The effect of fasting time on plasma total cholesterol concentration in Mongolian gerbils

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*This study investigated the effect of length of fasting time on plasma total cholesterol response of male Mongolian gerbils (*Meriones unguiculatus*). Plasma cholesterol levels from fed and fasted gerbils were also compared with those reported for humans under similar metabolic states. Plasma total cholesterol response showed a significant quadratic relationship with time over a 15-hour period. Between 6 and 9 hours of fasting (the time during which plasma triglyceride concentration became relatively constant), the average plasma total cholesterol concentration was 178 mg/dl, compared with a zero hour (fed) cholesterol level of 265 mg/dl. The difference in plasma cholesterol levels observed in fed and fasted gerbils is unlike what has been reported for humans. Results from most human studies show no differences in plasma total cholesterol concentrations for fed and fasted subjects. Failure to consider species differences in metabolic responses may have implications when results from animal experiments are extrapolated to humans.*

Keywords: Fasting; plasma cholesterol; lipid metabolism; gerbils

Introduction

The Mongolian gerbil has been used with increasing popularity as a small animal model in studies of lipid metabolism.[1,2] Previous reports have demonstrated that gerbils can tolerate diets containing fat and cholesterol contents typical of human intakes.[3,4] In contrast, rabbits have a poor tolerance for dietary cholesterol[5] and mortality rates are high for other rodents fed human-like levels of dietary fat.[6] The hypercholesterolemic response of gerbils fed cholesterol and different types of dietary fat approximates that of humans,[7] unlike what is observed in rats which are quite resistant to the development of hypercholesterolemia.[8] Recent studies have also shown that Mongolian gerbils are similar to humans in terms of their relative proportions of plasma free versus esterified cholesterol, their type of plasma cholesterol esters, and their plasma cholesterol response to dietary manipulation.[9,10]

Preliminary investigations in our laboratory have indicated that plasma total cholesterol concentrations from gerbils in the fasted state are significantly lower than their postprandial cholesterol levels.[11] This observation is unlike results from human trials which have indicated no significant differences between serum cholesterol levels from humans in the fasted or fed state.[12-14] This discrepancy may have implications when results from animal studies are extrapolated to humans. However, part of the discrepancy may be due to the duration of food deprivation and/or the timing of food removal. A 12- to 15-hour overnight fast, typical of the conditions used for most human metabolic studies, may have metabolic consequences for gerbils which are not similar to those for humans. Gerbils have a smaller body size relative to that of humans, and likely have a higher metabolic rate.[15] Also, by nature, these animals are nocturnal feeders and, therefore, a 12- to 15-hour overnight fast in gerbils may influence their cholesterol metabolism differently, especially since abstinence from food may have begun the previous morning. To compare results from animal

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and human studies, similar metabolic states must be ensured.

The aim of the present study, therefore, was to investigate the effect of length of fasting time on the plasma cholesterol response of male Mongolian gerbils (*Meriones unguiculatus*) under carefully controlled fasting/feeding conditions. This study investigated the plasma total cholesterol concentrations of gerbils over a 15-hour fasting period and compared plasma cholesterol levels from fed and fasted gerbils with those reported for fed and fasted humans.

Methods and Materials

Animals and diets

Male Mongolian gerbils (45 to 75 g; High Oak Ranch, Goodwood, Ontario, Canada) were paired according to body weight and were housed two per wire-bottomed stainless steel cage. They were maintained on a 12-hour light/dark cycle (illumination from 7:00 AM to 7:00 PM) at a constant temperature of 25°C, and had access to food and water ad libitum. After 1 week acclimatization on laboratory rodent chow, all gerbils were fed the semipurified diet (*Table 1*) for a period of 2 weeks. The composition of the experimental diet approximated the average North American diet in terms of energy contributions from protein, fat, and carbohydrate,[16-18] cholesterol content,[16-18] polyunsaturated to saturated fatty acid (P/S) ratio,[16] and proportion of dietary protein from animal and plant sources.[19]

Table 1 Composition of experimental diet

Constituent	Percent weight	Percent energy
Protein[a]	16.0	13.9
Fat[b]	20.0	39.0
Carbohydrate[c]	54.4	47.1
Mineral mixture[d]	4.0	
Salt mixture[e]	1.0	
Choline chloride	0.3	
Myo-inositol	0.1	
Alfa-floc	5.0	
Chromium acetate	0.00023	
Sodium selenite	0.000022	
DL-methionine	0.1	
Cholesterol[f]	0.1	

