

ARECA NUT SYMPOSIUM

Metabolic effects of the consumption of *Areca catechu*

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Abstract

Betel nut (Areca catechu) is chewed regularly by at least 10% of the world population, imported by immigrant users wherever they settle, and is the fourth most widely used addictive substance. It is thought, by users, to soothe the digestion and to be a stimulant and its use has a major role in social situations. Specific arecal alkaloids act as competitive inhibitors of GABA receptors and have widespread effects in the body, including actions on the brain, cardiovascular system, lungs, gut and pancreas. Nitrosated derivatives of arecal alkaloids, proven carcinogens inducing tumours throughout the upper gut and foregut derivatives in animals, are also associated with increased tumour risks in man. These nitrosated compounds are also diabetogenic in CD1 mice, producing a type 2 diabetes with obesity. Increased central obesity is found in association with betel usage in man as well as increases in circulating markers of inflammatory and cardiovascular damage. The effects of chronic betel usage in man are at least as diverse as those of smoking and the habit increases the risks of ill health.

Paan consumption is a psychoactive and addictive habit and is used by about 600 million people worldwide. Only three 'addictive' substances are used more widely: nicotine, ethanol and caffeine. Several mechanisms have been identified by which the specific alkaloids of the *Areca catechu* nuts, chewed alone or within Paan 'quids' or 'chews' made by wrapping chopped betel-nut, sea shells or slaked lime, and sometimes other ingredients, in a leaf of the piper betle vine produce physical and psychological effects. Users report increased well-being and stamina, a soothing effect on the digestion, protection of the mouth and gums and some euphoria. Acute ill effects are also reported at high rates of usage and include cardiac arrhythmia, exacerbation of asthma, acute psychosis and acute gut upset.^{1–3}

There are four main arecal alkaloids, arecoline, arecaidine, guvacine and guvacoline. Arecal alkaloids have anti-muscarinic effects on smooth muscle, especially arecoline. They also bind to GABA receptors in the brain, contributing to their psychoactive effects. GABA receptors are chloride channels, similar in structure to acetylcholine receptors and are found in many tissues of the body, including pancreatic islets, where arecal alkaloids can also be expected to have physiological effects; for example, arecaidine is as active as GABA in stimulating collagen synthesis by buccal fibroblasts.^{4,5}

Arecoline is not a simple activating ligand for the GABA receptor since it acts as a GABA receptor 'blocker', preventing normal GABA inhibition of neurotransmission.⁶ Since neuro-

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suppressant benzodiazepines such as diazepam (valium) are thought to potentiate GABA activity while arecal alkaloids act as GABA inhibitors the 'stimulant' or 'euphoriant' effects of betel consumption could be predicted to be diametrically opposite to the effects of anxiolytic benzodiazepines such as valium. It is not known whether arecal alkaloids would be as effective as the diazepam antagonist flumazenil in the treatment of overdose but on theoretical grounds people using areca nut might be resistant to benzodiazepines. On electroencephalogram (EEG) betel quid consumption leads to increased alpha activity in the occipital region, with more general and more marked increases in beta activity in man, these features being found together with reduction in theta activity. Together these findings support the suggestion that arecal alkaloids do in fact increase cerebral arousal with some lesser increase in 'relaxation'.⁷ Reaction latency time has been shown to be increased within minutes by betel chewing while visual information processing is not altered in habitual chewers.^{8,9} These neurological responses of betel quid are described in detail by Chu in this issue.

Betel chewing is thought to reduce the severity of symptoms in schizophrenia with reduction in both positive and negative symptoms. Extrapyramidal symptoms in schizophrenic patients on neuroleptic medication are not normally exacerbated by moderate betel quid usage, although there is a report of severe extrapyramidal symptoms in association with phases of unusually heavy betel quid usage thought to be due to antagonism of the anticholinergic agent procyclidine by arecoline.^{10,11}

The immediate effects of betel quid chewing include palpitations, sweating and facial flushing with a feeling of skin warmth. Objective studies have confirmed that facial skin temperatures rapidly increase by 0.5 to 2°C with betel use.¹² While the pulse rate increases, whether or not the user is habituated to betel use, increases in blood pressure are only seen in novices.¹³ The piper betle vine leaf, used as a wrap in making paan quids, appear to activate the sympathetic nervous system at low dosage and to increase production of adrenal medullary catecholamines. Areca nut has also been reported to induce higher basal secretion of catecholamines from adrenal chromaffin cells than other paan ingredients. Chewing juices can, *in vitro*, inhibit the secretion of catecholamines in response to carbachol or high

potassium concentrations, suggesting paan components may affect the entry of Ca²⁺ into cells through high-voltage channels.¹³⁻¹⁵ At modest dosage betel quid consumption affects the sympathetic nervous system but at high dosage, both sympathetic and parasympathetic nerves are activated. The latter effect may contribute to the reduction in RR interval rate variation seen on electrocardiogram with high consumption rates of plain betel nut in man.¹⁶ Other effects on the cardiovascular system reported include vasoconstriction of blood vessels; in the presence of narrowed vessels this could increase the risk of acute coronary events in betel quid users with ischaemic heart disease (IHD) although only a single case report so far suggests that this may occur.¹⁷

