High plasma cholesterol, but low triglycerides and plaque-free arteries, in Mexican free-tailed bats

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Widmaier, E. P., E. R. Gornstein, J. L. Hennessy,
J. M. Bloss, J. A. Greenberg, and T. H. Kunz. High plasma cholesterol, but low triglycerides and plaque-free arteries, in Mexican free-tailed bats. Am. J. Physiol. 271 (Regulatory Integrative Comp. Physiol. 40): R1101–R1106, 1996—Female mammals typically become hyperphagic from mid to late pregnancy and during lactation. Mexican free-tailed bats, Tadarida brasiliensis mexicana, double their nightly food intake from late pregnancy to peak lactation and consume an insect diet that is exceptionally high in fat. During late pregnancy and throughout lactation, fasting plasma levels of cholesterol in this insectivorous bat are high (215 ± 8 mg/dl) and are nearly 10-fold higher than in three species of Old World frugivorous bats. Fasting triglycerides were unexpectedly low in T. brasiliensis (25 ± 2 mg/dl), despite evidence of high fat intake during nightly feeding bouts (postprandial cholesterol and triglycerides, 268 ± 18 and 122 ± 20 mg/dl, respectively). High-density lipoprotein (HDL) cholesterol levels were extraordinarily high (124 ± 5 mg/dl) and unaffected by feeding. Low-density lipoprotein (LDL) cholesterol levels were correspondingly low (86 ± 7 mg/dl). This unusual plasma lipid profile was not associated with coronary or aortic atherosclerosis, nor was there evidence of hyperglycemia or hyperinsulinemia. A high-fat diet and high levels of cholesterol in T. brasiliensis are not correlated with cardiovascular disease or (possibly) insulin resistance. Among several possible factors that might account for these observations, nocturnal bouts of powered flight (commuting and foraging for food) may contribute to elevated HDL cholesterol, which may protect this species from developing atherosclerosis.

atherosclerosis; Chiroptera; insulin; high-density lipoprotein cholesterol

MOSST INVESTIGATIONS on the cardiovascular and metabolic consequences of hyperphagia, fat intake, and lipid deposition have used strains of rodents with genetically dependent aberrant feeding behavior or normal animals fed artificial diets supplemented with lipids. Results from these studies suggest that hyperphagia or the consumption of a high-fat diet is coupled with increases in body mass, hyperlipidemia, insulin resistance, and atherosclerosis (e.g., Refs. 1, 2, 4, 6–8, 17, 19, 27, 32). Most of these investigations, however, fail to account for the normal life histories of the animals in question. The consequences, if any, of excessive fat intake and gain in body mass would be ideally studied in a free-ranging mammal in its natural habitat.

There are numerous examples from mammalian species in which periods of hyperphagia and subsequent increases in body mass are integral parts of their life history (3). For example, hyperphagia and deposition of fat reserves are important survival strategies in preparation for annual hibernation and biannual migration (3). Similarly, hyperphagia occurs because of increased energy demands imposed by pregnancy and lactation. In the annual cycle of the Mexican free-tailed bat, Tadarida brasiliensis, hyperphagia occurs when females are pregnant and lactating (15), again before its southward migration in autumn, and again before its northward migration in spring. Dietary fat intake from insects during pregnancy and lactation may exceed 60% of the dry mass (15), and the deposition of fat reserves before the onset of migration (10, 18) may contribute up to 30% of the animal’s lean body mass (T. H. Kunz, unpublished observations). Because T. brasiliensis also has a relatively long life span, with some individuals reaching 15 years of age (5, 10), we tested the hypothesis that a high dietary fat intake may contribute to the development of cardiovascular and metabolic disorders.

In this study, we asked the following questions. 1) What is the circulating lipid profile of T. brasiliensis during late pregnancy and lactation? 2) Are the high intake of fat and the consequent gain in body mass associated with signs of coronary artery disease? 3) Do these dietary habits promote hyperinsulinemia and hyperglycemia (suggestive of insulin resistance)? The results suggest that, despite high levels of circulating cholesterol, T. brasiliensis is protected against atherosclerosis and insulin resistance, possibly because of a high high-density lipoprotein (HDL) cholesterol fraction coupled with powered flight associated with nightly commuting and foraging activity.