[a] Animal/plant = 2.3; as 11.15% Vitamin-free Test Casein (Teklad Test Diets, Madison, WI, USA) and 4.85% Isolated Soy Protein 620 (Ralston Purina, St. Louis, MO, USA).
[b] P/S = 0.38; as 19% Tenderflake Pure Lard (Canada Packers, Toronto, Ontario, Canada) and 1% Unico Sunflower Oil (Culinar Foods, Toronto, Ontario, Canada).
[c] Starch/surcrose = 2; as 35.2% cornstarch and 17.6% sucrose, plus 1.6% from casein-soy protein mix.
[d] William Briggs Modified (Teklad Test Diets).
[e] To supply in mg/kg diet: nicotinic acid, 25.0; DL-calcium pantothenate, 20.0; pyridoxine-HCl, 7.0; riboflavin, 7.0; thiamine-HCl, 6.0; folate, 1.0; menadione, 0.2; D-biotin, 0.2; vitamin B_{12}, 0.012; and in IU/kg diet: retinyl acetate, 17,500; ergocalciferol, 2, 000; DL-α-tocopherol acetate, 80.
[f] Equivalent to 220 mg/1,000 kcal.

Experimental procedures

Initial (time 0) body weights, blood samples, and hematocrit readings were obtained from all gerbils at 7:00 AM. The food cups were then removed from the cages, and subsequent measurements and samples were taken at 1, 2, 3, 4, 6, 9, 12, and 15 hours from time 0. Micro blood samples (150 μl) were collected from the orbital plexus[20] while gerbils were lightly anesthetized with methoxyflurane. Blood was drawn into heparinized microcapillary tubes and centrifuged for 5 minutes at 11,500 × *g*. Hematocrit readings (% packed red blood cell [RBC] volume) were obtained using a microcapillary reader. Plasma was then removed from the RBC layer, pooled with samples from gerbils within the same cage, and frozen at −80°C until lipid analyses were performed. Body weights and hematocrit readings were also averaged for the two gerbils in each cage. Plasma total cholesterol and triglyceride concentrations were determined using commercially available enzymatic kits (Boehringer Mannheim, 237574 and 240052, respectively). Final plasma total cholesterol concentrations represented values adjusted for a blood dilution effect (see below). Plasma triglyceride concentrations were used as a measure of when the gerbils were truly fasted.

Calculations and statistical analyses

Plasma total cholesterol concentrations were adjusted for a dilution effect resulting from repeated blood sampling. The dilution effect was estimated using packed RBC volume. It was assumed that RBCs are neither created nor destroyed (except by sampling) during the sampling period, but that the total blood volume returns to its natural level, V, shortly after each sampling. Under these assumptions, the hematocrit reading after n samplings will be $k(1-v/V)^n$, where k is the initial hematocrit reading and v (which equals 150 μl) is the volume of blood taken at each sampling. The natural log of hematocrit reading after n samplings is then $\log(k)+n\log(1-v/V)$. Thus, the slope of the regression of log hematocrit reading on n provided an estimate of $\log(1-v/V)$. In subsequent statistical analyses of plasma total cholesterol, the cholesterol values at the nth sampling were first adjusted for the dilution effect by dividing by $(1-v/V)^n$. All response variables (body weight, hematocrit, adjusted plasma total cholesterol, and plasma triglyceride) were plotted against hour of fasting. The effect of fasting time on plasma total cholesterol concentration was further investigated by polynomial regression analysis.[21] The final model for plasma total cholesterol included a block analysis for the number of gerbil pairs and used a quadratic regression on fasting time. An indication of the cholesterol level from fasted animals was obtained by observing the plasma total cholesterol response at the time for which plasma triglyceride concentration became relatively constant.