Despite the 'soothing' effects on the digestion reported by users, there appears to be an increase in peptic ulceration in betel quid chewers as in smokers and khat users.¹⁸ Chewers have an increased rate of salivary secretion with resultant dilution of amylase and K⁺. Increasing tobacco usage further reduces its K⁺ content.¹⁹ Effects on the mucosa include increased acid back-diffusion and reduction in mucous secretion, effects associated with haemorrhagic peptic ulceration in animals and likely to contribute to the increased risk of peptic ulceration in man. These effects are corrected by alkalization with sodium bicarbonate or the administration of therapeutic agents that block acid secretion.²⁰ Objective studies have shown that there is an acetylcholine-like effect of areca nut extract on the bowel due to the action of arecoline in particular, probably mediated through neuroreceptors in the autonomic nerve plexus of the bowel wall. Regular consumption of areca nut leads to abnormal liver function with increases in serum aminotransferases in rodents suggesting hepatocellular damage.²¹ It is not known whether such damage might increase the risk of cirrhosis of the liver, but this is an unusually common problem in users in east London, only sometimes accounted for by chronic viral hepatitis B and rarely, in this Muslim community, by the use of alcohol. The effects of paan usage on secretion of digestive juices and enzymes have also been studied. Two types of piper betle vine leaf are used; the pungent (Mysore) leaf and the non-pungent (Ambadi) leaf and both stimulate pancreatic lipase secretion. The Ambadi, but not the Mysore leaves, also increase intestinal enzyme secretion, especially of

lipase, amylase and disaccharidases, while both types of leaves reduce pancreatic trypsin and chymotrypsin secretion.²²

It was suggested early this century that areca nut chewing could aggravate asthma.²³ Patients seen at follow-up for asthma have reported noticing that their asthma was made worse by use of areca nut; over half of those who had given up using betel had done so because of adverse effects on their asthma. Observations on the effect of areca nut have shown minor reductions in FEV₁ in non-asthmatics, improvement in FEV₁ by up to +10% in asthmatics without worsening of symptoms with betel use and a reduction, averaging 22%, in FEV₁ in asthmatics reporting worsened symptoms after betel use. More recently this observation has been supported by studies of airway obstruction in asthmatics; inhalation of arecoline causing bronchoconstriction in a large proportion of asthmatic non-areca-using patients and in a small proportion of healthy non-asthmatic controls. In areca users with asthma a reduction in FEV₁ of up to 30% lasting several hours could be produced by inhalation of arecoline while *in vitro* it caused dose-related constriction of bronchial smooth muscle.²⁴⁻²⁶

It is possible that betel leaf extract may affect thyroid function, low doses increasing triiodothyronine to thyroxine ratios (suggesting increased activation of thyroxine) in rodents while high doses had the reverse effect.²⁷ Theoretically such changes, if found in man, could lead to hypothyroidism.

The consumption of arecoline, and perhaps of the leaves of the piper-betle, causes short-term hypoglycaemia.^{28,29} The mechanism by which this effect is produced is unclear and there do not appear to be any reports of clinical problems arising from this effect. The inhibitory neurotransmitter GABA is found, and its receptor expressed, in islet beta cells.³⁰ One of the GABA shunt enzymes, glutamate decarboxylase (GAD) is also found in islet cells and is an antigen strongly associated with the appearance of GAD antibodies at the onset of type 1 diabetes in man.³¹ Since arecal alkaloids act as GABA receptor inhibitors they could, by blocking the inhibitory effects of GABA on glucagon and somatotrophin secretion, increase their release. An immediate effect of a rise in glucagon is insulin release with subsequent hypoglycaemia but chronic hyperglucagonaemia, much as hypersecretion of growth hormone, is associated with

the development of diabetes. This is a mechanism, therefore, that might be capable of leading to hyperglycaemia, or eventual diabetes, over time.

