Original Article

Consumption of guava (*Psidium guajava* L) and noni (*Morinda citrifolia* L) may protect betel quid-chewing Papua New Guineans against diabetes

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Rapid increase in the incidence of type 2 diabetes (DM2) in Papua New Guinea, coupled with compelling epidemiological evidence supporting a diabetogenic association with betel quid (BQ) chewing has lead us to investigate dietary strategies that might offer protection from developing DM2. We investigated the dietary habits of Kalo residents from coastal Central Province who are avid BQ chewers yet have a relatively low incidence of DM2 compared to the ethnically similar and adjacent Wanigelans who abstain from BQ yet have an unusually high incidence of DM2. In Kalo, guava bud (*Psidium guajava* L) and noni (*Morinda citrifolia* L) were consumed much more frequently than in Wanigela, whereas the inverse was observed for mangrove bean (*Bruguiera gymnorrhiza* (L) Lam.). These plants, along with BQ and its component ingredients areca nut (*Areca catechu* L) and *Piper betle* L inflorescence, were assessed for their ability to mediate insulin-dependent and insulin-independent glucose transport in cultured 3T3-L1 adipocytes. A dose-dependent inhibition of glucose uptake from methanolic extracts of BQ, areca nut and *P. betle* inflorescence supports previous reports of pro-diabetic activity. Conversely, guava bud extract displayed significant insulin-mimetic and potentiating activity. Noni fruit, noni leaf, commercial noni juice and mangrove bean all displayed insulin-like activity but had little or no effect on insulin action. Habitual intake of guava and noni is proposed to offer better protection against DM2 development and/or betel quid diabetogenicity than cooked mangrove bean. These findings provide empirical support that DM2 risk reduction can be accomplished using traditional foods and medicines.

Key Words: Psidium, areca, 3T3-L1 cells, plants, medicinal

INTRODUCTION

Populations undergoing nutritional and social transition due to economic development and globalization are at greater risk of obesity and type 2 diabetes (DM2).[1] Compelling epidemiological and animal studies suggests that betel quid (BQ) chewing, popular in some Pacific nations, exacerbates this risk.[2-4] In Papua New Guinea, chewing BQ was reportedly the most significant independent risk factor for DM2 (odds ratio 3.4; 95% CI. 2.0-5.9).[5] However, DM2 is rare in Kalo, a coastal community where residents habitually and avidly chew BQ.[6] In contrast, the ethnically similar and geographically adjacent Wanigela people have been recorded as having an unusually high incidence of DM2 in both urban and rural settings,[7] yet for religious reasons abstain from chewing BQ. A plausible explanation for this apparent paradox is that some element(s) in the diet, lifestyle or environment is protecting Kalo residents from developing DM2 and from BQ diabetogenicity.

Beyond their macronutrient composition and glycemic index, several foods and medicinal plants have antidiabetic properties attributed to various phytochemicals that stimulate pancreatic insulin secretion, reduce peripheral insulin resistance or decrease carbohydrate absorption.[8] Conversely, some plant compounds such as the nitrosamines in areca nut can be diabetogenic.[4] Differential con-

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sumption patterns of pro- and antidiabetic traditional plants may mediate DM2 risk over and above the risk imposed by the nutrition transition.

The aim of this study was to select traditional plants that were prominent and/or distinct in Kalo and Wanigela using a pretested ethnomedical questionnaire, and to assess these plants' ability to mediate insulin-independent and -dependent glucose uptake in cultured 3T3-L1 adipocytes, a cell line used extensively to study peripheral tissue insulin responsiveness.[9] The study was designed as a preliminary screening of commonly used medicinal plants for antidiabetic activity, and multivariable analysis with community plant use frequency lead to our hypothesis that guava and noni consumption may counter diabetes risk. Focus was directed towards medicinal plants rather than food plants (with the exception of mangrove bean) since their role in non-communicable disease mediation is increasingly becoming an area of interest to researchers and public health workers who are developing culturally appropriate strategies to promote wellness and health.

SUBJECTS AND METHODS

Study area

A quantitative ethnobotanical survey of medicinal and food plants was conducted in Kalo (Central Province, 10.050° S, 148.200° E), Koki (National Capital District, 9.483° S, 147.167° E), and Wanigela (Central Province, 10.050° S, 147.783° E) from January to August, 2004. Wanigela is a lagoon stilt village located approximately 400 km east from the capital. Some residents engaged in subsistence fishing and farming, while others relied on city relatives to provide trade store goods. Koki, a permanent Wanigela settlement located in a relatively affluent suburb of Port Moresby, had direct access to grocery stores and open air markets. Both Wanigela and Koki practice the Seventh-Day Adventist religion which emphasizes health and diet by advocating vegetarianism and abstinence from stimulants such as betel quid, alcohol, caffeine and tobacco. Residents of Kalo, a road-connected village located roughly equidistant between Koki and Wanigela, are ethnically similar and are predominantly subsistence farmers and fishermen.

