Caffeine content of beverages as consumed

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Quantitative analysis of beverages prepared at home by staff of the Addiction Research Foundation revealed a lower and much more variable caffeine content of both tea and coffee than had been reported in earlier studies, most of which were based on analysis of laboratory-prepared beverages. Median caffeine concentration of 37 home-prepared samples of tea was 27 mg per cup (range, 8 to 91 mg); for 46 coffee samples the median concentration was 74 mg per cup (range, 29 to 176 mg). If tea and coffee as drunk contain less caffeine than generally supposed, the potency of caffeine may be greater than commonly realized, as may the relative caffeine content of certain commercial preparations, including chocolate and colas. The substantial variation in caffeine content emphasizes the need to establish actual caffeine intake in clinical, epidemiologic and experimental investigations of caffeine effects.

Dans une analyse quantitative de boissons préparées à la maison par les employés de l'Addiction Research Foundation on a constaté une proportion de caféine plus petite et plus variable que celles qu'on a trouvées dans les études d'avant, qui se sont occupées des boissons préparées au laboratoire. La concentration médiane de caféine de 37 échantillons de thé préparés à la maison était 27 mg par tasse (rangée, de 8 à 91 mg); de 46 échantillons de café la concentration médiane était 74 mg par tasse (rangée, de 29 à 176 mg). Si le thé et le café tels que bus comportent moins de caféine que l'on suppose, l'efficacité pharmacologique de la caféine peut être plus forte que l'on croit, et les préparations commerciales, y compris le chocolat et les colas, peuvent comporter plus de caféine, relativement, que l'on croit. La variation considérable de la proportion de caféine dans des préparations différentes donne de la force au besoin de déterminer la consommation actuelle de la caféine chez les sujets des études cliniques, épidémiologiques et expérimentales sur les effets de la caféine.

Caffeine is a psychotropic drug whose consumption has been associated with the occurrence of a variety of pathologic conditions, including ischemic heart disease,[1] gastrointestinal tract ulceration,[2] cancer of the urinary tract,[3] manifest anxiety,[4] premature birth[5] and chronic sleep disturbance.[6] Most of the associations are controversial. In some cases a disease state has been associated with consumption of one kind of caffeine-containing beverage and not another, which suggests that a substance other than caffeine might be responsible; however, analysis of the available data tends to implicate caffeine.[7] In no case are there sufficient data to make a firm judgement; information about caffeine consumption is especially deficient.

Caffeine is probably the most popular psychotropic drug in North America and in many other parts of the world.[7] In the Province of Ontario more than 90% of adults drink a caffeine-containing beverage each day.[8] As with most surveys of caffeine-beverage consumption, the information for Ontario is based on answers given by respondents (1883) to questions about the number of cups of each of various beverages consumed during a given period. Thus it can be estimated, for example, that over 25% of adult Ontarians drink 5 or more cups of coffee or tea, or both, each day.[8] If caffeine consumption is of interest, however, it is important to know how much of the actual drug is being consumed, rather than how much of a solution of the drug of unknown concentration. Epidemiologic studies that might implicate caffeine have also been based on consumption of fluid rather than of caffeine. For example, an influential report of a positive association between coffee consumption and acute myocardial infarction suggested that, compared with those who drink no coffee, persons who drink 6 cups of coffee a day have a 120% greater risk of infarction.[1] Although a positive correlation between caffeine consumption and coffee consumption is to be expected, implication of caffeine must remain uncertain until direct association between drug and disease is demonstrated. Moreover, as long as consumption data are largely in the form of reported numbers of cups of coffee or tea, the contribution of caffeine to disease could be obscured by substantial variation in the caffeine content of cups of these beverages.

