organ system in the body and the duration and severity of the disease may have a direct impact on organ involvement. Though gastrointestinal (GI) complications are common in longstanding diabetes, the awareness of these complications is low among physicians. Early identification and appropriate management of GI complications are important for improving both diabetic care and quality of life of the affected patient. This review aims to outline the GI complications of diabetes and the latest management options.

ESOPHAGEAL COMPLICATIONS

The thoracic esophagus and lower esophageal sphincter (LES) are composed of smooth muscle fibres innervated by myenteric plexus, and these autonomic nerves can be affected by diabetic neuropathy in patients with longstanding diabetes. Autonomic neuropathy and structural remodeling of the esophageal musculature in diabetes results in abnormal peristalsis, spontaneous contractions and reduced LES tone[2]. Morphological and biomechanical properties of the esophagus have been found to be altered significantly in animal models of diabetes[3]. The prevalence of esophageal dysmotility in diabetes has been reported to be as high as 63%[4]. The same study also found that there was no difference in dysmotility between patients with type 1 and type 2 diabetes or between genders and there was a strong association with retinopathy. Patients with dysmotility had longer duration of diabetes compared with those without dysmotility. Although the prevalence of esophageal dysmotility is high among patients with diabetes, only a minority present with the classical symptoms of dysphagia and heartburn[5].

The prevalence of gastroesophageal reflux symptoms in diabetes could be as high as 41%[6]. Erosive esophagitis (EE) was more frequent (66.7%) in diabetic patients with neuropathy than those without neuropathy (33.3%); also asymptomatic EE was significantly more frequent in the same group. In patients with type 2 diabetes, peripheral neuropathy is an independent risk factor for EE; however patients may be asymptomatic and a gastroscopy may be recommended in these patients[7]. Circulating levels of adiponectin, a potential anti-inflammatory adipocytokine is inversely related to visceral fat accumulation and it has been shown that the prevalence of gastroesophageal reflux disease is higher in type 2 diabetic patients with metabolic syndrome and low levels of serum adiponectin[8].

Diagnosis of reflux and dysmotility has relied on esophageal pH monitoring and conventional manometry for many years. The use of the wireless Bravo pH capsule, which allows catheter-free monitoring and impedance-pH measurement, a catheter-based technique which allows detection of acid and non-acid reflux have been major developments in the diagnostic field recently[9]. Two new procedures are available to assess esophageal motility: high resolution manometry which uses many pressure sensors and provides spatiotemporal plots of esophageal pressure changes; and impedance manometry, a test that directly measures bolus transit and provides conventional manometric data[9].

Gastroesophageal reflux disease was found to be inversely related to glycemic control and better glycemic control may improve esophageal dysmotility and reflux[10]. Management of reflux disease involves prokinetic drugs, such as metoclopramide and proton pump inhibitors. A two-week course of erythromycin has been shown to reduce mean esophageal transit time and gastric emptying time in type 2 diabetics[11]. Patients are also advised to drink fluids immediately after taking medications to avoid pill-induced esophagitis.

GASTROPARESIS

Gastroparesis, one of the commonest GI complications of diabetes mellitus, produces symptoms of gastric retention in the absence of physical obstruction[12]. The incidence of gastroparesis in a population with diabetes is reportedly low (5.2% over 10 years in type 1 and 1% in type 2 diabetes), but greater than in the general population (0.2%)[13]. Delayed gastric emptying can be demonstrated in 27%-65% of patients with type 1 diabetes and about 30% of patients with type 2 diabetes[14]. The incidence of gastroparesis is higher in women[15]. A recent study has reported obesity as a significant independent predictor of symptoms suggestive of gastroparesis in patients with type 2 diabetes mellitus (T2DM) and neuropathy[16].

Pathogenesis

The pathogenesis of diabetic gastroparesis is multifactorial and currently poorly understood. Delayed gastric emptying may be the first indication of gastroparesis in diabetes[15]. Elevated glycated hemoglobin level, duration of diabetes in excess of 10 years and the presence of macro- and microvascular complications are all accepted risk factors for the development of diabetic gastroparesis. Delayed gastric emptying contributes to poor glycemic control and may be the first indication that the patient is developing gastroparesis. Loss of the normal Migrating Motor Complexes, blunted antral contractions, spasm of the pylorus and small intestine and poor meal accommodation in the stomach are all demonstrable in diabetes[12]. Other factors that may have a role in the pathogenesis includes impaired inhibitory nitric oxide containing nerves, absent or dysmorphic interstitial cells of Cajal, smooth muscle fibrosis and abnormal macrophage- containing immune infiltrates[17,18]. Bezoar formation can contribute to the development of gastroparesis in some individuals. Endoscopic biopsies from diabetic gastroparesis demonstrate abnormal mucosal nerve density and morphology, reflecting possible potential for endoscopic diagnosis

of enteric neuropathy[19]. Neurohumoral factors including glucagon-like peptide-1 (GLP-1) can play a role in gastroparesis and the use of GLP-1 agonists Exenatide and Liraglutide can lead to symptoms of gastroparesis. A recent study showed that deficiency of apolipoprotein E can be a risk factor in diabetic gastroparesis in an animal model[20]. Extrinsic factors such as medications as well as concomitant disorders such as anxiety and depression may result in increased reporting of symptoms.

Clinical features

Symptoms of gastroparesis include nausea, vomiting, early satiety, postprandial fullness, bloating and upper abdominal pain. Worsening glycemic control along with frequent hypoglycemic episodes or unexplained alternating hyper- and hypoglycemia due to a mismatch between insulin action and carbohydrate absorption should prompt the clinician to evaluate the patient for diabetic gastroparesis. About 53% of patients may experience weight loss but 18%-24% may experience weight gain[14]. More than half of affected individuals present with acute onset of symptoms and the others insidiously. One third of cases have chronic symptoms with periodic exacerbations and one third have chronic worsening symptoms[14]. Epigastric distention and succussion splash may be observed in some patients but physical examination may not be always helpful.

Evaluation

A technical review from the American Gastroenterological Association recommends performing an initial evaluation consisting of careful history taking and physical examination, followed by complete blood count, thyroid stimulating hormone test, metabolic panel and optional amylase and pregnancy test[21]. History taking should particularly focus on macro- and micro-vascular complications of diabetes, although gastroparesis may occur in their absence. Additionally rumination syndrome should be excluded. Physical examination should focus on looking for evidence of peripheral and autonomic neuropathy, epigastric distension and the presence of succussion splash one hour post mealtimes. This is followed by upper GI endoscopy to rule out mechanical obstruction. Alternatively, an upper GI series with small bowel follow-through or small bowel magnetic resonance imaging can be performed. In the presence of significant abdominal pain, an abdominal ultrasound scan should be carried out to rule out biliary colic[16]. Presence of food in the stomach at endoscopy following a 12-h fast, in the absence of gastric outlet obstruction, is strongly suggestive of gastroparesis.

The diagnosis of gastroparesis is made by gastric emptying scintigraphy using ^{99m}Tc sulphur colloid bound to solid food[21]. This noninvasive, quantitative method is considered the gold standard test for diagnosing gastroparesis. The patient ingests a technetium-labeled egg meal and gastric emptying is then measured by scintiscanning at 15-min intervals for 4 h. However this test lacks standardization. A newer four image simplified scanning method has also shown comparable results[22]. The American Neurogastroenterology and Motility Society recommended a test meal of two slices of bread with jam plus two large eggs labeled with technetium-99m sulphur colloid and scintigraphy carried out at 0, 1, 2 and 4 h post prandially. A diagnosis of gastroparesis can be made if there is > 90% retention at 1 h, > 60% at 2 h and > 10% at 4 h[23].

An alternative method for gastric emptying study uses an indigestible wireless motility capsule (WMC), which senses intraluminal pH, temperatures and pressures as it traverses the gastrointestinal tract. The capsule wirelessly transmits the data to a receiver worn by the patient until it is excreted. WMC gastric emptying times greater than 5 h are said to be delayed, and this correlates with scintigraphic measurements[24]. Non-radioactive ^{13}C-breath tests quantify exhaled $^{13}CO_2$ after duodenal assimilation of a standardized substrate (octanoate, spirulina platensis) and are an alternative to scintigraphy[25]. The main advantage of these newer technologies is the lack of radiation exposure; however their general availability is limited.

Selected patients can be offered additional testing to exclude other contributions to the symptoms. Antroduodenal manometry excludes small bowel dysmotility, found in 17%-85% of gastroparetics[12]. Electrogastrography (EGG) can be used to detect rhythm disruptions and blunted postprandial responses. However a recent study has demonstrated the relative insensitivity of clinical EGG methodologies[26].

Treatment

Gastroparesis treatments include general measures, dietary modifications, medications that enhance emptying or lessen vomiting, non-medication interventions, psychological therapies and consideration of more invasive surgical treatment[12]. A grading system for assessing severity and guiding the management of gastroparesis has been suggested (Table 1)[27].

General approaches and dietary modifications

General approaches to management of gastroparesis include ensuring good hydration, correcting electrolyte imbalances, management of glycemic control and symptom reduction with pharmacotherapeutic agents. Any medications that can delay gastric emptying should be discontinued if possible. Dietary modifications include increasing liquid-based meals (as the rate of emptying liquid from the stomach is usually the same in diabetic gastroparesis), reducing fat and non-digestible fibre intake, avoiding large meals with high calorie contents and ensuring small frequent meals spread throughout the day.

Maintaining euglycemia has been one of the main principles of managing diabetic gastroparesis. Prolonged postprandial hyperglycemia has been observed in patients with diabetic gastroparesis compared to those with normal gastric emptying[28]. Another study observed a reduction of 1.8% in hemoglobin A_{1C} after initiating insulin

Table 1 Classification of severity of gastroparesis

Grade 1: Mild	Symptoms easily controlled Regular diet/minor dietary modifications helps to maintain normal nutritional status
Grade 2: Compensated	Moderate symptoms that are reasonably controlled with prokinetics and anti-emetics Maintenance of nutrition with diet/lifestyle changes Hospitalizations-infrequent
Grade 3: Gastric failure	Refractory symptoms Inadequate nutrition Needing hospitalization for therapy and nutritional supplementation (either enteral or parenteral) May need surgical or endoscopic intervention or gastric "pacemaker"

Originated from Abell *et al*[27].

Table 2 Drugs useful in treatment of diabetic gastroparesis

Drug/drug group	Mechanism of action	Common side effects	Efficacy
Metoclopramide 10 mg 4 times/d	Anti-emetic, reduces nausea and post-prandial fullness, increases gastro-esophageal sphincter tone and improves antro-pyloro-duodenal coordination	Tardive dyskinesia, drowsiness, irritability, extrapyramidal symptoms and dystonic reactions	Symptom control in 1/3 to 2/3 of patients
Domperidone 10 -20 mg 3 times/d	Similar to metoclopramide with fewer CNS side effects due to a predominant peripheral mechanism of action	May prolong QTc interval in ECG; in turn may provoke cardiac arrhythmia	Effective in up to 60% of cases; tachyphylaxis develops in a few weeks requiring discontinuation
Erythromycin 50-250 mg thrice daily	Motilin receptor agonist. Reduces gastric emptying time	Nausea and vomiting at high doses	Modest symptom control Intravenous form can be useful in refractory vomiting
Promethazine, prochlorperazine and chlorpromazine	Mechanism of antiemesis poorly understood	Drowsiness, liver injury and extrapyramidal effects	Marginal improvement of symptoms Intramuscular chlorpromazine is very effective in refractory vomiting
Ondansetron	Central serotonin receptor (5-HT3) antagonist Inhibits vagus nerve	Extrapyramidal effect	Modest efficacy

Originated from Hasler[12]. CNS: Central nervous system; ECG: Electrocardiogram; QTc: Corrected QT interval.

pump therapy[29]. This eventually reduced the number and length of hospitalizations for diabetic gastroparetics.

Prokinetics

Prokinetics are medications that augment gastrointestinal motility. In general these increase gastric motility and enhance stomach emptying. Medications commonly used in treatment are shown in Table 2.

Mosapride is a selective 5-HT$_4$ agonist that accelerates gastric emptying. Orally administered mosapride citrate has been associated with significantly increased food intake in ob/ob obese mice, with a tendency to decrease fasting blood glucose and fructosamine concentrations compared with controls[30]. A recent study reported symptom reductions in interferon induced gastroparesis in hepatitis C patients, treated with mosapride[31]. Other agents with gastric stimulating effects in gastroparesis include the new 5-HT$_4$ agonists prucalopride, velusetrag, naronapride and the acetycholinesterase inhibitor acotiamide, although their benefits are yet to be proven[12].

Ghrelin is peptide hormone secreted by the gastric fundic mucosa and pancreas. It is the first identified circulating hormone that controls hunger. One important physiological action of ghrelin is regulation of gastric motility[32]. Intravenous use of the ghrelin agonist TZP-101 was reported to reduce nausea and vomiting in patients with diabetic gastroparesis when compared to placebo[33]. Another study with the oral ghrelin analog TZP-102 also reported overall and individual reduction in the symptoms of diabetic gastroparetics[34].

Some published case reports have also claimed efficacy for the dopamine antagonist thiethylpeazine, the neurokinin NK1 antagonist aprepitant and the antidepressant mirtazapine. A retrospective study reported decreased symptoms in 88% of diabetics with tricyclic antidepressants. The herbal extract STW5 (iberogast) is also reported to be beneficial in functional dyspepsia and gastroparesis[12].

Endoscopic and surgical treatments

Mearin *et al*[35] proposed pyloric spasmodic contractions as one of the factors delaying gastric emptying. Endoscopic pyloric injections of botulinum toxin have been tried in the management of gastroparesis. This neurotoxin inhibits the release of acetylcholine at the neuromuscular junction, causing paralysis of the pylorus. Improved symptoms and accelerated gastric emptying persisting up to 3-6 mo were reported with pyloric botulinum toxin injections, especially in women and those with idiopathic gastroparesis[36]. It was also observed to be more beneficial in older men with vomiting[37]. However, small underpowered placebo-controlled trials did not show superior

responses for botulinum toxins *vs* placebo.

Gastric electrical stimulator implantations have also been shown to have benefits extending for more than 10 years and giving up to 80% reductions in nausea and vomiting. Additionally, there are reported improvements in nutritional and metabolic status, quality of life and health care utilizations[38]. Despite this, most studies show no effect on measured gastric emptying. One recent study showed improved symptoms in gastroparetics with gastric stimulators due to reduced gastric retention in diabetic patients[39]. Other newer technology in this field includes use of miniature wireless gastric stimulators inserted during endoscopy[40]. More studies are needed to ascertain the efficacy compared to other procedures.

Surgical treatments are rarely performed and are mainly reserved for patients with refractory gastroparetic symptoms who have failed to improve with other measures. A recent study demonstrated about 83% symptom reduction in gastroparetics after Heineke-Mikulicz pyloroplasty[41]. Completion gastrectomy was shown to give long-term symptom relief in some patients with post surgical gastroparesis, but data on patients with diabetic gastroparesis are limited. The possible benefits of pancreatic transplants for diabetic gastroparesis have not been proved[12].

Other measures include jejunostomy feeding and total parenteral nutrition. Jejunostomy feeding improves overall health and shows trends towards reduced healthcare utilization in diabetic gastroparesis[42]. The role of venting percutaneous gastrostomy in refractory idiopathic gastroparesis is controversial. One study reported symptom improvement as well as improvement in nutritional and functional status in patients with idiopathic gastroparesis[43]. Total parenteral nutrition can reverse rapid weight loss and ensure adequate sustenance and is usually used in patients with associated intestinal dysmotility[12].

ENTEROPATHY

Small intestinal and colorectal dysfunctions are common in patients with longstanding diabetes, especially in those with gastroparesis[44]. Diabetes-related enteropathy may present with diarrhea, constipation or fecal incontinence. The mechanism of development of enteropathy is similar to that of upper GI involvement in diabetes[45]. Advanced glycation end products (AGEs) cause damage to cellular DNA and tissues in diabetes. AGEs and their receptors are increased in the ganglia, crypt and brush border of diabetic jejunum and ileum as well as in the ganglia of diabetic colon in animal models[46]. Damage to the myenteric nerve plexus due to autonomic neuropathy and fibrosis of the intestinal muscular layers result in stasis of the intestinal contents. Reduced bowel motility results in constipation that may sometimes lead to overflow incontinence. Small intestinal bacterial overgrowth (SIBO), which can result in diarrhea, is usually a consequence of intestinal stasis.

