PROFILE OF DYSLIPIDEMIA IN PSAMMOMYS OBESUS, AN ANIMAL MODEL OF THE METABOLIC SYNDROME

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Objective. To investigate lipid profiles in *Psammomys obesus* and relationships between lipid profile and other components of the Metabolic Syndrome.

Methods. A total number of 49 adults with a wide range of body weight and glucose tolerance were studied in a cross-sectional analysis. Plasma cholesterol distribution profiles were measured by size exclusion lipid chromatography. Blood glucose was measured using an enzymatic glucose analyser, and plasma insulin was determined by radioimmunossay.

Results. Obese diabetic *Psammomys obesus* had elevated plasma cholesterol (P=0.003) and triglyceride levels (p>0.001) compared to their lean littermates. The hypercholesterolemia was mainly due to increased circulating levels of VLDL-cholesterol (P=0.003) and LDL-cholesterol (P=0.003) in these animals. Multiple linear regression analyses revealed that body weight was independently associated with plasma cholesterol (P=0.011) and LDL concentration (P=0.009), while plasma insulin was associated with VLDL-cholesterol concentration (P=0.005). All of the variables measured exhibited continuous distributions across a wide range of phenotypes, from a normal rodent lipid profile to profound dyslipidemia.

Conclusions. These data suggest that the dyslipidemia in obese, diabetic *Psammomys obesus* is closely associated with other components of the Metabolic Syndrome, including obesity and insulin resistance.

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The Metabolic Syndrome, previously called Syndrome X, comprises a range of cardiovascular risk factors which tend to occur simultaneously and significantly increase overall mortality (REAVEN 1988; STERN 1995; ZIMMET 1989). Components of the Metabolic Syndrome tend to cluster, suggesting a common, central causality (ZIMMET et al. 1999). For example, in the San Antonio Heart Study, a combination of three or more of the components was significantly more prevalent than the occurrence of each of the risk factors in isolation, and even more prevalent than a combination of any pair of the factors. Similar data is also available from studies in Mauritius (ZIMMET et al. 1994).

Dyslipidemia, along with insulin resistance, obesity and Type 2 diabetes, comprises an important component of the Metabolic Syndrome. Dyslipidemia increases the risk of cardiovascular disease and overall mortality (CARLETON et al. 1991; HOKANSEN et al. 1996; LA ROSA et al. 1990; RIFKIND 1990), and lipid-lowering therapy can substantially improve clinical outcomes (FRICK et al. 1986; the Lipid Research Clinics Coronary Primary Preventation Trial: Results 1 1984; the Lipids Research Clinics Coronary Primary Preventation Trial: Results 11 1984; Report from the Committee of Principal Investigators 1978; The Scandinavian Simvastatin Survival Study 1994;

Shepherd et al. 1995).The development of new pharmacological treatments to improve lipid profile could further enhance our ability to effectively prevent cardiovascular disease in at risk individuals. This would be greatly facilitated by the identification and characterisation of a polygenic animal model exhibiting dyslipidemia as part of the Metabolic Syndrome in a manner similar to that seen in human populations.

