PLASMA LIPIDS AND LIPOPROTEINS IN THE GRASS-CUTTER, *THRYONOMYS SWINDERIANUS*, IN CAPTIVITY[1]

Parra, H-J.*; Kadiatou Kamian* (1); Lucie Ayi-Fanou; Akpona, S.A.***; Ahissou, H.**; Yewadan. L.T.****; Mouray, H.***** y Fruchart, J-C.***

* Département de Recherche sur les Lipoprotéines et l'Athérosclérose, INSERM U325, Institut Pasteur de Lille, Lille, France.

** Faculté des Sciences et Techniques, BP 04-0320, Cotonou, Bénin.

*** Faculté des Sciences de la Santé, BP 188, Cotonou, Bénin.

**** Projet Bénino-Allemand d'Aulacodiculture, BP 504, Cotonou, Bénin.

***** Faculté de Médecine, Tours, France.

(1) Present address: B.P. 2042 - Bamako (Mali)

Recibido: 14 Junio 1995
Aceptado: 12 Junio 1996

Correspondence to: Dr HJ. Parra. Service d'Expertise Pharmacologique. Institut Pasteur de Lille. 1 rue du Pr Calmette, BP 245. 59019 Lille Cédex, France. Tél. +33 (0)3 20 87 73 06. Fax +33 (0)3 20 87 78 23

ABSTRACT

The grass-cutter, *Thryonomys swinderianus* (TEMMINCK 1827), an african hystricomorph rodent, represents a popular meat, much sought after in Africa south of the Sahara. Unfortunately, breeding of this animal is far from being acceptable in terms of efficiency, probably because of the insufficient information available on its biology and physiology.

Lipid and lipoprotein profiles were done on the plasma or serum of 49 male grass-cutters. We showed that:

1. The concentrations of lipids and lipoproteins were a quarter to half the values found in man;

2. The serum lipoproteins could be separated into 4 distinct fractions by discontinuous polyacrylamide gel electrophoresis;

3. The grass-cutter could be classified as «HDL mammal»;

4. Human and grass-cutter apolipoproteins A-IV, B, C-III, and E carry partial immunological cross-reactivity.

1 In memory of Professor Ibrahim KORA (1949-1995).

Further characterization of the grass-cutter lipoproteins is needed as is the investigation of the role of diet composition on these profiles.

Key words: *Thryonomys swinderianus*, grass-cutter, plasma, lipids, lipoproteins, apolipoproteins, electrophoresis.

RESUMEN

La rata de cañaveral, *Thryonomys swinderianus* (TEMMINCK 1827), roedor histricomorfo africano representa una popular fuente de alimentación, muy apreciada en África al sur del Sahara. Desgraciadamente, la eficacia de la crianza de este animal está lejos de ser aceptada, probablemente debido a que no se tiene suficiente información sobre su biología y su fisiología.

En este estudio, se ha realizado un perfil lipídico y lipoproteico plasmático sobre 49 ratas de cañaveral machos. Los resultados muestran que:

1. Las concentraciones en lípidos y lipoproteínas de la rata de cañaveral se sitúan entre 1/4 a 1/2 de los valores encontrados en el hombre.
2. Las lipoproteínas del animal pueden separarse en 4 fracciones distintas por electroforesis sobre gel de poliacrilamida en gradiente discontinuo.
3. La rata de cañaveral puede ser considerado como un «mamífero de HDL».
4. Las apolipoproteínas A-IV, B, C-III y E del hombre y de la rata de cañaveral presentan una identidad antigénica común.

Un análisis más detallado de las lipoproteínas de este animal deberá ser realizado, así como la investigación de la influencia que tiene el régimen alimentario en estos perfiles.

Palabras claves: *Thryonomys swinderianus*, rata de cañaveral, plasma, lípidos, lipoproteínas, apolipoproteínas, electroforesis.

INTRODUCTION

Certain mammalian species are very important, not only as experimental models for lipid and lipoprotein metabolism in diseases afflicting man, but also as direct contributors to human diet and nutrition (ARMSTRONG and HEISTAD 1990; AJAYI 1971; PETERS 1987).

The grass-cutter, *Thryonomys swinderianus* (TEMMINCK 1827) (Fig. 1), which is localized in Western Africa is one of two genera of Aulacoda in Africa south of the Sahara; it is an Old World hystricomorph rodent commonly encountered in the herbaceous and tree-studded savannas, and in marshy areas along river banks (ROSEVEAR 1969). The other Aulacoda genus is *Thryonomys gregorianus* (THOMAS 1894), which inhabits Central and Eastern Africa (MORKRAMER 1988).

