

1 **Original research article**2 **An examination of consumer exposure to caffeine from**  
3 **commercial coffee and coffee-flavoured milk**4 Ben Desbrow\*<sup>a,b</sup>, Michael Henry<sup>c</sup>, Pieter Scheelings<sup>c</sup>5 <sup>a</sup> School of Public Health, Griffith University, Queensland, Australia6 <sup>b</sup> Research Centre for Clinical and Community Practice Innovation, Griffith Health  
7 Institute, Griffith University, Queensland, Australia8 <sup>c</sup> Queensland Health Forensic and Scientific Services, Queensland, Australia

9 \*Corresponding Author. Tel.: +61 7 5552 9110; fax: +61 7 5552 8679.

10 E-mail: [b.desbrow@griffith.edu.au](mailto:b.desbrow@griffith.edu.au).11 **Abstract**

12 A cross-section of Australian “Espresso/short black” coffee and coffee-flavoured milk  
13 samples were purchased and analysed for their caffeine content using micellar  
14 electrokinetic capillary chromatography (MEKC). Coffees were collected using  
15 convenience cluster sampling across four major cities. Packaged coffee-flavoured milks  
16 were collected from national grocery distributors. In all, 131 espresso samples and 20  
17 coffee-flavoured milks were analysed. The mean ( $\pm$ SD) quantity of caffeine from espresso  
18 coffee was  $107\pm 37$  mg/serving with a concentration of  $2550\pm 1030$  mg/L. The mean ( $\pm$ SD)  
19 quantity of caffeine from coffee-flavoured milk was  $99\pm 50$  mg/carton with a concentration  
20 of  $193\pm 90$  mg/L. There was considerable variation in caffeine content across both  
21 categories and within the same espresso brand purchased at different locations. In total, 42

22 samples (27.5%) contained  $\geq 120$  mg per serving of caffeine, and 20 samples (13.1%)  
23 exceeded 165 mg per serving. The expanded caffeine data supports our original findings  
24 which indicated that the probability of consumer exposure to high caffeine doses from  
25 popular coffee beverages in Australia is greater than previously reported.

26 *Keywords:* Caffeine; Coffee; Variability; Consumer exposure; Food safety; Food analysis;  
27 Food composition

## 28 **1 Introduction**

29 Caffeine is probably the most widely used pharmacologically active substance in the world,  
30 and its influence on human health has been studied and discussed extensively (Committee  
31 on Toxicology, 2001; Desbrow et al. 2007; Nawrot et al. 2003; Scientific Committee on  
32 Food, 1999; Smith et al. 2000). The debate regarding the impact of caffeine on health  
33 relies initially on the strength of establishing an epidemiological link between caffeine  
34 consumption level and concurrent health events which occur across a defined population  
35 and period. The accurate estimation of caffeine consumption relies, in turn, on having  
36 reliable information on the frequency of consumption and caffeine content of commonly  
37 consumed dietary items.

38 Although caffeine is found in a number of foods, it is most frequently consumed in coffee,  
39 tea and cola beverages (Frary et al. 2005). It is recognised that beverages with caffeine as a  
40 natural component will have a wide range of caffeine levels. These variances are likely the  
41 result of many factors which include the species of plant origin (Mazzafera and Silvarolla  
42 2010), growing environment, effects of commercial processing and storage along with  
43 variances at the retail level such as amount of coffee or tea used, the extraction method (e.g.  
44 percolated, drip etc) and the temperature and amount of water used in beverage preparation.

45 Interestingly, preliminary data from the US demonstrated that a large caffeine variance  
46 (259-564 mg/dose) was observed when the same specialty coffee (Starbucks® Breakfast  
47 Blend) was purchased on consecutive days (i.e. intra-drink variance) from a single outlet  
48 (McCusker et al 2003). This indicates that a significant proportion of caffeine variance  
49 remains despite a number of the supply variables being controlled.