METHODS

Study species. T. brasiliensis (Molossidae) is an insectivorous species that ranges from the southwestern United States to southern South America (31). Its diet consists largely of moths, beetles, and flying ants (18). Analysis of stomach contents has revealed that the ingested dry mass is composed of ~60% fat (15). In the present study, bats were collected from a large maternity colony (estimated population, ~6 million) in the Eckert James River Cave, Mason County, Texas. With the use of hand nets, bats were captured at the main opening to the cave at the time of nightly emergence (1800–2000) and again on return to the cave (0600–0730) after the second of two nightly feeding bouts. A few of the captured bats were in late stages of pregnancy, but most were lactating when these collections were made (20 June–3 August, 1995). Bats captured at emergence were judged to be fasting, because at this time of day ~12 h have elapsed since the last feeding bout. Immediately on collection, bats were weighed and the reproductive condition of each female was noted. Pregnancy was determined by abdominal palpation, and lactation was determined by condition of the nipples and manual expression of milk. Blood was withdrawn by venipuncture through a wing or ureteral vein and collected in microcapillary tubes. In some instances, individual bats were decapitated and trunk blood was collected as described above.
is currently unknown. A diet rich in triglycerides containing polyunsaturated fats might be expected to impart cardiovascular protection. However, at present we have no evidence to support any such possibilities and have confined ourselves to more conservative explanations.

We are grateful to Keith Atkinson, John Seyjagat, and Darryl Heard for collecting blood from Old World fruit bats housed at The Lubee Foundation and to Dr. William Eldred and Floyd Craft for assistance with histological procedures.

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lipoprotein lipase activity is generally increased during lactation (26). Notwithstanding, pregnant bats had cholesterol and triglyceride levels comparable to lactating individuals. Other mechanisms also may contribute to triglyceride clearance. The pelage and wing membranes of *T. brasiliensis* appear to be very oily, and this condition could be caused by the sequestration of circulating lipids and secretion by skin glands (14). In addition, the activities of the other lipoprotein lipases could be unusually active. Unfortunately, postmigratory locations of these animals are not well known, and we cannot yet test the intriguing hypothesis that seasonal changes in triglyceride clearance may occur.

Most of the circulating cholesterol was found in the higher-density form, with circulating HDL cholesterol levels of 100–160 mg/dl. The typical LDL/HDL ratio in an individual animal was in all but one case <1.0, and sometimes <0.5. This lipid profile may partly result both from lactation, which may elevate certain subfractions of HDL cholesterol in women (11), and from the prolonged nightly flight activity engaged in by *T. brasiliensis*. Lactating Mexican free-tailed bats feed twice nightly, averaging 5 and 3 h, respectively, for each bout (15). Epidemiological data for humans suggest that exercise is a good predictor of elevated HDL cholesterol (e.g., Ref. 25). Thus the present results using a mammalian model in its natural habitat are at least consistent with the hypothesis that chronic exercise results in an improved LDL/HDL ratio. It is interesting to suggest that very high circulating HDL levels may be characteristic of small mammals with high metabolic rates, although the number of species examined to date is too small to confidently make such a generalization (16, 24).

The elevated HDL cholesterol/LDL cholesterol ratio suggested that, despite the high-fat diet of *T. brasiliensis* during the summer before autumn fattening and migration, atherosclerosis would be absent. The rationale for this hypothesis arose from epidemiological literature that suggests that HDL cholesterol protects against plaque formation in humans (e.g., Ref. 12). To test this hypothesis, thin sections of left and right coronary arteries, anterior and posterior descending branches of the coronary arteries, and aortas were analyzed for signs of plaque formation. In humans and other mammals, these vessels are the most common sites of atherosclerotic damage (23). In support of our hypothesis, no thickening of the intima layer nor smooth muscle hypertrophy was observed at any site (proximal or distal) along any vessel from the 10 randomly chosen animals, all of whom had fasting cholesterol levels >200 mg/dl. Thus our results strongly suggest that, despite their relatively great longevity, moderately elevated cholesterol, high fat intake, and seasonal mass gain, female *T. brasiliensis* are protected against the development of classical atherosclerosis. Although we have yet to determine the nature of this protection, it is possibly related to the high percentage of cholesterol present in the HDL fraction (so-called good cholesterol). Because estrogen is believed to protect against the development of severe atherosclerosis, it would be of great interest to compare the lipid and histological profile of female and male *T. brasiliensis*. Unfortunately, adult males of this species are not common at maternity roosts and otherwise are difficult to locate when females are rearing young.