Constipation alternating with diarrhea is one of the most common symptoms of diabetic enteropathy. The diarrhea is typically painless, may be associated with fecal incontinence and occurs during the day but more often at night[47]. Characteristically, it is seen in patients with poorly controlled diabetes who have peripheral and autonomic neuropathy[48]. Other causes of diarrhea in diabetics include pancreatic insufficiency, bile salt malabsorption, steatorrhea and drugs (Metformin). These should be excluded by appropriate investigations before making a diagnosis of diabetic enteropathy.

Constipation is a common problem affecting up to 60% of patients with long-standing diabetes mellitus[49]. Severe constipation leading to megacolon or colonic intestinal pseudo-obstruction occurs rarely. Stercoral ulcer, perforation and overflow diarrhea are encountered infrequently.

Fecal incontinence, particularly nocturnal, due to internal and external sphincter dysfunction secondary to autonomic neuropathy is a troublesome symptom. Acute hyperglycemia has been shown to inhibit external anal sphincter function and decrease rectal compliance, potentially increasing the risk of fecal incontinence[50].

Patients should undergo endoscopic examination, ultrasound or computed tomography to exclude other diagnosis. Although aspiration and direct culture of jejunal contents are regarded by many as the gold standards for the diagnosis of SIBO[51], these methods have several limitations, including the potential for contamination by oropharyngeal bacteria during intubation, and the fact that bacterial overgrowth may be patchy and may be missed by a single aspiration. Non-invasive diagnostic tests for SIBO are largely based on excretion of hydrogen in exhaled breath, following metabolism of carbohydrate by luminal bacteria. These tests have a specificity of 80%, but lack sensitivity (40%) and have their own limitations[52]. A radio opaque marker test is useful for excluding possible slow transit constipation. Tests for fecal incontinence include endoanal ultrasound and anorectal manometry.

Treatment of diabetic diarrhea mainly involves symptom relief, correction of fluid and electrolyte deficits, improvement of nutrition and glycemic control, and management of underlying causes[53]. Anti-diarrheal agents should be used with caution as there is a risk of toxic megacolon. Rifaximin is a minimally absorbed oral antimicrobial agent that is concentrated in the gastrointestinal tract, has broad spectrum *in vitro* activity against gram-positive and gram-negative aerobic and anaerobic bacteria, and has low risk of inducing bacterial resistance[54,55]. It has been shown to eradicate bacterial overgrowth in up to 84% of patients[56]. Other antibiotics used to treat this condition include amoxicillin-clavulanic acid, doxycycline, ciprofloxacin, metronidazole, neomycin and norfloxacin. There are anecdotal reports of successful treatment with somatostatin analogues of otherwise intractable secretory diarrhea in diabetic patients with autonomic neuropathy[57,58].

Loperamide may prove useful in fecal incontinence. Constipation may be treated with prompt hydration, reg-

ular exercise and increased intake of dietary fibre. Lactulose and osmotic laxatives may be necessary in more severe cases. Newer drugs for treatment of chronic constipation include prucalopride, a selective 5-HT$_4$ receptor agonist that enhances colonic transit and lubiprostone, which stimulates colonic water and electrolyte secretion through activation of type 2 chloride channels in enterocytes. They may prove useful in the future for treatment of chronic constipation in diabetes mellitus due to autonomic neuropathy and slow transit.

NONALCOHOLIC FATTY LIVER DISEASE

The definition of nonalcoholic fatty liver disease (NAFLD) requires that there is evidence of hepatic steatosis, either by imaging or by histology, and that there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders[59]. NAFLD is considered to be the hepatic manifestation of metabolic syndrome[60]. Metabolic syndrome encompasses the clinical tetrad of hyperinsulinemia with insulin resistance, visceral obesity, dyslipidemia and hypertension. In the majority of patients, NAFLD is associated with metabolic risk factors such as obesity (60%-95%), diabetes mellitus (28%-55%) and dyslipidemia (27%-92%) and, less clearly, with raised arterial pressure[61]. Histologically, NAFLD is further subdivided into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH).

Data from various studies indicate that the prevalence of NAFLD in the general population ranges from 6.3% to 33%. NAFLD is now the most common cause of chronic liver disease in North America, and it is estimated that 30% of the population of the United States has NAFLD[62]. In an ultrasonographic study, 69% of patients with T2DM had NAFLD[63]. Another study showed a prevalence of 62.3% (127 of the 204 diabetes patients had a fatty infiltration on ultrasound) and 87% of these patients with fatty infiltration who consented to biopsy had histological confirmation of the condition[64].

Clinical features, course and prognosis

Although the majority of patients with NAFLD are asymptomatic, some may present with nonspecific symptoms such as malaise and right upper quadrant pain. Clinical disease in NAFLD ranges from mild elevation of liver enzymes to severe liver disease with fibrosis and nodular degeneration. A recent study identified that approximately 30% of NAFLD cases with isolated steatosis will progress to NASH and, of these, approximately 20% will develop cirrhosis. About 40% of these cirrhotic patients develop decompensated liver disease[65].

Patients with simple fatty change had no increase in mortality, whereas patients with NASH had reduced survival and more cases died from cardiovascular disease (15.5% *vs* 7.5%) than liver related disease (2.8% *vs* 0.2%)[66]. Another long term study, conducted in Minnesota United States, of 420 patients in the community with NAFLD showed higher mortality in patients with impaired fasting glucose and cirrhosis, when compared with the general population. Liver-related mortality was also higher in this group than in the general population (13% *vs* < 1%)[67].

Hepatocellular carcinoma (HCC) is a well recognized complication of cirrhosis due to NAFLD[68-71]. Diabetes, obesity and cirrhosis-associated carcinogenic factors may have roles in the development of HCC in patients with NAFLD[68,70,72]. Presence of diabetes, elevated body mass index and liver fibrosis were identified as risk factors for progression to HCC among NAFLD cases[73]. Recent evidence from animal models shows that metabolic syndrome itself is high risk state for the development of NASH and HCC[74].

Pathogenesis

The development of NAFLD involves complex mechanisms and the relationship between T2DM and NAFLD is depicted in the Figure 1. Obesity, insulin resistance and metabolic syndrome are linked to the development of NAFLD[75]. It is now postulated that a combination of "multi hits" leads to development of steatohepatitis. This concept has replaced the earlier two hit hypothesis[76,77]. There is strong association between NASH, insulin resistance and increased level of free fatty acids in the liver[78,79]. Several factors including tumor necrosis factor alpha, oxidative stress, adiponectin, leptin, apoptosis and genetic factors are believed to have a role in the pathogenesis of NAFLD and NASH.

Evaluation

According to guidelines from the American Association for the Study of Liver Diseases (AASLD), the diagnosis of NAFLD requires that there is hepatic steatosis by imaging or histology, there is no significant alcohol overconsumption, there are no competing etiologies for hepatic steatosis, and there are no co-existing causes for chronic liver disease[59].

The following conditions should be excluded: history of alcohol intake > 20 g/d, nutritional causes (*e.g.,* total parenteral nutrition and rapid weight loss), metabolic disorders (glycogen storage disorders), chronic hepatitis C (particularly genotype 3), other causes of chronic liver diseases (autoimmune liver disease, Wilson's disease and hemochromatosis) and endocrine disorders such as polycystic ovary syndrome, hypopituitarism and hypothyroidism. Drug-induced steatosis can be caused by a number of agents including glucocorticoids, synthetic estrogens, amiodarone, methotrexate and highly active antiretroviral drugs. NAFL is considered benign whereas NASH can progress to cirrhosis, liver failure, and liver cancer.

Liver biopsy is considered the most reliable approach for identifying the presence of steatohepatitis and fibrosis in patients with NAFLD, but its limitations include cost, sampling error, and procedure-related morbidity and mortality. Features of the metabolic syndrome can predict the presence of steatohepatitis in patients with

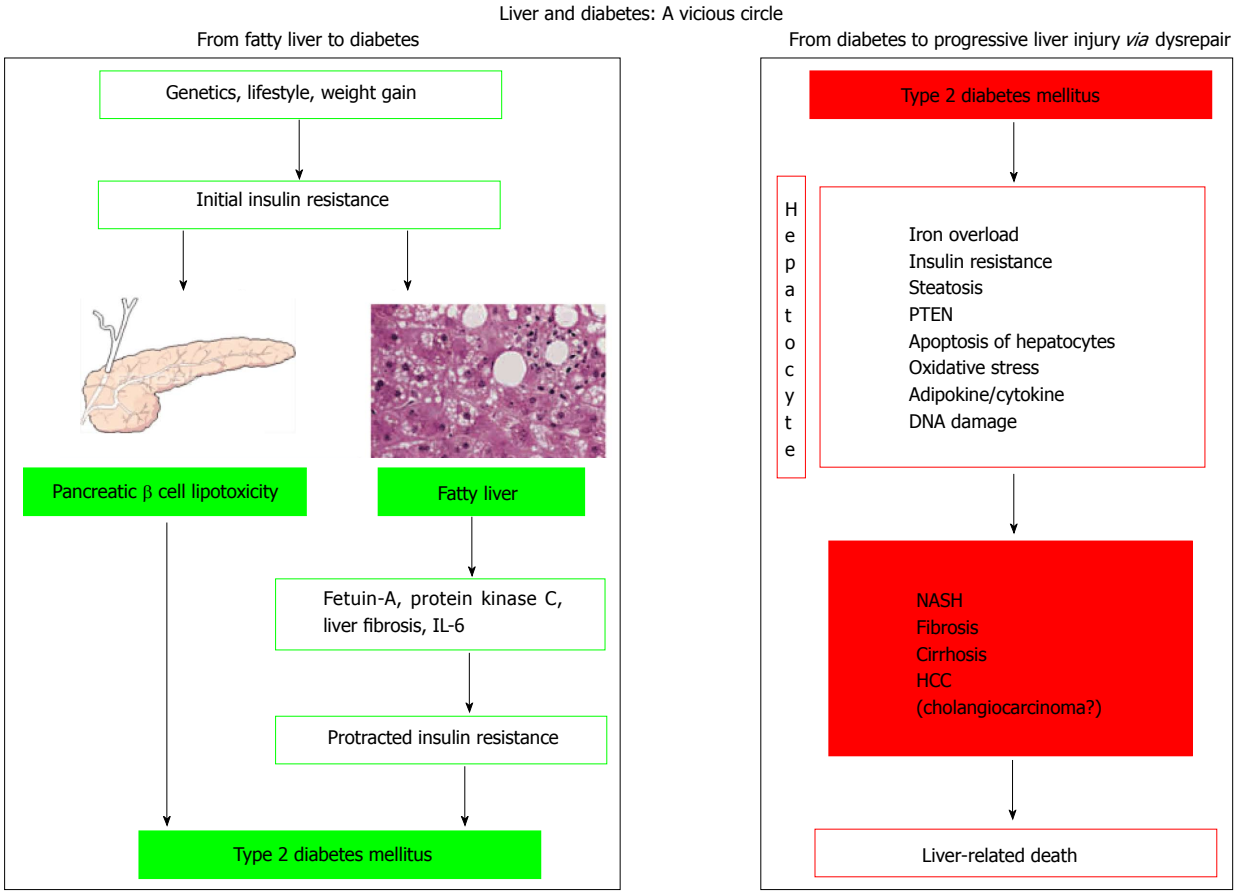

Figure 1 Molecular mechanisms involved in the vicious circle linking fatty liver to diabetes and diabetes to progressive liver injury. Left: The first part of the journey, leading from initial insulin resistance to fatty liver and eventually to the development of type 2 diabetes mellitus (T2DM) in those predisposed individuals in whom pancreatic lipotoxicity occurs; Right: The mechanism that (triggered by long-lasting/decompensated T2DM) may be conducive to progressive liver disease including primary liver cancer in predisposed individuals. HCC: Hepatocellular carcinoma; IL: Interleukin; NASH: Non-alcoholic steatohepatitis; PTEN: Phosphatase and tensin homolog. Reproduced from Loria *et al*[121].

NAFLD. Hence, liver biopsy is recommended in patients with NAFLD who have the metabolic syndrome[80-84]. There has been increasing interest in developing non-invasive methods to identify fibrosis in patients with NAFLD. NAFLD Fibrosis Score is a clinically useful tool for identifying NAFLD patients with higher likelihood of having bridging fibrosis and/or cirrhosis. In a meta-analysis of 13 studies consisting of 3064 patients, it was shown that NAFLD Fibrosis Score has a 90% sensitivity and 60% specificity to exclude advanced fibrosis and 67% sensitivity and 97% specificity to identify the presence of advanced fibrosis[83]. The NAFLD Fibrosis Score is based on six variables [age, body mass index (BMI), hyperglycemia, platelet count, albumin, aspartate aminotransferase/alanine aminotransferase ratio] and it is calculated using the published formula (http://nafldscore.com).

A novel biomarker that has been investigated for the presence of steatohepatitis in patients with NAFLD is circulating levels of cytokeratin-18 fragments[85,86]. This has a sensitivity of 78% and specificity of 87% for identifying steatohepatitis in patients with NAFLD. Transient elastography (TE), which measures liver stiffness non-invasively, showed high sensitivity and specificity for identifying fibrosis in NAFLD in a recent meta-analysis[85]. However, TE has a high failure rate in individuals with a higher BMI. There is some evidence that the Enhanced Liver Fibrosis test which uses the fibrosis markers hyaluronic acid, amino-terminal propeptide-of-type-Ⅲ-collagen and tissue-inhibitor of matrix-metalloproteinase-1, compares favourably with the use of TE[87].

Management

The management of patients with NAFLD consists of treating liver disease and the associated metabolic co-morbidities such as obesity, hyperlipidemia, insulin resistance and T2DM.

Lifestyle modification and weight reduction: Modifications in diet and lifestyle along with weight reduction and exercise are the cornerstones of treatment of NAFLD, as it is a disease related to excess weight and sedentary lifestyle. Many studies have shown that lifestyle modification can reduce aminotransferase levels and improve hepatic steatosis when measured either by ultrasound[88-91] or MR imaging and spectroscopy[92-95]. A randomized study of 31 obese persons with NASH who

underwent intensive lifestyle changes (diet, behaviour modification and 200 min a week of moderate physical activity for 48 wk) *vs* structured basic education alone showed improvement in steatosis, necrosis and inflammation in the obese group and participants with 7% weight loss had significant improvement in steatosis, lobular inflammation, ballooning, and NAFLD Activity Score[96].

Insulin sensitizing agents: Insulin resistance plays a key role in the pathogenesis of NAFLD. The two main classes of insulin-sensitizing drugs used in the management of patients with NAFLD/NASH are biguanides (metformin) and the thiazolidinediones (pioglitazone).

Metformin increases insulin sensitivity by decreasing hepatic gluconeogenesis and decreasing triglyceride production[97]. Early small, open-label studies showed a reduction in insulin resistance and serum levels of aminotransferases[98-100] but no significant improvement in liver histology[99,100]. A recent meta-analysis examining effects of medical treatment and/or lifestyle intervention did not show significant benefit of metformin in NAFLD[101]. Metformin showed no effect on liver histology and is not recommended as a specific treatment for liver disease in adults with NASH.

Pioglitazone has been available for over a decade for the treatment of T2DM. It acts by promoting peripheral and hepatic insulin sensitivity and increasing circulating levels of adiponectin[102]. A recent meta-analysis showed that pioglitazone improved histological disease activity, glucose, lipid and inflammatory variables and delayed fibrosis progression in patients with NAFLD[101]. The current recommendation by AASLD is that Pioglitazone can be used to treat steatohepatitis in patients with biopsy-proven NASH, although the long term safety and efficacy of pioglitazone in patients with NASH is unknown.

Vitamin E: The antioxidants vitamin E and betaine were investigated as potential therapeutic agents in NASH[103,104]. When administered for 2 years vitamin E improved liver histology, but increased insulin resistance and plasma triacylglycerols[101]. Therefore, the current recommendation by the AASLD is that vitamin E (α-tocopherol), administered at a daily dose of 800 IU/d should be considered as first-line pharmacotherapy for non-diabetic adults with biopsy-proven NASH. However, vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis or cryptogenic cirrhosis due to lack of supporting evidence.

Incretin mimetics: Incretins are a group of gastrointestinal hormones released after food intake that enhance insulin release from pancreatic beta cells. The most studied among these hormones is GLP-1. The role of the GLP-1 analogues exenatide and liraglutide in the management of T2DM in obesity is well established. These drugs may emerge as new options in management of NAFLD because of similar mechanisms in its pathogenesis.