Because of its large size (3-7 kgs.), the grass-cutter is valued for food and has become a popular meat, much sought after, in Benin and generally in Western Africa (AJAYI and TEWE 1980; MORKRAMER 1988). As a consequence of the increasing demand, poaching and hunting have contributed greatly to the dramatic disappearance of the grass-cutter species (MARTIN 1985; ANADU *et al.* 1988). So, for the conservation of a natural resource providing a significant amount of animal protein, attempts at breeding have been made in many countries of Western Africa (HEYMANS and MENSAH 1984; MENSAH 1985; BAPTIST and MENSAH 1986; ADENIJI 1986). Unfortunately and in spite of some positive results, breeding efficiency is far from being acceptable, not only because little is known about the grass-cutter's life in captivity, but also because insufficient information is available on the animal's biology and physiology.

In order to contribute to the knowledge of its biology, the aim of this paper is therefore to

FIGURE 1. The grass-cutter, *Thryonomys swinderianus* (TEMMINCK 1827), in captivity.

present basic data on plasma lipids and lipoproteins in the grass-cutter, *Thryonomys swinderianus*.

MATERIALS AND METHODS

Animals and sampling blood

Forty nine randomly selected male grass-cutters were studied; they were bred on the Projet Bénino-Allemand d'Aulacodiculture's animal farm in Cotonou (Benin) and were at least 1 year old, with body weights ranging between 1 and 3 kgs. They were kept individually in 1 m^3 parks under natural conditions of light and temperature, and were fed a standardized fodder diet that contained fresh-cut grasses (*Pannicum maximum* and *Pennisetum purpureum*) eaten by these animals in the wild (EWER 1969; AJAYI and TEWE 1980), supplemented with a stock concentrate feed developed at the farm (43.4% wheat starch, 26.0% brewery draff, 21.0% maize grain, 5.0% sugar-cane molasses, 3.0% dry leaves of *Leucaena leucocephala*, 1.0% oyster shell, 0.5% cooking salt and 0.1% vitamin mix). For chemical compositions see Table I (YEWADAN 1992). Water was provided *ad libitum*.

To obtain blood samples, the animals were fasted overnight and then restrained for about 20 mn by anaesthesia with a solution of equal volumes of Imalgene 500 (Rhône Mérieux) and Rompun (Bayer) (100 µl/kg). Subsequently, blood was collected by intracardiac puncture into dry or citrate-containing tubes. Serum or plasma was then separated by low-speed centrifugation and, after addition of preservatives (final concentrations: EDTA, 0.27 mmol/l; ε-amino-*n*-caproic acid, 0.9 mmol/l; chloramphenicol, 0.6 mmol/l; and glutathione,

TABLE I
Chemical composition of the standard grass-cutter diet (% of dry matter)

Nutrient	Stock concentrate feed (granulated feed)	Fodder
Carbohydrates	59.2	43.2
Proteins	19.0	10.6
Fats	6.2	2.3
Crude cellulose	10.8	34.3
Minerals	5.2	7.2
Calcium	0.7	0.4
Phosphate	0.9	0.3

0.3 mmol/l), immediately mailed to Lille, at +4°C, for lipid and lipoprotein analyses within 48 h. Human serum was collected by venipuncture from 10-12 h fasting ostensibly healthy normolipidemic men recruited at the Center for Preventive Medicine at the Institut Pasteur in Lille. Rat serum was obtained from fasted (14 h) male Wistar rats (Iffa-Credo, L'Arbresle, France) by retro-orbital venous plexus puncture.

Analytical methods

Plasma total cholesterol, free cholesterol and free fatty acids were determined by enzymatic colorimetric methods (Boehringer-Mannheim, Germany). Cholesterol in high density-lipoproteins (HDL-cholesterol) was measured in the supernatant after precipitation of VLDL, IDL and LDL (very low-, intermediate- and low-density lipoproteins) with phosphotungstate/$MgCl_2$ (Boehringer-Mannheim). Total triglycerides and phospholipids were determined by enzymatic kits from Biotrol (Paris, France) and from BioMérieux (Marcy l'Etoile, France), respectively.

Electrophoresis of whole serum or lipoprotein fractions was performed on ready-to-use polyacrylamide gel slabs marketed by Sébia (Issy-les-Moulineaux, France), and constructed to give a discontinuous gradient from 2% (at point of sample loading) to 3% (running gel).

Monospecific antibodies against human apolipoproteins (apo) A-I, A-II, A-IV, B, C-III, and E were raised in rabbits; the antigens injected were purified by preparative ultracentrifugation and anion-exchange chromatography (MEZDOUR *et al.* 1987). Antibodies against lipoprotein (a) were from Immuno Diagnostika (Vienna, Austria). Immunological cross-reactivity of grass-cutter apolipoproteins with these anti-human apolipoprotein antibodies was checked by counter-immunoelectrophoresis (Immuno Diagnostika).