Participants were selected by stratified random sampling according to gender and age and administered a quantitative questionnaire modeled after the Expanded Diabetes Diagnostic Criteria (EDDC) developed by Carlson.[10] The questionnaire invited participants to list all plants indicated for DM2 symptoms. All individuals above 16 years were eligible to participate. The survey covered a final sample of 365 participants roughly divided between the three villages. Approval from the head of each local level government was obtained, followed by a public information session and individual prior informed consent before initiation of the study. Permission and ethics approval was obtained from the McGill University Ethics Committee, the Papua New Guinea Medical Advisory Board and the Papua New Guinea Department of Environment and Conservation.

Anthropometric measurements and blood glucose determination

Participants were weighed without shoes and in light clothing to the nearest 100g on a Seca Dial Scale (Vogel & Halke, Germany). Standing height was measured using wooden boards with a measuring tape (0.1 cm precision) that were built locally based on the UNICEF model. Waist and hip circumference was measured using a fiberglass measuring tape. Skin-fold thickness was obtained using Lange calipers and body fat % calculated according to formulae supplied by the manufacturer for four sites: tricep, bicep, subscapular and suprailiac. Blood pressure was taken in duplicate after a participant was asked to sit quietly for five minutes. In cases where hypertension was detected (>120/80), another reading was taken after ten minutes. If still hypertensive, the participant was referred to the Community Health Worker. Participants were encouraged to retest their blood pressure at any time during the study. Fasting blood glucose (FBG) concentration was determined using a portable glucometer (CardioChek™ Analyzer, Polymer Technology Systems Inc, Indiana) after an overnight 12-hour fast. Readings were obtained within 2 minutes. Using current WHO diagnostic criteria, participants with glucose concentrations above 6.1 mmol/L were considered to have impaired glucose tolerance and those above 7.0 mmol/L to have diabetes.[11] Newly identified hyperglycemic individuals were retested for confirmation and referred to the Port Moresby General Hospital Diabetes Clinic for further testing and treatment. Participants with known diabetes were excluded from the analyses since this may have influenced dietary and lifestyle patterns.

Plant selection and extract preparation

Plants included in the present study were selected from a combination of quantitative and qualitative measurements determined by the frequency of their usage, the EDDC questionnaire and their local sociocultural importance. Plants were extracted in methanol using the Soxtec™ system as described elsewhere.[12] Voucher specimens were deposited at the University of Papua New Guinea and McGill University herbarium. Betel quid (BQ) was prepared by combining approximately 66.6% areca nut extract (AN), 26.6% *Piper betle* extract (PBI) and 6.6% calcium hydroxide before extraction. Cooked mangrove bean (MBC) was prepared according to traditional methods: thin slices were soaked for 1 h and boiled in two changes of sea water. Noni juice (NJ) was purchased from a local health food store and used in bioassays as purchased (Flora Manufacturing & Distributing Ltd, Burnaby, BC).

Cell Culture

3T3-L1 murine pre-adipocytes were purchased from American Type Cell Collection (ATCC; Manassas, VA) and cultured in a humidified 37 °C 5% CO_2: 95% air atmosphere in Dulbecco's modified eagle medium containing 10% fetal bovine serum (FBS) and penicillin-streptomycin antibiotics (Invitrogen Life Technologies, Burlington, ON) as described previously.[13] Upon 80% confluence, differentiation was initiated by adding 250 µmol/L 3-isobutylmethylxanthine, 1 µmol/L dexamethasone, and 500 nmol/L insulin for 2 d and further

continued with FBS and insulin until at least 90% of the adipocytes developed visible lipid droplets.

Determination of plant extract concentration for cell culture

Plant extracts were solubilized in dimethyl sulfoxide (DMSO) at a concentration of 200 mg/mL, filter-sterilized, aliquoted, and stored at -20°C. Stock solutions were diluted in culture medium to produce a final concentration of 200 µg/mL and a final DMSO concentration of 0.1%. Additional concentrations of 100 and 50, as well as 25 and 12.5 µg/mL for PBI, were obtained by serial dilution and addition of DMSO to keep solvent concentration constant. A trial to determine maximal non-toxic concentrations by observing cellular morphological changes showed that all plants except PBI were well-tolerated at concentrations up to 200 µg/mL (data not shown).

Glucose Uptake Assay

Following the methods of Martineau et al.[13] differentiated and confluent 3T3-L1 adipocytes grown in 12-well plates were incubated with vehicle (DMSO), plant extract or positive control for 18 hours. Thereafter, cells were rinsed twice with Krebs Ringer phosphate buffer (KRBS) solution (20 mM HEPES, 4.05 mM Na_2HPO_4, 0.95 mM NaH_2PO_4, 136 mM NaCl, 4.7 mM KCl, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM glucose, 0.5% BSA, pH 7.4) at 37°C and then treated with 0, 1, or 100 nM insulin in this buffer for 30 minutes in the continued presence or absence of plant extract. Cells were then washed twice with glucose-free KRPB and treated with 0.5 µCi/mL 2-deoxy-D-[1-^{3}H] glucose (TRK-383, Amersham Biosciences, Baie d'Urfé, QC) for 10 minutes at 37°C without extracts. After incubation, cells were placed on ice and immediately rinsed three times with ice-cold KRPB, lysed with 0.1 mol/L NaOH for 30 minutes and scraped. The lysate and additional rinse were added to 4 mL of liquid scintillation gel (Ready-Gel 586601; Beckman Coulter Inc, Fullerton, CA) and incorporated radioactivity was measured in a scintillation counter. A well-recognized hypoglycemic plant extract, fenugreek seed (*Trigonella foenum-graecum* L) ethanolic extract[14], was used as a positive control at a maximal non-toxic dose of 75 µg/mL (data not shown). After this period, cells were incubated for an additional 3 h in serum-free medium. Results are derived from the average of three independent experiments performed in triplicate and expressed as the change in glucose uptake activity relative to basal levels obtained from incubation with the vehicle.