Results

The results for mean body weight, hematocrit, and plasma total cholesterol and triglyceride concentra-

Table 2 Body weight, hematocrit, and plasma total cholesterol and triglyceride responses[a] (mean ± SD[b]) for gerbils, by hour of fasting

Fasting time (hr)	Body weight (g)	Hematocrit (% PCV)	Plasma cholesterol (mg/dl)[c]	Plasma triglyceride (mg/dl)
0	80.1 ± 9.0	45.8 ± 1.3	265.2 ± 24.0	363.8 ± 216.6
1	79.2 ± 9.1	44.7 ± 1.2	252.8 ± 26.1	253.8 ± 144.0
2	78.5 ± 9.0	42.2 ± 1.5	237.3 ± 31.5	173.6 ± 71.7
3	77.9 ± 9.0	40.1 ± 1.3	225.3 ± 31.1	121.3 ± 53.5
4	77.2 ± 9.0	38.7 ± 1.1	210.4 ± 35.4	84.0 ± 22.1
6	76.4 ± 8.9	37.3 ± 0.9	185.4 ± 32.3	58.7 ± 19.4
9	75.4 ± 8.9	35.9 ± 0.9	170.1 ± 26.4	51.3 ± 12.2
12	74.6 ± 8.7	34.7 ± 1.1	168.2 ± 27.5	65.8 ± 20.3
15	74.2 ± 8.7	32.8 ± 1.3	162.8 ± 25.5	79.7 ± 34.5

Abbreviation: PCV, packed RBC volume.
[a] Individual responses pooled by cage; therefore, n = 23 is the number of cages.
[b] Standard deviations reflect among-animal variation; generally too large for between time comparisons.[d]
[c] Adjusted for dilution effect.
[d] For between time comparisons for plasma cholesterol, use standard error (SE) for difference between two treatment means; SE = 4.0.

tions are shown in *Table 2*. In general, all responses decreased as the hours of fasting increased; however, plasma triglyceride concentration reached a minimum at 9 hours postprandial, after which time triglyceride levels began to rise. These trends are presented graphically in *Figures 1, 2,* and *3*. *Figure 3* also shows the plasma total cholesterol response corresponding to the plasma triglyceride response. Between 6 and 9 hours of fasting, the average plasma total cholesterol concentration was approximately 178 mg/dl, compared with the time 0 (fed) cholesterol level of 265 mg/dl (*Figure 3*). In *Figure 4*, the actual plasma total cholesterol response curve over time is compared with the predicted response curve obtained from the quadratic regression analysis, providing evidence that the quadratic model was an appropriate fit for the data over the time period examined.

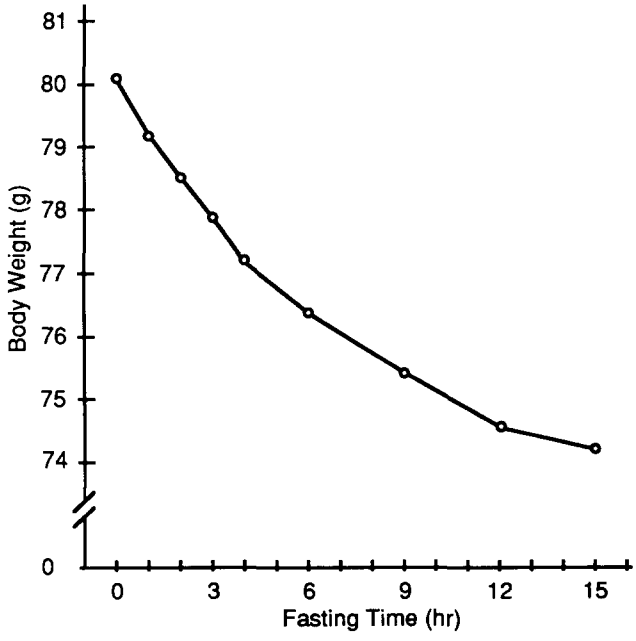

Figure 1 Body weight response curve of gerbils over 15 hours of fasting.