Dipeptidyl-peptidase Ⅳ (DPP4) inhibitors were introduced as an alternative means to increase GLP-1 activity. There is increased serum DPP4 activity in patients with NASH, and this has a positive correlation with the histological grade and degree of liver steatosis[105] DPP4 inhibitors are already established oral treatments for type 2 diabetes[106], and data from experimental studies suggest that they may also reduce liver inflammation and steatosis[107]. Incretin mimetics may, in the future, represent a novel therapeutic option for slowing the progression of NAFLD.

Omega-3 fatty acids: So far, there is no clear evidence for the use of omega-3 fatty acids for the specific treatment NAFLD and NASH[108]. A large multicenter study of omega-3 fatty acid (eicosapentanoic acid) for treatment of NASH is ongoing in the United States.

Other agents: Orlistat, Sibutramine and Rimonabant (a cannabinoid receptor antagonist) have all been investigated for their potential as weight loss medications in NAFLD/NASH, although Sibutramine and Rimonabant have been withdrawn due to their side effects[109]. A single large multicenter randomized controlled trial showed that ursodeoxycholic acid offers no histological benefit over placebo in patients with NASH[110]. Recent data from animal models showed that consumption of hydrogen-rich water may be an effective treatment for NASH by reducing hepatic oxidative stress, apoptosis, inflammation, and hepatocarcinogenesis[111].

Bariatric surgery: AASLD recommends that foregut bariatric surgery is not contraindicated in otherwise eligible obese individuals with NAFLD or NASH. In a study of 381 adult obese patients by Mathurin *et al*[112] there was a significant improvement in the prevalence and severity of steatosis and ballooning at 1 and 5 years following bariatric surgery. A recently published Cochrane review concluded that lack of randomized clinical trials or quasi-randomized clinical studies precludes definitive assessment of the benefits and harms of bariatric surgery as a therapeutic approach for patients with NASH[113].

GLYCOGENIC HEPATOPATHY

Glycogenic hepatopathy is defined as pathological overloading of hepatocytes with glycogen leading to hepatic enlargement and/or derangement of liver enzymes and is usually seen in patients with longstanding poorly-controlled type 1 diabetes mellitus (T1DM)[114]. Glycogen accumulation in the liver was first described in 1930 as a component of Mauriac's Syndrome. This syndrome was characterized by unstable diabetes, hepatomegaly, hyperlipidemia, dwarfism, cushingoid features and delayed sexual maturity. It is now recognized that glycogen accumulation within hepatocytes can be present without all the findings described in Mauriac's Syndrome. Inadequate

control of T1DM results in concomitant presence of insulin and excess glucose that increases glycogen storage in the liver. Insulin activates the enzyme glycogen synthase phosphatase which dephosphorylates and activates glycogen synthase, another enzyme that is required for the conversion of glucose-1-phosphate to glycogen[115]. This results in increased glycogen storage in the liver and blocks glycogenolysis. The histological picture is characterized by pale appearance of the hepatocytes with compression of the sinusoids, glycogenated nuclei and giant mitochondria. Steatosis may be present, usually mild, or absent. Glycogen accumulation, the hallmark of this condition is demonstrated by PAS-diastase staining[114].

The disease is under-recognized and usually presents with abdominal pain, nausea, vomiting and abnormalities in liver function tests. While hepatic dysfunction is usually due to NAFLD in T2DM, liver dysfunction in T1DM usually results from glycogenic hepatopathy. It cannot be distinguished from NAFLD clinically or by ultrasound and confirmation requires a liver biopsy. The disorder should be suspected when liver dysfunction occurs in patients with T1DM, especially when viral, autoimmune and metabolic liver diseases are excluded by laboratory investigations. The hallmark of this condition is its reversibility with improved glycemic control. Unlike hepatic steatosis, glycogen overload is not known to progress to fibrosis distinct from fatty liver disease[116]. Prompt improvement with optimal diabetes control by insulin treatment within 4 wk is usually seen in these patients[117,118].

HEPATOGENOUS DIABETES

Up to 79% of cirrhotic subjects can have abnormalities of glucose metabolism[119]. T2DM is usually associated with metabolic syndrome that can lead to NAFLD and cirrhosis. The term "hepatogenous diabetes" (HD) is used to describe diabetes developing in patients with cirrhosis[119]. Numerous factors, including reduced insulin clearance, peripheral hyperinsulinemia and down-regulation of insulin receptors, lead to development of diabetes in cirrhosis[120]. HD is clinically different from T2DM in that it is less frequently associated with microangiopathy and patients suffer from complications of cirrhosis more frequently. However, HD is not yet recognized by the American Diabetes Association and the World Health Organization.

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BRIEF ARTICLE

Cardiometabolic health among gastric bypass surgery patients with polycystic ovarian syndrome

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Abstract

AIM: To examine the effect of gastric bypass surgery on cardiometabolic health among women with polycystic ovarian syndrome (PCOS).

METHODS: Retrospective medical chart review identified women (n = 389) with PCOS who underwent Roux-en-Y gastric bypass surgery from 2001-2009 in one surgical practice. Separate repeated measures linear mixed models were fit using the MIXED procedure to assess mean change in cardiometabolic disease risk factors from before to 1-year after surgery and were evaluated by ethnicity [Hispanic, non-Hispanic black (NHB) and white (NHW)].

RESULTS: The majority of the sample was Hispanic (66%, 25% NHB, 9% NHW). Mean body mass index significantly improved 1 year post-surgery for all ethnic groups (45.5 to 35.5 kg/m^2 for Hispanics, 46.8 to 37.7 kg/m^2 for NHB and 45.7 to 36.7 kg/m^2 for NHW, P < 0.001). Among Hispanic women mean total cholesterol (198.1 to 160.2 mg/dL), low-density lipoproteins (LDL) cholesterol (120.9 to 91.0 mg/dL), triglycerides (148.6 to 104.8 mg/dL), hemoglobin A1c (6.2% to 5.6%), alanine aminotransferase (28.1 to 23.0 U/L) and aspartate aminotransferase (23.5 to 21.6 U/L) decreased significantly (P < 0.001). Among NHB, mean total cholesterol (184.5 to 154.7 mg/dL), LDL cholesterol (111.7 to 88.9 mg/dL) and triglycerides (99.7 to 70.0 mg/dL) decreased significantly (P < 0.05). Among NHW, mean total cholesterol (200.9 to 172.8 mg/dL) and LDL cholesterol (124.2 to 96.6 mg/dL), decreased significantly (P < 0.05). Pairwise ethnic group comparisons of all cardiometabolic outcomes adjusted for age and type of surgery before and 1 year after surgery showed no statistical difference between the three groups for any outcome.

CONCLUSION: Cardiometabolic disease risk improvements vary by ethnicity and obesity may impact glucose tolerance and liver function changes more in Hispanic women with PCOS *vs* non-Hispanic women.

Key words: Bariatric surgery; Polycystic ovary syndrome; Cardiometabolic outcomes; Weight outcomes; Morbid obesity

Core tip: Morbidly obese women with polycystic ovary syndrome (PCOS) who have undergone Roux-en-Y

gastric bypass surgery show improvement in cardiometabolic disease risk at one year after the procedure. All women demonstrated a decrease in cardiometabolic disease risk factors including body mass index, glycated hemoglobin A1c (HgA1c), alanine aminotransferase (ALT), total cholesterol (TC), low-density lipoproteins (LDL) and triglyceride (TG). However, only Hispanic women showed a significant decreasing HgA1c and ALT, TC, LDL and TG one year after surgery. These ethnic group differences suggest there may be more comprehensive benefits in terms of cardiometabolic disease risk reduction after bariatric surgery in Hispanic women with PCOS *vs* other ethnic groups.

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex condition strongly associated with obesity, diabetes, coronary heart disease, and certain cancers[1-3]. Often presents in adolescents and is probably the most common endocrinopathy in women. PCOS affects 5%-10% of women of reproductive age and up to 28% of overweight or obese women[4-6]. Diagnostic features consist of clinical evidence of anovulation and clinical or biochemical evidence of hyperandrogenism after exclusion of other etiologies of hyperandrogenism. Consequently, it is the most common cause of anovulatory infertility, oligomenorrhoea, amenorrhoea, and hirsutism[7].

It is widely reported that women with PCOS have a higher prevalence of cardiometabolic disease risk factors and overt disease compared to non-PCOS women[7]. Specifically, obesity, insulin resistance, metabolic syndrome, diabetes, dyslipidemia and nonalcoholic fatty liver disease (NAFLD) are commonly seen in women with PCOS[7]. Limited studies have shown that weight loss after bariatric surgery in women with PCOS results in improvement or elimination of the symptoms of PCOS and associated cardiometabolic risk factors[8-10]. For example, women with PCOS following surgery have demonstrated normalization of menses, hirsutism score, serum androgens, and insulin sensitivity. However, it is less clear how effective bariatric surgery is on the resolution of pre-diabetes, hypertension and dyslipidemia in affected women with PCOS following bariatric surgery, and in multiethnic women in particular. Hispanics have a higher prevalence of obesity, impaired glucose tolerance, type 2 diabetes and NAFLD compared to their NHW counterparts, and consequently a higher risk for subsequent cardiovascular disease[11-13]. Therefore, weight loss in obese Hispanic women with PCOS may improve the risk of diabetes more substantially compared to non-Hispanic black (NHB) and NHW subjects but these comparisons are largely unknown. The objective of this analysis was to examine the effect of weight loss after bariatric surgery on cardiometabolic disease risk factors in a primarily Hispanic population of women with PCOS.

MATERIALS AND METHODS

A retrospective medical chart review identified women with a history or diagnosis of PCOS who underwent Roux-en-Y gastric bypass surgery (n = 389) in one private practice from 2001-2009. Diagnosis of PCOS was self reported and confirmed by an endocrinologist or gynecologist. Pre-surgery and 12 mo post-surgical cardiometabolic risk factors and ethnicity were collected for comparative analysis. Cardiometabolic risk factors included weight, height, body mass index (BMI), total cholesterol (TC), high- and low-density lipoproteins (HDL and LDL), triglyceride (TG), glycated hemoglobin A1c (HbA1c), and alanine aminotransferase (ALT) and aspartate aminotransferase (AST). This study was approved by and in accordance with the ethical standards of the Institutional Review Board.

Data collection

We abstracted data collected before and 1 year after surgery. In addition to data on sex, race, and ethnicity, these data included BMI and concentrations of HbA1c, HDL, LDL, TC, TG, AST and ALT. Pre-operative comorbidities and surgical complications were also recorded.

Measurements

Height and weight were measured during routine clinical visits by practice nursing staff. Weight was measured to the nearest 0.1 pound (and converted to kilograms for analyses) using a digital scale with the participants wearing light clothing and no shoes. Height was measured to the nearest 0.5 cm using an Accustat Genentech stadiometer. Body mass index was calculated as body weight in kilograms divided by height in meters squared (kg/m^2). Excess weight loss (EWL) was calculated as show in the following equation: EML (%) = (Preoperative weight - follow up weight)/(Preoperative weight - ideal body weight) × 100%.

Ideal body weight was calculated in base of the 1983 Metropolitan Life Insurance tables for gender and height. All blood samples were collected, processed, stored at -20 ℃, and shipped to the laboratory for analysis. HbA1c was analyzed by turbidimetric immunoinhibition with commercially available reagents. TC, LDL, HDL and TG were analyzed enzymatically with commercially available reagents. ALT and AST were determined by kinetic-spectrophotometric technique.

Surgery criteria

All patients met the National Institutes of Health criteria for bariatric surgery[14]. Thus, all patients had a BMI

Table 1 Mean body mass index and cardiometabolic disease risk factor values among women with polycystic ovary syndrome before and 1 year after gastric bypass surgery by ethnic group

		Pre-surgery	1-yr follow up
	n[4]	Estimate (95%CI)	Estimate (95%CI)
Hispanic (*n* = 257)[1]			
BMI (kg/m^2)	257	45.5 (44.7-46.4)	35.5 (34.6-36.3)
Weight (kg)	257	118.4 (115.8-120.9)	92.3 (89.8-94.7)
EWL (%)	257		54.5 (1.9)
Percent weight loss (%)	257		22.0 (0.6)
Total cholesterol (mg/dL)	28	198.1 (190.4-205.8)	160.2 (154.0-166.4)
HDL cholesterol (mg/dL)	28	49.5 (46.8-52.1)	48.7 (46.4-51.0)
LDL cholesterol (mg/dL)	24	120.9 (112.9-128.9)	91.0 (86.0-95.9)
Triglycerides (mg/dL)	28	148.6 (128.7-168.6)	104.8 (93.9-115.8)
Hemoglobin A1c (%)	58	6.2 (6.0-6.3)	5.6 (5.5-5.6)
ALT (U/L)	99	28.1 (25.8-30.4)	23.0 (20.7-25.4)
AST (U/L)	100	23.5 (22.0-25.0)	21.6 (19.8-23.4)
Non-Hispanic black (*n* = 97)[2]			
BMI (kg/m^2)	97	46.8 (45.5-48.2)	37.7 (36.2-39.2)
Weight (kg)	97	128.2 (123.5-132.8)	103.1 (98.5-107.8)
EWL (%)	97		47.5 (3.1)
Percent weight loss (%)	97		19.5 (1.0)
Total cholesterol (mg/dL)	6	184.5 (166.6-202.3)	154.7 (140.2-169.2)
HDL cholesterol (mg/dL)	6	50.6 (45.0-56.1)	53.0 (47.4-58.6)
LDL cholesterol (mg/dL)	6	111.7 (97.0-126.5)	88.9 (76.4-101.3)
Triglycerides (mg/dL)	6	99.7 (80.0-119.5)	70.0 (56.8-83.3)
Hemoglobin A1c (%)	15	6.4 (5.9-6.8)	5.8 (5.3-6.3)
ALT (U/L)	23	18.6 (16.3-20.9)	16.0 (12.6-19.4)
AST (U/L)	23	19.2 (17.4-20.9)	18.6 (16.4-20.7)
Non-Hispanic white (*n* = 35)[3]			
BMI (kg/m^2)	35	45.7 (43.2-48.1)	36.7 (34.4-39.0)
Weight (kg)	35	123.9 (116.5-131.2)	99.58 (92.3-106.9)
EWL (%)	35		50.2 (5.2)
Percent weight loss (%)	35		20.2 (1.7)
Total cholesterol (mg/dL)	7	200.9 (178.1-223.7)	172.8 (160.5-185.2)
HDL cholesterol (mg/dL)	7	49.7 (44.3-55.1)	49.6 (43.8-55.4)
LDL cholesterol (mg/dL)	6	124.2 (104.2-144.2)	96.6 (80.4-112.9)
Triglycerides (mg/dL)	7	160.5 (120.9-200.1)	137.8 (104.9-170.7)
Hemoglobin A1c (%)	5	6.3 (5.8-6.8)	5.9 (5.0-6.8)
ALT (U/L)	10	30.0 (23.7-36.0)	22.0 (16.6-27.4)
AST (U/L)	10	23.7 (19.7-27.8)	20.8 (16.6-25.0)

[1]Differences were statistically significant ($P < 0.001$), except for high-density lipoprotein (HDL) cholesterol and aspartate aminotransferase (AST); [2]All differences were statistically significant ($P < 0.05$), except for HDL cholesterol, hemoglobin A1c (HbA1c), alanine aminotransferase (ALT) and AST were not significant; [3]All differences were statistically significant ($P < 0.05$) except for HDL cholesterol, triglycerides, HbA1c, ALT and AST; [4]Number of patients with data at baseline and 1 year follow up. BMI: Body mass index; EWL: Estimated weight loss; LDL: Low-density lipoprotein.

greater than 35 kg/m^2 and at least one other risk factor for cardiometabolic disease (*e.g.*, elevated blood pressure, hypercholesterolemia, and so on) or a BMI greater than 40 kg/m^2. Patients received group and individual education from an interdisciplinary group of providers about surgical and nonsurgical treatments, outcomes, complications, and necessary lifestyle changes. All patients underwent psychological and nutritional evaluation and testing before surgery.

Surgical procedure

All patients underwent Roux-en-Y gastric bypass surgery. The majority of patients spent one night in the hospital after surgery. All patients were scheduled for routine post-operative visits at 1, 3, 6 and 12 mo post-surgery.