Statistical Analysis

Differences in consumption patterns, anthropometric and clinical measures between sexes and between villages were assessed using independent Student's t-tests. Spearman's correlation for non-normally distributed data was used to find associations between consumption patterns and health parameters. Fasting blood glucose was first normalized by inverse transformation (100-1/FBG) and multivariate linear regression performed with age, waist circumference and weight as covariates. The interaction of plant extract concentration on insulin dose response was assessed using 2-factor ANOVA with pair-

Table 1. Characteristics of plants, the area of collection, ethnomedical indications and methanolic extract yield.

Family, species, Voucher Number	Plant part	English, local name	Ethnomedical indications†	Area collected	Extract yield (%)
Arecaceae *Areca catechu* L (AN), KAL-12	Seed	Betelnut, Buai	Stimulant/ sedative, appetite suppressor/ stimulant, anti-malarial	Koki, Kalo	24.4
Myrtaceae *Psidium guajava* L (GB), KAL-01	Bud	Guava, Tuava	Antidiabetic, antibacterial, anti-malarial, anti-inflammatory, antidiarrheal, gastrointestinal tonic	Kalo	18.4
Piperaceae *Piper betle* L (PBI), KAL-24	Inflores-cence	Pepper, Daka	Antibacterial	Koki, Kalo	12.5
Rhizophoraceae *Bruguiera gymnorrhiza* (L.) Lam. (MBC), WAN-01	Cooked hypocotyls	Mangrove bean, Kavela	Antimicrobial, insecticidal	Wanigela	2.1
(MBR)	Raw hypocotyls			Wanigela	23.5
Rubiaceae *Morinda citrifolia* L (NF) KAL-03	Fruit	Noni, Nono	Analgesic, anti-inflammatory, hypotensive, antibacterial, tonic	Kalo	29.4
(NJ)	Juice			Commercial	
(NL)	Leaf			Kalo	23.3
(NR)	Root			Koki	17.3
Betel quid (BQ)					13.3

†Ethnomedical information was obtained from interviews with participants and traditional healers.

Table 2. Population characteristics of adult men and women of Kalo, and the Wanigelan urban and rural communities Koki and Wanigela, Papua New Guinea. Data expressed as mean ± SD. The proportion of people who have consumed the plant at least once during the survey week is also included.

	Kalo (semi-rural)		Wanigela			
			Koki (urban)		Wanigela (rural)	
	Male	Female	Male	Female	Male	Female
N	60	61	60	60	60	65
Age (y)	43.2 ± 17.9	42.6 ± 15.3	35.8 ±17.8	42.0 ± 16.5	40.7 ± 16.2	40.7 ± 19.4
Fasting blood glucose (mmol/L)	3.5 ± 1.3	3.6 ± 1.3	4.2 ± 3.5	3.8 ± 1.3	4.8 ± 3.5	3.8 ± 1.3
Diabetes (%)[†]	3.3*	1.6	10.2[a]*	5.0	13.3a*	4.6
Weight (kg)	68.3 ± 15.3*	59.1 ± 13.2	67.0 ± 17.9	61.7 ± 17.4	59.1 ± 10.2[ab]*	48.7 ± 8.3[ab]
Stature (m)	1.7 ± 0.6*	1.6 ± 0.1	1.6 ± 0.7[a]*	1.5 ± 0.1[a]	1.6 ± 0.1[a]*	1.5 ± 0.1[a]
Body mass index (kg/m2)	23.6 ± 4.8	23.1 ± 4.4	25.3 ± 5.4	26.0 ± 6.2[a]	22.3 ± 3.1[b]*	21.0 ± 3.0[b]
Overweight (%)[‡]	26.6	26.2	48.3[a]	46.6[a]	6.7[ab]	8.5[ab]
Waist circumference (cm)	85.6 ± 13.6	85.6 ± 14.7	87.4 ± 14.43	85.4 ± 13.2	80.4 ± 8.85	77.14 ± 8.3
Abdominal Obesity (%)[§]	15.0*	32.8	13.6[a]*	35.0[a]	1.7[ab]*	10.8ab
Waist: hip ratio (cm)	0.93 ± 0.11	0.90 ± 0.13	0.94 ± 0.09	0.87 ± 0.10[a]	0.92 ± 0.07b	0.89 ± 0.06[ab]
% Body fat	21.6 ± 5.5*	32.5 ± 6.7	23.6 ± 7.7*	36.3 ± 6.9[a]	18.4 ± 4.7[ab]*	29.4 ± 5.4[ab]
Tricep skinfold thickness (mm)	19.0 ± 6.7	19.0 ± 6.7	13.9 ± 8.2	22.5 ± 9.3	9.4 ± 4.0	13.5 ± 4.9
Excess body fat (%)[¶]	49.2*	49.2	67.2[a]*	75.0[a]	33.3[ab]*	27.7[ab]
Systolic blood pressure (mm Hg)	131 ± 18.5	128 ± 14.6	129 ± 15.9	130 ± 18.1	128 ± 16.5	133 ± 16.7
Diastolic blood pressure (mm Hg)	75.9 ± 10.2	75.5 ± 7.8	81.4 ± 7[a]	81.1 ± 12.3[a]	79.9 ± 11.3	80.6 ± 7.5[a]
Hypertension (%)[††]	15.0*	13.1	22.0*	28.3[a]	16.7	23.1[a]
Proportion who consumed the plant (%)						
Betel quid	93.3	93.4	33.3[a]	1.7[a]	6.7[ab]*	0.00[a]
Guava bud	15.0	14.8	1.7[a]	2.0[a]	3.3[a]*	0.00[a]
Noni	21.7	18.0	2.0[a]*	8.2[a]	11.7[a]*	0.00[a]
Mangrove bean	0.0	0.0	48.3[a]*	86.7[a]	93.3[ab]*	100.[a]