50 To assess likely consumer exposure to caffeine from popular commercial coffee beverages  
51 we initially quantified the content and range of caffeine found in commercial espresso  
52 coffee (n=97) purchased from different outlets across one geographical region of Australia  
53 (i.e. the Gold Coast) (Desbrow et al 2007). These caffeine values were similar to data  
54 collected on commercial coffee prepared throughout the United Kingdom (Food Standards  
55 Agency, 2004). The wide range of caffeine concentrations and number of heavily  
56 caffeinated samples differentiated these findings from frequently cited textbook values  
57 reporting the caffeine content of commercial coffee. Consequently, we concluded that  
58 individual or population based quantification of caffeine intake when those under  
59 consideration purchase retail coffee or use ground coffee varieties at home was prone to  
60 large errors and, more importantly, a potential underestimation of caffeine intake because  
61 of early lower estimates of caffeine dose.

62 In Australia, caffeine is now added (often in the form of guarana) to some already  
63 caffeinated foods (e.g. coffee-flavoured milks, which are popular pre-packaged, ready-to-  
64 consume drinks) as a “functional” ingredient by some manufacturers. This example of a  
65 change to the food supply will only serve to further complicate our ability to clarify  
66 consumer level exposure to caffeine.

67 Given the natural variation in caffeine content of coffee, the increasing national popularity  
68 of coffee consumption (in all its forms) (ABS 2007) and the current food labelling  
69 regulations (Australian manufacturers are not required to state caffeine concentrations on  
70 products with naturally occurring caffeine (FSANZ 2010) there is a need to have accurate  
71 and independent information on the caffeine content of common coffee beverages.

72 Therefore the aims of this study were to a) broaden the geographical spread of espresso  
73 coffee samples collected within Australia; b) describe the extent of caffeine variance  
74 occurring when the same espresso brand is purchased at different locations (intra-drink  
75 variance); and c) to quantify the caffeine content of popular commercially prepared coffee-  
76 flavoured milk beverages and to reconcile these values against the manufacturer's reported  
77 values. The clarification of caffeine content and the extent of its variance in retail coffees  
78 will a) improve the accuracy of estimations of caffeine consumption in Australia and  
79 consequently any likely association with ill-health, b) result in more reliable advice from  
80 health care providers concerning beverage recommendations and health risks and c) enable  
81 greater awareness within the population as to potential exposure of an individual to a given  
82 caffeine dose.

## 83 **2 Methods**

### 84 **2.1 Study Design**

85 A cross-section of "Espresso/short black" coffee and coffee-flavoured milk samples were  
86 purchased. Hot espresso coffees were collected using convenience cluster sampling across  
87 four major cities. Cold coffee-flavoured milks were collected from national grocery  
88 distributors.

## 89      **2.2 Sample Collection**

90      *Espresso Samples:* A single “Take-away Espresso/short black” coffee sample was  
91 purchased from retail vendors housing an espresso machine. The original data sample  
92 included 97 espressos collected on the Gold Coast (Desbrow et al 2007). An additional 34  
93 samples were subsequently collected using the same sampling method from retail outlets in  
94 the central business districts of Brisbane (n=10), Sydney (n=10) and Melbourne (n=14).  
95 Briefly, the samples were served in the vendors’ own cups and subsequently decanted into  
96 vials that were chilled then weighed and volume determined prior to freezing and  
97 subsequent caffeine analysis. To standardise the sampling method, the researchers, if asked,  
98 would indicate “single shot” espressos, otherwise no further instruction was provided. The  
99 rationale for only collecting espresso coffee was that it limits many variables (e.g. serving  
100 size, milk, plain water, sugar etc) in the coffee making procedure and that the espresso  
101 “shot” often forms the basis of many other types of retail coffee (e.g. lattes, cappuccinos  
102 etc) and therefore the caffeine dose found in an espresso will be equal to (if not less than)  
103 those found in larger beverages.