Finally, because a high-fat diet is often associated with obesity and an increased likelihood of the onset of insulin resistance and its complications (hyperinsulinemia, hyperglycemia), we determined circulating insulin and glucose levels in bats that were randomly chosen at the time of emergence (fasting state) and in the postprandial period. The few available data on Chiropteran insulin and glucose concentrations suggest that circulating insulin and sugar levels in bats are similar to other mammals. Plasma glucose levels in fruit-eating bats, for example, fall within the identical range as in man, dogs, rodents, and other mammals (28, 30), and those of at least one insectivorous bat species are in this range (21). Circulating glucose and insulin levels in *T. brasiliensis* were within the range of concentrations found in most mammals, with no evidence of hyperglycemia or hyperinsulinemia.

Lack of a significant rise in glucose or insulin after feeding may reflect the fact that these animals are captured immediately on reentering the cave and thus had not yet begun to digest food (because digestion is slowed by exercise and sympathetic activity). Thus, although we have yet to directly determine insulin sensitivity of target tissues such as muscle in *T. brasiliensis*, the euglycemia and low insulin levels argue against the presence of diabetic-like symptoms secondary to fat intake and mass gain. Use of a heterologous assay, however, may underestimate insulin levels, although the samples in the assay diluted roughly in parallel with the standards. Confirmation of these results will await development of a homologous assay for Chiropteran insulin. In addition, these results do not rule out the possibility that insulin resistance and hyperglycemia would be present in the postpractical period when the rate of fat deposition is expected to increase.

Thus, contrary to hyperphagic and genetically obese strains of rodents and other mammals, females of *T. brasiliensis* do not appear to suffer the classic consequences of hyperphagia and high fat intake. The prolonged nightly flight activity characteristic of bats may contribute to a high rate of production of HDL cholesterol by the liver, which is believed to protect against atherosclerosis. In addition, exercise has been postulated to delay or attenuate the symptoms of insulin resistance in humans at risk for development of non-insulin-dependent diabetes mellitus and in diabetic rats (13, 29). The present results support this hypothesis. There are other potential explanations for the apparent resistance to the deleterious effects of hypercholesterolemia and mass gain, such as the presence of unidentified circulating factors that inhibit LDL attachment to endothelium or prevent oxidation of LDL particles, which is believed to result in a more atherogenic form of the lipoprotein (22). Finally, the fat composition of the insects consumed by *T. brasiliensis*
Fig. 3. Light microscopic appearance of sections through a representative coronary artery (A) and aorta (B) from 2 bats. Thin sections were cut on an ultramicrotome and stained with Toluidine blue. Note typical ruffled appearance of elastic fibers in the aortic media and the homogeneity of appearance and thickness of intima and media. Higher-magnification view of a portion of another aorta is shown in C. Note uniform subendothelial thickening and several layers of smooth muscle and elastic fibers. Arrowhead points to an endothelial cell nucleus. Scale bars represent 20, 100, and 10 µm in photographs A, B, and C, respectively.
Table 1. Fasting plasma lipid levels in four species of bats

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Values are means ± SE; no. of individual animals is indicated in parentheses. ND, not determined. Blood samples were obtained by venipuncture, and plasma was analyzed for either total cholesterol or triglycerides or both (when sufficient plasma was available). Data for lactating *T. brasiliensis* are included from Fig. 1 for the sake of comparison.

Ten hearts were chosen at random for histological analyses from 10 bats (8 fasting, 2 postprandial) whose complete lipid profile was known (total cholesterol 218 ± 15 mg/dl; HDL cholesterol 117 ± 7 mg/dl; triglycerides 42 ± 16 mg/dl; LDL cholesterol 88 ± 11 mg/dl). Some of the hearts were encased in thick layers of fat, especially around the pulmonary trunk. The histological appearance of coronary and aortic vessels was unremarkable except for the thinness of the media, compared with arteries of larger mammals, which may have 40–50 layers of elastic fibers. Coronary arteries, for example, had only 3–4 layers of smooth muscle around the intima, and the aortas had only ~12 layers of elastic fibers. All aortas had evident subendothelial thickening that did not stain with osmium. Lumens of the coronary arteries and aortas were completely open and free of visible signs of smooth muscle hypertrophy and plaque formation (Fig. 3, A-C).