In this study we describe a detailed characterisation of dyslipidemia in *Psammomys obesus* (Israeli Sand Rats). *Psammomys obesus* is a unique rodent model of obesity, insulin resistance and Type 2 diabetes, and the characterisation of dyslipidemia is required to define the full spectrum of the Metabolic Syndrome in these animals. The natural habitat of *Psammomys obesus* is the desert regions of the Middle East, where it subsists on a diet of saltbush and remains lean and normoglycemic (Shafrir et al. 1993).However, when housed in laboratory conditions and fed *ad libitum* chow, a diet on which many other rodent species remain healthy, a range of metabolic responses have been observed (Barnett et al. 1994; Kalderon et al. 1996). By 16 weeks of age approximately one third of the animals have normal glucose tolerance, one third are hyperinsulinemic and normoglycemic, and one third develop diabetes (Barnett et al. 1994). Retrospective analysis showed no difference in body weight, blood glucose or plasma insulin at 4 weeks of age between animals that would progress to the different adult phenotypes. At 16 weeks of age, the relationship of blood glucose and plasma insulin concentrations forms a continuous curve in *Psammomys obesus* similar to "Starling's curve of the pancreas" in human populations (DeFronzo 1988; Zimmet et al. 1979). This heterogeneous response indicates that *Psammomys obesus* represents a polygenic animal model of human diabetes. The body weight distribution in *Psammomys obesus* approximates a normal distribution and closely resembles that observed in human populations (Walder et al. 2000). The obesity described in these animals is familial in nature and was significantly associated with Type 2 diabetes (Walder et al. 2000). Therefore, *Psammomys obesus* is a unique animal model of obesity and Type 2 diabetes that exhibits a phenotypic pattern closely resembling that observed in human population studies. However, to establish *Psammomys obesus* as a polygenic animal model of the Metabolic Syndrome, it is necessary to investigate lipid profiles and how these are related to other components of the Metabolic Syndrome in these animals.

Materials and Methods

Experimental animals. A colony of Psammomys obesus is maintained at Deakin University, Geelong, Australia. Breeding pairs were fed ad libitum a diet of lucerne and chow. Experimental animals were weaned at 4 weeks of age and given a diet of standard laboratory chow, from which 12% of energy was derived from fat, 63 % from carbohydrate and 25 % from protein (Barastoc, Pakenham, Australia). Animals were housed in a temperature-controlled room (22 ± 1 °C) with a 12-12 h light-dark cycle (light 06:00-18:00 h).

The animals were classified according to blood glucose and plasma insulin concentrations in the fed state, at 16 weeks of age, as follows:

Group A – blood glucose <8.0 mmol/l, plasma insulin <150 mU/l

Group B – blood glucose <8.0 mmol/l, plasma insulin >150 mU/l

Group C – blood glucose > 8.0 mmol/l, plasma insulin >150 mU/l.

These groupings correspond to normal glucose tolerant (Group A), insulin resistant (Group B) and Type 2 diabetic (Group C). Group C animals were also obese relative to Group A, while Group B could be considered overweight (Table 1) (Walder et al. 2000). There was no difference in the gender distribution between the 3 groups of animals in this study (c^2=2.6, P=0.28).

Biochemical assays. Whole blood was collected from the tail vein of the animals at 16 weeks of age in the fed state, and glucose concentration was measured immediately using an enzymatic glucose analyzer (Model 27, Yellow Springs Instruments, Ohio). Plasma insulin concentrations were determined using a double-antibody solid phase radioimmunoassay (Phadeseph, Kabi Pharmacia Diagnostics, Sweden).

Plasma cholesterol distribution profiles were measured in 10 ml plasma samples using a size exclusion high performance liquid chromatography system (SMART) with a Superose 6 PC 3.2/30 column

TABLE 1: Metabolic characteristics of *Psammomys obesus* (mean ± SE [range]).

	n	Body weight (g)	Glucose (mmol/L)	Insulin (mU/L)
Group A	25	160 ± 5 [128-212]	4.4 ± 0.2 [3.1-6.6]	56 ± 8 [15-140]
Group B	15	195 ± 5 [160-243]*	4.3 ± 0.2 [2.4-6.8]	317 ± 46 [152-879]*
Group C	9	212 ± 9 [175-259]*	12.8 ±1.1 [8.4-16.6]*	637 ± 58 [282-877]*
Total	49	181 ± 5	5.9 ± 0.5	242 ± 36

*p<0.05 compared with Group A animals.

(Amersham Pharmacia Biotech, Uppsala, Sweden) as previously described (Sartipy et al. 1999). The various peaks in the profiles are designated "VLDL", "LDL" and "HDL" for simplicity, by analogy with the nomenclature used for the human profile.