[†]Newly diagnosed diabetes was defined as fasting blood glucose >7.0 mmol/L; [‡]BMI>25.0; [§]Waist circumference >102 cm for men, >80 cm for women; [¶]% body fat >20% for men; >32% for women according to guidelines from the American Council on Exercise; [††]Systolic/diastolic blood pressure >140/90 mm Hg

[a]$p<0.05$ between same sex vs from Kalo

[b]$p<0.05$ between same sex vs from Koki

*$p<0.05$ between sexes of the same village

wise comparisons. All data are presented as mean ± SEM from at least 3 independent experiments performed in triplicate with significance set at $p\leq0.05$. Analysis was performed with SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

Plant and human population characteristics

Collection details of the plants used in this study, their ethnopharmacological indications and extract yield are presented in Table 1. Although Papua New Guineans consider the classification of foods and medicines as a continuum, most recognize mangrove bean as principally a food, noni and guava bud as principally medicines, and betel quid as a habit akin to chewing gum, but with recognized medical properties. Mangrove bean, although considered a food plant, was included in this study because of its distinctiveness as being one of the world's few remaining human dietary staples that are still harvested from wild populations. It was also included since little of its phytochemistry and pharmacology is known.

Consumption patterns of these plants along with the health characteristics of males and females of each community are presented in Table 2. Both sexes living in rural Wanigela were significantly less heavy, had a lower BMI, a lower WHR indicating less prevalence of abdominal obesity and a lower total body fat percentage compared to age and sex-matched residents of urban Koki and semi-rural Kalo. Despite this, Wanigelans had a prevalence of DM2 (males: 13.3%; females: 2.8%) and hypertension (males: 18.3%; females: 23.1%) equal to that of their urban counterparts. One dietary factor that is shared between the two communities is the consumption of mangrove bean. Almost all of the Wanigelans interviewed relied on mangrove bean as their primary source of energy, and roughly half of the males and 87% of the females in urban Koki had consumed it at least once during the week. Considered more of a famine food in Kalo, none of the villagers had consumed mangrove bean during the survey period. Virtually all Kalo villagers regularly chewed betel quid, roughly 15% of the population had consumed an infusion of guava buds and young

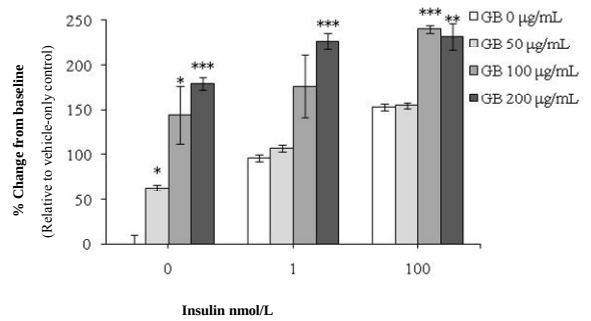

Figure 1. Percent change in glucose uptake from vehicle (DMSO)-treated cells in 3T3-L1 adipocytes treated with guava bud (GB) MeOH extract in the absence (0 nmol/L) and presence (1 and 100 nmol/L) of insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. *p<0.05; **p<0.01; ***p<0.0001 vs. insulin (control).

leaves at least once during the week, and about a fifth had used noni (part not specified). Urban and rural sex differences in noni use differed in that slightly more women in Koki had used noni (8.2 %), while in rural Wanigela, males were more frequent users (11.7 %).