104      *Coffee-Flavoured Milk Samples:* 20 varieties of commercial iced coffee were purchased on  
105 3 occasions (i.e. different production dates) from national grocery distributors located on  
106 the Gold Coast. Samples were kept cold prior to subsequent caffeine analysis and standard  
107 serving sizes were recorded to estimate likely consumer exposure (i.e. concentration x  
108 volume). Drink manufacturers were contacted to provide the reported caffeine content of  
109 each drink.

## 110      **2.3 Sample preparation**

111      A milk coffee sample (10 mL) was added to a centrifuge tube (50 mL), followed by ethanol  
112 (10 mL), gently but thoroughly mixed and allowed to stand (15 minutes) until precipitation

113 of solids occurs. The tube was centrifuged at 2000 rpm for 3 minutes. The supernatant (2  
114 mL) was placed in a 13x100 mm culture tube and the ethanol was evaporated under a  
115 stream of nitrogen at room temperature. To the resultant residue of around 1 mL was added  
116 approximately 2 mL of deionized water followed by 0.75mL of 0.1M H<sub>3</sub>PO<sub>4</sub>, 0.75mL  
117 0.05M Sodium dodecyl sulphate and 0.5mL dehydroacetic acid as internal standard  
118 (500ppm) and finally made up to 5.0mL with deionized water. The solutions were mixed  
119 thoroughly and filtered through a polyvinylidenedifluoride (Millex HV) membrane (0.45  
120 µm) before analysis.

#### 121 **2.4 Standards preparation**

122 Standard solutions of caffeine containing constant amounts of dehydroacetic acid were  
123 prepared. Caffeine concentrations of 10, 20, 30, 40 and 50 milligram/kilogram (mg/L)  
124 together with a constant 50 (mg/L) of dehydroacetic acid were employed.

#### 125 **2.5 Sample Analysis**

126 The caffeine analysis was performed using Micellar Electrokinetic Capillary  
127 Chromatography (MEKC) according to the procedure reported by Thompson and co-  
128 workers (Thompson et al. 1995). The instrument employed was a Beckman Coulter MDQ  
129 Capillary Electrophoresis Unit fitted with an uncoated fused-silica capillary column (57cm  
130 x 75 µm) with an inlet to window length of around 50cm and employing a diode array  
131 detector.

132 The run buffer consisted of SDS (25 mM) and disodium tetraborate (12.5 mM, pH 9.2).  
133 The test sample was loaded under a pressure of 0.5 psi for 5 seconds and the instrument  
134 operated at 20 kV and 27 degrees C. Analytical quality control included the analysis of

135 duplicate and spiked samples, whilst recent participation in interlaboratory proficiency  
136 studies on caffeine has provided a good measure of analytical competence.

## 137 **2.6 Statistical Analysis**

138 Descriptive statistics of caffeine dose and concentration (mean, SD, coefficient of variation  
139 (CV), range) were determined. Variance from manufacturers reported caffeine intake in  
140 coffee-flavoured milk beverages is illustrated via Bland-Altman plot. One way analysis of  
141 variance (ANOVA) was applied to assess if mean espresso caffeine concentrations of  
142 samples differed between geographical collection points.

## 143 **2.7 Ethics**

144 The study was reviewed and approved by the Griffith University Human Research Ethics  
145 Committee.

## 146 **3 Results**

147 *Espresso Samples:* **Table 1** provides a descriptive summary (mean, median, SD, CV and  
148 range) of the espresso analysis results; in all, 131 espresso samples were collected. The  
149 mean ( $\pm$ SD) caffeine concentration was  $2550\pm 1030$  mg/L, and ranged from 580 to 7000  
150 mg/L. Thirty four samples (25.9%) contained 120 mg/serving of caffeine or higher. No  
151 statistical significance was demonstrated ( $p=0.719$ ) between caffeine content and its  
152 location of origin. **Table 2** describes the variability in caffeine when the same espresso  
153 sample was ordered at different outlets of the same retail vendor (note: data are included  
154 only where  $\geq 4$  outlets were sampled).