Data are expressed as mean ± SD. The Mann-Whitney U-test was used to test differences for significance.

RESULTS

The data for the plasma lipid and lipoprotein parameters in grass-cutters, compared to man and rat, are shown in Table II. There was a wide individual variation within animals for most parameters, giving large standard deviations. Free and total plasma cholesterol, triglycerides, phospholipids and free fatty acids in grass-cutters were approximately a quarter to a half the values present in man, while HDL-cholesterol was about half the concentrations found in man;

TABLE II

Concentrations of plasma lipid and lipoprotein in grass-cutters compared to man and rat (values are expressed as mean ± SD)

Parameters (g/l)	Grass-cutter n=49	Man n=30	Rat n=10
Total cholesterol	0.542±0.200	1.751±0.219[b]	0.655±0.104[a]
Free cholesterol	0.140±0.055	0.445±0.075[b]	0.113±0.028
(% of total)	(26.1)	(25.4)	(17.3)
HDL-cholesterol	0.291±0.149	0.516±0.176[b]	0.491±0.113[b]
(% of total)	(52.7)	(29.8)	(74.4)
Triglycerides	0.347±0.158	0.845±0.340[b]	0.829±0.394[b]
Total phospholipids	0.643±0.227	1.964±0.256[b]	1.230±0.487[b]
Free fatty acids*	0.187±0.151	0.648±0.345[b]	0.701±0.189[b]

* mmol/l; [a,b] Significantly different from values for grass-cutters: [a] p=0.025; [b] p=0.0001.

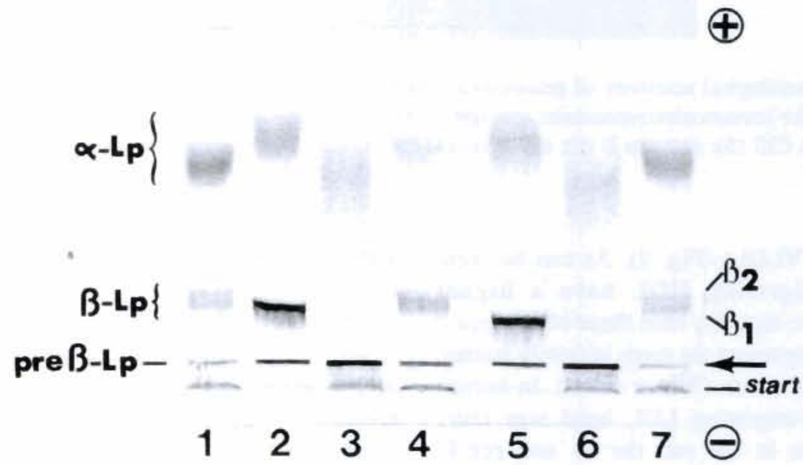

FIGURE 2. Comparison of the polyacrylamide gel electrophoresis patterns of grass-cutter (1,4,7), human (2,5) and rat (3,6) whole serum. Samples were prestained with Sudan black. The arrow indicates the junction of the 2 and 3% gels. Lp: Lipoprotein.

however, the proportions of free to total cholesterol in grass-cutters and man were similar (21.6 vs 25.4%). On the other hand, the concentrations of free and total cholesterol in grass-cutter and rat were of the same magnitude, while triglycerides and phospholipids were lower and the proportion of free cholesterol higher in the former. In grass-cutter, as in rat, but unlike man, the main amount of cholesterol was in the HDL fraction (on average 52.7% of total cholesterol).

After electrophoresis of grass-cutter serum, at least 4 bands were visible: one with α-mobility (HDL), two with ß-mobility (LDL_1 and LDL_2) and one blocked right at the start of the running gel corresponding to human and rat pre-ß