Insulin-like activity of plant extracts in 3T3-L1 adipocytes

The ability of plant extracts to stimulate glucose uptake in 3T3-L1 adipocytes was first assessed in the absence of insulin (unshaded left-most group of bars in Figures 1-3). Of the extracts tested, GB had the highest insulin-like activity, increasing glucose uptake in a dose-dependent manner up to more than 150% at a concentration of 200 µg/mL (Figure 1). All noni part extracts, except for the root, had significant insulin-like activity at all concentrations; the commercially prepared fruit juice having had the largest effect (92% increase from baseline at 100 µg/mL) (Figure 2). In contrast, the root had no effect at 50 µg/mL and inhibited uptake at 100 and 200 µg/mL (Figure 2d). Raw mangrove bean (MBR) exhibited strong activity at all tested concentrations, however cooking of MBR reduced its activity by 65% suggesting that removal of tannins was responsible for the loss in activity (data not shown). Of the BQ ingredients, AN elicited a 40-60% increase in glucose uptake (Figure 3a) while PBI had no effect at 12.5 µg/mL and at higher concentrations inhibited glucose transport (Figure 3b). As expected, BQ exhibited a combination of these effects corresponding to the proportion of its PBI and AN content (Figure 3c).

Insulin potentiating activity of plant extracts in 3T3-L1 adipocytes

The ability of plant extracts to potentiate insulin-stimulated glucose uptake was assessed at insulin concentrations of 1 and 100 nmol/L (shaded middle and right-most group of bars, respectively in Figures 1-3). Guava bud extract displayed significant insulin potentiating activity at 200 µg/mL, increasing glucose uptake by a factor of 2.5 compared to insulin alone at 1 nmol/L and 1.6 times greater than at 100 nmol/L (Figure 1). No insulin potentiating effect was observed for any of the noni extracts (Figure 2a-d), and in the case of NL and NR 200 µg/mL inhibited glucose uptake. A potent insulin-potentiating effect was observed for uncooked MBR, where 50 µg/mL produced a 140% increase in glucose transport at an insulin concentration of 1 nmol/L and a 45% increase at 100 nmol/L (data not shown). The cooked form of mangrove bean however had no effect on insulin-mediated glucose uptake (data not shown), further indicating a participation of tannins in MBR bioactivity. Areca nut (AN) extract reduced insulin's action on glucose uptake in 3T3-L1 adipocytes in a dose-dependent manner (Figure 3a) while all tested concentrations of PBI inhibited insulin-mediated glucose uptake to below base-

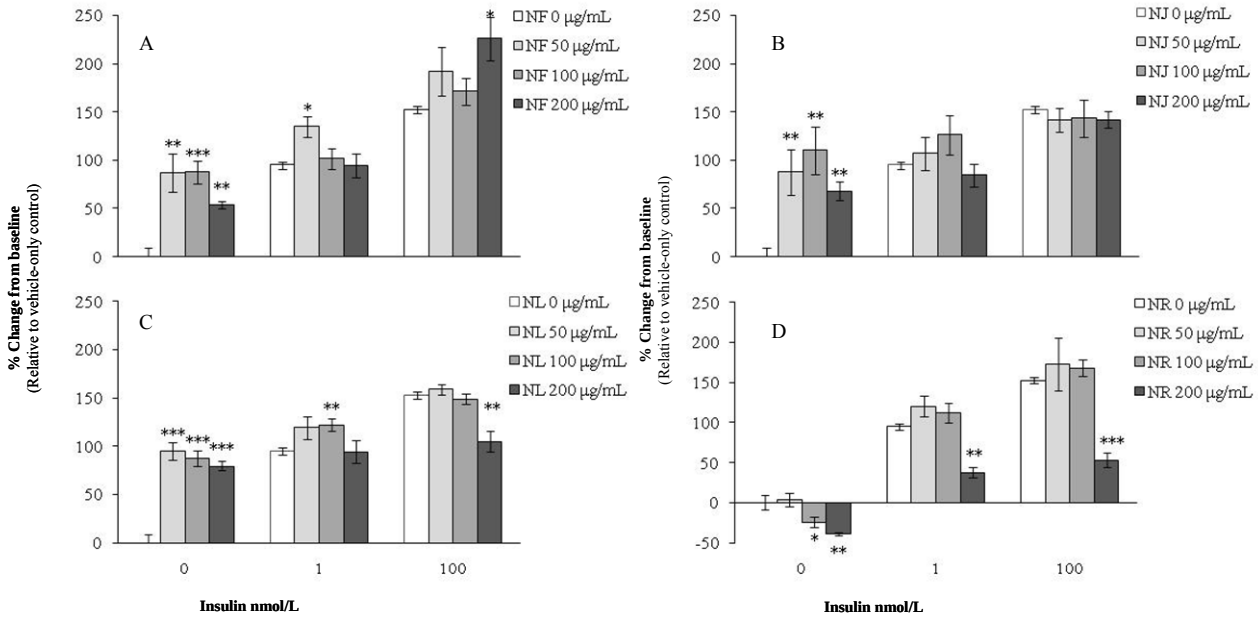

Figure 2. Percent change in glucose uptake from vehicle (DMSO)-treated cells in 3T3-L1 adipocytes treated with A: noni fruit (NF), B: commercial noni fruit juice (NJ), C: noni leaf (NL), and D: noni root (NR) MeOH extracts in the absence (0 nmol/L) and presence (1 and 100 nmol/L) of insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. *p<0.05; **p<0.01; ***p<0.0001 vs. insulin (control).