Literature references to the caffeine content of beverages are confusing. A sample appears in Table I. Only three of these reports describe laboratory determinations. Many other statements about caffeine content have their origin in one or another of these three assays. For example, the values given in Greden's recent paper[4] are taken from Truitt's chapter in *Drill's Pharmacology in Medicine*,[17] values that in turn were taken from the results achieved in Wolman's laboratory.[9,10] An obvious feature of Table I is the range of reported values for caffeine content per cup or serving, for cups or serving size, and for caffeine concentration. Clearly, reliance on the existing literature would

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be inadvisable for someone interested in estimating the range of caffeine consumption in the population. Moreover, the laboratory estimates may not bear much relation to what people prepare for themselves in their homes, where, according to some surveys,[18,19] most coffee is drunk. It seemed important to have some indication of the caffeine content of beverages *as consumed*. What follows is the report of an investigation into the caffeine content of home-prepared beverages.

Methods

Collection of samples

Of the approximately 750 persons listed in the spring 1974 staff telephone directory of the Addiction Research Foundation and working at various centres in Ontario, 80 were selected by a randomization process and asked to complete a short questionnaire and to submit two samples of home-prepared beverage in provided vials. The 47 respondents provided 86 usable samples (Table II). Respondents were asked to provide a sample from the "very first cup of coffee or tea that you drink today" and a sample from "a cup of coffee or tea *other than* the very first one of the day". They were enjoined to take each sample "from the cup, not from the coffee pot or tea pot". Thus, samples were submitted complete with sugar, cream, sweetener, creamer, lemon, etc. The questionnaire requested personal data, information about regular caffeine-beverage consumption, method of preparation, including quantities used, and cup size. Method of preparation and cup size were considered in the analysis of the caffeine-content data. The other information was used to assess the degree of representativeness of the respondents and to provide an independent (although crude) means of determining caffeine concentration. Further checks on our procedures included preparation and analysis of beverages of typical strengths in the laboratory, and submission of samples from one source at different points in the study, unknown to laboratory personnel.

Extraction of caffeine from coffee and tea

A 1.0-ml sample of coffee or tea in a 10-ml tube fitted with a Teflon-lined cap was extracted with 2.0 ml of a chloroform solution of n-tetracosane, 0.25 mg/ml, for 1 min on a Vortex mixer, and then centrifuged at 2000 rpm for 5 min. This extraction process was repeated with 2.0 ml of chloroform, and the combined extracts were dried under nitrogen at room temperature and redissolved in 1 ml of chloroform. Aliquots of 2 μl were analysed by gas chromatography.

Preparation of standard curves

Standard coffee samples were prepared by adding a standard aqueous solution of caffeine to previously prepared coffee containing no caffeine detectable by the above-described method. The final caffeine concentrations of these standard samples were 0.05, 0.1, 0.25, 0.5, 1.0 and 1.5 mg/ml. The standard samples were extracted

Table I—Summary of selected literature estimates of the caffeine content of beverages

	Ground coffee			Instant coffee			Decaffeinated coffee			Tea			Colas		
Reference no.	Stated caffeine content (mg/cup)	Stated cup size (ml)	Estimated caffeine concentration (µg/ml)	Stated caffeine content (mg/cup)	Stated cup size (ml)	Estimated caffeine concentration (µg/ml)	Stated caffeine content (mg/cup)	Stated cup size (ml)	Estimated caffeine concentration (µg/ml)	Stated caffeine content (mg/cup)	Stated cup size (ml)	Estimated caffeine concentration (µg/ml)	Stated caffeine content (mg/serving)	Stated serving size (ml)	Estimated caffeine concenration (µg/ml)
9*	88 – 119	250	352 – 476	55 – 62	250	220 – 248	13 – 35	250	52 –140	43 – 110	250	172 – 440	–	–	–
10*	–	–	–	86 – 99	250	344 – 396	2 – 4	250	8 – 16	–	–	–	–	–	–
11	90 – 120	140	643 – 857	66 – 74	140	471 – 529	1 – 6	140	7 – 43	70	140	500	19	140	136
12*	100	155	645	30 – 75	155	194 – 483	2 – 6	155	13 – 39	–	–	–	25 – 37	280	89 – 132
13	100 – 150	225	444 – 667	–	–	–	–	–	–	30 – 50	225	133 – 222	–	–	40
14	96	140	686	–	–	–	–	–	–	19	140	136	–	–	–
15	170 – 190	–	–	–	–	–	–	–	–	–	–	–	–	–	–
16	90 – 125	140	643 – 893	60 – 80	140	429 – 571	30 – 75	140	214 –536	30 – 70	140	214 – 500	30 – 45	335	90 – 134
17	100 – 150	–	–	?	–	–	–	–	–	100 – 150	–	–	35 – 55	335	104 – 164
Range: Low	88	140	352	30	140	194	2	140	7	19	140	133	19	140	40
Range: High	190	250	893	80	250	571	75	250	536	150	250	500	55	335	164

*These reports describe laboratory determinations.