Patients had a Roux limb length of either 125 or 150 cm, depending on the patient's baseline BMI. The Roux limb was brought up in an antecolic, antegastric manner. The jejunal-jejunal mesenteric defect was closed with a permanent running suture. The entire gastrojejunostomy was over-sewn with a circumferential permanent running suture. All patients went to the bariatric floor postoperatively and were started on liquids after a normal upper gastrointestinal study on post-operative day 1. All patients were discharged home on a standard bariatric liquid diet for 1 wk.

Statistical analysis

Separate repeated measures linear mixed models were fit using the MIXED procedure to assess change all cardiometabolic disease risk factors (TC, HDL, LDL, BMI, HbA1c, AST and ALT) before and 1-year after bariatric surgery were evaluated by ethnicity (Hispanic, NHB and NHW).

RESULTS

The majority of the sample was Hispanic ($n = 257$, 66%), 25% ($n = 97$) were NHB and 9% ($n = 35$) were NHW; mean age was 40.9 years, (SD 12.9, range 16-74 years). BMI significantly improved from before to 1 year after surgery for all ethnic groups ($P < 0.0001$). Among Hispanic women all before-after differences in cardiometabolic disease risk factors were statistically significant ($P < 0.001$) with the exception of HDL cholesterol and AST (not significant). Among NHB, HDL Cholesterol, HbA1c, ALT and AST did not decrease significantly 1-year after surgery. Among NHW, all differences were statistically significant ($P < 0.05$), except for HDL cholesterol, triglycerides, HbA1c and AST (not significant) (Table 1). Pairwise ethnic group comparisons adjusted for age and type of surgery of before and 1 year after surgery cardiometabolic outcomes showed no statistical difference between the three groups for any outcome (Table 2).

Limitations

Our study has some limitations. In the evaluation of hepatic steatosis, we were not able to evaluate liver ultrasound or liver biopsy prior to and after bariatric surgery which may be more specific to detect pathology. Blood pressures were not available in all patients to assess hypertension as an additional cardiovascular disease risk factor. Finally, we were unable to assess potential confounders such as lifestyle factors (physical activity and diet) because this information was not assessed in patients.

DISCUSSION

This analysis shows that Roux-en-Y gastric bypass surgery results in a significant decrease in BMI among Hispanic, NHWs and NHBs with a history or diagnosis

Table 2 Ethnic group comparison of change in mean cardiometabolic disease risk factors among women with polycystic ovary syndrome 12 mo, after gastric bypass surgery performed between 2001 and 2010

Anthropometric measures	Group	Estimated change 12 mo after surgery (SE)	*P* value	*P* value Ethnic group comparisons	
BMI (kg/m^2)	Overall	9.7 (0.3)	< 0.0001	NHB	NHW
	Hispanic	10.1 (0.3)	< 0.0001	0.09	0.19
	NHB	9.1 (0.5)	< 0.0001	-	0.87
	NHW	9.0 (0.8)	< 0.0001	0.87	-
Weight (kg)	Overall	25.7 (0.7)	< 0.0001	NHB	NHW
	Hispanic	26.1 (0.8)	< 0.0001	0.49	0.40
	NHB	25.0 (1.3)	< 0.0001	-	0.76
	NHW	24.3 (2.0)	< 0.0001	0.76	-
Total cholesterol (mg/dL)	Overall	35.1 (3.6)	< 0.0001	NHB	NHW
	Hispanic	37.9 (4.2)	< 0.0001	0.36	0.21
	NHB	29.7 (7.8)	0.01	-	0.63
	NHW	28.1 (10.8)	0.04	0.63	-
HDL cholesterol (mg/dL)	Overall	-0.1 (1.2)	0.95	NHB	NHW
	Hispanic	0.7 (1.5)	0.64	0.26	0.96
	NHB	-2.4 (2.6)	0.40	-	0.36
	NHW	0.1 (2.9)	0.98	0.36	-
LDL cholesterol (mg/dL)	Overall	27.8 (3.4)	< 0.0001	NHB	NHW
	Hispanic	29.9 (4.3)	< 0.0001	0.32	0.89
	NHB	22.9 (6.2)	0.01	-	0.51
	NHW	27.6 (7.3)	0.01	0.51	-
Triglycerides (mg/dL)	Overall	40.1 (7.6)	< 0.0001	NHB	NHW
	Hispanic	43.8 (10.4)	0.0003	0.36	0.26
	NHB	29.7 (7.5)	0.01	-	0.64
	NHW	22.7 (12.8)	0.13	0.64	-
Hemoglobin A1c (%)	Overall	0.6 (0.1)	< 0.0001	NHB	NHW
	Hispanic	0.6 (0.1)	< 0.0001	0.77	0.38
	NHB	0.5 (0.3)	0.11	-	0.66
	NHW	0.37 (0.3)	0.31	0.66	-
ALT (U/L)	Overall	4.9 (1.1)	< 0.0001	NHB	NHW
	Hispanic	5.1 (1.3)	0.0002	0.16	0.38
	NHB	2.6 (1.4)	0.08	-	0.16
	NHW	7.9 (3.7)	0.06	0.16	-
AST (U/L)	Overall	1.8 (0.8)	0.04	NHB	NHW
	Hispanic	1.9 (1.1)	0.07	0.37	0.66
	NHB	0.6 (1.1)	0.56	-	0.40
	NHW	2.9 (2.8)	0.33	0.40	-

NHB: Non-Hispanic black; NHW: Non-Hispanic white; BMI: Body mass index; EWL: Estimated weight loss; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

of PCOS. Several ethnic group differences in pre-post change in individual cardiometabolic disease risk factors were found. Specifically, while all ethnic groups showed improvement in HbA1c and ALT, the decrease was significant only in Hispanic subjects. Additionally, all ethnic groups showed improvement in TC, LDL and TG one year after surgery. However, improvement in TC and TG were only significant for Hispanics and NHB subjects. Decrease in LDL was only significant in Hispanic and NHW subjects.

Our results are similar to previous studies of much smaller sample sizes that have shown that weight loss after bariatric surgery improves cardiometabolic disease risk among women with PCOS including insulin resistance and dyslipidemia, as well as biochemical features we did not include such as hyperandrogenism and oligomenorrhea[8-10]. Specifically, Escobar-Morreale *et al*[8] followed 12 Caucasian women of European ancestry with PCOS after bariatric surgery and found significant improvements in hirsutism and normalization of serum androgens in 91% of subjects at follow up (mean 12 mo, range 7-26 mo). All patients had regular menses after weight loss and 10 subjects had confirmed ovulation. They also found normalization of insulin sensitivity based on homeostasis model assessment (HOMA) in all patients. Similarly, Eid *et al*[9] followed 24 American women, whom were primarily Caucasian, with PCOS after Roux-en-Y gastric bypass (mean follow up 27 mo, range 12-57 mo). All subjects had normalization of menses and 77% had significant improvement or resolution of hirsutism. Eleven of the 24 subjects had type 2 diabetes prior to surgery and all 11 were normoglycemic at follow up without glucose lowering medications. Likewise, eleven patients with dyslipidemia prior to surgery no longer required cholesterol lowering medications at follow up. The most recent study by Jamal et al followed 20 American women with PCOS after Roux-en-Y gastric bypass (mean follow up 46.7 mo, range 15-123 mo)[10]. They found similar improvements in menses, hirsutism and type 2 diabetes. Six patients that desired pregnancy were able to conceive. These studies indicate weight loss through bariatric surgery significantly improved symptoms of PCOS and associated cardiometabolic disease risk and confirm our findings.

Hispanic ethnicity is associated with an increased cardiometabolic disease risk. In the Diabetes Prevention Trial, Hispanic adults with impaired glucose tolerance were found to have a higher HbA1c and HOMA after adjusting for BMI compared with white subjects with similar fasting plasma glucose levels[15]. Similarly, in the Nurse's Health Study the relative risk of type 2 diabetes among Hispanic women was 2.18 compared to white women after adjusting for BMI[13]. In addition, relative risk for type 2 diabetes is higher in Hispanics than in African-Americans[13]. Obesity further amplifies this risk; it has been reported that for every 5 point increase in BMI the relative risk for diabetes in Hispanic women is 2.36 *vs* 1.96 in NHW women[13]. Furthermore, obese Hispanic women are at a higher risk of diabetes compared to NHW and NHB women. Therefore, weight loss in obese Hispanic women with PCOS may improve the risk for overt diabetes more substantially compared to NHB and NHW subjects. Our results here showed significant ethnic group differences in the post-surgery improvement in HbA1c. While HbA1c improved in all groups, the improvement was only significant among Hispanic women with PCOS. This specific finding suggests that obesity may impact glucose tolerance more in Hispanic women with PCOS *vs* non-Hispanic women.

Hispanics also have a higher prevalence of NAFLD which is associated with obesity, insulin resistance and increased metabolic risk[11]. NAFLD represents a spectrum of fat accumulation in the liver from steatosis to cirrhosis and is the most common cause of elevated liver

enzymes[16]. The estimated United States prevalence of NAFLD is approximately 25% in the general population, but has been found to be as high as 45% in some Hispanic populations *vs* 33% among NHW and 25% among NHB[11,17]. The prevalence of hepatic steatosis on ultrasound among NHW women with PCOS is 55% and may be as high as 90% in some obese cohorts[17,18]. Hispanic women with PCOS also had a higher prevalence of NAFLD compared to Caucasian subjects[11,12]. Women with PCOS have a low prevalence of elevated transaminases despite radiographic evidence of hepatic steatosis. Gambarin-Gelwan *et al*[18] determined that only 15% of women with PCOS and steatosis on ultrasound had abnormal liver enzymes. We found in our population of women with PCOS that Hispanic and NHW subjects had a higher baseline ALT compared to NHB subjects. Post-surgery, Hispanic women with PCOS had a significant decrease ALT, whereas the decrease in ALT in NHW and NHB subjects was not significant. This decreased in ALT in the Hispanic population may indicate improvement of fatty infiltration after surgery and therefore, weight loss in obese Hispanic women with PCOS may have a greater impact on hepatic steatosis compared to NHW and NHB subjects.

We also found differential improvements in cholesterol among the Hispanic and NHB subjects. The majority of American women with PCOS have a lipid abnormality[19]. Most display an atherogenic lipid profile including high triglyceride concentrations, high LDL and low HDL[20]. Browning *et al*[11] found in a sample of 12 women the majority with PCOS that required cholesterol lowering medication prior to surgery were able to discontinue medication at follow up based on improvements in total cholesterol and triglycerides. We found that all ethnic groups showed improvement in total cholesterol, LDL and TG after surgery. However, total cholesterol and triglycerides showed significant improvement only for Hispanics and NHB subjects, and improvement in LDL was only significant for Hispanics and NHW subjects one year after surgery. We also found NHB subjects had significantly lower baseline triglycerides compared to Hispanics and NHW subjects. This is consistent with other adult[21-23] and adolescent studies which found lower triglycerides levels among NHB subjects compared to Hispanics and NHW subjects[24-26].

In conclusion, morbidly obese women with PCOS who have undergone Roux-en-Y gastric bypass surgery show improvement in cardiometabolic disease risk at one year after the procedure. All women demonstrated a decrease in cardiometabolic disease risk factors including BMI, HgA1c, ALT, TC, LDL and TG. However, only Hispanic women showed a significant decreasing HgA1c and ALT, TC, LDL and TG one year after surgery. These ethnic group differences suggest there may be more comprehensive benefits in terms of cardiometabolic disease risk reduction after bariatric surgery in Hispanic women with PCOS *vs* other ethnic groups.

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COMMENTS

Background

It is widely reported that women with polycystic ovarian syndrome (PCOS) have a higher prevalence of cardiometabolic disease risk factors and overt disease compared to non-PCOS women. However, it is less clear how effective bariatric surgery is on the resolution of pre-diabetes, hypertension and dyslipidemia in affected women with PCOS following bariatric surgery, and in multiethnic women in particular.

Research frontiers

This analysis shows that Roux-en-Y gastric bypass surgery results in a significant decrease in body mass index (BMI) among Hispanic, non-Hispanic black (NHB) and white (NHW) with a history or diagnosis of PCOS. Several ethnic group differences in pre-post change in individual cardiometabolic disease risk factors were found. Specifically, while all ethnic groups showed improvement in hemoglobin A1c (HbA1c) and alanine aminotransferase (ALT), the decrease was significant only in Hispanic subjects. Additionally, all ethnic groups showed improvement in total cholesterol (TC), low-density lipoproteins (LDL) and triglyceride (TG) one year after surgery. However, improvement in TC and TG were only significant for Hispanics and NHB subjects. Decrease in LDL was only significant in Hispanic and NHW subjects.

Innovations and breakthroughs

This is one of the first studies to demonstrate improvement in cardiometabolic health at one year after the procedure in a large multiethnic sample of morbidly obese women with PCOS. All women demonstrated a decrease in cardiometabolic disease risk factors including BMI, HbA1c , ALT, TC, LDL and TG. However, only Hispanic women showed a significant decreasing HgA1c and ALT, TC, LDL and TG one year after surgery. These ethnic group differences suggest there may be more comprehensive benefits in terms of cardiometabolic disease risk reduction after bariatric surgery in Hispanic women with PCOS *vs* other ethnic groups.

Applications

Post-surgery, Hispanic women with PCOS had a significant decrease ALT, whereas the decrease in ALT in NHW and NHB subjects was not significant. This decreased in ALT in the Hispanic population may indicate improvement of fatty infiltration after surgery and therefore, weight loss in obese Hispanic women with PCOS may have a greater impact on hepatic steatosis compared to NHW and NHB subjects. This finding suggests multiple improvements post-bariatric surgery among Hispanic women in particular not previously reported.

Terminology

Obesity, insulin resistance, metabolic syndrome, diabetes, dyslipidemia and nonalcoholic fatty liver disease are commonly seen in women with PCOS. Limited studies have shown that weight loss after bariatric surgery in women with PCOS results in improvement or elimination of the symptoms of PCOS and associated cardiometabolic risk factors. For example, women with PCOS following surgery have demonstrated normalization of menses, hirsutism score, serum androgens, and insulin sensitivity.

Peer review

The paper may add some new knowledge to the literature.

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BRIEF ARTICLE

Caribbean maitotoxin elevates $[Ca^{2+}]_i$ and activates non-selective cation channels in HIT-T15 cells

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Abstract

AIM: To investigate the cytotoxic mechanism of caribbean maitotoxin (MTX-C) in mammalian cells.

METHODS: We used whole-cell patch-clamp techniques and fluorescence calcium imaging to determine the cellular toxic mechanisms of MTX-C in insulin secreting HIT-T15 cells, which is a system where the effects of MTX have been observed. HIT-T15 cells stably express L-type calcium current, making it a suitable model for this study. Using the fluorescence calcium indicator Indo-1 AM, we found that there is a profound increase in HIT-T15 intracellular free calcium 3 min after application of 200 nmol/L MTX-C.

RESULTS: About 3 min after perfusion of MTX-C, a gradual increase in free calcium concentration was observed. This elevation was sustained throughout the entire recording period. Application of MTX-C did not elicit the L-type calcium current, but large cationic currents appeared after applying MTX-C to the extracellular solution. The current-voltage relationship of the cation current is approximately linear within the voltage range from -60 to 50 mV, but flattened at voltages at -80 and -100 mV. These results indicate that MTX-C induces a non-voltage activated, inward current under normal physiological conditions, which by itself or through a secondary mechanism results in a large amount of cationic influx. The biophysical mechanism of MTX-C is different to its isoform, pacific maitotoxin (MTX-P), when the extracellular calcium is removed.

CONCLUSION: We conclude that MTX-C causes the opening of non-selective, non-voltage-activated ion channels, which elevates level of intracellular calcium concentration and leads to cellular toxicities.

Key words: Maitotoxin; Calcium fluorescence; High voltage gated Ca^{2+} channels; Whole cell patch clamp; Insulin secreting cells

Core tip: The toxicity of maitotoxin is estimated to affect over 50000 people annually. Baracuda, snapper, grouper, jacks, and moray eel are particularly notorious for their potential to carry high toxin load. The symptoms of the toxicity include numbness of the perioral area and extremities, reversal of temperature sensation, muscle and joint aches, headache, itching tachycardia, hypertension, blurred vision, and paralysis. Our study aims to elucidate the cellular toxic mechanism of caribbean maitotoxin in mammalian cells. We conclude that it causes opening of non-selective, non-voltage activated ion channels, which elevates level of intracellular calcium concentration and leads to cellular toxicities.