Table 3. Spearman's correlation coefficients for the relationship between intake of betel quid, guava and mangrove bean, and fasting blood glucose, diabetes status and selected anthropometric and vascular antecedents.

	Betel quid (g/wk)	Guava fruit (g/wk)[†]	Mangrove bean (g/wk)
Fasting blood glucose (mmol/L)	-0.154**	-0.213***	0.137*
Type 2 diabetes[‡]	-0.111*	-0.116*	0.047 [ns]
Standardized β[§]	-0.01 [ns]	-0.120*	0.140*
Weight (kg)	0.273***	0.092[ns]	-0.315***
Stature (m)	0.409***	0.233***	-0.397***
Body Mass Index (kg/m2)	0.101*	-0.040 [ns]	-0.149**
Waist circumference (cm)	0.109*	-0.032 [ns]	-0.190***
% Body fat	-0.08 [ns]	-0.109*	-0.023 [ns]
Tricep skinfold thickness (cm)	0.176**	-0.008 [ns]	-0.154**
Systolic blood pressure (mm Hg)	-0.081 [ns]	-0.091 [ns]	0.071 [ns]
Diastolic blood pressure (mm Hg)	-0.184***	-0.132*	-0.190***

[†]Guava bud tea intake data was supplemented with guava fruit because of infrequent intake.

[‡]Nondiabetic=0; diabetic=1.

[§]Continuous dependent variable fasting blood glucose was normalized by inverse transformation (100-1/p) and food intake was entered in multivariable analysis with age, weight and waist circumference as covariables.

*p<0.05; **p<0.005; ***p<0.0001; ns =non-significant

line levels (Figure 3b). When considered together in the BQ admixture, glucose uptake gradually declined with increased dose (Figure 3c). With the exception of MBR, a significant interaction was observed in all cases between extract concentration and insulin dose response (data not shown).

Association between plant intake and population health parameters

Associations between the frequency and amount of plant ingested and selected health parameters were assessed using Spearman's correlation for non-normally distributed data (Table 3). Since medicinal plants were not ingested as frequently as food plants, but rather used to relieve symptoms as they arose, correlations could not be reliably calculated for GB and noni. As an alternative, guava bud tea intake data was replaced with frequency data of guava fruit, and this was found to have an inverse correlation with FBG (r=-0.21, p<0.0001) and prevalence of DM2 (r=-0.12, p<0.05). This relationship remained significant when FBG was inverse-transformed and controlled for age, central adiposity and weight (β=-0.117, p=0.024). A simple inverse correlation between BQ and FBG and DM2 was also observed (r=-0.15, p<0.005; r=-0.11, p<0.05, respectively), but this disappeared when covariates were included (β=-0.-014, p=0.787). In contrast, a significant relationship between MBC and FBG was observed (r=0.14, p<0.05) and remained significant when controlled for age, weight and central adiposity (β=0.139, p=0.010). There was no correlation between MBC consumption and DM2 prevalence.

Mangrove bean consumption was associated with lighter body weight, shorter stature, smaller BMI, a thinner waistline and less subcutaneous fat as assessed by TSF thickness. In contrast, all these parameters increased significantly with BQ consumption. Those who consumed more guava fruit tended to have less % body fat (r=-0.11, p<0.05) and lower DBP (r=-0.13, p<0.05), although the latter was more strongly correlated with BQ (r=-0.18, p<0.0001) and MBC (r=-0.19, p<0.0001) intake.