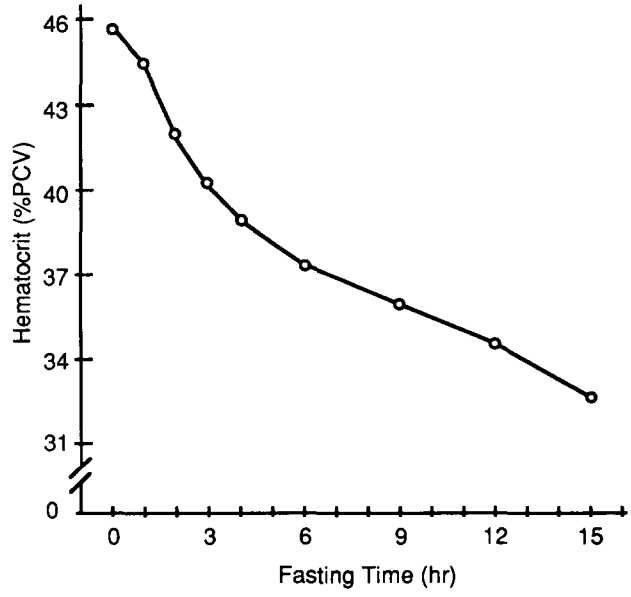

Figure 2 Hematocrit response curve of gerbils over 15 hours of fasting.

Discussion

In the present study, length of fasting time was shown to significantly affect the plasma total cholesterol concentrations of male Mongolian gerbils. The quadratic nature of the cholesterol response curve demonstrated that plasma cholesterol levels decreased over time, and that the decline occurred at a slower rate as the length of fasting increased. Although a complete quadratic curve would eventually begin to rise, this was not observed in the plasma total cholesterol response for the gerbils by 15 hours of fasting. The statistical quadratic model is, however, appropriate, considering the homeostatic regulation of cholesterol metabolism. De novo synthesis of cholesterol is a major contributor to body cholesterol levels for both humans and animals.[8] The rate of cholesterol biosynthesis in mammals is highly responsive to the amount of dietary cholesterol available[22] and, hence, a lack of dietary

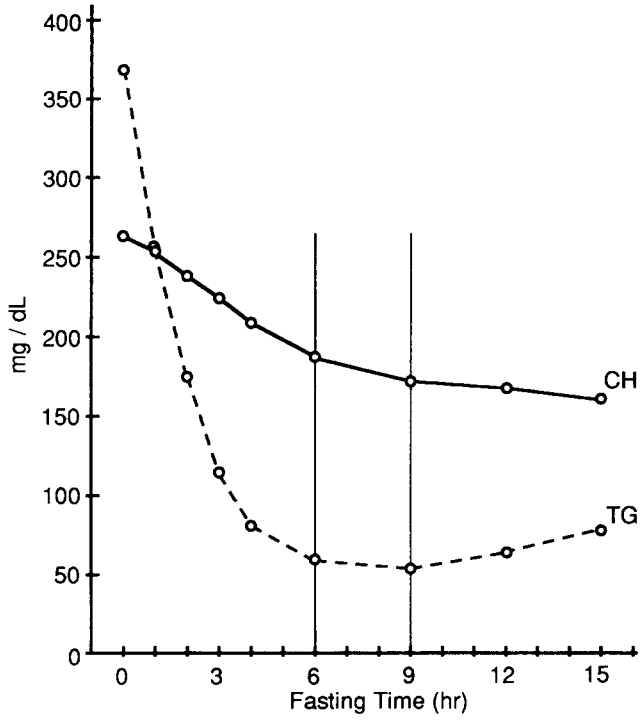

Figure 3 Plasma lipid responses of gerbils over 15 hours of fasting; plasma total cholesterol adjusted for dilution effect (CH) —— and plasma triglyceride (TG) ---; vertical lines delineate fasting state.

cholesterol (or fasting) would lead to an increase in de novo cholesterol synthesis, such that a maintenance of prefasted plasma cholesterol levels could be expected. In the present study, it appears that a compensatory increase in cholesterol biosynthesis either does not occur or did not occur during the 15-hour fasting period. Alternatively, de novo cholesterol synthesis may have occurred, but not at a rate sufficient to maintain the plasma cholesterol levels observed in the fed state. If the plasma cholesterol levels from fasted gerbils are physiologically appropriate, then the concentrations observed as a result of cholesterol feeding were perhaps artificially high. Plasma lipids were not examined beyond 15 hours, since prolonged fasting would have resulted in animals closer to a state of starvation. Additional effects of starvation, such as accelerated body weight loss and increased catabolic processes for supplying the body with fuel, may have confounded the fasting effect. In addition, more direct measures of cholesterol homeostasis other than just plasma total cholesterol concentration must also be considered. One study demonstrated a reduction in HMG-CoA reductase activity when gerbils were fed diets containing polyunsaturated fatty acids with or without cholesterol.[23]