Plasma triglyceride concentration was measured using reagents from a commercial enzymatic colorimetric kit (Boehringer Mannheim, GmbH, Germany).

Statistical analysis. All data are expressed as mean ± sem. Distribution of data was tested using Kolmogorov-Smirnov test for normality, and data was normalized by transformation if required for further analysis. Group means were tested by one-way ANOVA with a *post hoc* LSD test, and relationships between continuous variables were tested using multiple linear regression analysis. All tests were performed using SPSS version 9.0 (SPSS Inc., Chicago, IL). Results were considered significant at P<0.05.

Results

The characteristics of the animals included in the study are detailed in Table 1. Group C *Psammomys obesus* were hyperglycemic, hyperinsulinemic and obese compared with Group A (control) animals. Group B animals were hyperinsulinemic and mildly obese compared with Group A animals. The distribution of blood glucose and plasma insulin concentrations for all animals included in this study is shown in Figure 1. As shown previously in this animal model (Barnett et. al. 1994), the distribution forms a continuous inverted U-shaped curve similar to "Starling's curve of the pancreas" observed in cross-sectional data from human populations (DeFronzo 1988; Zimmet et al. 1979).

Plasma cholesterol (total) and triglyceride concentrations were significantly different between the three

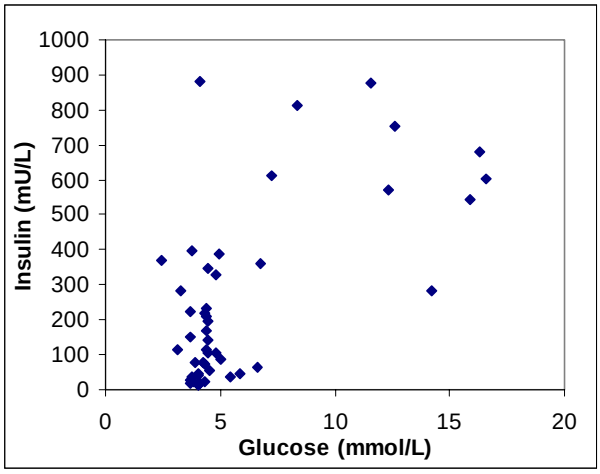

Fig. 1 Cross-sectional data displaying inverted U-shaped relationship between blood glucose and plasma insulin concentration in Psammomys obesus.

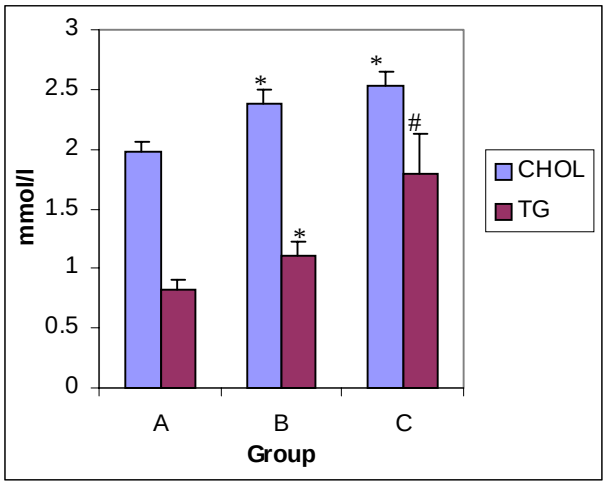

Fig. 2 Total plasma cholesterol and triglyceride concentration in groups of Psammomys obesus (mean ± s.e.m.). Group A – lean, normal glucose tolerance; Group B – overweight, impaired glucose tolerance; Group C – obese, diabetic. *p<0.05 compared with Group A animals, # p<0.05 compared with Group A and Group B animals.