Table II—Caffeine content of 86 home-prepared beverage samples

	Coffee					
	Ground		Instant			
	Percolated (n = 11)	Drip or filter (n = 14)	Regular (n = 21)	Decaffeinated (n = 3)	All coffee except decaffeinated (n = 46)	Tea (n = 37)
Caffeine per cup (mg)						
Lowest	39	56	29	1	29	8
Median	74	112	66	1	74	27
Highest	168	176	117	2	176	91
Cup size (ml)						
Lowest	140	170	170	225	140	115
Median	200	225	225	255	225	225
Highest	285	255	285	255	285	300
Caffeine concentration (µg/ml)						
Lowest	195	218	102	2	102	43
Median	436	621	328	5	360	144
Highest	1170	753	559	8	1170	400

quantitatively using the method described above. Linearity was confirmed over the range 0.05 to 1.5 mg/ml.

To determine the efficiency of extraction of caffeine from coffee, we prepared a standard curve with chloroform solutions of caffeine in the following concentrations: 0.1, 0.25, 0.5, 1.0 and 1.5 mg/ml — each with n-tetracosane, 500 μg/ml. Aliquots of 2 μl were analysed by gas chromatography. Standard curves were plotted as Area caffeine/ Area n-tetracosane v. mg caffeine per ml solution. Comparison of the slopes of the standard curves for coffee and for solution in chloroform indicated a mean extraction recovery of 94.9%.

Conditions for gas–liquid chromatography

A Varian Model 2100 Gas Chromatograph was fitted with a flame ionization detector, Hewlett-Packard Model 3380 A with recorder. A 1.83-m pyrex column (internal diameter, 2 mm) packed with 3% OV-17 on Chromosorb Q 100/120 mesh was used in an on-column injection system. Temperatures were maintained as follows: column, 215°C; detector, 275°C; injector, 250°C. Flow rates were 250, 24 and 56 ml/min for air, hydrogen and nitrogen, respectively.

Results

For all types of beverage the amount of caffeine per cup in our analysis tended to be lower and even more variable than the amounts reported in other studies (Tables I and II).

There were considerable differences between tea and coffee samples and between samples of coffee prepared by different methods. The median caffeine concentration of nondecaffeinated coffee samples was 2.5 times that of tea samples, and the median estimated caffeine content of the cups of nondecaffeinated coffee was 2.74 times that of the cups of tea. Both methods of preparing ground coffee yielded significantly higher caffeine concentrations than appeared in the instant coffee samples (Mann-Whitney $U = 54$; $P < 0.01$ for both comparisons). Although the median caffeine content per cup of percolated coffee was closer to that of instant coffee than to that of filtered or dripped coffee (as might be expected from an analysis of preparation procedures[12]), the difference in content resulting from the two methods of preparation from ground coffee was not significant ($U = 62$; $P > 0.05$).

The caffeine content of coffee samples did not vary significantly with the reported coffee consumption of the respondents. The caffeine concentration of tea was also independent of reported tea consumption. Other comparisons between different categories of respondent, including those based on age and sex, also yielded no significant differences with respect to caffeine concentration.

Discussion

The caffeine content of beverages sampled in this investigation was generally lower than has been reported. A variety of reasons can be given for the discrepancy, including many that are peculiar to the respondents, but we are inclined to accept just two. The main reason is likely that home-prepared beverages are generally lower in caffeine content than those prepared in laboratories because smaller quantities of leaf and bean are used at home than in laboratories, where manufacturers' instructions are usually followed. This reason is supported by scrutiny of the details submitted by our respondents about their preparation procedures. It is also possible that the dilution of beverages by cream and other additives causes a substantial decrease in caffeine concentration.

