

What is already known on this topic

It has been extremely difficult to investigate anatomical changes during the act of coitus and the female sexual response

Modern magnetic resonance imaging allows exploration of aspects of living anatomy

What this paper adds

Taking MR images of the male and female genitals during coitus is feasible

During 'missionary position' intercourse the penis has the shape of a boomerang

During female sexual arousal without intercourse the uterus rises and the anterior vaginal wall lengthens

The size of the uterus does not increase during sexual arousal

the typescript; and Professor W Mali for offering the use of equipment at the University Hospital Utrecht. P van Andel does not want to be acknowledged for his idea of using MRI to study coitus. He excuses himself by quoting the French romantic poet Alphonse de Lamartine (1790-1869): "C'est singulier! Moi, je pense jamais, mes idées pensent pour moi."

Contributors: WWS initiated and coordinated the formulation of the study hypothesis, designed the protocol, and participated in data collection, interpretation of the findings, and writing of the paper; he is guarantor of the study. PvA had the original idea for the present study, and participated in formulation of the study hypothesis, data collection, interpretation of the findings, and writing of the paper. IS, together with her partner, participated in the first two experiments and helped design the

protocol. EM participated in the execution of the study, particularly data collection and interpretation of the magnetic resonance findings.

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Shaken, not stirred: bioanalytical study of the antioxidant activities of martinis

C C Trevithick, M M Chartrand, J Wahlman, F Rahman, M Hirst, J R Trevithick

Department of Biochemistry, Faculty of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada N6A 5C1

C C Trevithick
research assistant
M M Chartrand
research assistant
J Wahlman
research assistant
F Rahman
research assistant
M Hirst
professor
J R Trevithick
professor

Correspondence to: J R Trevithick
trejohn@julian.uwo.ca

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Abstract

Background Moderate consumption of alcoholic drinks seems to reduce the risks of developing cardiovascular disease, stroke, and cataracts, perhaps through antioxidant actions of their alcohol, flavonoid, or polyphenol contents. "Shaken, not stirred" routinely identifies the way the famous secret agent James Bond requires his martinis.

Objectives As Mr Bond is not afflicted by cataracts or cardiovascular disease, an investigation was conducted to determine whether the mode of preparing martinis has an influence on their antioxidant capacity.

Design Stirred and shaken martinis were assayed for their ability to quench luminescence by a luminescent procedure in which hydrogen peroxide reacts with luminol bound to albumin. Student's *t* test was used for statistical analysis.

Results Shaken martinis were more effective in deactivating hydrogen peroxide than the stirred variety, and both were more effective than gin or

vermouth alone (0.072% of peroxide control for shaken martini, 0.157% for stirred *v* 58.3% for gin and 1.90% for vermouth). The reason for this is not clear, but it may well not involve the facile oxidation of reactive martini components: control martinis through which either oxygen or nitrogen was bubbled did not differ in their ability to deactivate hydrogen peroxide (0.061% *v* 0.057%) and did not differ from the shaken martini. Moreover, preliminary experiments indicate that martinis are less well endowed with polyphenols than Sauvignon white wine or Scotch whisky (0.056 mmol/l (catechin equivalents) shaken, 0.060 mmol/l stirred *v* 0.592 mmol/l wine, 0.575 mmol/l whisky).

Conclusions 007's profound state of health may be due, at least in part, to compliant bartenders.

Introduction

James Bond, the well known fictional secret agent ("007") of the British intelligence services, not only is



THE KOBAL COLLECTION

Is it martinis that help James Bond stay so healthy?

astute in matters of clandestine affairs at a personal and international level but may also possess insights of interest to medical science. Take for example his insistence on having his martini “shaken, not stirred.” Does this straightforward direction to the barman merely yield a crisper drink, more to James Bond’s taste, or is there more to it?