TABLE 2: Distribution of cholesterol subfractions in *Psammomys obesus.*

	VLDL (%)	LDL (%)	HDL (%)
Group A	5.5	19.8	74.7
Group B	10.6	20.8	68.6
Group C	11.2	22.4	66.4

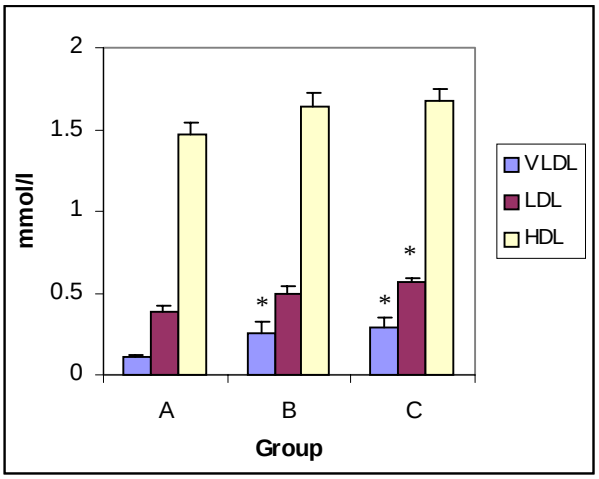

Fig. 3 Concentrations of cholesterol subfractions (VLDL, LDL and HDL) in Psammomys obesus from Groups A, B and C (mean ± s.e.m.). * p<0.05 compared with Group A animals.

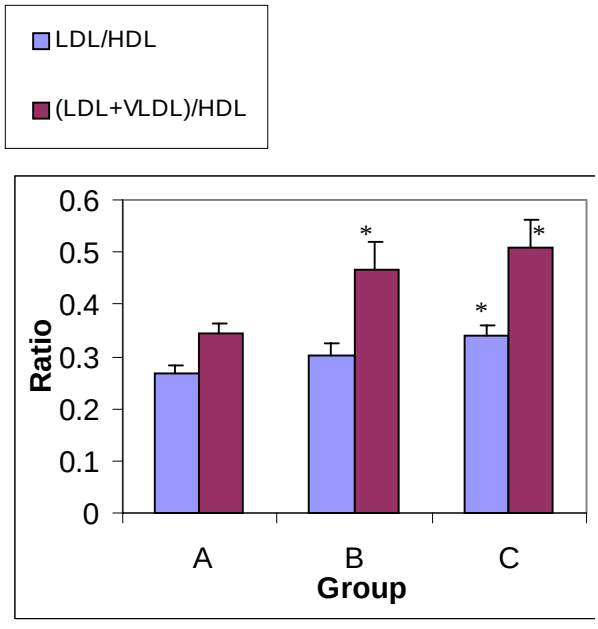

Fig. 4 Ratios of LDL:HDL and (LDL + VLDL):HDL in Psammomys obesus from Groups A, B and C (mean ± s.e.m.). * p<0.05 compared with Group A animals.

groups (P=0.003 and P<0.001 respectively, by ANOVA; Figure 2). Group C *Psammomys obesus* had elevated plasma cholesterol (2.53 ± 0.12 v. 1.97 ± 0.09 mmol/l, P=0.003) and triglycerides (1.79 ± 0.34 v. 0.83 ± 0.08 mmol/l, P<0.001) compared to Group A animals (Figure 2), and were hypertriglyceridemic compared to Group B animals (1.79 ± 0.34 v. 1.11 ± 0.12 mmol/l, P=0.021). Group B animals were also hypercholesterolemic (2.38 ± 0.12 v. 1.97 ± 0.09 mmol/l, P=0.008) and hypertriglyceridemic (1.11 ± 0.12 v. 0.83 ± 0.08 mmol/l, P=0.026) compared to Group A animals (Figure 2).