The plasma triglyceride results from the gerbils of the present study indicate that a fasting state was reached between 6 and 9 hours postprandial and that, after this time, the gerbils were catabolizing body tissues to maintain physiologic function. The method used for plasma triglyceride analysis involved an enzymatic hydrolysis step such that the actual measure was of free glycerol, including not only the glycerol that was cleaved from plasma triglycerides, but also a measure of the free glycerol already present in the plasma. During certain physiologic conditions such as prolonged fasting or starvation, adipose tissue lipolysis occurs at an accelerated rate,[24] and one of the products of lipolysis is the glycerol backbone of stored triglycerides. Since the gerbils of this study did not have access to any exogenous triglyceride, the observed increase in plasma triglyceride concentration, per se, after 9 hours of fasting was therefore more likely a reflection of increased plasma-free glycerol.

The plasma total cholesterol concentration for fasted gerbils in this study was therefore established as 178 mg/dl, the average between responses at 6 and 9 hours. Regression analysis demonstrated that plasma cholesterol concentration of fasted gerbils declined significantly from the fed level of 265 mg/dl. The decrease in plasma cholesterol concentrations for fasted gerbils is different from observations in humans. Several investigators have reported no significant differences in serum cholesterol concentrations obtained from human subjects during fed or 1- to 12-hour fasted states.[12,13] Slight variations (of 3% to 3.8%) between cholesterol levels of fasted or fed humans have been reported[25,26]; however, these differences are more likely within the range for intrasubject or intersubject variability. A more recent study of postprandial plasma lipid changes in human subjects has also demonstrated no differences in plasma cholesterol levels obtained from zero to 12 hours after a test meal for the sample population as a whole.[27] Furthermore, other

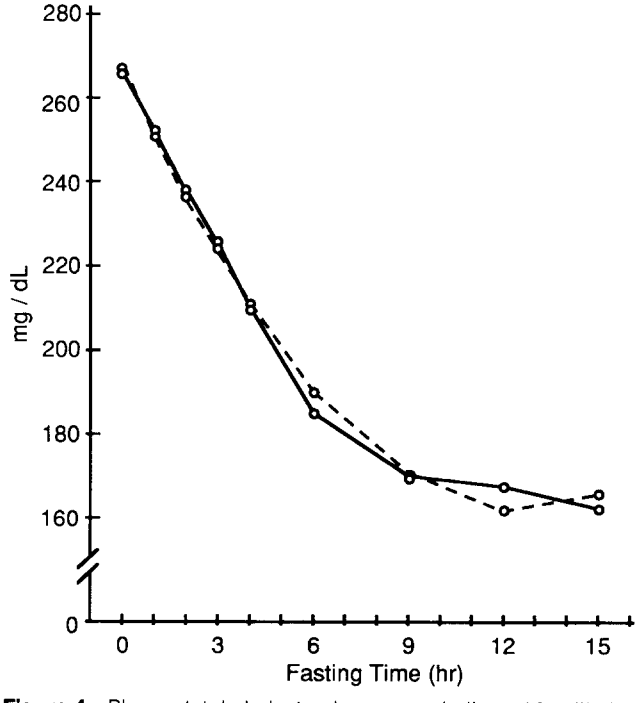

Figure 4 Plasma total cholesterol response (adjusted for dilution effect, ——) of gerbils over 15 hours of fasting, and predicted response curve from the quadratic equation (---).

investigators have concluded that it is not necessary to obtain blood samples from fasted subjects for cholesterol determinations,[14] and standard plasma or serum cholesterol methodologies do not include a requirement for fasted blood samples.[28,29]

Part of the difference in gerbil and human plasma cholesterol responses to fasting time may be explained by the type of meal ingested prior to fasting. When differences due to fasting were reported for human subjects, the experimental protocol consisted of feeding a fat-rich meal relative to usual fat intakes.[27] Gerbils in the present study were fed a diet typical of the average North American fat intake, yet the fat content was high relative to the habitual fat intake of gerbils. An explanation for the observed difference in plasma cholesterol levels for fed and fasted gerbils remains to be elucidated. Some insight into this phenomenon could be obtained by investigating the cholesterol homeostatic mechanisms in gerbils in both the fed and fasted states.

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