Lu XZ, Deckey R, Jiao GL, Ren HF, Li M. Caribbean maitotoxin elevates $[Ca^{2+}]_i$ and activates non-selective cation channels in HIT-T15 cells. *World J Diabetes* 2013; 4(3): 70-75 Available

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INTRODUCTION

Ciguatera fish poisoning is caused by ladder-like polyether toxins[1]. Ciguatera occurs circumglobally in tropical coral reef regions, and results from the consumption of fish that have accumulated toxin through the food web[2-4]. It is estimated to affect over 50000 people annually, and is no longer a disease limited to the tropics, due both to travel to the tropics and to shipping of tropical fish species to markets elsewhere in the world[5]. Large carnivorous fishes associated with coral reefs are the most frequent source of ciguatera[6]. Baracuda, snapper, grouper, jacks, and moray eel are particularly notorious for their potential to carry high toxin load[7]. The symptoms of ciguatera comprise early onset (2-6 h) gastrointestinal disturbance, including nausea, vomiting, and diarrhea, and may be followed by a variety of later onset (18 h) neurological sequelae, including numbness of the perioral area and extremities, reversal of temperature sensation, muscle and joint aches, headache, itching tachycardia, hypertension, blurred vision, and paralysis[7-9]. Ciguatera symptoms in the Caribbean differ somewhat from those in the Pacific in that gastrointestinal symptoms are dominant in Caribbean cases, while in Pacific cases neurological symptoms tend to dominate[10].

Maitotoxin (MTX) is one of toxins implicated in ciguatera. This toxin is the most potent marine toxin known today, with a lethal dose of 0.17 ug/kg[11]. MTX elicits calcium influx in all cells and tissues tested[12,13]. This calcium influx however, is not mediated by the MTX itself but by activating existing non-selective cation ion channels[14-16]. MTX induced calcium influx is observed in pancreatic β-cells[17-19]. In β-cells, MTX-induced nonselective cation current is indistinguishable from the insulin stimulating hormone glucagon-like peptide-1 and PACAP-activated current[20]. It was reported that the MTX-induced calcium current is dependent on extracellular calcium[21].

We have isolated a novel Caribbean isoform of MTX (MTX-C) from tropical fish. In the present study, we used whole-cell patch-clamp techniques and fluorescence calcium imaging to determine the cytotoxic mechanisms of MTX-C in a hamster pancreatic islet cell line, HIT-T15, which stably expresses L-type calcium currents and thus is a suitable model[22]. We also used a pacific maitotoxin (MTX-P) to perform parallel comparisons for all experiments.

MATERIALS AND METHODS

Materials

The Caribbean and Pacific maitotoxins were gifts from the Food and Drug Administration of the United States, Mobile, AL.

Cell culture

Transformed hamster pancreatic islet cells HIT-T15 (American Type Culture Collection CRL-1777) were routinely cultured in Dulbecco's modified Eagle's medium (25 mmol/L glucose) supplemented with 10% fetal bovine serum (Gibco). For patch clamp recording in the whole-cell and cell-attached configurations, the cells were grown to 30%-80% confluence in 35-mm dishes (Corning). All culture media contained penicillin-G (25 U/mL) and streptomycin (25 mg/mL). Cultures were maintained at 37 ℃ in 5% CO_2 atmosphere incubator.

Electrical recording

Whole-cell patch clamp recording followed standard techniques. Calcium currents were recorded with an EPC-9 patch clamp amplifier (HEKA Electronics, Lambrecht, Germany). All data were analyzed with PULSE/PULSFIT acquisition and analysis software (HEKA Electronics). The filter frequency was 2.8 kHz and sample frequency was 5 kHz. Patch pipettes were pulled by a two-stage puller (PC-10; Narishige, Greenvale, NY), and heat-polished with a microforge (MF-200; World Precision Instruments, Sarasota, FL) before use. For high voltage gated calcium current measurement, the extracellular solution contained (in mmol/L) 90 TEA-Cl, 40 $BaCl_2$, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES and 40 sucrose, with pH adjusted to 7.4. The pipette solution contained (in mmol/L) 130 TEA-Cl, 20 EGTA, with pH adjusted to 7.3. For non-selective cation current recording, the extracellular solution contained (in mmol/L): 120 NaCl, 20 TEA-Cl, 5.6 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, with pH adjusted to 7.4; the pipette solution contained (in mmol/L): 130 CsCl, 10 NaCl, 5 Cs-EGTA, 2 $MgCl_2$, 5 HEPES, with pH adjusted to 7.4. For cation substitution experiments, bath solutions were the same as the pipette solution except CsCl (120 mmol/L) was replaced by the same amount of KCl or NaCl, accordingly.

Intracellular calcium fluorescent imaging

Intracellular free calcium concentration was measured in HIT-15 cells using Indo-1 fluorescence imaging. Cells were loaded with 2.5 mmol/L Indo-1 for 30 min. The cells were incubated at 37 ℃ for 45 min to allow for de-esterification. Fluorescent measurement was conducted by using an ACAS 570 confocal laser scanning microscope. The measurement solution contained (in mmol/L) 135 NaCl, 2 $CaCl_2$, 5 KCl, 10 HEPES, with pH adjusted to 7.3. The loading solution consisted of measurement solution augmented with indo-1 AM (2.5 μmol/L), DMSO (0.4%) and pluronic F-127 (0.1%). The de-esterification washing solution contained (in mmol/L): 82 Na_2SO_4, 30 K_2SO_4, 5 $MgCl_2$, 10 HEPES, 10 glucose, and 1 mg/mL BSA. Cells were illuminated with monochromatic light (350 nm), and emitted light was detected at wavelengths of 405 and 485 nm using photomultiplier tubes. The signals from the photomultipliers and force transducer were digitized and stored on a dedicated computer. Data were acquired and analyzed with a separate

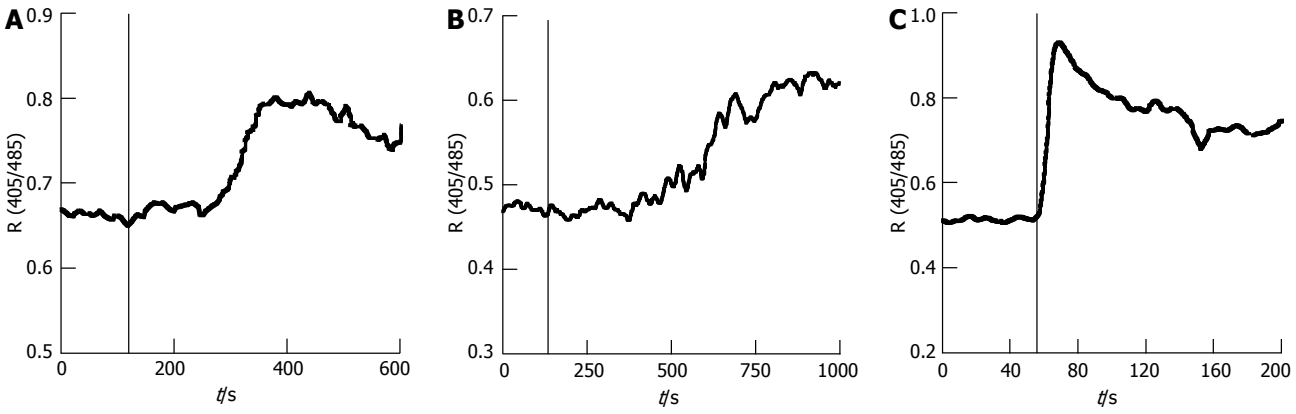

Figure 1 Indo-1 calcium fluorescence measurements of caribbean maitotoxin, pacific maitotoxin and 40 mmol/L of KCl in HIT-T15 cells. The vertical axis represents relative free intracellular calcium concentration as estimated by the ratio of fluorescent emissions at 405 and 485, respectively. The vertical bars in the figure indicate the time when the toxins or KCl were perfused.

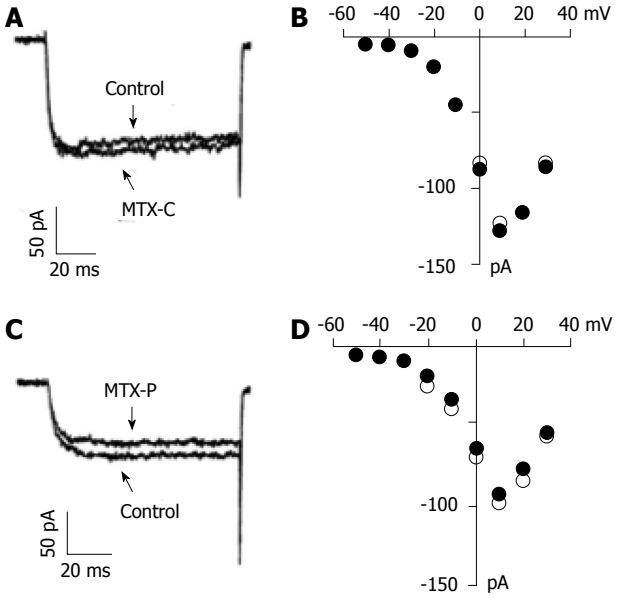

Figure 2 Effect of caribbean maitotoxin (A) and pacific maitotoxin (C) on voltage gated calcium channels in HIT-T15 cells. Representative barium current traces recorded at 10 mV when held at -70 mV in patch clamp. Extracellular solution contains 40 mmol/L $BaCl_2$. B and D show I-V relationship of voltage gated calcium currents elicited by caribbean maitotoxin (B) and pacific maitotoxin (D), respectively. Open and solid circles represent values of current before and after application of maitotoxins, respectively.

data analysis work station.

RESULTS

It is known that MTX-P's cytotoxicity is related to disturbing of intracellular calcium homeostasis. In order to determine the effect of MTX-C on intracellular calcium regulation, experiments using the fluorescent dye indo-1 to directly measure the intracellular free calcium concentration were performed in insulin secreting HIT-T15 cells. Both MTX-C and MTX-P induce a sustained elevation in intracellular free calcium concentration. Figure 1A shows the intracellular free calcium concentration before and after the application of 200 nmol/L MTX-C. About 3 min after perfusion of MTX-C, a gradual increase in free calcium concentration was observed. This elevation was sustained throughout the entire recording period. Similar results were obtained from a MTX-P (10 μmol/L) perfusion experiment, as shown in Figure 1B. The increase of free intracellular calcium started after a delay, and then increased to a plateau over 4-5 min. The pattern of maitotoxin-induced intracellular calcium elevation is noticeably different than the calcium elevation caused by opening of voltage-gated calcium channels where a sharp peak of calcium is followed by a quick decay, as was observed in the same type of cells (Figure 1C).

Although the calcium fluorescence experiments suggest that MTX-C might not activate voltage gated calcium channels directly, it is important to determine whether MTX-C alters the kinetics of voltage gated calcium channels. To address this question, whole cell patch clamp experiments were employed to record voltage calcium channel currents and analyze the I-V relationship under conditions with or without the presence of MTX-C or MTX-P. Figure 2A and C show barium current traces measured at 10 mV when held at -70 mV. Figure 2B and D show the I-V plots of the channels before and after adding MTX-C and MTX-P, respectively. These results show that the currents measured in these experiments are generated from the activation of high voltage gated calcium channels. Neither MTX-C nor MTX-P has significant effect on the current amplitude or the voltage dependence of these channels.

Since voltage gated calcium channels are not the primary targets of MTX-C, could its effect of intracellular calcium elevation result from the activation of non-voltage activated cation channels? Experiments were performed to delineate the effect of MTX-C on non-voltage activated cation currents, which were measured using extracellular sodium and intracellular cesium solutions. In these whole cell patch clamp experiments, the membrane potential was held at 0 mV, and the test pulses were from -100 to 50 mV in increments of 10 mV. As shown in Fig-

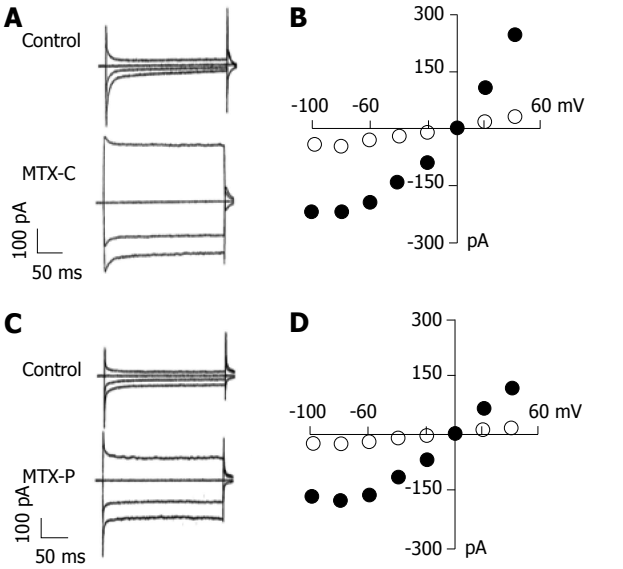

Figure 3 Effect of caribbean maitotoxin and pacific maitotoxin on non-voltage activated cation currents in HIT-T15 cells. Representative traces of cation current elicited recorded at -80, -40, 0 and 40 mV before and after adding caribbean maitotoxin (MTX-C) (A) or pacific maitotoxin (MTX-P) (C). The holding potential was at 0 mV. I-V relationships of MTX-C- and MTX-P-elicited currents are shown in B and D, respectively. The solid circles represent current amplitudes measured after MTX-C (B) or MTX-P (D) administration. The open circles represent the current recorded under the control condition.

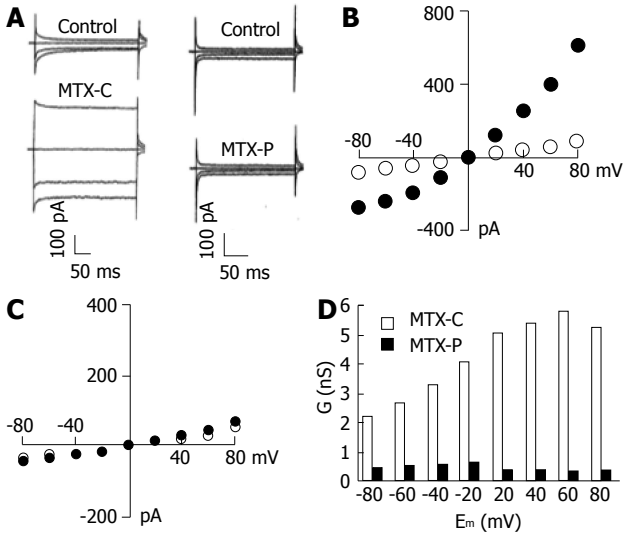

Figure 4 Non-voltage activated cation current induced by caribbean maitotoxin or pacific maitotoxin in the calcium free extracellular solution. A: Current traces measured before and after caribbean maitotoxin (MTX-C) or pacific maitotoxin (MTX-P) administration at -80, -40, 0 and 40 mV when held at 0 mV; B and C: I-V relationships of MTX-C (B) and MTX-P (C) elicited currents. The solid circles represent current amplitudes measured after MTX-C (B) or MTX-P (C) administration. The open circles represent the current recorded under the control condition; D: Current conductance measured at different test potentials in cells treated with MTX-C or MTX-P.

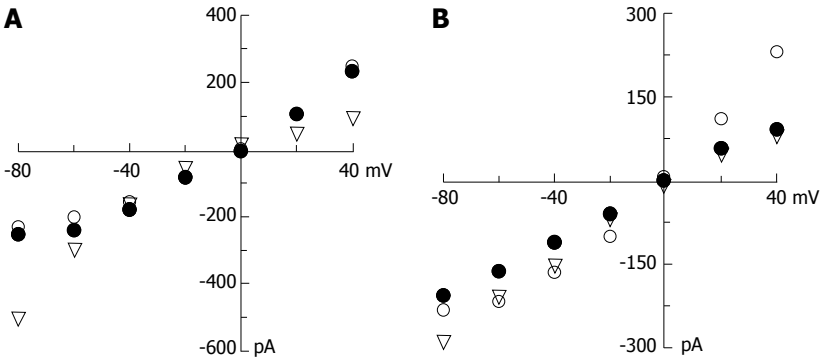

Figure 5 Current and voltage (I-V) relationships of caribbean maitotoxin (A) and pacific maitotoxin (B) opened cation currents. The pipette solution contains 130 mmol/L CsCl; the bath solutions contain 120 mmol/L of CsCl (open circle), NaCl (solid circle) or KCl (triangle).

ure 3, both MTX-C and MTX-P caused profound cation currents, which are approximately linear within the voltage range from -60 to 50 mV, but reduced or flattened at voltages at -80 and -100 mV (Figure 3B and D). Since the electrical driving force for calcium ions are higher at these voltages, this rectification may due to a partial closure of non-voltage gated calcium channels.