DISCUSSION

Several strains of rodents have been described that are genetically predisposed to obesity. Life histories of these mammals are characterized by hyperphagia, excessive increase in body mass, and several endocrine and metabolic disorders (hyperinsulinemia, insulin resistance, hyperglycemia, hypercholesterolemia, hypercorticoidism) (2, 8, 19, 27, 32). In addition, normal rats and other animals on high-fat diets develop a similar syndrome (4, 7, 9). In virtually all animal models of high fat intake and hypercholesterolemia, atherosclerosis is a common finding (7, 9, 17).

In the present study, we asked whether the Mexican free-tailed bat, which has a high-fat diet and undergoes deposition of fat in spring and autumn in preparation for migration, is also subject to the same metabolic, endocrine, and cardiovascular consequences observed in laboratory rodent models. We examined *T. brasiliensis* because its dietary composition and life history have been well documented (14, 15, 31) and its life span is sufficient to permit the onset and progression of atherosclerosis and other disorders. Before its southward migration in autumn, individuals of *T. brasiliensis* may increase their body mass by up to 30%. The diet of this species consists largely of moths, beetles, and flying ants with a high fat content, and its postprandial stomach contents consist of ~60% fat (dry mass) (15, 20).

One complication in considering free-ranging *T. brasiliensis* as a mammalian model for investigating the adverse effects of a high-fat diet is that only adult females (and their young) were available for study at maternity roosts. On arrival in spring and before the onset of autumn migration, adult females at these sites are either pregnant, lactating, or postlactating. Unfortunately, we were unable to sample bats during the lactation period and during the hyperphagic period before the onset of spring and autumn migration.

Regardless of reproductive status, all adult females of *T. brasiliensis* demonstrated moderately to very high levels of circulating cholesterol. These results are consistent with observations in a related insectivorous bat, *Molossus sp.*, in which total cholesterol levels approach 250 mg/dl (21). The elevated levels of cholesterol in *T. brasiliensis* are not unexpected in light of its high-fat diet, nor do they simply reflect a general characteristic of the Chiroptera (Table 1). The low levels of total cholesterol in the three species of Old World fruit-eating bats (Pteropodidae) are similar to those reported for fruit-eating members of the New World family Phyllostomidae, but less than those reported for omnivorous (Phyllostomus sp.) and a sanguivorous (Desmodus sp.) species (21). More remarkable, however, is that in all cases circulating triglycerides were extremely low in *T. brasiliensis* and similar to levels more typical of herbivores. That these bats were indeed consuming fatty insects each night is evident not only from analyses of stomach contents (15) but also by the significant postprandial rise in circulating cholesterol and triglycerides. Clearly, this species possesses mechanisms that lead to rapid clearance of triglycerides from the general circulation. In part, this may result from lactation and the sequestration of triglycerides into milk, because the milk of lactating *T. brasiliensis* is very high in fat content (14) and mammary gland.

![Fig. 2. Mean fasting plasma levels of glucose and insulin in *T. brasiliensis*. Blood samples were collected from bats at emergence from ("fasting") and return to ("fed") the cave by venipuncture. These animals were collected on July 25 and 26, 1995, and are from a separate population from those in Fig. 1. Bars represent SE; n = 11–13/group.](image-url)

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Carcasses were subsequently frozen for use in other studies. Whole blood was stored on ice for ~30 min before centrifugation at 12,000 revolutions/min for 3 min; plasma was frozen until it was used for lipid or hormone assays.

Blood also was obtained by wing venipuncture from non-breeding females of three species of Old World fruit bats (Pteropus hypomelanus, Pteropus pumilus, and Pteropus vampyrus) housed at the Lubeck Foundation, Gainesville, FL, and centrifuged as described above. Plasma from these bats was frozen until assayed for total cholesterol.

**Cholesterol assays.** Plasma cholesterol (total) and HDL cholesterol were determined using a modification of the procedures recommended in Sigma procedure no. 332. HDL cholesterol was determined after precipitation of lower density forms of cholesterol, followed by determination of the remaining total cholesterol in the supernatant. Precipitation was accomplished by adding 20 μl plasma to 4 μl Sigma HDL cholesterol reagent (phosphotungstic acid-MgCl₂; procedure no. 352–4). In some preliminary trials, the precipitating reagent was increased to 8 μl.

