

## Short Communication

### **Boiled coffee does not increase serum cholesterol in gerbils and hamsters**

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*Summary:* In contrast to drip filter coffee, boiled coffee increases the serum cholesterol level in man. To identify the substance(s) responsible for this effect, it is necessary to find an animal model sensitive to boiled coffee. In this study, three groups of 20 male gerbils and three groups of six male hamsters were fed a control diet or a control diet supplemented with either freeze-dried boiled coffee or freeze-dried filtered coffee. At the end of the 5-week feeding period serum cholesterol levels were not different in either species fed the different diets. These results suggest that these animal species are not sensitive to boiled coffee, or that the cholesterol-raising factor in boiled coffee is lost during the process of freeze-drying.

*Zusammenfassung:* Im Gegensatz zu filtriertem Kaffee erhöht gebrühter Kaffee den Serumcholesterinspiegel im Menschen. Um die für diesen Effekt verantwortliche(n) Substanz(en) zu identifizieren, wird ein Tiermodell gesucht, das auf gebrühten Kaffee entsprechend reagiert. In dieser Studie wurden an drei Versuchsgruppen zu je 20 Gerbilmäusen und drei Versuchsgruppen zu je 6 Hamstern eine Kontrolldiät und eine Kontrolldiät supplementiert mit entweder gefriergetrocknetem filtriertem Kaffee oder gefriergetrocknetem gebrühtem Kaffee verabreicht. Nach Beendigung der fünföchigen Fütterungsperiode konnte kein signifikanter Unterschied in den Serumcholesterinwerten der Gerbilmäuse und der Hamster in den verschiedenen Versuchsgruppen festgestellt werden. Diese Ergebnisse weisen darauf hin, daß entweder diese Tiermodelle für diese Studien nicht geeignet sind oder daß die cholesterinerhöhende Substanz im gebrühten Kaffee durch das Gefrier-trocknen verlorenght oder unwirksam wird.

*Key words:* Hamster, gerbil, boiled coffee, filtered coffee, serum cholesterol

*Schlüsselwörter:* Hamster, Gerbilmäuse, gebrühter Kaffee, filtrierter Kaffee, Serumcholesterin

## Introduction

Studies in man have shown that Scandinavian-style boiled coffee has a powerful serum cholesterol-raising effect compared with filtered coffee (1, 2). Boiled coffee is made by boiling ground coffee beans in water, and decanting the fluid into a cup. One liter of boiled coffee contains 1–1.5 g of lipid material as opposed to about 0.01 g for drip filter coffee (7). We have recently shown that the hypercholesterolemic factor from boiled coffee is a lipid-soluble compound (7). To purify and identify this substance, an animal species sensitive to boiled coffee is indispensable. Serum cholesterol in gerbils and hamsters shows a sensitivity to dietary fatty acids paralleling that in man (4, 6). This makes these animals an obvious choice for assaying the coffee lipid factor. Here, we describe the effects of freeze-dried boiled coffee on serum cholesterol levels in male gerbils and in hamsters.

## Methods

Male gerbils (*Meriones unguiculatus*) and male hamsters (*Mesocricetus auratus*), 4 to 6 weeks of age were used. They were housed four gerbils or three hamsters to a polycarbonate cage, with a layer of sawdust as bedding. Cages were located in rooms with controlled lighting (light on, 0700–1900 hours) and a constant temperature (19–21 °C). Relative humidity varied between 35 and 55 %.

The basal diet consisted of (g/kg): casein, 151; taurine, 5; corn oil, 50; coconut fat, 50; corn starch, 333.9; dextrose, 300; cellulose, 50; CaCO<sub>3</sub>, 12.4; NaH<sub>2</sub>PO<sub>4</sub> · 2 H<sub>2</sub>O, 15.1; MgCO<sub>3</sub>, 1.4; KCl, 1; KHCO<sub>3</sub>, 7.7; cholesterol, 0.5; vitamin premix, 12, and mineral premix, 10. The composition of the vitamin and mineral premix has been described (5).