Moderate consumption of alcoholic beverages has been associated with a decreased risk of several age related diseases, including cardiovascular disease,^{1,2} stroke,³ and cataract.^{4,5} This effect has been tentatively ascribed to the antioxidant activities of alcohol,⁶ flavonoids, or polyphenols⁷ in the beverages, since it has been established that the antioxidant vitamin E reduces the risk of cardiovascular disease^{8,9} and cataract development.¹⁰

Methods

Mini-martinis were prepared by mixing two parts (vol/vol) gin (6 ml) with one part vermouth (3 ml). They were either shaken vigorously (9 ml in a 100 ml medicine bottle for one minute), or stirred (9 ml in a 20 ml glass vial, using a vortex mixer). Aliquots of the martinis were then added into a luminescent assay^{11,12}

to see if they altered the luminescence resulting from the addition of a standard amount of hydrogen peroxide. In both cases, the addition of the martinis decreased the net luminescence to a small percentage of control values. When we analysed the net luminescence statistically, using the *t* test function of Microsoft’s Excel spreadsheet program, the luminescence remaining after we added the shaken mixture to the peroxide was virtually half that afforded by the stirred mixture (table 1, $P=0.0057$). This indicates that there was twice as much peroxide remaining after treatment with the stirred martinis than after the shaken variety. Thus, shaken martinis are better able to “neutralise” peroxide than stirred martinis.

The normalised luminescent count rate is reduced by the addition of martinis. Counts per minute were obtained with a Lumac Biocounter M2010 (Celsis, Chicago) when hydrogen peroxide was incubated with the luminol bound to albumin, as described below. Samples consisted of an aqueous portion (0.3 ml), and a dimethyl sulphoxide portion (0.4 ml). The aqueous portion contained luminol and albumin (0.02 ml, 10 mg/ml of each) prepared as described,^{11,12} and phosphate buffered saline (0.11 ml) containing martini (0.07 ml) or distilled water (for control). Hydrogen peroxide (0.1 ml of 1.0%, final concentration 42 mmol/l) was added after the addition of dimethyl sulphoxide (0.4 ml), just before the tube was placed in the counter to begin counting of the emitted light. We determined the probability that the means of each set of samples are identical by using Student’s *t* test in the Excel program.

We considered that a difference might arise from air oxidation of reactive martini components during the vigorous shaking. This may, however, not be the reason because when, as an alternative to shaking or stirring, we vigorously bubbled air or nitrogen at similar rates through the martinis (for one minute), the two treatments showed no significant difference in net luminescence (table 1). Nevertheless, the martinis bubbled with both air and nitrogen showed net luminescent counts equivalent to the shaken rather than to the stirred martinis.

Results

To ascertain the relative contribution of the gin and vermouth components, both were assayed in a preliminary experiment for their abilities to reduce luminescence produced by the peroxide challenge. Vermouth was much more potent, causing a 98.1% (SE 0.5%) ($n=3$) decrease in count rate, while gin reduced the count rate after challenge with peroxide by only 41.7% (14.1%) ($n=36$). This implies that the vermouth contributes more to the antioxidant properties of martinis. Even so, the combination of gin and vermouth is

Table 1 Remaining luminescence after addition of martinis to the luminescent assay containing peroxide

Manoeuvre	No of samples	Percentage of peroxide control value			Significance (shaken v stirred)
		Mean	SE	95% CI	
Shaken	7	0.072	0.020	0.023 to 0.121	$t=3.418(df=11)P=0.0057$
Stirred	6	0.157	0.016	0.113 to 0.201	
Air bubbled through mix	5	0.061	0.007	0.044 to 0.077	$t=0.126(df=8)P=0.904$
Nitrogen bubbled through mix	5	0.057	0.030	-0.027 to 0.140	

Table 2 Polyphenol content of martinis compared with white wine and blended Scotch whisky

Sample	No of samples	Catechin equivalent concentration (mmol/l)		
		Mean	SE	95% CI
Shaken martini	8	0.056	0.005	0.044 to 0.068
Stirred martini	9	0.060	0.009	0.039 to 0.081
Sauvignon white wine	9	0.592	0.030	0.523 to 0.661
Scotch whisky	9	0.575	0.025	0.517 to 0.633