If the caffeine concentration of beverages as actually consumed tends to be lower than hitherto reported, it follows that it may be possible to attribute certain effects of caffeine to lower doses of the drug than had been previously assumed. For example, Goldstein and associates found that physical dependence on caffeine was associated with the regular consumption of 5 or more cups of coffee a day,[20] having noted earlier that an average cup of coffee contains 130 mg of caffeine.[6] From these figures it might be supposed that dependence requires a daily dose of 650 mg of caffeine, but if 74 mg is a more typical amount of caffeine in a cup of coffee, dependence may be associated with the use of much smaller daily amounts of caffeine — that is, upwards from 370 mg.

Another implication of generally lower caffeine content of tea and coffee as consumed is that commercially prepared sources of caffeine appear to contain relatively more caffeine. Colas, which are legally required to contain caffeine in both Canada and the United States, typically contain about 40 mg of the drug per serving, an amount well in excess of the mean amount found by us in a cup of tea and more than half the median amount found in a cup of coffee. A small chocolate bar contains about 25 mg of caffeine.[12] Thus, a 27-kg child ingesting three colas and the chocolate equivalent of three small bars a day would be self-administering approximately 7.2 mg/kg caffeine. A 79-kg man would have to drink nearly 8 cups of coffee at 74 mg of caffeine per cup to ingest the same daily dose of caffeine.

What may be the more important aspect of our findings is not the lower caffeine content but the extremely wide range of caffeine content. Available evidence suggests that there may be considerable risk to the health of an adult of average weight who consumes upwards of 600 mg of caffeine a day[7] — that is, more than about 8 cups of average-strength coffee, or about 22 cups of average-strength tea, or about 15 285-ml bottles of cola, or about nine of certain headache pills (Exedrin contains 65 mg caffeine per tablet), or the equivalent in combinations. The range of caffeine-beverage consumption that could be equivalent to 600 mg caffeine is enormous. The caffeine content of a cup of coffee could be as high as 333 mg according to the above findings (i.e., coffee of maximum caffeine concentration in a maximum-sized coffee cup). One such cup consumed daily by a person half the average weight would provide more caffeine per kilogram than 600 mg consumed by a person of average weight. The lowest caffeine content could be 14 mg per cup. A person twice the average weight would have to drink 86 such cups to exceed the caffeine consumption per kilogram of an average-weight person drinking 8 typical cups of coffee. Because of this potential range — and a similar one exists with respect to tea consumption — difficulty must be expected in establishing associations between caffeine-beverage consumption and the occurrence of pathologic conditions, especially in view of the apparent absence of correlation between consumption and concentration of caffeine. Clearly, further epidemiologic and clinical work on the pathologic effects of caffeine must be concerned with caffeine consumption rather than with caffeine-beverage consumption. Moreover, experimental studies into the effects of caffeine should include accurate assessment of the habitual caffeine intake so that the relative impact of a given dose of the drug can be gauged and between-subject differences anticipated and allowed for.

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Addendum

Since the preparation of this article we have become aware of a report by Burg in the January 1975 issue of the US-based *Tea & Coffee Trade Journal*, reviewing 46 recent commercial analyses of the caffeine content of beverages and concluding that the industry should adopt as standards 85 mg of caffeine per 150-ml cup for roast and ground coffee, 60 mg/cup for instant coffee, 30 mg/cup for instant tea and, tentatively, 50 mg/cup for other tea. We found that the median reported cup size was approximately 225 ml (do Canadians really use larger cups?); therefore, our values for both coffee and tea concentrations remain below those based on analyses of laboratory preparations.

Another investigation of the caffeine content of home-prepared beverages has just been reported in abstract form (Al-Samarrae W, et al: *Proc Nutr Soc* 34: 18A, 1975). This study, conducted in Britain, revealed narrower ranges (58 to 125 mg/cup for coffee; 51 to 87 mg/cup for tea) and higher mean values for the caffeine content of both coffee and tea than those found in our study. The main difference is that the British seem to make much stronger tea than Canadians, although, because cup size was not reported, proper comparison between the two studies is not possible.