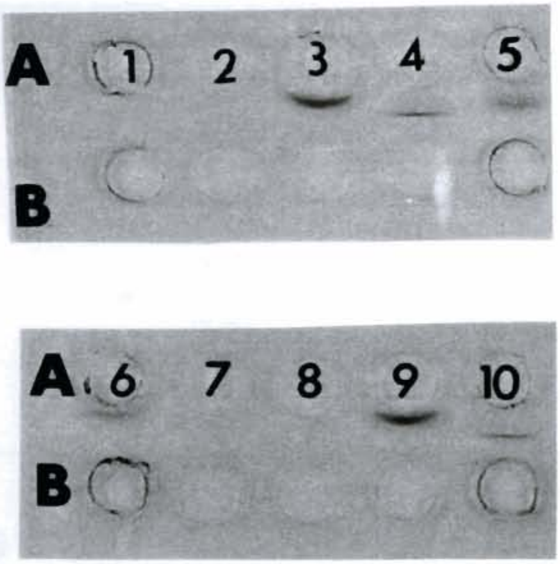

FIGURE 3. Immunological reactivity of grass-cutter whole serum (B) with antisera to human apolipoproteins (A) upon counter-immunoelectrophoresis: anti apo AI (1,8); anti apo AII (2); anti apo AIV (3,9); anti apo B (4,10); anti apo CIII (5); anti apo E (6); anti apo (a) (7). The slides were stained for protein with Ponceau S.

lipoproteins (VLDL) (Fig. 2). As can be seen, human α-migrating HDL have a higher electrophoretic mobility than those of the grass-cutter which represent the more intensely stained band, as in rat serum (Wistar strain). In human serum, the ß-migrating LDL band was more intense, while in the rat, the α- and pre-ß-migrating bands were the dominant lipoprotein fractions. Unlike the rat, α-migrating LDL bands ($ß_1$ and $ß_2$) in the grass-cutter's serum were clearly visible on the electrophoresis gel (Fig. 2).

Immunological cross-reactivity of grass-cutter apolipoproteins with various anti-human apolipoprotein antibodies was represented in Fig. 3. Grass-cutter's plasma reacted very positively with anti-human apo A-IV and B, and less strongly with anti-human apo C-III and E. No positive immunological reactivity was observed with anti-human apo A-I, A-II and lipoprotein (a).

DISCUSSION

Plasma lipids and lipoproteins in rodents —such as mouse, rat, rabbit, guinea pig— are amply described in the literature, including their isolation, origin and function (CHAPMAN 1986). However, and to our knowledge, no information is available concerning the lipid and lipoprotein profile of the grass-cutter *Thryonomys swinderianus* (TEMMINCK 1827). So, this is the first study providing basic information on the plasma lipid and lipoprotein parameters in this african rodent which represents a very high nutritional feature in many countries of Africa, south of the Sahara.

Our results showed that, as in the rat, lipid and lipoprotein concentrations in the grass-cutter were approximately a quarter to half the concentrations found in man. Furthermore, the grass-cutter appears grossly similar to other rodents since it carries a very large portion (52.7%) of its cholesterol in its HDL fraction; it could be classified as a «HDL mammal».

This last aspect was in accordance with the electrophoretic profile in that the α-migrating fraction (HDL) was the major component of the grass-cutter's serum. The slower migration of this fraction, when compared with human serum, might indicate that the animal's HDL were larger than their human counterparts, which generally corresponds to a richer triglyceride content. As can be observed in Fig. 2, the grass-cutter had two slow-moving LDL bands ($ß_1$ and $ß_2$ lipoproteins) which were of much lower relative concentration than the relative LDL concentration of humans, but of a much higher relative concentration than those of the rat. LDL represent the lipoprotein class which is of great interest in atherosclerosis studies, and the rat, a widely used animal model for studying lipoprotein metabolism, is almost totally lacking in this component.

The major purpose of the present study was to contribute to the knowledge of grass-cutter's biology. However, it should be kept in mind that animal models continue to have important roles in human disease research. Unfortunately, no single species fulfills the scientific and practical requirements for being a good model, and it is often necessary to use several animal models to investigate all aspects of a given disease. With regard to lipoprotein metabolism and atherosclerosis research, the grass-cutter might have advantages over the rat because its basal lipoprotein profile showed 4 distinctly stained bands; furthermore, another advantage of the grass-cutter is its large body size allowing more frequent and important blood sampling.

It is well known that high levels of serum cholesterol are associated with increased development of atherosclerosis in most species and that a positive association exists between dietary saturated fat and atherosclerotic vascular disease. The role of diet composition in cholesterol metabolism has been studied extensively in animals. In rabbits, for example, it has been found that changes in the levels of serum cholesterol due to modulations of the protein sources in the diet, paralleled the degree of aortic lesions (HUFF *et al.* 1982). Thus, the effects of the nature and the proportion of protein in the diet on the concentration of serum cholesterol might be of great importance in the development of atherosclerosis.While the rat is very resistent to the development of atherosclerosis, except in case of consumption of a very high-chlolesterol diet together with the administration of thiouracil and cholic acid (HADJIISKY *et al.* 1991), this kind of information is not available for the grass-cutter. In this study, our animals were fed a standardized diet developed at the farm. Further studies are therefore necessary to investigate the role of diet composition on the lipid and lipoprotein profile of the grass-cutter.