Assay performed by adding 20 µl sample to 980 µl Folin reagent diluted 1/10 with distilled water; colour allowed to develop for 0.5–2.5 hours the absorbance of the solution at 750 nm was read. Samples were compared with a standard curve prepared by adding 10–50 µl of 1 mmol/l catechin solution to Folin reagent. Standard curves measured on three days had R² values of 0.9984–0.9999.

better than either gin or vermouth alone, resulting in a much lower net luminescent count rate (0.072% of peroxide control for the shaken martini) than those found after either gin (58.3% of control) or vermouth (1.9% of control) alone. The remaining luminescence with martinis is substantially lower than that of the vermouth itself.

Since much of the antioxidant activity of wine and whisky has been ascribed to the polyphenols they contain,⁷ the polyphenol content in the martinis was investigated using Folin reagent.⁷ As shown in table 2, the phenolic concentrations in the martinis, in catechin equivalents, were an order of magnitude lower than those in white wine or 12 year old Scotch whisky, and there was no significant difference between the phenolic contents of shaken and stirred martinis.

The martinis, when undiluted, are capable of suppressing counts from 42 mmol/l peroxide by over 99.9%. We calculate that after ingestion an absorbed martini may be able to react with 210 µmol/l of hydrogen peroxide. We have previously determined that 5 mmol/l ethanol, a blood concentration of ethanol found after absorption of one or two typical alcoholic drinks, would eliminate 131 µmol/l peroxide.⁶ The peroxide concentrations detected in the aqueous humour have ranged from 14 µmol/l to 31 µmol/l: mean 24 (SE 7) µmol/l for normal humans, with higher concentrations in cataract patients (82 (155) µmol/l and 198 (88) µmol/l¹³). Both vitamin E and ethanol decrease the risk of cataract^{1 5} and atherosclerosis^{1 3} by about half. The residual peroxide concentrations in the aqueous humour possibly reflect those in the serum, from which the aqueous humour is formed by ultrafiltration at the ciliary body. After the consumption of shaken martinis, peroxide concentrations of serum and aqueous humour could be half those found after ingestion of stirred martinis.

Discussion

Although the reason for the superior antioxidant activity of shaken martinis is not clear, is it possible that James Bond chose shaken (not stirred) martinis because of the improved antioxidant potential? This added antioxidant effect could result, of course, in a healthier beverage. There is no indication in the literature that 007 suffered from cataracts or cardiovascular disease, hence he must be considered a moderate consumer of alcoholic drinks. The authors have not examined any antioxidant contributions from olives.

Contributors: CCT originated the idea and performed preliminary experiments on the antioxidant activity of martinis. FR and MMC performed the Student's *t* tests using the Excel

spreadsheet program, and MMC performed the Folin phenol content determinations and statistical analysis. JW and FR, with assistance from Darin Lawrence, Adrian Lee, and Ashley MacDonald, prepared the mini-martinis and performed antioxidant assays using the luminometer. MH and JRT coordinated the study, aided in the statistical analysis, suggested appropriate tests and controls to perform in group meetings, and were mainly responsible for writing the paper. CCT and MMC suggested editorial changes to the text. JRT and MH are guarantors of the paper.

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Competing interests: The research grants from Labatt Breweries were used for a portion of the laboratory supplies, a portion of expenses incurred by CCT, MMC, JW, and JRT in attending the conference of the Association for Research in Vision and Ophthalmology (1999), and a portion of the expenses of MH in attending the fourth international conference on toxicology in developing countries (1999).

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Endpiece

Essential reading matter

From the plenary session at the National Assembly for Wales, 1 December 1999. Dr Brian Gibbons (Labour, Aberavon): "Is Jane [secretary for health and social services] aware of the editorial in the *BMJ* two years ago . . . ?"

Jane Hutt (Labour, Vale of Glamorgan): "No, but I read it regularly now."

Submitted by John Jenkins,
BMA public affairs officer, Cardiff