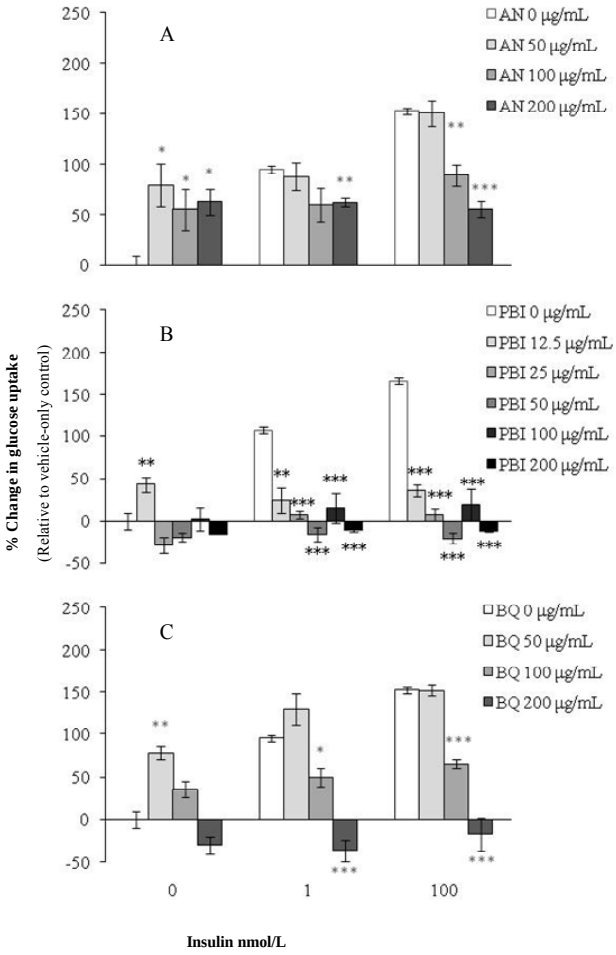

Figure 3. Percent change in glucose uptake from vehicle (DMSO)-treated cells in differentiated 3T3-L1 adipocytes treated with components of betel quid. A: areca nut (AN), B: *P betle* inflorescence (PBI) and C: betel quid (BQ) MeOH extracts in the absence (0 nmol/L) and presence (1 and 100 nmol/L) of insulin. Bars represent the mean ± SEM of n=9 samples from 3 independent experiments. *p<0.05; **p<0.01; ***p<0.0001 vs. insulin (control). Bars higher than the control indicate insulin-like or insulin-potentiating activity; lower bars indicate inhibition of glucose uptake.

DISCUSSION

Despite the social perturbations affecting all residents of Papua New Guinea, relatively high rates of adiposity, as well as habitual BQ utilization, the low incidence of DM2 in Kalo residents suggests the mediation of protective environmental factors. Our findings indicate that consumption of certain plants, in particular guava and noni, may confer anti-diabetic effects via enhanced insulin-independent and insulin-dependent glucose uptake despite representing only a minor component of the diet. While only insulin-mimetic and insulin-sensitizing activities were tested, plant products may exert additional antidiabetic properties through other biological mechanisms such as increased insulin secretion, reduced glucose production, and reduced glucose absorption, as well as cytoprotective, antioxidant, anti-glycation, and anti hypertensive effects.[8]

Betel quid chewing is an integral component of Papua New Guinean culture, particularly in strengthening social bonds between individuals and clans. Although the use of BQ as an herbal therapy should not be surprising given its socio-cultural importance, a growing trend of prescribing it as a treatment for DM2 by some traditional medical practitioners is alarming considering its association with increased DM2 risk.[5] Areca nut, the main component of BQ, is believed to promote DM2 by damaging islet β-cells via the unstable free-radical generating nitrosated derivates of its alkaloids.[15] This is supported epidemiologically by a 1995-1999 survey in Papua New Guinea which reported BQ chewing as an independent predictor of high fasting capillary blood glucose (β=1.032, p=0.005), surpassing the effect of age (β=0.554, p=0.028), BMI (β=0.507, p=0.061) and region of origin (β=-1.582, p=0.056).[5] Considering that BQ chewing has been practiced for centuries without any association with DM2, a relationship may be justified if the myriad of dietary, economic and social confounders related to modernization is considered. A previous study suggests that BQ chewing may indirectly increase susceptibility to DM2 by promoting weight gain and increased waist size.[2] Such a relationship has been observed in the present study with weight (r=0.27, p<0.0001), BMI (r=0.10, p<0.05) and waist circumference (r=0.11, p<0.05), despite an inverse correlation with DM2 (r=-0.11, p<0.05). However, the latter was rendered non-significant when age, weight and central obesity were accounted for in the analysis (β=-0.014, p=0.787). Our cell culture experiments suggest a novel diabetogenic mechanism for BQ whereby its individual components inhibit the effects of insulin (Figure 3c). Inhibition of insulin-mediated glucose uptake in 3T3-L1 adipocytes may be caused by the cytotoxic nature of PBI observed here at concentrations as low as 25 μg/mL and also observed in other cell lines.[12,16-18]

Guava bud extract displayed particularly strong insulin-like and insulin-potentiating activity in our study. Assuming good bioavailability, this plant product may be useful for the prevention and treatment of DM2. Indeed, antidiabetic effects of guava leaf have been reported in genetically[19] and chemically-induced[20,21] animal models of DM2. Guava juice was also reported to be hypoglycemic in healthy and alloxan diabetic mice as well as in healthy and diabetic humans.[22] A bioactive constituent of guava may be gallic acid since this common phenolic molecule has been reported to stimulate glucose uptake and enhance insulin sensitivity by activating peroxisome proliferator-activated receptor (PPAR)-γ.[23] In addition, guava fruit may exert indirect benefits through its rich antioxidant and soluble fibre content.[24] A variety of other bioactive compounds found in guava leaves including essential oils, saponins, and flavonoids, could contribute to an antidiabetic effect, requiring further research towards identification of responsible agents.