155 *Coffee-Flavoured Milk Samples:* **Table 3** provides a descriptive summary (mean, SD and  
156 range) of the analysis results. 58 samples were analysed (i.e. 19 varieties on 3 occasions + 1

157 variety on 1 occasion). The mean ( $\pm$ SD) caffeine concentration was 1930 $\pm$ 90 mg/L, the  
158 median was 1745 mg/L and values ranged from 660 to 3290 mg/L. The average CV across  
159 production dates was 8.4% and ranged from 1.0 to 19.0%. Caffeine per serving  
160 (concentration x volume) ranged from 33 to 197 mg/serving; 17 of 20 beverages had  
161 caffeine concentrations in excess of the manufacturer's claims. **Figure 1** is a Bland-Altman  
162 plot indicating the strength of relationship between the measured and manufacturer's  
163 claimed caffeine content. Five of these beverages (25% of the sample) had >19% more  
164 caffeine than claimed (that is, in excess of the maximum CV observed with production date  
165 variance).

#### 166 **4 Discussion**

167 This study aimed to expand the database of independently tested caffeine values derived  
168 from popular coffee-based products available to Australian consumers. The main finding of  
169 this study was that the caffeine content of popular coffee-based sources was highly variable  
170 and could result in higher than anticipated caffeine intakes. The expanded caffeine analysis  
171 conducted on commercial espresso coffee confirms that the mean caffeine content (107  
172 mg/serving) is higher than earlier international reports of 78, 35, 85 and 62 mg/serving,  
173 respectively by Barone and Roberts (1996), Harland (2000), Knight et al. (2004) and  
174 Mandel (2002). These Australian values are in agreement with values found in retail  
175 coffees from the United Kingdom (105 mg/serving) (Food Standards Agency, 2004) and on  
176 home-prepared retail market coffee in Portugal (98mg/serving) (Candeias et al 2009).  
177 Consequently, any individual or population based quantification of caffeine intake where  
178 those involved purchase retail coffee or use ground coffee varieties at home is prone to  
179 potential errors. Furthermore, the potential for a significant underestimation of caffeine  
180 intake if relying on historical caffeine content values clearly exists.



181 The extent of caffeine variance observed when a consumer purchases a commercial coffee  
182 (inter-drink variance) is large (current range 25-214 mg/serving). This range is likely to  
183 reflect the variable nature of the retail coffee environment (i.e. the numerous coffee  
184 roasting wholesalers who supply a wide variety of coffee blends) as well as any variance in  
185 the method and technique of the barista (i.e. amount of coffee, amount and temperature of  
186 water etc). It now appears that the intra-drink caffeine variance (i.e. the variance observed  
187 when the same drink is either purchased from different locations (current study) or from the  
188 same location on different days (McCusker et al 2003) is also considerable. The current  
189 results indicate when the same brand of espresso coffee was purchased on  $\geq 4$  occasions  
190 from different locations the coefficient of variation was typically  $>15\%$  and that for 5  
191 brands this variation was  $\geq 30\%$ . Intra-drink variances of this magnitude make any estimates  
192 of caffeine consumption at the individual level very difficult, even when the individual's  
193 commercial coffee purchasing patterns are consistent.