Reduced expression of GAD is produced by GABA inhibition and this in turn reduces autoimmune responses to GAD in rat islets and brain.³² It has been suggested that such inhibition of GAD antigenicity might reduce the risk of the development of type 1 diabetes in those at risk. Since arecal alkaloids are likely to inhibit GABA receptors in the islets, as they do in the brain, it is possible that areca chewing provides protection from progression from type 2 to type 1 diabetes.

Nitrosamine derivatives from each of the four major arecal alkaloids are produced by nitrosation of the alkaloids in dried stored nuts, in the mouth and especially in the acid conditions found in the stomach in the presence of nitric oxide generated by bacterial action. Two of these derivatives are accepted as carcinogenic in animal studies, MNPN (methylnitrosaminopropionitrile) being the most carcinogenic.³³ The administration of arecal nitrosamines induces growths, often at multiple sites including the naso- and oropharynx, the stomach, liver, lungs and pancreas and also the forebrain. It is of interest that the same parts of the body develop these tumours whether arecal nitrosamines are given by mouth or by intravenous injection and that all the tissues and organs affected originate during development from the embryonic foregut.³⁴ The targeting of specific organs by specific nitroso-compounds is a well-known phenomenon thought to be determined by the tertiary structure of the nitrosated parent molecule. Damage to cell nuclei with increased methylation of DNA and increases in mutation rates then follow, largely as a result of free radical cascades generated by these unstable nitroso-compounds. The evidence for the carcinogenicity of arecal nitrosamines has not been felt to be as strong in man as it is in animals, the evidence reviewed by the IARC in the late 1980s being adduced to be 'suggestive but not compelling'.³⁴ Further evidence continues to accrue on this difficult subject but it is perhaps unlikely that man will prove to differ much from other animals in his vulnerability to these toxins.

Insulin-secreting beta cells of the pancreatic islets are derived from the embryological foregut so that they too may be targets for arecal

nitrosamines. Many nitrosated compounds have been found to be diabetogenic and have been used experimentally to induce type 1 diabetes. The best-known and most widely used of these is probably streptozotocin (STZ) which has also been used in the treatment of malignant insulin-secreting tumours of the pancreatic islets in man. High-dose treatment with STZ induces permanent type 1 diabetes in these patients, just as it does in animals, with dependence on insulin injections thereafter. Since it became clear that there was also a considerable risk of developing primary adenocarcinoma of the liver after STZ therapy this form of treatment is still used widely but in very specific ways.^{35,36}

One feature common to diabetogenic nitroso-compounds, including STZ, is that they each contain a moiety with a 'ring' structure similar in configuration to the chair-shaped 'ring' of hexameric glucose. It is this part of the molecule that may, by binding to islet beta cell glucose receptors, account for their diabetogenicity.³⁷ Damage or, at high dosage, destruction of the beta cell then develops and this probably results from the local generation of free radical cascades by these unstable nitrosated compounds. While high doses of STZ lead to permanent type 1 diabetes and early death in the absence of insulin replacement, low doses can be used to produce a type 2 diabetes-like syndrome, treated animals not being dependent on insulin for survival.³⁸

Similar findings are reported for dietary sources of nitroso-compounds. Increased maternal intake of nitrosamines being associated with increases in childhood type 1 diabetes in man.³⁹ Smoked cured mutton, a local delicacy popular in Iceland, contained nitrosamines in larger amounts in the 1980s than are now permitted. Diabetes developed, as a permanent condition, in significant numbers of normally fed offspring of adult CD1 mice (fathers or mothers) that had been fed Icelandic smoked cured mutton over a 5-day period more than 2 weeks before mating. Similar changes were also found after feeding pure smoked cured mutton nitrosamines.⁴⁰ There was no disturbance of glucose homeostasis in the fed adults in either of these mouse studies. While smoked cured mutton was never confirmed to be diabetogenic in man the reduced incidence of childhood diabetes seen in Iceland compared to that seen in other European countries in the years since the regulation of smoked mutton nitrosamines is of interest.⁴¹

There is additional evidence for the diabetogenicity of another specific nitroso-compound in man. Survivors of accidental ingestion of the rat poison Vacor develop either type 1 or type 2 diabetes according to the magnitude of the dose taken. Survivors of the larger doses of this material sold in the United States develop type 1 diabetes while only 20% of survivors of the smaller doses sold in Korea develop type 1 diabetes, 80% of survivors developing type 2 diabetes.⁴² Considered against this background of knowledge the fact that the reported structure of arecal alkaloids, and hence their nitrosated derivatives, appear to contain a ring-shaped moiety similar in configuration to that of glucose suggested the possibility that betel nut consumption might be diabetogenic.