Further experiments revealed that these profound non-voltage activated currents induced by MTX-C and MTX-P had a different characterization in extracellular calcium dependency. Under the condition in which EGTA was used to chelate the free extracellular calcium, MTX-P failed to elicit non-voltage activated cation current, as shown in Figure 4A and C. In contrast, this current was continually detected in MTX-C treated cells bathed in the calcium free extracellular solution (Figure 4A and B). However, the inward current conductance was non-linear at voltages lower than 0 mV, as shown in Figure 4D. The discrepancy in calcium-dependent activation of non-voltage activated current between MTX-C and MTX-P indicates that the toxicological mechanisms in activating non-voltage activated cation currents by the two isoforms of maitotoxin have small but distinct differences.

The selectivity of MTX-C and MTX-P sensitive channels for other cations were also examined. The I-V relationship analyses were performed under conditions of symmetrical CsCl in the pipette and bath solutions, or the bath cesium was replaced by potassium or sodium ions. Since the reversal potentials across at 0 mV for both toxin-elicited currents in all cation solutions, the selectivity is the same for Na^+, Cs^+ and K^+. These results suggest that maitotoxins elicit a non-selective, non-voltage activated current. However, for MTX-C, while the I-V relationships in extracellular CsCl were similar to that in NaCl as shown above (Figure 3B), it was very different when the extracellular solution contained KCl, where a clear inward rectification was observed (Figure 5A). In MTX-P sensitive channel experiments, unlike in the NaCl extracellular

solution, this channel showed non-linear reduction at very negative test potential (Figure 3D). The I-V relationships were linear in all voltages between 100 to 50 mV when the bath solutions contain CsCl or KCl (Figure 5B). These results suggest that MTX-C and MTX-P may act on different channel proteins or act on different parts of the same channel protein.

DISCUSSION

In conclusion, MTX-C causes an opening of non-selective, non-voltage activated ion channels, which permits or elicits further abnormal calcium influx. The elevated level of intracellular calcium concentration resulting from this calcium influx may lead to cellular toxicities.

The toxic potency of MTX-P exceeds that of ciguatera toxins (LD50 0.05/kg *ip* in mice). Its biological activity is strictly calcium dependent and causes both membrane depolarization and calcium influx in many different cell types. It was originally believed to be an activator of voltage dependent calcium channels[23]. However, voltage dependent calcium channel antagonists can block MTX-P-stimulated calcium influx, but not MTX-P-induced membrane depolarization[24]. The results in the present study show that the opening of non-selective cation channels will result in a net positive ion influx due to the higher electrochemical driving force for sodium ions than for potassium ions. Such a net sodium ion influx can depolarize cell membrane potential and caused activation of voltage gated calcium channels in HIT-T15 cells. This depolarization is a gradual process, which could explain why there was a time delay in the intracellular calcium experiments (Figure 1A and B). MTX-C-induced membrane depolarization also likely causes anomalies of cellular functions and possibly reduces chances of cell survival.

Although the structure of MTX-P has been described previously[25,26], the mechanism of calcium dependence in MTX-P-induced current activation is unclear. It is possible that extracellular calcium creates a significant surface potential that provides a necessary influent to help MTX-P open non-selective cation channels, or it may affect an enzymatic mechanism on the cell surface to achieve the same purpose. The fact that MTX-C could effectively activate these channels regardless of the presence of extracellular calcium indicates that it may be a more potent isoform than MTX-P. However, this likely has little physiological significance since living cells are exposed to an extracellular solution containing calcium ions.

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COMMENTS

Background

Ciguatera occurs circumglobally in tropical coral reef regions, and results from the consumption of fish that have accumulated toxin through the food web. Maitotoxin (MTX) is one of the toxins implicated in ciguatera. MTX is the most potent marin toxin which causes severe illness in gastrointesting system. MTX presents primarily as an acute neurologic disease manifested by a constellation of gastrointestinal, neurologic and cardiovascular signs and symptoms. Acute fatality, usually due to respiratory failure, circulatory collapse or arrhythmias, ranges from 0.1% to 12% of reported cases. Although the mechansim of pacific isoform of MTX induced cell damage has been studied extensively, the mechanism of caribbean isoform of MTX toxicity is largely unknown.

Research frontiers

MTX activates Ca^{2+} permeable, non-selective cation channels, leading to an increase in levels of cytosolic Ca^{2+} ions, which triggers a cell death cascade, resulting in membrane blebbing and eventually cell lysis. MTX is known to activate cytosolic calcium-activated proteases calpain-1 and calpain-2, contributing to necrosis. The molecular characters of MTX activated ion channels, however, remains unknown. This study charactered the electrophysiogical differences between currents induced by two isoforms of MTX, provided more insights to the physiological properties of the channel.

Innovations and breakthroughs

The study shows that pacific and caribben isoforms of MTX share similar potency in eliciting calcium influx, carried by similar non-selective, non-voltage activated outward-rectified calcium currents. However, the currents induced by two isoforms of MTX had a difference in extracellular calcium dependency: function of pacific MTX is extracellular calcium dependent whereas the caribbean MTX is not. This difference indicates that the toxicological mechanisms is different between the two isoforms of MTX.

Applications

The conclusion of this study can be applied to further characterize the molecular nature of the MTX activated ion channels and to eventually elucidate the molecular mechanism of MTX toxicity.

Terminology

Rectified current-voltage relationship: The changes in the size of current is non-linear with the changes of voltage values.

Peer review

This succinct paper characterizes the effects of caribbean maitotoxin (MTX-C) on Ca^{2+} influx in insulin-secreting HIT-T15 cells by means of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) measurement and whole-cell patch-clamp analysis. $[Ca^{2+}]_i$ measurement reveals that this toxin induces a marked increase in $[Ca^{2+}]_i$. Whole-cell patch-clamp analysis verifies that MTX-C had no influence on voltage-gated Ca^{2+} currents, but evidently activated non-selective cation channels. The data are interesting. They indicate that MTX-C may exert their cellular toxicity by promoting Ca^{2+} influx through non-selective cation channels in insulin-secreting HIT-T15 cells.

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BRIEF ARTICLE

Hemoglobin A1c in early postpartum screening of women with gestational diabetes

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Abstract

AIM: To assess the utility of hemoglobin A1c (HbA1c) in the early postpartum screening of women with gestational diabetes mellitus (GDM).

METHODS: Over a 3 years period, HbA1c estimations were undertaken in addition to and simultaneously with the traditional oral glucose tolerance test (OGTT), in 203 women with GDM as a part of early postpartum screening for dysglycaemia, at 6 wk post-partum. World Health Organization criteria was used for diagnosing diabetes: fasting blood glucose (FBG) ⩾ 7.0 mmol/L and/or 2-h postprandial blood glucose (PPBG) ⩾ 11.1 mmol/L and/or HbA1c ⩾ 48 mmol/mol; and impaired glycaemiastate: impaired fasting glucose 6.1-6.9 mmol/L and/or impaired glucose tolerance 7.8-11.0 mmol/L and/or HbA1c: 42-47 mmol/mol.

RESULTS: Mean FBG, 2-h PPBG and HbA1c were 4.9 ± 0.7 mmol/L, 5.6 ± 2.0 mmol/L and 38 ± 5 mmol/mol respectively. FBG, 2-h PPBG and HbA1c detected 6 (3%), 7 (3.5%) and 11 (5.4%) cases of diabetes respectively, and 11 (5.4%), 25 (12.3%) and 23 (11.3%) cases of pre-diabetes state respectively. HbA1c values ⩾ 48 mmol/mol (⩾ 6.5%) showed a diagnostic sensitivity of 71.4% and specificity of 98.5% for diabetes in comparison to OGTT in receiver operating characteristics curve analysis. At HbA1c cut-off 44 mmol/mol, sensitivity and specificity were 100% and 92.3% respectively [area under the curve: 0.98 (95%CI: 0.96-1.00)]. Sensitivity and specificity for detecting high risk "impaired glycaemia" state [HbA1c 42 mmol/mol (6.0%)] were 28% and 80%, respectively.

CONCLUSION: HbA1c level ⩾ 48 mmol/mol (⩾ 6.5%) has reasonable sensitivity and high specificity in comparison to OGTT for early postpartum screening of diabetes in GDM. At 6th week postpartum screening, if FBG is normal and HbA1c < 44 mmol/mol OGTT is not recommended.

Key words: Postpartum screening; Glycated haemoglobin; Gestational diabetes mellitus; Impaired glycaemia

Core tip: Hemoglobin A1c (HbA1c) though accepted as a screening tool for diagnosis of diabetes by professional bodies, its role in early postnatal screening of women with gestational diabetes mellitus is not known, which is explored in this study. Analysing the results of simultaneous oral glucose tolerance test (OGTT) and HbA1c estimations undertaken as a part of postpartum screen-

ing at 6 wk, we note that HbA1c has a high negative predictive value and can help in excluding diabetes (but not impaired glycaemia). We thus propose that HbA1c could potentially be used with fasting blood glucose estimation to avoid OGTT in those women with an HbA1c < 44 mmol/mol.

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INTRODUCTION

The global prevalence of diabetes mellitus continues to escalate with the force of an approaching tsunami that affects about 350 million individuals worldwide[1]. Increasing prevalence of overweight and obesity in both developed and developing countries are the main factors for the alarming rise in the diabetic epidemic. Alterations in the glucose homeostasis among obese/overweight children and adolescents lead to the peculiar phenomenon of emergence of prediabetes and type 2 diabetes in young adults in the recent years[2]. Consequently the prevalence of diabetes among women of child-bearing age is also expected to rise.

Diabetes mellitus is a major cause of perinatal morbidity and mortality, as well as maternal morbidity that complicates about 3%-14% of all pregnancies[3-7]. About 85% of diabetes among pregnant women is due to gestational diabetes mellitus (GDM) and the remainder are due to type 2 or type 1 diabetes. It is well recognised that women with GDM have a higher risk of development of impaired glycaemicstate or type 2 diabetes over time, with studies suggesting a 17% to 63% risk of developing type 2 diabetes within 5 to 16 years of the index pregnancy[8]. The recurrence rate of GDM in a second consecutive pregnancy was found to be about 41% in a recent major study[9]. Despite majority of women with GDM becoming normoglycaemic immediately after delivery of the baby, the prevalence of persistent abnormal glucose metabolism (diabetes and impaired glycaemia) in the early postpartum period has been reported to be as high as 46%[10]. Identifying these high risk women with persistently impaired glucose regulation and frank diabetes would help in instigating early intervention strategies for better health outcomes. Thus, early postpartum diabetes screening is recommended by professional organizations, for all women with the diagnosis of GDM during pregnancy[11-15].

The American Diabetes Association (ADA) recommends diabetes screening with a 75 g oral glucose tolerance test (OGTT) at 6-12 wk after delivery in women with GDM[11] and the World Health Organization (WHO) recommends similar screening at least 6 wk after delivery[12]. A fasting glucose test, instead of the traditional OGTT, at the 6th week postpartum visit is recommended by the United Kingdom's National Institute for Health and Clinical Excellence (NICE) for GDM patients as a screening tool[13]. Though estimation of glycated hemoglobin A1c (HbA1c) level is accepted as a screening tool for diagnosis of diabetes by WHO and other professional bodies, there is no official recommendation for its use for diabetes screening in the postpartum period.

Because of the ease of estimation of HbA1c in comparison to the cumbersome OGTT, it is being utilized increasingly for screening and diagnosis of diabetes and prediabetes states in the recent years. In this background we conducted a study to explore the utility of HbA1c in the early post-partum screening of women with gestational diabetes in a large university hospital in the United Kingdom.

MATERIALS AND METHODS

All women who were diagnosed with GDM, managed by diet/lifestyle modifications and/or medical treatment, in the combined antenatal diabetes clinic (with obstetrician, diabetologist, dietician and diabetic specialist nurse) between January 2010 and August 2012, were offered postpartum screening in the 6th week postpartum visit. Along with the OGTT, HbA1c estimation was undertaken as a part of the post-partum screening test. All such women were identified and included, and there were no exclusion criteria. These women were given counselling by the diabetic team, during their antenatal follow up, regarding the implications of GDM diagnosis and the need for screening in the post-partum period.

OGTT was performed after a minimum of 8 h of overnight fast, by measuring fasting blood glucose (FBG) and 2-h postprandial blood glucose (PPBG). After taking blood sample for FBG check each participant was given a glucose drink (75 g of D-dextrose powder dissolved in 200 mL of water). Samples for FBG and 2-h PPBG were obtained by taking 2 mL of venous blood in tubes containing sodium fluoride. 3 mL each of venous blood samples were collected in tubes containing EDTA for HbA1c estimation while taking sample for fasting blood glucose. HbA1c was measured using high performance liquid chromatography on a Tosoh G7 analyser (Tosoh Bioscience Ltd., Worcestershire, United Kingdom). The performance scores in the United Kingdom National External Quality Assurance Scheme were: A scores < 100 and B scores < 2%. The between batch coefficient of variation was 1.8% and 1.4% for an HbA1c of 5.7% and 9.5% respectively.

The International Federation of Clinical Chemistry (IFCC) units for HbA1c levels were introduced in the United Kingdom since 1st June 2009. Locally, the IFCC reference system was adopted and the dual reporting of HbA1c with IFCC units and the corresponding calculated Diabetes Control and Complications Trial (DCCT)

Table 1 Categorisation of glycaemia into diabetes, impaired and normal based on the different tests undertaken: fasting blood glucose, oral glucose tolerance test and hemoglobin A1c (*n* = 203) *n* (%)

Category	FBG	OGTT	HbA1c
Diabetes	6 (3.0)	7 (3.5)	11 (5.4)
Impaired	11 (5.4)	25 (12.3)	23 (11.3)
Normal	186 (91.6)	171 (84.2)	169 (83.3)

FBG: Fasting blood glucose; OGTT: Oral glucose tolerance test; HbA1c: Hemoglobin A1c.

value was available during the period and utilised for the analysis of data among the participants. The equation describing the relationship between the IFCC and the DCCT units used was: IFCC-HbA1c (mmol/mol) = [DCCT - HbA1c (%) - 2.15] × 10.929[16].

Data of the test results from participants were collected and they were grouped into categories according to the values as normal, impaired glycaemiaor diabetes. FBG values less than 6.1 mmol/L was taken as normal; FBG values between 6.1 mmol/L and 6.9 mmol/L as impaired fasting glucose (IFG); and FBG ⩾ 7.0 mmol/L as diabetes. The OGTT results were classified by the WHO criteria: normal glucose tolerance (FBG < 6.0 mmo/L and/or 2-h PPBG < 7.8 mmol/L); impaired glucose tolerance (FBG ⩾ 6.1 mmol/L and < 7.0 mmol/L, and/or 2-h PPBG between 7.8 and 11.0 mmol/L); and diabetes (FBG ⩾ 7.0 mmol/L and/or 2-h PPBG ⩾ 11.1 mmol/L). Glycaemic categorization was also undertaken according to the HbA1c criteria recommended by the WHO in diagnosis of diabetes in the general population: Diabetes (HbA1c ⩾ 48 mmol/mol or ⩾ 6.5% DCCT) and non-diabetic (HbA1c < 48 mmol/mol or < 6.5%). Those with HbA1c values between 42 mmol/mol and 47 mmol/mol (6.0% and 6.4%) were considered as having an impaired glycaemicstate (high risk)[17].

Statistical analysis

Data was analysed using computer software SPSS Version 19.0 for Windows (SPSS Inc, Chicago, IL, United States). The continuous variables are presented as mean (± 2SD). Differences in the classifications between normal, impaired glycaemia and diabetes using FBG, OGTT and HbA1c were assessed using a non-parametric sign test. Receiver operating characteristics (ROC) curve analysis was used to determine the sensitivity and specificity of HbA1c in comparison with OGTT (as the gold standard) for postpartum screening of diabetes or high risk pre-diabetes state. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 408 women with gestational diabetes were identified during the study period. However, only 203 women (49.8%) had simultaneous OGTT and HbA1c estimation as a part of the postpartum screening at the 6th postpartum week, the cohort which we used in the analysis for this study. Demographic characteristics were as follows: mean age: 29 ± 4.6 years; ethnic origin: 142 were Caucasians (70%) and 61 belonged to other racial groups (Asian: 60, Afro-Caribbean: 2, others: 9); body mass index: 30 ± 6.4 kg/m^2 (Caucasians: 32 ± 5.1 kg/m^2 and Asians 26 ± 4.2 kg/m^2).