194 In Australia, pre-packaged coffee-flavoured milks are supplied by a number of  
195 manufacturers and are likely to contribute to the caffeine intake of many individuals  
196 including children and adolescents (personal observation). National food labelling  
197 requirements do not make it mandatory for manufacturers of such drinks (with naturally  
198 occurring caffeine) to quantify the caffeine content of beverages, making it problematic for  
199 consumer to compare products on a caffeine content basis. It has been suggested however,  
200 that the caffeine levels in foods with naturally occurring caffeine will not normally exceed  
201 100 mg in a standard serving (Smith et al. 2000). Fifteen (75%) of the 20 coffee-flavoured  
202 cold milk varieties included within present comparison contained  $\leq 120$  mg/serving of  
203 caffeine. This indicates that these varieties are in accordance with the previous suggestion  
204 as they contain similar amounts of caffeine to that found in the hot coffee drinks. Of some

205 public health concern is that the remaining five samples contained concentrations of  
206 caffeine approaching (or exceeding) the upper limit of caffeine permissible within  
207 Australian formulated energy drinks (i.e. 32 mg/100mL) (FSANZ 2010). Given that these  
208 coffee beverages are served in large volumes ( $\leq 500$  ml) they represent a significant caffeine  
209 source that may not be recognised as such by inadvertent consumers or their guardians.

210 It appears that the production of commercial coffee-flavoured milks produces substantially  
211 less intra-drink caffeine variance (average CV 8.4%) than commercial espresso coffee. This  
212 result is not surprising and most likely reflects the reduction in methodological variables  
213 associated with mass-scale coffee production. However, despite this relatively small day-  
214 to-day variance in caffeine content, manufacturers of coffee-flavoured milks were typically  
215 poor at reporting (usually underestimating) the caffeine content of their drinks. Eight (40%)  
216 of the drinks contained more caffeine than could be explained by the CV of that beverage  
217 when purchased on three separate occasions. Five (25%) drinks contain more caffeine than  
218 the maximum 19% CV observed by the most “inconsistent” drink sampled. Taken  
219 collectively, some coffee-flavoured milks provide the potential for consistently high  
220 caffeine exposures to unwitting consumers. Consequently it is our recommendation that  
221 coffee-flavoured milk beverages be required to conform to the same labelling requirements  
222 as those required for formulated energy drinks. This change would increase consumer  
223 capacity to make informed choices regarding the caffeine content of beverages and for the  
224 accuracy of the such labelling to be regulated by independent authorities.

225 Of further public health concern is the potential for exposure to higher than recommended  
226 amounts of caffeine in vulnerable population groups (e.g., reproductive-age women and  
227 children). In an environment of uncertainty regarding the effects of caffeine on health the  
228 prudent public health approach is to remain conservative. The present data further indicate

229 that it is difficult for health care professionals to provide any reliable advice other than to  
230 “abstain, drink decaffeinated beverages or have no more than one caffeinated retail ground  
231 coffee per day” if, for example, they wish to ensure their pregnant clients stay below a level  
232 of 300 mg/day of caffeine (Nawrot et al 2003), or when providing advice to parents for  
233 children to “avoid cold iced coffee beverages” if they wish to keep caffeine intakes  $\leq 2.5$   
234 mg/kg body weight as is also recommended (Nawrot et al 2003).

## 235 **5 Conclusion**

236 This study provides further information on the caffeine content and the extent of its  
237 variance in popular Australian retail coffee products. The data will enable health  
238 professionals and their clients to have a more informed understanding of the likely caffeine  
239 exposures associated with commercial coffee consumption. Furthermore, the reliability of  
240 estimations of caffeine consumption in Australia and consequently its relationship with ill-  
241 health can henceforth be achieved with greater confidence.

## 242 **Acknowledgements**

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287 other foods. *Journal of Chromatography. A*, 694, 507-514.
- 288

289 **Figure captions**

290

291 **Figure 1: Bland-Altman plot (95% limits of agreement) between the measured and**  
292 **manufacturer's claimed caffeine content.**

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**Table 1****Sample numbers, mean, standard deviation and range of caffeine (mg/serve) content of espresso coffees purchased at different locations.**

	N	Mean	Median	SD	Min	Max
Gold Coast	97	106	96	38	25	214
Brisbane	10	113	107	37	73	189
Sydney	10	112	105	34	54	168
Melbourne	14	108	105	33	63	168
<b>TOTAL</b>	<b>131</b>	<b>107</b>	<b>99</b>	<b>37</b>	<b>25</b>	<b>214</b>