The effect of areca feeding on glucose tolerance and on the pancreatic islets has been investigated in the mouse.⁴³ Ground dried cured areca nut was bought in shops used by the Bangladeshi community in Tower Hamlets and fed to normal young adult CD1 mice at 20% in a standard low nitrosamine feed for 5 days. Ninety-minute plasma glucose concentrations on intraperitoneal glucose tolerance tests were used for comparison. A diagnosis of diabetes was defined by 90-minute plasma glucose levels >3 standard deviations above the mean values found in groups of healthy pair fed control mice of the same age, sex and generation; 8.3% of betel-fed adults developed permanent hyperglycaemia, central obesity and islet enlargement with beta-cell changes typical of human type 2 diabetes in contrast to an incidence of diabetes of 0.5% in the colony as a whole and in control-fed animals. When the areca-fed animals, especially areca-fed F0 males, were mated with controls 4 weeks after completion of test-feeding similar abnormalities of body build, glycaemia and islet morphology developed at 3% to 10.2% in each of the subsequent non-areca fed generations studied (F1-F3). The rates at which these abnormalities appeared were independent of maternal hyperglycaemia in each generation studied. Diabetes also appeared at a greater rate in the offspring of areca-fed F0 fathers than of areca-fed F0 mothers. The mechanism for the inheritance of the diabetes is not known. It may be due to direct damage to germ cells since sperm heads, and their nuclei, show obvious damage on light microscopy at the same interval of time after completion of 5 days' betel feeding as the interval before mating in our betel feeding studies.⁴⁴

We have more recently examined the effects of feeding the most carcinogenic of the arecal nitrosamines (MNPN), in CD1 mice at 35 mg/kg of feed for 5 days. Since paan leaves contain beta-carotene, which is known to reduce the carcinogenic effect of paan usage in man as well as in animals, we have also examined the effect of MNPN administration with added beta-carotene. Significant hyperglycaemia was found in male but not female adult MNPN-fed CD1 mice by the age of 18 weeks and this effect was reduced by added beta carotene.⁴⁵

Thus there is a theoretical basis for suspecting that areca nut might well prove to be a diabotogenic item of diet. Since areca consumption could increase the risk of the development of diabetes in man a study was made of glycaemia in relation to paan usage and other recognized risk factors for type 2 diabetes in ~1000 adult Bangladeshis living in Tower Hamlets, east London, between 1991 and 1993.⁴⁶ Increases in waist-size and weight, the major markers for hyperglycaemia in this population,⁴⁷ were found to be in direct relation to paan usage. These findings were independent of the effects of other risk factors for central obesity such as age or parity. A study of anthropomorphic features in relation to areca, paan, smoking and tobacco usage in SE Asia has reported reduction in body mass index and an increase in resting metabolic rate (RMR) in relation to increasing areca and paan usage but only in association with increases in ambient temperature.⁴⁸ Increases in RMR could be accounted for by increased heat loss since areca chewing increases skin temperature, as discussed above. The differences between the findings in these two studies may reflect the fact that changes in ambient temperature in the United Kingdom are not large enough to affect RMRs.

One other additional risk factor for diabetes present in the east London Bangladeshi study population was the high prevalence of vitamin D deficiency.⁴⁹ This was found in 90% of those 'at-risk' of diabetes (spot blood glucose > 6.6 mmol/l < 2 hours pc or > 4.4 mmol/l > 2 hours pc on two separate occasions) and in 45% of those not at risk. Insulin secretion was directly related to vitamin-D status independently of other factors and glycaemia was inversely related to vitamin D status independently of other factors. These findings confirm previous reports on vitamin D deficiency as a risk factor for diabetes both

experimentally and in man. A subgroup of vitamin D-deficient subjects, supplemented by a depot injection of calciferol, 100 000 i.u. was re-studied 12 weeks later and insulin secretion was found to be markedly increased.