Cholesterol distribution profiles were also different between the groups for "VLDL" (P=0.006) and "LDL" (P=0.009) but not "HDL" concentration (Figure 3). Plasma "VLDL" was increased in Group C (0.28 ± 0.07 v. 0.11 ± 0.01 mmol/l, P=0.003) and Group B (0.25 ± 0.07 v. 0.11 ± 0.01 mmol/l, P=0.027) compared with Group A animals. Plasma "LDL" concentration was significantly elevated in Group C (0.57 ± 0.03 v. 0.39 ± 0.03 mmol/l, P=0.003) but not Group B (P=0.055) *Psammomys obesus* (Figure 3). The distribution of cholesterol subfractions, expressed as a percentage of the total plasma cholesterol, for the 3 groups is shown in Table 2.

The ratio of "LDL":"HDL" was higher in Group C (0.34 ± 0.02 v. 0.27 ± 0.02, P=0.028) compared to Group A (Figure 4), while ("VLDL"+"LDL"):"HDL" ratios were significantly increased in both Group C (0.51 ± 0.05 v. 0.34 ± 0.02, P=0.006) and Group B (0.47 ± 0.05 v. 0.34 ± 0.02, P=0.013) *Psammomys obesus*.

Composite traces representing the cholesterol distribution profile for animals in each of the three groups are shown in Figure 5. As stated previously, the various peaks in the profiles are designated "VLDL", "LDL", and "HDL" for simplicity, even though it is clear that the separation is determined primarily by the size of the lipoproteins.

Multiple linear regression analyses were conducted with covariates including body weight, plasma insulin, blood glucose and gender. The only independent factor associated with total plasma cholesterol, "LDL" and "HDL" concentrations was body weight (P=0.011, 0.009 and 0.034, respectively). Plasma insulin was associated with "VLDL" concentration (P=0.005) independent of body weight,