Boiled coffee was brewed by adding 100 g of coarsely ground coffee to 2 l of boiling tap water. This mixture was allowed to boil for 2 min; then the grounds were allowed to settle for 15 min and the brew was decanted. Regular filtered coffee was prepared using an automatic coffee maker and a paper filter. Per liter of tap water 50 g of extra-finely ground coffee was used. The coffee brews were freeze-dried for 24 h at 30 °C. One liter of boiled or filtered coffee yielded 17 g of freeze-dried material. Per kg of basal diet 35 g of freeze-dried boiled or filtered coffee was added at the expense of 35 g of starch. The diets were pelleted and stored at 4 °C until feeding.

The gerbils and hamsters were divided into three groups, stratified for body weight and for serum cholesterol levels as measured 1 week before the start of the study. One group of each species received the basal diet, the second group the basal diet with the freeze-dried boiled coffee, and the third group the basal diet supplemented with the freeze-dried filtered coffee. The experiment lasted 35 days. Animals had free access to feed and tap water.

Blood was sampled by orbital puncture on days -7, 0, and 35 of the study between 0900 and 1100 hours in the nonfasting state under light diethyl-ether anesthesia. Serum was obtained by low-speed centrifugation and stored at -80 °C until analysis of cholesterol (3).

## Results and Discussion

Body weight gain did not differ in response to the dietary regimens for either the gerbils or the hamsters (Table 1). On average, the gerbils consumed 5 g feed per day with no differences between the three diet groups.

Table 1. Effect of freeze-dried boiled and filtered coffee on body weight and serum cholesterol in male gerbils and hamsters.

	Dietary supplement		
	None	Boiled coffee	Filtered coffee
<i>Gerbils</i>			
Body weight (g)			
Baseline	48.8 ± 8.6	48.4 ± 9.7	49.1 ± 9.2
Change	14.2 ± 7.0	12.5 ± 6.0	13.6 ± 5.9
Serum cholesterol (mmol/l)			
Baseline	3.83 ± 0.44	3.70 ± 0.47	3.81 ± 0.41
Change	0.50 ± 0.72	0.59 ± 0.43	0.62 ± 0.46
<i>Hamsters</i>			
Body weight (g)			
Baseline	102.4 ± 14.8	107.3 ± 7.9	107.2 ± 7.1
Change	24.6 ± 9.1	35.4 ± 9.3	29.3 ± 8.7
Serum cholesterol (mmol/l)			
Baseline	4.87 ± 0.32	4.74 ± 0.51	4.53 ± 0.49
Change	1.41 ± 0.27	0.93 ± 0.59	1.66 ± 0.92

Means ± SD, n = 20 (gerbils) or n = 6 (hamsters) for each treatment group. Change refers to difference between days 0 (baseline) and 35 (final).

Feed intake of the hamsters was not measured. Table 1 shows that, in both the gerbils and the hamsters, changes in serum cholesterol were not affected by the addition of freeze-dried boiled coffee to the diets. The level of freeze-dried coffee extract in the diets amounted to 2.2 g per MJ of diet. This is equivalent to one 125-ml cup of coffee per MJ of diet, using coffee brewed with 50 g of ground coffee per 1 l of water. For a human consuming 10 MJ of energy per day the corresponding intake of coffee would be 10 cups a day. Such amounts have given rise to increase in serum total cholesterol levels of more than 0.5 mmol/l in man (1, 2).

In conclusion, our results show that, unlike in man (1, 2), serum cholesterol levels in the gerbils and hamsters were not affected by the addition of boiled coffee to the diets. Whether these results indicate that the serum cholesterol-raising factor in boiled coffee is lost during the process of freeze-drying or that these two animal species are not sensitive to boiled coffee warrants further investigation.

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