ANOVA Caffeine vs Location P=0.719

**Table 2**

**Sample numbers, mean, standard deviation, coefficient of variation (CV) and range of caffeine (mg/serve) content of espresso coffees purchased from the same retailer across different locations.**

<b>Coffee Chain*</b>	<b>n</b>	<b>Mean</b>	<b>SD</b>	<b>CV(%)</b>	<b>Max</b>	<b>Min</b>
Coffee Club <sup>®</sup>	7	113	38	33.6	177	82
Gloria Jeans <sup>®</sup>	6	145	11	7.6	162	130
Muffin Break <sup>®</sup>	6	137	49	35.8	186	68
Donut King <sup>®</sup>	6	134	51	38.1	214	82
BB's <sup>®</sup>	5	115	42	36.5	189	81
Starbucks <sup>®</sup>	4	79	13	16.5	91	63
McDonalds <sup>®</sup>	4	70	13	18.6	83	54
Zarrafas <sup>®</sup>	4	62	11	17.7	75	49
Goldstein's <sup>®</sup>	4	91	27	29.7	114	54

\* Only samples collected at  $\geq 4$  locations were included within this analysis.

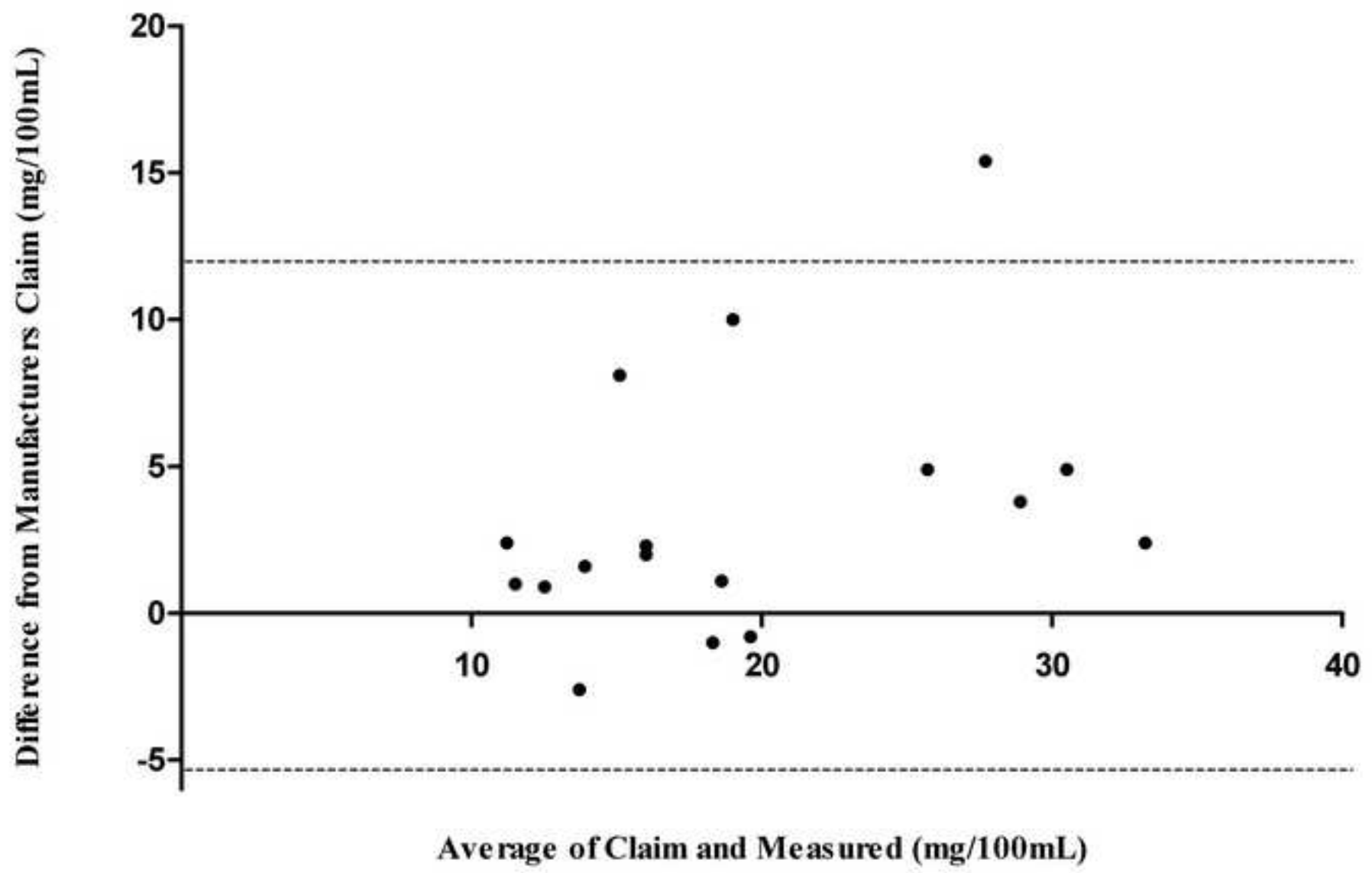


**Table 3**  
**Mean, standard deviation and range of caffeine (mg/100mL and mg/serve) content of coffee-flavoured milk purchased from national grocery distributors.**