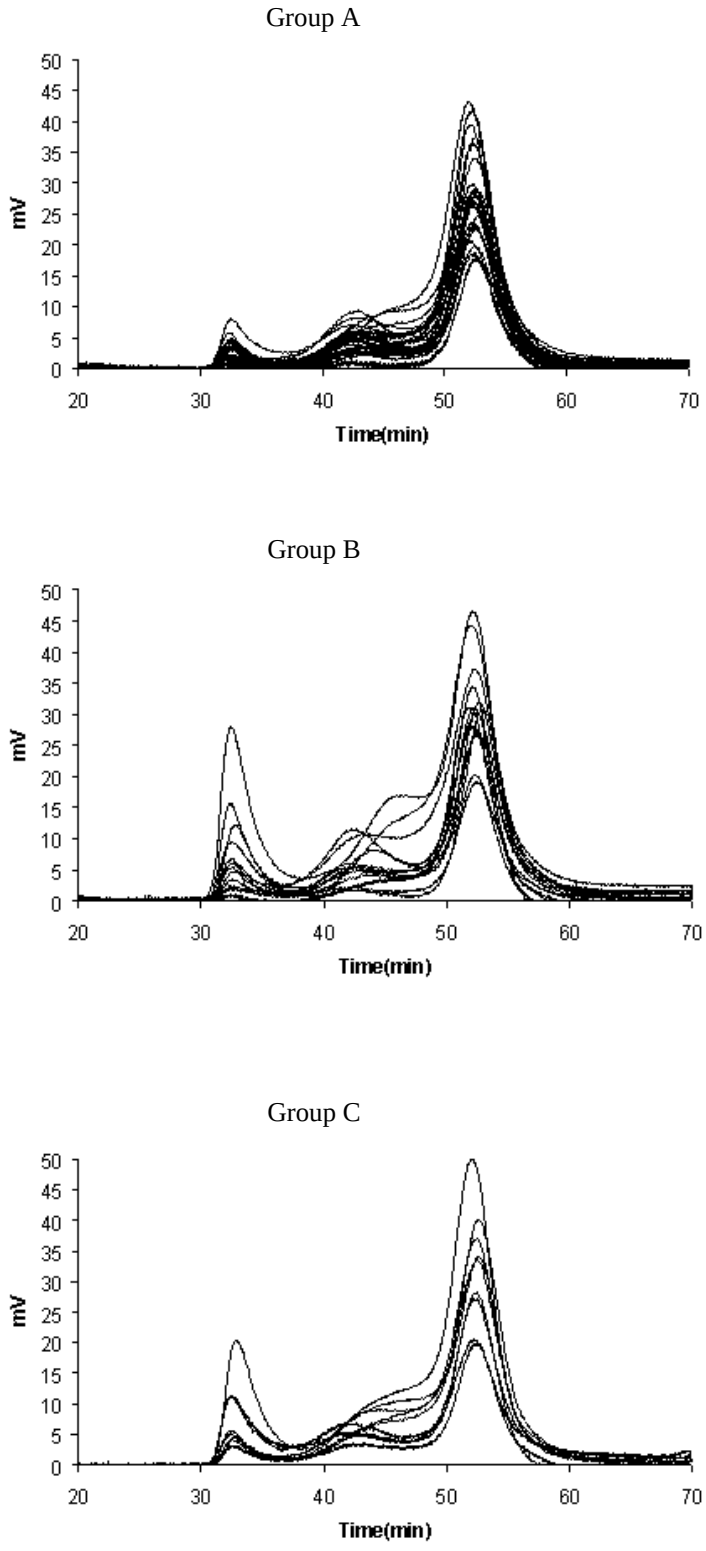

Fig. 5 Size exclusion HPLC lipid profile results for each animal separated into Groups A, B and C. The 3 peaks seen in this output correspond to (from left to right) "VLDL", "LDL" and "HDL". The integrated area of the fractions was calculated and expressed in molar concentration for comparisons between groups.

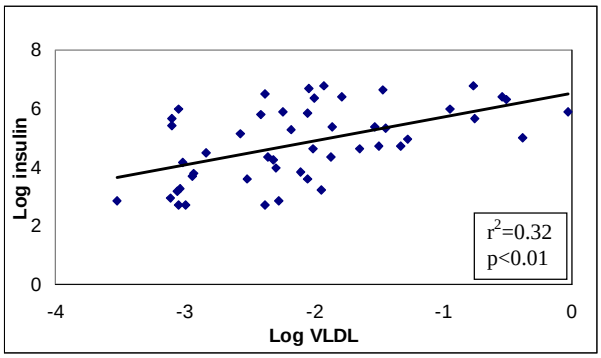

Fig. 6 Relationship between plasma insulin and VLDL cholesterol concentrations in Psammomys obesus.

blood glucose and gender. Figure 6 shows the relationship between these two variables, unadjusted for the covariates listed above. The bivariate correlation coefficient was calculated to be 0.54 ($P<0.001$). A partial correlation, controlling for the effects of gender, improved the correlation coefficient to 0.57 ($P<0.001$).

Discussion

Here we describe in detail the lipid profiles observed in *Psammomys obesus*. The previously characterised obesity and Type 2 diabetes found in a proportion of these animals was associated with hypercholesterolemia and hypertriglyceridemia. Plasma VLDL and LDL cholesterol subfractions were elevated in obese, diabetic animals, with no change in plasma HDL concentration, resulting in increased LDL:HDL and (LDL+VLDL):HDL ratios. Therefore, a proportion of *Psammomys obesus* exhibit a cluster of 4 components of the Metabolic Syndrome (obesity, insulin resistance, Type 2 diabetes and dyslipidemia) while other animals show a range of lesser responses including some that are free of any Metabolic Syndrome components. This distribution and clustering of components is analogous to the pattern observed in human populations (Reaven 1988; Stern 1995; Zimmet 1989; Zimmet et al. 1999; Ferranini et al. 1991; Zimmet et al. 1994), suggesting that *Psammomys obesus* is an excellent polygenic model of the Metabolic Syndrome.