A further study carried out between 1995 and 1997, designed to look specifically at vitamin D deficiency as a risk factor for both type 2 diabetes and for ischaemic heart disease, surveyed nearly 700 'healthy' adult British Bangladeshis aged 30–65 years. People with ongoing illness, including diabetes, were excluded. Paan usage was again recorded and similar relationships between body build, glycaemia and paan usage were found (unpublished data). Assessments made on 171 of the 174 subjects found to be at risk of diabetes included anthropometry, questionnaire records of diet, smoking rates and betel chewing tobacco usage. Subjects at risk of diabetes underwent a full oral glucose tolerance test together with measurement of other known risk markers for diabetes and IHD. Rates of paan usage were virtually unchanged from those found in the earlier study reaching > 80% in men aged 30–50 and > 90% in women aged 40–60 in both studies. The use of paan by those in their 30s increased from 81.7 to 85.2% in men and from 81% to 93.4% in women between the two studies. Smoking prevalence was much lower in women than men in both surveys but had increased in women in their 50s and 60s from 13.4% to 21.5% and 15.8% to 19.2%, respectively, between the first and second study. Smoking in men had fallen in each age group other than in those in their 30s, the maximum reduction being from 88.5% to 64.1% in men in their 40s. Tobacco use in women, recorded only in the second study, ranged from 44.5% to 80.0% with peak usage by those in their 60s whilst in men it ranged from 14.7% to 30.5% with peak usage by those in their 60s.

In addition to glycaemia, insulin profiles and standard risk factors for diabetes and ischaemic heart disease (IHD) vitamin D status, vitamin D receptor (VDR) genotypes, serum homocysteine, plasma matrix metalloproteinases (2 and 9) and the MMP inhibitor TIMP-1 were measured. Each of the recognised risk markers for type 2 diabetes and IHD was examined in relation to vitamin D status and to the use of paan, of chewing tobacco and cigarettes smoked in addition to other recognized risk factors such as age and central obesity.

Forty-two of the 44 vitamin-D deficient subjects [serum 25(OH) vitamin D <11 ng/ml] agreed to be vitamin D-supplemented for a year (by depot calciferol injection 3-monthly) and were re-assessed at the end of supplementation. Significant reductions were found in circulating apolipoprotein-b and in PAI-1.^{50,51}

Increases in circulating homocysteine are found in people with active IHD and are more common in those with folate deficiency although genetic disorders contribute to this abnormality.⁵² The healthy Bangladeshi group we studied was free of known diabetes or IHD but had a remarkably high prevalence of both folate insufficiency and of abnormally high levels of homocysteine.⁵³ On multiple regression analysis independent increases in homocysteine were found with reductions in serum folate ($p < 0.000$), as expected, but also with the numbers of cigarettes smoked per day ($p = 0.003$) and the number of paan quids used daily ($p = 0.025$), although there was no relationship with tobacco chewing. Smoking and paan usage together had as great an effect as that of folate status in the determination of serum homocysteine.⁵³ While the need for adequate folate fortification is under debate, and already in place in products such as cereals, it may be that the avoidance of smoking and of paan usage could be equally effective in this regard.

Instability of arterial plaque contributes to the risk of arterial occlusion with resultant myocardial infarction. It is also known that increases in activity of matrix metalloproteinases (MMPs), enzymes destroying collagen and other specific support structures in the interstitium are found in unstable plaque, both in the arterial wall and in the invading foamy macrophages.⁵⁴ MMP2 and MMP9, not normally present in the bloodstream, appear in the circulation with acute coronary events.⁵⁵ Suppression of MMPs by ACE I inhibition is thought to reduce vascular damage through this mechanism.⁵⁶ Inhibition of MMP activity, by pharmacological agents or increased expression of tissue inhibitors of MMP activity (TIMPs), is thought to be likely to reduce plaque destruction in atheromatous disease.⁵⁷ We have found MMP9 to be present in the plasma in our healthy but vitamin D-insufficient subjects [serum 25(OH) vitamin D <20 ng/ml; <50 mcu/l]. We have also found that MMP9 levels increase in direct relationship to the degree of vitamin D insufficiency present while plasma MMP9 levels in vitamin D supple-

mented subjects were reduced by -68%.⁵⁸ In contrast, plasma TIMP-1 concentration was found, using multiple regression analysis, to increase in direct relation to daily paan quid usage, independently of other risk factors in nearly 100 vitamin-D insufficient subjects. Increases of TIMP-1, independent of MMP9 with which it is normally co expressed, are thought to contribute to the risk of disorders where increased fibrosis contributes to disease progression, including cirrhosis of the liver and hypertensive ventricular hypertrophy: common problems in this population.⁵⁹ This mechanism may also prove to contribute to the development of submucous fibrosis (SMF) in man. Since we have found plasma TIMP-1 to vary with Taq-1 polymorphisms of the VDR gene, independent of other factors such as vitamin D status, the VDR gene could itself be a risk marker for SMF.⁶⁰

It is most unlikely that the pathogenic effects of betel use, alone or in paan quids, has a role limited to its effects in the mouth since it is clear that its actions affect many processes in many tissues. Indeed, the disorders where areca usage plays a part are likely to be at least as diverse as those in which smoking is now known to play a role.

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