Table 2 Cross tabulation between hemoglobin A1c and oral glucose tolerance test or fasting blood glucose in categorisation of glycaemic status *n* (%)

Glycaemic status		HbA1c		
		Normal	Impaired glycaemia	Diabetes
OGTT	Normal	149 (73.4)	17 (8.4)	5 (2.5)
	Impaired glycaemia	20 (9.9)	4 (2.0)	1 (0.5)
	Diabetes	0 (0)	2 (1.0)	5 (2.5)
FBG	Normal	162 (79.8)	19 (9.4)	5 (2.5)
	Impaired glycaemia	7 (3.4)	3 (1.5)	1 (0.5)
	Diabetes	0 (0)	1 (0.5)	5 (2.5)

FBG: Fasting blood glucose; OGTT: Oral glucose tolerance test; HbA1c: Hemoglobin A1c.

Mean FBG was 4.9 ± 0.7 mmol/L; mean 2-h PPBG 5.6 ± 2.0 mmol/L and HbA1c 38 ± 5 mmol/mol (5.6 ± 0.5%). The correlation for HbA1c *vs* FBG was $r = 0.42$ ($P < 0.001$) and for HbA1c *vs* 2-h PPBG was $r = 0.42$ ($P < 0.001$).

Categorization of the cohort into normal (= 1), impaired glycaemia (= 2) or diabetes (= 3) according to blood glucose and HbA1c criteria (WHO criteria) are shown in Table 1. Using the ADA criteria the prevalence of IGT in OGTT increased from 12.3% to 16.7% without altering the total number diagnosed diabetes[18]. The diabetes prevalence using FBG alone and OGTT were 3.0% and 3.5%. Further, OGTT diagnosed a higher proportion with impaired glycaemia compared to FBG (5.4% *vs* 12.3%). Of the 186 women with normal FBG, 15 (8.1%) had IGT on OGTT and none had diabetes based on the 2-h PPBG value on the OGTT. Amongst the 11 women with IFG, 1 had the 2-h PPBG in the diabetes range. Differences in the classifications using FBG and OGTT were assessed using a non-parametric sign test. The misclassifications identified 0 positive, 16 negative differences and 187 ties ($P < 0.001$), suggesting that OGTT is inclined to diagnose a significantly higher proportion of patients with impaired glycaemia and full diabetes compared to FBG.

The categorisation of glycaemia into normal (= 1), impaired glycaemia (= 2) and diabetes (= 3) using HbA1c identified different individuals compared to those identified using the OGTT and FBG (Table 2). HbA1c was in the diabetes range in more women when compared to the OGTT criteria (5.4% *vs* 3.5%, $P > 0.05$). Differences in the classifications using OGTT and HbA1c were also assessed using a non-parametric sign test. The misclassifications identified 22 positive, 23 negative differences and 160 ties ($P > 0.05$), suggesting that HbA1c classified different individuals with normal, impaired glycaemia and diabetes compared to those identified using the OGTT.

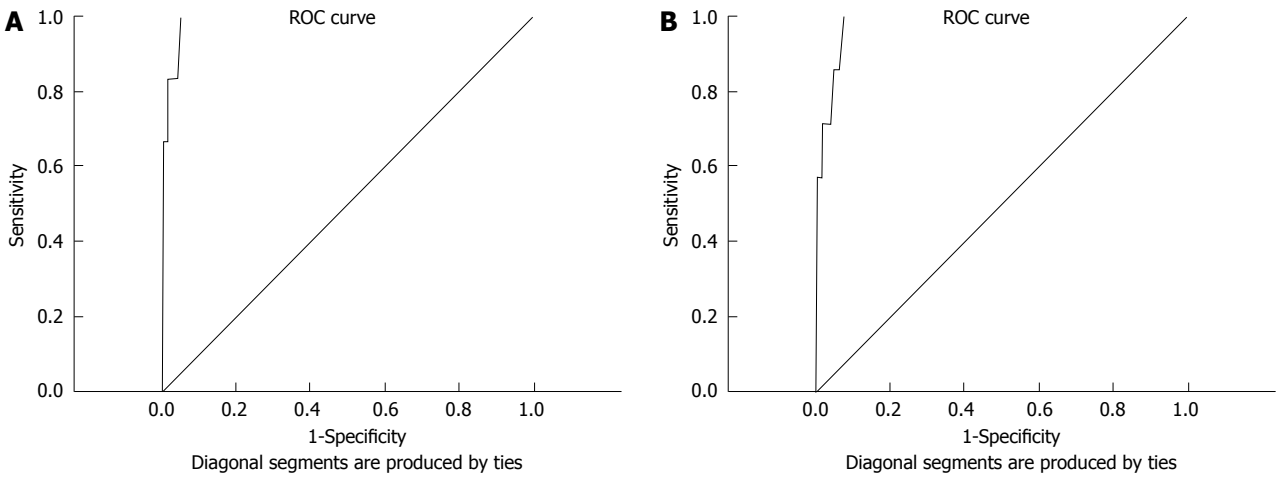

Figure 1 Receiver operating characteristics curve for hemoglobin A1c used for detection of diabetes by glucose criteria. A: Hemoglobin A1c (HbA1c) *vs* fasting blood glucose [area under the curve 0.99 (95%CI: 0.97-1.00)]; B: HbA1c *vs* oral glucose tolerance test [area under the curve 0.98 (95%CI: 0.96-1.00)]. ROC: Receiver operating characteristics.

For the diagnosis of diabetes, in the ROC curve (Figure 1), HbA1c values ≥ 48 mmol/mol (≥ 6.5%) showed a diagnostic sensitivity 71.4% and specificity 98.5% in comparison to the gold standard OGTT. When the HbA1c cut-off was lowered to 44 mmol/mol the sensitivity 100% and specificity 92.3% [area under the curve 0.98 (95%CI: 0.96-1.00)]. The sensitivity and specificity for detecting high risk impaired glycaemia using cut-off HbA1c 42 mmol/mol (6.0%) were 28% and 80% respectively.

DISCUSSION

In this study during early post-partum screening at 6 wk for women with GDM all women diagnosed to have diabetes by the OGTT had an abnormality in their fasting blood glucose. Thus, this supports a strategy to undertake FBG as the initial screening test, and a follow-on OGTT only in those with IFG, that would be sufficient to identify all women with diabetes. This is also in line with the current NICE recommendations for the use of FBG as the initial screening test in these women with GDM during early postpartum period. This would be a very cost-effective strategy avoiding OGTT in majority of women with GDM, but is likely to miss a significantly high proportion of women with IGT (8.1% in our study) who potentially may have a higher risk of developing diabetes earlier, than those with normalised postpartum glycaemia, in whom more aggressive interventions have been recommended by some authorities. However, others would argue that gestational diabetes itself is a significant risk for future diabetes, and that having IGT or with normalisation of glycaemia in postpartum period, there should be no practical difference in the degree of intervention and future screening for diabetes.

This study explored the utility of HbA1c in the early postpartum screening of women with gestational diabetes mellitus. Applying the recent WHO HbA1c criteria, the yield of diabetes was significantly higher compared to the blood glucose (OGTT) criteria (5.4% *vs* 3.5%) and the two tests diagnosed different women with diabetes with concordance only in 2.5%. Previous studies tested the utility of HbA1c in postpartum screening of glucose abnormalities at variable time periods from 6 wk to 36 mo[10,19-21]. Our data is unique in that estimation of all the 3 parameters for dysglycaemia (abnormal FBG, OGTT and HbA1c) were done in a single setting during the first postpartum visit at 6 wk. Many patients would have done postpartum screening at other health centres and there remained a variation in the test undertaken in a proportion of women making the response rate low (49.8%).

Use of HbA1c has been recommended as a test to diagnose diabetes by various international organisations[22-25]. Advantage of blood sampling in the non-fasting state without the need for OGTT makes HbA1c check as a promising tool for diabetes screening. However, the reliability of HbA1c as a true reflection of glycaemic status at 6 wk post-partum could be affected by several factors including glucose control in the later stages of pregnancy and/or the use or discontinuation of hypoglycaemic therapies peri-partum. HbA1c represents average glucose levels over the last 3 mo, not 6 wk, and therefore HbA1c at 6 wk postpartum partly reflects glucose levels during the pregnancy and hence the concern to use it at 6 wk post-partum as a screening test. The performance of HbA1c is likely better at about 12 wk or 3 mo postpartum. In addition, altered red cell turnover, anaemia of pregnancy and potential factors altering glycation - deglycation rates in the erythrocyte might affect the HbA1c value independent of prevailing glucose control that affect the categorisation of glycaemic status[26]. In our study higher proportion of patients were found to have HbA1c in the diabetes range compared to the previous reports, which may be due to the impact of prevailing glucose control just prior to delivery on the HbA1c estimation at 6 wk post-partum[19,20].

Though HbA1c did not appear to help in the diagno-

sis of diabetes in this study, it was noted that HbA1c had a high negative predictive value/sensitivity, with HbA1c cut-off < 44 mmol/mol practically excluding all those with diabetes (but not IGT). Thus HbA1c may be potentially used with FBG estimation to avoid OGTT in those women with an HbA1c < 44 mmol/mol. Further studies with larger cohort may be required to confirm this and prior to recommending HbA1c on its own as the test for early post-partum screening for women with gestational diabetes mellitus.

COMMENTS

Background

Hemoglobin A1c (HbA1c) has been accepted as a screening tool for diabetes mellitus by various professional bodies in the recent years. However, there is no data available on the efficacy of HbA1c in comparison to oral glucose tolerance test (OGTT) for screening diabetes and impaired glycaemia at the 6th postpartum week in patients with gestational diabetes mellitus (GDM).

Research frontiers

OGTT, the gold standard test for postpartum screening of GDM cases, is cumbersome and the patients need to fast overnight, whereas HbA1c estimation needs only random blood testing. Limited data on postpartum screening with HbA1c at different timescales after childbirth showed variable results in terms of the sensitivity and specificity. It remains unclear if HbA1c estimation can be used as a screening tool for diabetes and impaired glycaemia at the 6th week postpartum clinic visit.

Innovations and breakthroughs

This study demonstrated that HbA1c has reasonable sensitivity and high specificity in comparison to OGTT for postpartum screening of diabetes in patients with GDM.

Applications

Results of the study shows that GDM patients with HbA1c values less than 44 mmol/mol and normal fasting blood glucose levels may not need the cumbersome OGTT to exclude diabetes mellitus.

Peer review

HbA1c levels reflect the average glycaemic burden over 3-mo period and estimation of HbA1c at the 6th postpartum week may overestimate prevalence of diabetes in those with high glucose levels towards term of pregnancy and therefore may limit the diagnostic utility in screening patients with GDM. However, the study demonstrated reasonable sensitivity, high specificity of HbA1c as a postpartum screening tool.

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BRIEF ARTICLE

Atherosclerotic cardiovascular disease among patients with type 2 diabetes in Basrah

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Abstract

AIM: To determine the frequency of atherosclerotic cardiovascular disease and its risk factors among patients with type 2 diabetes in Basrah, Iraq.

METHODS: Participants in this cross-sectional study were patients who had type 2 diabetes for at least 1 year, presenting at the Al-Faiha Diabetes Endocrine and Metabolism Center in Basrah (Southern Iraq) over the period from January to December 2008.

RESULTS: The series included 1079 patients (58.8% men), of whom 25.0% were smokers. The prevalence of symptomatic cardiovascular disease and hypertension was 16.0%, and 44.3% respectively. Those who were overweight or obese constituted 70.5%. Insulin was used in only 26.9% despite 56.1% having had diabetes for 6 years and more. The mean glycated hemoglobin (HbA1c) was 9.46% ± 2.0% and only 5.5% achieved the target of HbA1c of < 7%. We had 68.7% of patients with total cholesterol of ⩾ 200 mg/dL, 21.5% with high density lipoprotein cholesterol of < 40 mg/dL, 84.1% with low density lipoprotein cholesterol of ⩾ 100 mg/dL and 71.6% with triglyceride of ⩾ 150 mg/dL.

CONCLUSION: Among adults with type 2 diabetes mellitus, there was increased frequency of cardiovascular disease and its modifiable risk factors. This finding necessitated urgent work to modify these risk factors in a population based setting.

Key words: Cardiovascular risk factors; Diabetes mellitus; Type 2 diabetes mellitus; Cardiovascular disease

Core tip: Atherosclerotic cardiovascular complications are mainly responsible for the high morbidity and mortality in people with diabetes. We studied 1079 patients with type 2 diabetes mellitus to determine the frequency of atherosclerotic cardiovascular disease and its risk factors. The main finding of this study was an increased frequency of cardiovascular disease and its modifiable risk factors among patients with type 2 diabetes in Basrah, Iraq.

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INTRODUCTION

In 2004, the World Health Organization projected that by 2030, the global prevalence of diabetes would double, to affect an estimated 366 million individuals, representing 4.4% of the world population[1].

Atherosclerotic cardiovascular complications are mainly responsible for the high morbidity and mortality in people with diabetes[2-4]. Cardiovascular disease (CVD), which includes coronary heart disease (CHD), and stroke, accounts for up to 80% of all deaths among patients with

diabetes[5], compared with one-third of all deaths in the general population[6]. This CVD rate in people with type 2 diabetes also varies according to ethnicity[7].

Atherosclerosis in diabetes is more precocious, aggressive and multi-focal than in the non-diabetic population[8]. That is why it is recommended to decrease low density lipoprotein cholesterol (LDL-C) < 100 mg/dL (< 70 mg/dL in people with overt CHD with diabetes)[9]. From a cardiovascular point of view, it has been suggested that diabetic patients should be considered as about 15 years older than the general population[10]. The chance of cardiovascular events in diabetic patients is as high as in patients with a previous history of myocardial infarction but without diabetes[11]. Risk factors for CVD in people with diabetes include age, duration of diabetes, smoking, abnormal lipid profiles, raised blood pressure and albuminuria[12,13].

The aim of this study was to determine the frequency of atherosclerotic CVD and its risk factors among patients with type 2 diabetes in Basrah, Iraq.

MATERIALS AND METHODS

Participants in this cross-sectional study were patients who had type 2 diabetes for at least 1 year, presenting at the Al-Faiha Diabetes, Endocrine and Metabolism Center in Basrah (Southern Iraq) over the period from January to December 2008 and who had not received lipid lowering agents prior to enrollment. This center received patients from all over Basrah and it is the only qualified center treating diabetes in Southern Iraq. Participants gave written consents and the ethical committee of Basrah College of Medicine approved the study.

The definition of CVD included CHD and cerebrovascular accidents(CVA; stroke). CHD diagnosis was based on a history of admission to CCU with elevated cardiac biomarkers, electrocardiographic evidence of ST elevation, Q wave myocardial infarction or left bundle branch block, echocardiographic segmental wall motion abnormalities, abnormal angio-cardiography, percutaneous coronary intervention or coronary artery bypass surgery. The CVA was diagnosed on the basis of sudden neurological deficit that lasted for 24 h, with or without neuroimaging changes.

Variables risk factors for CVD

Potential risk factors for CVD included age, gender, smoking status, waist circumference (WC), body mass index (BMI), hypertension, duration of diabetes, glycated hemoglobin (HbA1c), insulin use, serum total cholesterol and high density lipoprotein cholesterol (HDL-C), triglycerides (TG), and LDL-C. Current smoker was defined as any person who had smoked cigarettes in the preceding three months, regardless of the number.

Hypertension was defined as systolic blood pressure of 140 mmHg or higher, diastolic blood pressure of 90 mmHg or higher, use of antihypertensive agents or a combination of these. Blood pressure was measured in a seated position after 5 min at rest, three times. The average of the second and third readings was defined as the subject's blood pressure.

Diabetes mellitus was defined as plasma glucose level being 200 mg/dL or higher independent of fasting time, fasting plasma glucose of ⩾ 126 mg/dL on two occasions, use of anti-diabetes agents or a combination of these[14]. After failure of oral antihyperglycemic agents failed we started with basal human insulin neutral protamine Hagedorn at bedtime then basal prandial or premixed twice.