Noni, perhaps the most popular herbal medicine in the Pacific Islands, displayed decent insulin-mimetic activity, especially in the form of the commercial fruit juice (92.0 ± 22.3 % increase at 100μg/mL compared to basal glucose intake levels). None of the noni extracts however, were able to stimulate insulin-mediated glucose uptake, and at 200 μg/mL, root extract inhibited glucose uptake. This bell-shaped pattern could help explain a report for a related species, *Morinda officinale*, where the ethanolic root extract displayed both hypo- and hyperglycemic effects in STZ-diabetic mice depending on the dose.[25] Noni is known to be rich in anthraquinones, especially the roots, and studies suggest that these may be anti-diabetic bioactive compounds.[26]

Cooked mangrove bean extract lost roughly 80% of total phenol content and 65% of insulin-like activity compared to the raw plant extract. Nevertheless, a modest yet significant insulin-mimetic effect suggests that cooked mangrove bean could be of some benefit in preventing DM2. This, however, was not supported in the present study since our survey suggests a positive correlation between MBC frequency intake and FBG despite an otherwise favourable association with weight, waist circumference, TSF, and DBP. That DM2 had only been detected in the village from a 1986 survey,[27] concurrent with the introduction of non-staple items such as polished rice, refined flour, sugar and edible oils and not in earlier diabetes surveys dating from 1977,[28] suggests the association between MBC and FBG is due to other factors that have statistical collinearity with residence in Wanigela, thereby limiting our ability to distinguish the effect of a single food item on chronic disease development.

Medicinal plants constitute only a minor component of total dietary intake and habitual food habits clearly have a greater influence on non-communicable disease risk. Dietary analysis showed that Wanigela residents consumed significantly less rice than Kalo and as vegetarians, abstained from meat. There were no significant differences in the amount of fruits and vegetables consumed between the communities except for guava which was consumed in greater quantities in Kalo.[29] The impact of consuming the plants included in the present study on metabolic health is unknown when considered holistically with diet and physical activity, although it is plausible that they may have an influence on nutrient metabolism and long-term health. Our next objective is to assess this interaction in a study designed specifically to test whether guava and noni consumption could effectively counter DM2 and betel quid diabetogenicity.

CONCLUSION

The present study is a first step in determining whether consumption of particular plants can protect against DM2 risk or its comorbidities in light of the risks associated with nutritional transition and those purported to be incurred by chewing BQ. Of the plants that form Kalo's traditional food and medical systems, guava bud and noni stood out as being distinctive from those of rural Wanigela. Using cultured 3T3-L1 adipocytes as a model of insulin resistance, we demonstrated that guava bud and noni extract possessed potent insulin-mimetic activity, the former also having effective insulin-potentiating activity. Habitual ingestion of these may protect against BQ diabetogenicity or DM2 associated with socioeconomic transition. A large prospective study that controls for confounders is required to reliably discern any dietary association with lifestyle-related diseases, especially for infrequently consumed plants such as guava and noni. Likewise, different experimental models, particularly animal studies and clinical studies are needed to more accurately explore antidiabetic potential and mechanisms of action for these plants.

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AUTHOR DISCLOSURES

The authors declare that there are no conflicts related to the publication of this study.

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Original Article

Consumption of guava (*Psidium guajava* L) and noni (*Morinda citrifolia* L) may protect betel quid-chewing Papua New Guineans against diabetes

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攝取番石榴(*Psidium guajava* L.)及諾麗果(*Morinda citrifolia* L.)可能保護嚼食檳榔的巴布亞新幾內亞人對抗糖尿病

第二型糖尿病(DM2)的發生率在巴布亞紐幾內亞快速的上升，而幾個令人注目的流行病學證據支持糖尿病致病與咀嚼檳榔(BQ)具有相關性，這促使我們研究某些膳食對策或許對 DM2 的發展具有保護作用。我們調查沿海中央省份 Kalo 居住者的飲食習慣，他們習慣咀嚼檳榔卻有相對較低的 DM2 發生率；與他們種族相近且是緊鄰的 Wanigelans，拒吃檳榔但有不尋常的高 DM2 發生率。在 Kalo 地區，番石榴芽(*Psidium guajava* L.)及諾麗果(海巴戟天)(*Morinda citrifolia* L.)的攝取是較 Wanigela 地區頻繁，反之木欖豆(*Bruguiera gymnorrhiza* (L.) Lam.)的攝取則是較少的。評估這些植物與檳榔嚼塊及其組成物-檳榔果核(*Areca catechu* L.)與荖藤花(*Piper betle* L.)，它們對培養的 3T3-L1 脂肪細胞之胰島素依賴型及胰島素非依賴型的葡萄糖運輸能力之調節。檳榔嚼塊、檳榔果核及荖藤花的甲醇萃取物對葡萄糖攝入有抑制作用並呈劑量效應，這結果支持之前的致糖尿病的說法。相反地，番石榴芽萃取物呈現顯著的類胰島素及加強胰島素的活性。諾麗果、諾麗葉、諾麗果汁商品及木欖豆全部顯示類似胰島素活性，但是對於胰島素效能僅輕微或是沒有影響。習慣性食用番石榴或是諾麗果比起煮過的木欖豆，在對抗 DM2 的發展或檳榔的糖尿病致病性，提供較佳的保護作用。這些發現提供實驗上的支持，亦即使用傳統食物及藥物可達成降低 DM2 的風險。

關鍵字：番石榴屬、檳榔屬、3T3-L1 細胞、植物、藥用