"HDL" cholesterol concentration was not decreased in obese, diabetic *Psammomys obesus*, although this is recognised as a component of the dyslipidemia observed in patients with the Metabolic Syndrome. However, the ratio of "LDL": "HDL" was significantly increased in these animals, and, as shown in Table 2, the percentage of total cholesterol made up by "HDL" was lower in obese, diabetic animals. Both of these factors are consistent with the deleterious cholesterol profile associated with the Metabolic Syndrome.

A number of rodent models of dyslipidemia have been described (for review see Bocan 1998). The most widely used are rabbits and hamsters, fed diets rich in cholesterol and/or fat. These animals typically have increased VLDL-cholesterol, and the degree of hypercholesterolemia can be manipulated by dietary alterations. However, dyslipidemia does not generally spontaneously arise in rabbits or hamsters, with the exception of the Watanabe Heritable Hyperlipidemia Rabbit, the homozygotes of which lack functional LDL-receptors (Rosenfeld et al. 1987). These animals, along with other single-gene defective models, such as JCR:LA (cp/cp) rats (Dolphin et al. 1987), exhibit hypercholesterolemia characterised by increased VLDL-cholesterol.

Recently, a number of gene-knockout or transgenic mice have been described that exhibit lipid profiles closely resembling those observed in dyslipidemic humans. Examples include apo $E^{-/-}$ and $apoE^{-/-}/LDLR^{-/-}$ mice, which have hypercholesterolemia with elevated LDL and VLDL (Ishibashi et al. 1994; Zhang et al. 1992). Other genetically modified mice show variations on this lipid profile, including LDLR knockouts, and transgenic mice overexpressing human apoB, apoA, Lp(a), or CETP (Calow et al. 1995; Lawn et al. 1992; Marotti 1983; Pirell-Huynh 1995).

In contrast to all of these animal models of dyslipidemia, *Psammomys obesus* does not contain a known single-gene defect resulting in dyslipidemia, nor is a high-cholesterol/high-fat diet required to elicit the dyslipidemic phenotype. *Psammomys obesus* fed a normal rodent chow diet exhibit a wide spectrum of lipid profiles, ranging from normal for a rodent to hypercholesterolemic and hypertriglyceridemic with elevated LDL and VLDL-cholesterol. The heterogeneity observed suggests that *Psammomys obesus* is a polygenic model of spontaneous dyslipidemia in response to a standard rodent chow diet.

Although clear differences in cholesterol metabolism are known to exist between rodents and humans (Gibbons et al. 1982; Kane 1977), the finding of dyslipidemia in *Psammomys obesus* as part of the Metabolic Syndrome will be useful for further investigations in these animals. It should be noted that *Psammomys obesus* have the majority of their circulating cholesterol in HDL particles, which is similar to other rodents and different to humans (Gibbons et al. 1982; Kane 1977). It is likely that the "VLDL", "LDL" and "HDL" particles in *Psammomys obesus* may have different structural and metabolic properties to the corresponding particles in humans. Indeed, we were unable to detect significant plasma levels of CETP in *Psammomys obesus* (data not shown), suggesting that reverse cholesterol transport from HDL to LDL probably does not occur in these animals as it does in humans.

Despite this, we have demonstrated that obese, diabetic *Psammomys obesus* have altered lipid profiles relative to their lean littermates. The possible differences in cholesterol metabolism do not detract from the similarities between humans and *Psammomys obesus* with respect to the distribution patterns of obesity, insulin resistance and Type 2 diabetes.

In summary, this study has demonstrated that *Psammomys obesus* exhibits 4 components of the Metabolic Syndrome (obesity, insulin resistance, Type 2 diabetes and dyslipidemia) that tend to be clustered in affected animals. The unique nature of this animal model, which is not transgenic or fed a high-fat/high-cholesterol diet, but spontaneously develops a range of metabolic profiles in response to a normal rodent chow diet, makes it an ideal candidate for future studies investigating the pathogenesis, prevention and treatment of the Metabolic Syndrome.

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