Plasma triglycerides were determined in 10 μl of nonheparinized plasma using the Sigma triglyceride (INT) reagent kit. Low-density lipoprotein (LDL) cholesterol levels were calculated from the following formula:

\[
[LDL] = \left[ \text{total cholesterol} \right] - \left[ \frac{\text{HDL cholesterol}}{5} \right] - \left[ \text{triglycerides} \right]
\]

where brackets indicate concentration.

Plasma insulin was determined in 25-μl aliquots of plasma using a radioimmunoassay kit from ICN Biomedicals. Plasma glucose levels were determined from the same animals in 10 μl of plasma by the glucose oxidase method (Sigma trinder reagent kit).

**Histology.** Hearts were dissected from decapitated animals within 5 min and washed twice in phosphate-buffered saline (PBS, pH 7.4), then fixed by immersion in 10 ml of 4% paraformaldehyde-0.1% picric acid. After fixation, 10 hearts were selected at random and immersed in PBS for histological examination. In each case, hearts whose hearts were examined histologically had plasma cholesterol levels >200 mg/dl. Segments (~2–3 mm) of coronary arteries were dissected under a dissecting microscope. Representative segments were taken from the left and right coronary arteries, the proximal regions of both the anterior (left) and posterior (right) descending arteries (particularly at bifurcation points), and occasionally more distal parts of the descending branches. In cases in which it remained intact, the initial 2- to 3-mm segment of the aortic arch was also dissected and examined separately. Clusters of ~10 sections were obtained at 20- to 50-μm intervals along each vessel. Dissected segments were washed several times in cold PBS for 1 h, then postfixed with osmium tetroxide to stain and preserve lipids. Segments were fixed in osmium for either 1 or 24 h. The extended fixation was employed to better visualize minor lipid inclusions within the arterial walls, because osmium not only fixes lipids but also stains them brown. Tissues were then dehydrated through a graded series of acetone washes and embedded in Spurr's low-viscosity embedding medium (Polycell). One-micron-thin sections were cut using an automated ultramicrotome, layered onto gelatin-coated microscope slides, and briefly stained with Toluidine blue or left unstained.

**RESULTS**

Before analysis of lipid levels in individual bats, the cholesterol, HDL cholesterol, and triglyceride assays were validated for bats, using pooled blood samples from *T. brasiliensis*. Serial dilutions of bat plasma gave approximately linear readings in the assays, suggesting the absence of nonspecific matrix effects. The addition of extra precipitant reagent to an equivalent volume of plasma did not cause a reduction in detectable HDL cholesterol, verifying that the precipitation and removal of lower-density forms was complete.

Plasma levels of cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides in lactating bats are shown in Fig. 1. Fasting total cholesterol was >200 mg/dl and in some animals approached 450 mg/dl postprandial. Most of the cholesterol existed in the HDL fraction. Fasting triglycerides were extremely low and were significantly increased after feeding. Body mass increased significantly (P < 0.001) from 11.5 ± 0.2 to 13.5 ± 0.3 g after feeding. Estimates from other studies indicate that average nightly intake of insects by *T. brasiliensis* may exceed 50% of its prefeeding (fasting) body mass, especially during peak (mid) lactation (15).

Fasting levels of cholesterol and triglycerides of nonlactating *T. brasiliensis* are shown in Table 1 for comparison. Circulating cholesterol was high and triglycerides were very low in pregnant bats (Table 1). Total fasting cholesterol levels were very low in each of three nonmigratory Old World fruit-eating species, with some individuals having nearly undetectable levels (Table 1).

Fasting (dusk/early evening) and postprandial (early morning) plasma levels of insulin and glucose are shown in Fig. 2. Plasma glucose ranged from 44 to 127 mg/dl and from 48 to 123 mg/dl in fasting and postprandial animals, respectively. Insulin levels were low and statistically indistinguishable at both times of day (Fig. 2).

![Fig. 1. Fasting and postprandial plasma levels of total cholesterol (total-chol), high-density lipoprotein cholesterol (HDL-chol), low-density lipoprotein cholesterol (LDL-chol), and triglycerides in *Tadarida brasiliensis*. For fasting values, n = 15–18 bats for each determination. For postprandial values, n = 6 for LDL and triglycerides, 16 for HDL, and 20 for total cholesterol. All values are in mg/dl. *Significantly different (at least, P < 0.05, unpaired Student's t-test) from fasted animals for that lipid determination.](image-url)