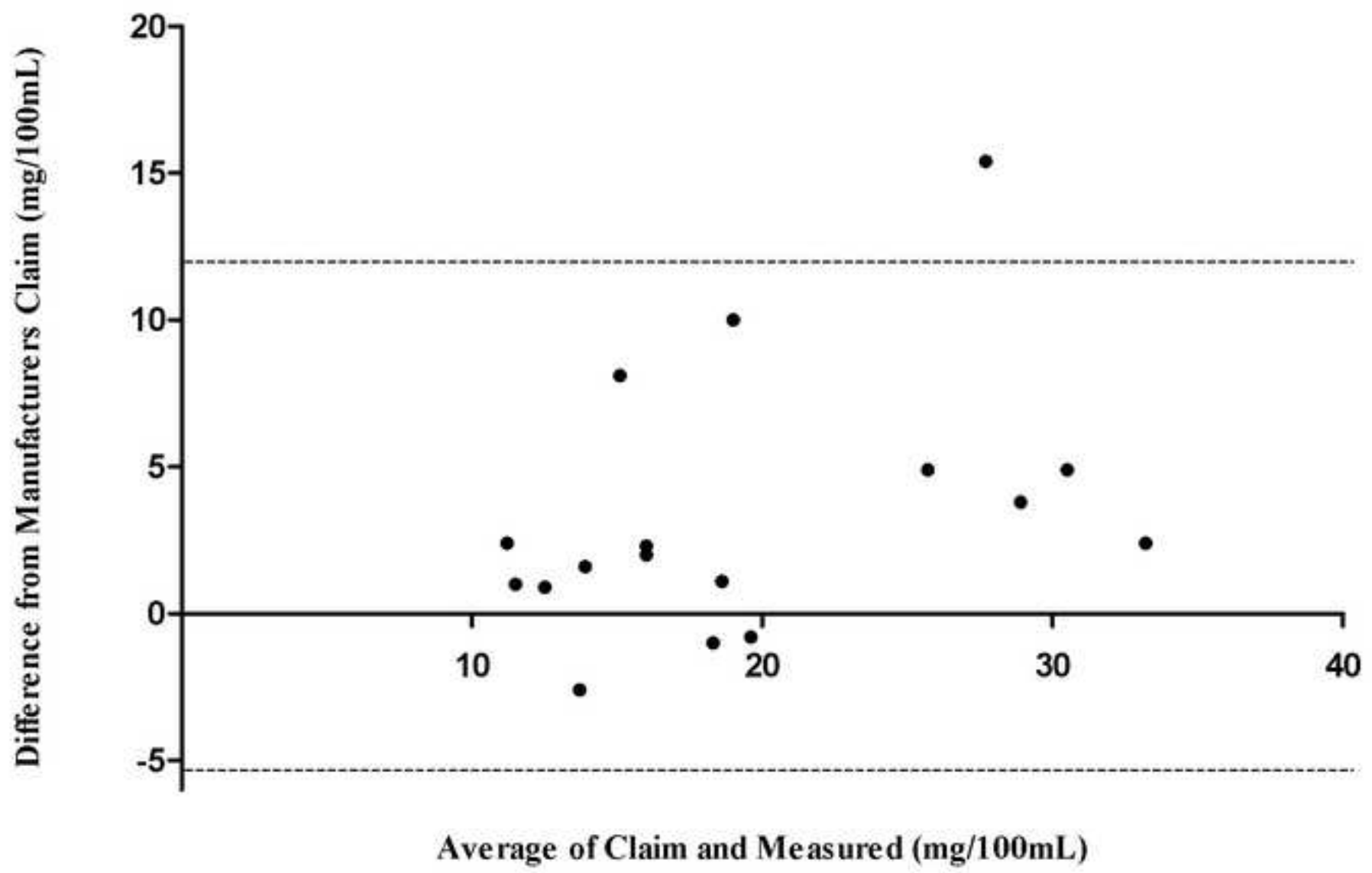
<b>Brand</b>	<b>Mean (Caffeine(mg)/100mL)</b>	<b>SD*</b>	<b>Container Volume</b>	<b>Caffeine/serve</b>	<b>Manufacturer's Report (Caffeine(mg)/100mL)</b>	<b>% Difference to Manufacturer</b>
Breaka Strong®	32.9	0.8	600	197.4	28	17.5
Dare Double Espresso®	35.4	1.9	500	177.2	20	77
Ice Break Loaded®	34.4	2.0	500	171.8	32	7.5
Rush Intense Coffee®	30.8	3.5	500	153.8	27	14
Ice Break®	28.1	2.6	500	140.7	23.2	21
Dare Espresso®	24.0	2.4	500	119.8	14	71
Farmers Union®	19.1	1.2	600	114.8	18	6
Jacaranda®	19.1	1.1	600	114.6	11	74
Farmers Union Light®†	17.8	-	600	106.8	18.8	-5.5
Big M Double Strength®	19.2	2.7	500	96.0	20	-4
Breaka®	17.1	2.7	500	85.5	14.8	15.5
Big M Edge®	17.0	0.7	500	84.8	15	13
Breaka Lite®	14.7	2.8	500	73.5	13.1	12
Dare Cappuccino®	12.9	1.4	500	64.5	12	7.5
Rush Wicked Latte®	12.4	1.4	500	61.8	10	24
Rush Mocha Kenya®	12.0	0.1	500	60.2	11	9
Dare White Chocolate Mocha®	9.7	1.1	500	48.7	N/A	N/A
Oak®	12.4	0.9	300	37.3	15	-17
Woolworth's Iced Coffee®	11.2	0.3	300	33.6	N/A	N/A
Browne's Cappuccino®	6.6	0.4	500	33.0	N/A	N/A
<b>Average(SD)*</b>	<b>19.3(8.7)</b>			<b>99(50)</b>		

\*Indicates SD of samples from one brand across three production dates. N/A indicates Not available from manufacturer. † Indicates only purchased on one occasion.

scrip



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## Highlights

- Analysis was conducted on popular hot and cold coffee beverages in Australia.
- The probability of exposure to high caffeine doses greater than historical reports.
- Estimations of caffeine consumption can now be achieved with greater accuracy.
- Assessment of link between caffeine and health can be made with greater confidence.

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