Finally, in this report giving basic data on the plasma lipids and lipoproteins of the animal, we have shown that, as in other rodents, the levels were approximately a quarter to half the values found in man. We also showed by electrophoresis that the grass-cutter has four distinct bands of plasma lipoproteins. However, more sensitive procedures will be required to isolate and characterize these bands; this work is being conducted presently in our laboratory.

ACKNOWLEDGEMENTS

We thank Mrs Anne Montpellaz for expert secretarial assistance, Miss Nadège Béthencourt and Mr Séraphin L. M'Pili for verifying and improving the English of the manuscript, and Mr Oscar Perez Mendez for the spanish abstract. This investigation was supported in part by the «Projet Campus» of the «Ministère Français de la Coopération».

REFERENCES

ADENIJI, K.O. 1986: Livestock breeding in Africa. *World Review of Animal Production*, 21(1), 65-72.

AJAYI, S.S. 1971: Wild life as a source of protein in Nigeria: some priorities for development. *Nigerian Field*, 36(3), 115-127.

AJAYI, S.S. and TEWE, O.O. 1980: Food preference and carcass composition of grass-cutter (*Thryonomys swinderianus*) in captivity. *African Journal of Ecology*, 18, 133-140.

ANADU, P.A., ELAMAH, P.O. and OATES, J.F. 1988: The bushmeat trade in southwestern Nigeria: a case study. *Human Ecology*, 16(2), 199-208.

ARMSTRONG, M.L. and HEISTAD, D.D. 1990: Animal models of atherosclerosis. *Atherosclerosis*, 85, 15-23.

BAPTIST, R. and MENSAH, G.A. 1986: The cane rat - Farm animal of the future? *World Animal Review*, 60, 2-6.

CHAPMAN, M.J. 1986: Comparative analysis of mammalian plasma lipoproteins. *Methods in Enzymology*, 128, 70-143.

EWER, R.F. 1969: Form and function in the grass-cutter *Thryonomys swinderianus* Temminck (*Rodentia, Thryonomidae*). *Ghana Journal of Science*, 9(2), 131-141.

HADJIISKY, P.; BOURDILLON, M.C. and GROSGOGEAT, Y. 1991: Modèles expérimentaux d'athérosclérose. Apports, limites et perspectives. *Archives des Maladies du Coeur*, 84, 1593-1603.

HEYMANS, J.C. and MENSAH, G.A. 1984: Sur l'exploitation rationnelle de l'aulacode - rongeur Thryonomyidé - en R.P. Bénin - Données préliminaires. *Tropicultura*, 2(2), 56-59.

HUFF, M.W.; ROBERTS, D.C.K. and CARROLL, K.K. 1982: Long-term effects of semipurified diets containing casein or soy protein isolate on atherosclerosis and plasma lipoproteins in rabbits. *Atherosclerosis*, 41, 327-334.

MARTIN, G.H.G. 1985: Carcass composition and palatability of some wild animals commonly used as food. *World Animal Review*, 53, 40-44.

MENSAH, G.A. 1985: Rapport final des études préliminaires sur l'élevage de l'aulacode (*Thryonomys swinderianus*) au Bénin. *Notes techniques sur l'élevage N°.0.2.3* Cotonou, M.D.R.A.C.

MEZDOUR, H.; CLAVEY, V.; KORA, I.; KOFFIGAN, M.; BARKIA, A. and FRUCHART, J.C. 1987: Anion-exchange fast protein liquid chromatographic characterization and purification of apolipoproteins AI, AII, CI, CII, CIII0, CIII1, CIII2 and E from human plasma. *Journal of Chromatography*, 414, 35-45.

MORKRAMER, G. 1988: L'aulacode comme source de ravitaillement de l'Afrique en viande. *Trypanotolérance et Production Animale*, 5: 41-48.

PETERS, K.J. 1987: Classification et utilisations possibles du bétail non conventionnel. *Bulletin du CIPEA*, 27, 37-43.

ROSEVEAR, D.R. 1969: *The rodents of West Africa*. London: Trustees of the British Museum (Natural History) 541-588.

TEMMINCK, C.I. 1827: *Aulacodus swinderianus* Temminck 1827. *Monographies de mammalogie I*, Sierra Leone, 248.

THOMAS, O. 1894: Description of a new species of reed-rat (*aulacodus*) from East Africa, with remarks on the milk-dentition of the genus. *Annals and Magazine of Natural History* XIII, Ser 6, 202-204.

YEWADAN, L.T. 1992: *1st Conference on grass-cutter production*. Achievements and prospects 17-19 February, Cotonou (Republic of Benin).