Anthropometric measurements

The waist circumference, was measured in centimeters using a plastic anthropometric tape from the horizontal plane at the umbilical level. Measurement was made with the participant standing erect, abdomen relaxed, arms at the side and feet together with weight equally divided over both legs. Participants were told to breathe normally and to breathe out gently at the time of measurement and not to hold in their abdomen or hold their breath. BMI was calculated as body weight in kilograms divided by the squared value of body height in meters (kg/m^2).

Biochemical analysis

Fasting serum samples were analyzed in the same local laboratory. Blood (10 mL) was drawn into a tube after the subject had fasted for at least 10 h. Serum total cholesterol, HDL-C, and TG were measured by the enzymatic method. LDL-C concentrations were calculated using the Friedewald formula. Risk factors in the lipoprotein profile of participants would be a total cholesterol of equal or more than 200 mg/dL, LDL-C equal or more than 100 mg/dL, HDL-C less than 40 mg/dL and TG level equal or more than 150 mg/dL[14,15]. HbA1c was measured using a fully automated glycohemoglobin analyzer which uses high performance liquid chromatography.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS) version 15 (SPSS Inc, Chicago, Illinois, United States). Characteristics of the sample are reported as mean ± SD or frequencies and percentages. Pearson's χ^2 was used to examine if there is an association between two categorical variables and Student's *t* test were used for continuous variables.

RESULTS

Table 1 shows the basic socio-demographic and cardiovascular risk factors. The series included 1079 patients (58.8% men, mean age 56.3 ± 11.37 years; age range, 26-94 years) with a mean qualification period of 6.0 ± 6.0 years and 25.0% were current smokers. The mean duration of type 2 diabetes was of 7.4 ± 6.5 years.

The prevalence of symptomatic CVD was 16.0% and hypertension was seen in 44.3%. The mean WC was 100.2 ± 12.3 cm and BMI 28.0 ± 5.4 kg/m^2. Those who

Table 1 Sociodemographic and cardiovascular risk factors of 1079 patients *n* (%)

Variable		
Sex	Men	634 (58.8)
	Women	445 (41.2)
Age (yr)	mean ± SD	56.3 ± 11.37
	< 40	83 (7.7)
	40–49	220 (20.4)
	50–59	330 (30.6)
	⩾ 60	41.3 (41.3)
Education (yr)	mean ± SD	6.0 ± 6.0
	Illiterate	455 (42.2)
	⩽ 9	326 (30.2)
	⩾ 10	298 (27.6)
Current smoking	Yes	270 (25.0)
	No	809 (75.0)
Duration of diabetes (yr)	mean ± SD	7.4 ± 6.5
	⩽ 5	474 (43.9)
	6-10	361 (33.5)
	> 10	244 (22.6)
Known cardiovascular disease	Yes	173 (16.0)
	No	906 (83.9)
Hypertension	Yes	478 (44.3)
	No	601 (55.7)
WC (cm)	mean ± SD	100.2 ± 12.3
BMI	mean ± SD	28.0 ± 5.4
	< 25	318 (29.5)
	25-29	396 (36.7)
	⩾ 30	365 (33.8)
Insulin use	Yes	290 (26.9)
	No	789 (73.1)
HbA1c (% total Hb)	mean ± SD	9.46 ± 2.0
	< 7	59 (5.5)
	⩾ 7	1020 (94.5)
Total cholesterol (mg/dL)	mean ± SD	206.8 ± 42.6
	< 200	338 (31.3)
	⩾ 200	741 (68.7)
HDL cholesterol (mg/dL)	mean ± SD	46.5 ± 13.0
	< 40	232 (21.5)
	⩾ 40	847 (78.5)
LDL cholesterol (mg/dL)	mean ± SD	122.6 ± 35.9
	< 100	172 (15.9)
	⩾ 100	907 (84.1)
Triglycerides (mg/dL)	mean ± SD	193.5 ± 98.3
	< 150	306 (28.4)
	⩾ 150	773 (71.6)

WC: Waist circumference; BMI: Body mass index; HbA1c: Hemoglobin A1c test; HDL: High density lipoprotein; LDL: Low density lipoprotein.

were overweight or obese constituted 70.5%. Insulin was used in 26.9% despite the fact that 56.1% had diabetes for 6 years and more. The mean HbA1c was 9.46 % ± 2.0% and only 5.5% achieved the target of HbA1c of < 7%. We had 68.7% of patients with total cholesterol of ⩾ 200 mg/dL, 21.5 % with HDL-C of < 40 mg/dL, 84.1% with LDL-C of ⩾ 100 mg/dL and 71.6 % with TG of ⩾ 150 mg/dL.

In Table 2, the sociodemographic and cardiovascular risk factors are shown according to gender. There were no differences between the two groups in the age or duration of diabetes. Qualification levels were statistically higher among men, as was the smoking rate ($P < 0.0001$). Among the 25% current smokers, 87.8% were men. Established CVD and hypertension levels were higher in

Table 2 Sociodemographic and cardiovascular risk factors of 1079 patients according to gender *n* (%)

Variable		Men	Women	*P* value
Age (yr)	mean ± SD	56.6 ± 11.5	55.8 ± 11.0	0.276
	< 40	44 (53.0)	39 (47.0)	0.650
	40-49	133 (60.5)	87 (39.5)	
	50-59	191 (57.9)	139 (42.1)	
	⩾ 60	266 (59.6)	180 (40.4)	
Education (yr)	mean ± SD	8.2 ± 2.8	3.0 ± 4.7	< 0.0001
	Illiterate	160 (35.2)	295 (64.8)	< 0.0001
	⩽ 9	228 (69.9)	98 (30.1)	
	⩾ 10	246 (82.6)	52 (17.4)	
Current smoker	Yes	237 (87.8)	33 (12.2)	< 0.0001
Duration of	mean ± SD	7.6 ± 6.9	7.1101 ± 6.02	0.149
diabetes (yr)	⩽ 5	267 (56.3)	207 (43.7)	0.314
	6-10	222 (61.5)	139 (38.5)	
	> 10	145 (59.4)	99 (40.6)	
Known cardiovascular disease		125 (72.3)	48 (27.7)	< 0.0001
Hypertension		265 (55.4)	213 (44.6)	0.048
WC (cm)	mean ± SD	100.15 ± 12.45	100.46 ± 12.16	0.681
BMI	mean ± SD	27.7 ± 5.0	28.5 ± 5.9	0.029
	< 25	190 (59.7)	128 (40.3)	0.193
	25-29	243 (61.4)	153 (38.6)	
	⩾ 30	201 (55.1)	164 (44.9)	
Insulin use	Yes	129 (44.5)	161 (55.5)	< 0.0001
HbA1c	mean ± SD	9.32 ± 2.0	9.61 ± 2.05	0.100
(% total Hb)	< 7	35 (59.3)	24 (40.7)	0.928
	⩾ 7	599 (58.7)	421 (41.3)	
Total cholesterol	mean ± SD	206.2 ± 42.6	207.7 ± 42.6	0.658
(mg/dL)	< 200	221 (65.4)	117 (34.6)	0.003
	⩾ 200	413 (55.7)	328 (44.3)	
HDL cholesterol	mean ± SD	45.85 ± 14.58	47.76 ± 9.84	0.040
(mg/dL)	< 40	173 (74.6)	59 (25.4)	< 0.0001
	⩾ 40	461 (54.4)	386 (45.6)	
LDL cholesterol	mean ± SD	122.2 ± 35.5	123.2 ± 36.5	0.723
(mg/dL)	< 100	106 (61.6)	66 (38.4)	0.447
	⩾ 100	528 (58.2)	379 (41.8)	
Triglycerides	mean ± SD	201.0 ± 107.1	181.3 ± 80.60	0.004
(mg/dL)	< 150	179 (58.5)	127 (41.5)	0.913
	⩾ 150	455 (58.9)	318 (41.1)	

WC: Waist circumference; BMI: Body mass index; HbA1c: Hemoglobin A1c test; HDL: High density lipoprotein; LDL: Low density lipoprotein.

men ($P < 0.0001$ and $P = 0.048$ respectively). There was no difference in the WC but the BMI was higher among women ($P = 0.029$). Insulin was used more commonly among women ($P < 0.0001$), but no differences were seen in the mean HbA1c. No significant differences were seen between the two groups regarding total cholesterol or LDL-C. The HDL-C was higher among women ($P = 0.040$) while TG was higher among men ($P = 0.004$). Subgroup analysis of the two groups showed total cholesterol ⩾ 200 mg/dL and HDL-C < 40 mg/dL was more common among men ($P = 0.003$ and $P < 0.0001$ respectively).

The percentage of patients with hypertension, current smoker status and levels of HbA1c, LDL-C, and HDL-C outside the target levels according to the studied variables are summarized in Table 3. Men were more likely to be current smokers and have HDL-C < 40 mg/dL, whereas women were more likely to be hypertensive. There were no differences in risk factors between age groups. Illiterates were more likely to be hypertensive and have HbA1c

Table 3 Percentages of patients with type 2 diabetes (n = 1079) with cardiovascular risk factors outside target range

Variable (%)	Current smoking	Blood pressure (≥ 140/90 mmHg)	HbA1c (≥ 7%)	LDL cholesterol (≥ 100 mg/dL)	HDL cholesterol (< 40 mg/dL)
All	25.0	44.3	86.1	84.1	27.6
Gender					
Men	37.4	41.8	84.4	83.3	32.8
Women	7.4	47.9	87.8	85.2	18.7
P value	< 0.0001	0.048	0.267	0.447	< 0.0001
Age (yr)					
< 40	27.7	45.8	87.2	84.3	33.9
40-49	26.8	39.5	87.3	81.4	26.2
50-59	21.2	45.5	84.4	83.9	27.4
≥ 60	26.5	45.5	86.4	85.4	27.3
P value	0.291	0.469	0.905	0.610	0.727
Education (yr)					
Illiterate	19.3	52.3	86.6	87.9	26.1
≤ 9	31.0	35.3	90.7	82.8	27.8
≥ 10	27.2	41.9	79.4	79.5	29.2
P value	0.001	< 0.0001	0.020	0.007	0.693
Duration (yr)					
≤ 5	25.3	28.7	85.4	84.6	24.6
6-10	26.3	58.4	85.2	84.8	28.5
> 10	22.3	55.8	88.6	82.0	31.7
P value	0.545	< 0.0001	0.672	0.596	0.207
BMI (kg/m^2)					
< 25	25.8	31.1	84.0	84.9	28.4
25-29.9	23.2	47.0	84.6	81.6	26.6
≥ 30	26.3	52.9	89.7	86.0	27.9
P value	0.579	< 0.0001	0.254	0.216	0.873
WC (cm)					
< 80	25.9	15.5	88.6	81.0	23.7
≥ 80	25.0	45.9	85.9	84.2	27.7
P value	0.879	< 0.0001	0.656	0.466	0.585
Insulin use					
Yes	18.3	40.0	92.8	79.7	26.3
No	27.5	45.9	80.7	85.7	28.0
P value	0.002	0.085	< 0.0001	0.019	0.624

WC: Waist circumference; BMI: Body mass index; HbA1c: Hemoglobin A1c test; HDL: High density lipoprotein; LDL: Low density lipoprotein.

> 7%. Those with a duration of diabetes greater than 5 years and those with high BMI and WC ≥ 80 cm were more likely to be hypertensive. Insulin was used less in those who were smokers, and had LDL-C ≥ 100 mg/dL but was used more in those with HbA1c ≥ 7%.

DISCUSSION

The prevalence of symptomatic CVD in our study was 16.0%; Current smokers were 25.0%; Hypertension was seen in 44.3% and 70.5% were overweight or obese. In Kuwait, 59.3% of patients with type 2 diabetes were hypertensive, 26.3% were overweight, 65.0% were obese, history of CVD was present in 10.0% of patients and 13.3% were smokers[16]. Among centers for diabetes, at the University of Jordan, Amman, in patients with type 2 diabetes approximately 70.0% of the patients had hypertension[17].

In a cohort study of diabetic patients in Lebanon, 43.8% patients were obese, 35.0% were current smokers and hypertension was present in 53.2%[18]. Among 1846 patients with diabetes in Spain, obesity and high blood pressure were present in > 50.0%, and established CVD in 24.0%[19]. Of patients with type 2 diabetes in a district of Madrid, Spain, only 13.0% had a BMI < 25 kg/m^2[20].

In patients with type 2 diabetes mellitus at primary care settings from the North Catalonia Diabetes Study, CVD prevalence was 22.0%, hypertension 74.5%, smoking 14.9% and obesity 44.9%[21]. Overall, 27.0% of diabetic American Indians reported a history of CVD and 57.0% had hypertension[22]. Zhang *et al*[23] found that in 3469 type 2 diabetic in-patients in China hypertension was seen in 41.8%, CHD in 25.1%, cerebral vascular disease in 17.3%.

In our study the mean HbA1c was 9.46% ± 2.0% and only 5.5% achieved the target HbA1c of < 7% while in Kuwait it was 16.2%[16], in Spain 41%[20], in Germany 46.6%[24] and in Lebanon, 32% of patients with type 2 diabetes had HbA1c of ≤ 7%[18]. The association between HbA1c and CVD in type 2 diabetes is well known with a relative risk of 1.18 (95%CI: 1.10-1.26) for CVD with a 1% increase in HbA1c[25].

The insulin was used in our study by 26.9 % of patients despite the fact that 56.1 % had diabetes for 6 years and more. In Lebanon, only 20.0% of patients were taking insulin[18].

In this study, majority of our patients had lipid profiles outside of the target range. In one study on lipid

profile in apparently healthy volunteers in Mosul, Iraq, 42% of the study population had TG > 150 mg/dL, 58% had LDL-C > 100 mg/dL, and 50 % had HDL-C < 40 mg/dL[26]. There was no difference in the prevalence of elevated LDL-C by the studied variables except for less use of insulin, a finding that was also seen in Kuwaiti patients[16]. In Kuwait, 86.2% overall and 63.2% of the patients with type 2 diabetes had LDL-C > 100 mg/dL and HDL-C outside the target levels[16]. The North Catalonia Diabetes Study showed dyslipidemia in 77.7%[21]. In Jordan, patients with type 2 diabetes had high LDL-C (≥ 100 mg/dL) in 91.5%, low HDL-C in 83.9%, high TG in 83.1% and high total cholesterol in 77.2%[17]. In Augsburg Diabetes Family Study, total cholesterol was seen as < 200 mg/dL in 44.1%, and LDL-C was < 100 mg/dL in 16.0% of the participants[24].

In this study the smoking rate among men was higher than in other studies. Established CVD and hypertension were found more in men, while BMI was higher among women. In Kuwait, hypertension was more prevalent among women than men and smoking was only present among men[16]. The BMI, and HDL-C were significantly higher in women than men, while TG and total cholesterol was higher among men in Kuwait.

In conclusion, our study demonstrated that among adults with type 2 diabetes mellitus, there was increased frequency of cardiovascular disease and its modifiable risk factors. This finding suggests a need for urgent work to modify these risk factors in the population-based setting.

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COMMENTS

Background

Atherosclerotic cardiovascular complications are mainly responsible for the high morbidity and mortality in people with diabetes. Cardiovascular disease (CVD), which includes coronary heart disease, and stroke, accounts for up to 80% of all deaths among patients with diabetes, compared with one-third of all deaths in the general population. The CVD rate in people with type 2 diabetes also varies according to ethnicity.

Research frontiers

The prevalence of symptomatic CVD was 16.0% and hypertension was seen in 44.3% of patients. Those who were overweight or obese constituted 70.5%. Only 5.5% achieved the target of hypertension, duration of diabetes, and glycated hemoglobin (HbA1c) of < 7%. The authors found 68.7% of patients with total cholesterol of ≥ 200 mg/dL, 21.5% with high density lipoprotein cholesterol (HDL-C) of < 40 mg/dL, 84.1% with low density lipoprotein cholesterol (LDL-C) of ≥ 100 mg/dL and 71.6 % with triglycerides (TG) of ≥ 150 mg/dL.

Innovations and breakthroughs

Potential risk factors for CVD included age, gender, smoking status, waist circumference, body mass index, hypertension, duration of diabetes, HbA1c, insulin use, serum total cholesterol and HDL-C, TG and LDL-C. This study demonstrated that among adults with type 2 diabetes mellitus, there was increased frequency of cardiovascular disease and its modifiable risk factors.

Applications

This finding suggests a need for urgent work to modify these risk factors in the population-based setting.

Peer review

Well written study report provides epidemiological data from a cohort of type 2 diabetes patients in Iraq.

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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 200201 03498. 2002 Aug 1

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