Z. Säugetierkunde 59 (1994) 224–229

ISSN 0044-3468

Lipid deposits in pregnant and non-pregnant bats (*Pipistrellus pipistrellus*)

By Pilar López-Luna, F. Arevalo, M. J. Burgos, and N. del Hoyo

Departamentos de Fisiología y Farmacología, Biología Animal y Bioquímica y Biología Molecular, Universidad de Alcalá, Alcalá de Henares, Madrid, Spain

Receipt of Ms. 15. 12. 1993
Acceptance of Ms. 1. 3. 1994

Abstract

Studied the white and brown adipose tissues of female bats (*Pipistrellus pipistrellus*) in order to ascertain differences in relative lipid, fatty acid and phospholipid contents that might be related to pregnancy. Relative lipids were higher in the white adipose tissue of pregnant bats than in that of non-pregnant bats, and the same was true for phospholipids in the brown adipose tissue. Pregnancy in *P. pipistrellus* could be responsible for the changes in the length and unsaturation of some fatty acids in both kinds of adipose tissue.

Introduction

Reproduction is accompanied by an increase in energy demands (Towsend and Callow 1981). The total energy investment of pregnancy involves many components such as production of fetal, uterine, placental, and mammary tissue, as well as the production and increased maintenance costs associated with the new tissues. It has been suggested that suppressing metabolic energy expenditure increases energetic efficiency during pregnancy (Schneider and Wade 1987). Although in many mammals the energy thus conserved is first stored as white adipose tissue and later mobilized to meet the energy demands of lactation (Naismith et al. 1982; Sadleir 1984), this reserve is apparently not used by small insectivorous bats (Racey and Speakman 1987). However, fat storage may be important in bats because of their unavoidably high activity levels and obvious flight costs (Gittleman and Thompson 1988).

Maternal body fat accumulation is one of the most striking features of gestation in both women (Hytten et al. 1966; Hytten and Leitch 1971) and experimental animals (Beaton et al. 1954; Lopez-Luna et al. 1986; Herrera et al. 1988). Fat storage may also be important in pregnant bats since, when fat is unavailable, fetal growth rates and pregnancy rates often decline (Racey 1973; Kurta 1986).

The main purpose of this study is to learn whether pregnant females of *Pipistrellus pipistrellus* show differences in body weight, total lipids, fatty acids and phospholipids in the white and brown adipose tissues compared with non-pregnant female individuals.

Material and methods

Animals

The active bats used in this study were pregnant and non-pregnant *Pipistrellus pipistrellus* females captured alive in eastern Madrid, between June 28 and July 31 and between March and September, respectively. The pregnant bats were gathered from their maternity roosts (an inhabited building) between 19:00 and 22:00 h.

Bats were placed in a wet sack for transportation to the laboratory and weighed and killed by exsanguination under diethyl ether anaesthesia 2–3 hours after collection. The abdomen was immediately opened and conceptus (fetus and placenta) were delivered by hysterectomy and weighed.

U.S. Copyright Clearance Center Code Statement: 0044-3468/94/5904-0224 $ 02.50/0

The subcutaneous white and interscapular brown adipose tissues were quickly removed, stripped of connective tissue, excised, weighed and immediately frozen in liquid nitrogen. They were then kept frozen at –30 °C until lipid analysis. Standard fatty acids were purchased from Carlo Erba (Milan, Italy) and Alltech Associates (Deerfield, IL, USA).

Lipid analysis

Lipids were extracted from frozen tissues and purified with chloroform:methanol (2:1), according to the method of Folch et al. (1957). After evaporation of the solvent, the lipids were stored in a N_2 atmosphere at –30 °C.

The method of Rouser et al. (1966) was used for quantitative determinations of total phospholipid from both tissues. Purified total lipids from both tissues were directly treated with BF_3/CH_3OH to obtain the fatty acid methyl esters as previously described by Pulido et al. (1986). A Pye Unicamp Philips gas chromatograph equipped with a flame ionization detector was used to assay the products in a 5 m × 2 mm glass column packed with 20 % Silar 10 C on 80/100 Chromosorb WHP (Alltech Associates); the flow rate of the nitrogen carrier gas was 32 ml/min.

Statistical treatment of the results

Absolute and relative values are expressed as mean ± SE. The statistical significance of differences between groups was determined by Student's t-test and differences were considered significant when $p < 0.05$.

Consent for capturing the animals was given by the Consejeria de Medio Ambiente (Comunidad de Madrid) in accordance with the Bern Agreement (1979) ratified by the Spanish Government in 1986.

Results

As shown in table 1 the conceptus-free body weight of pregnant bats was significantly higher ($p < 0.05$) than the body weight of non-pregnant bats. The pregnant bats studied had each one fetus which weighed between 0.76 and 1.12 g. However, the subcutaneous

Table 1. **Absolute body weights and relative tissue weights, relative lipid content and relative phospholipids in white and brown adipose tissues in non-pregnant and pregnant females of *P. pipistrellus***

The results are given as mean ± SE. Comparisons of pregnant vs non-pregnant

	Non-pregnant (n = 28)	pregnant (n = 15)
Body weight		
(g)	3.9 ± 0.04	4.2 ± 0.1*
White adipose tissue		
weight (mg wet tissue/g body wt)	32.9 ± 2.4	28.6 ± 5.4
lipids (g lipid/g wet tissue)	0.19 ± 0.02	0.32 ± 0.06**
Phospholipids (µmol/g wet tissue)	2.2 ± 0.2	2.5 ± 0.6
Brown adipose tissue		
weight (mg wet tissue/g body wt)	10.9 ± 0.6	12.0 ± 0.8
lipids (g lipid/g wet tissue)	0.26 ± 0.02	0.27 ± 0.02
Phospholipids (µmol/g wet tissue)	18.9 ± 1.9	27.5 ± 4.7***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

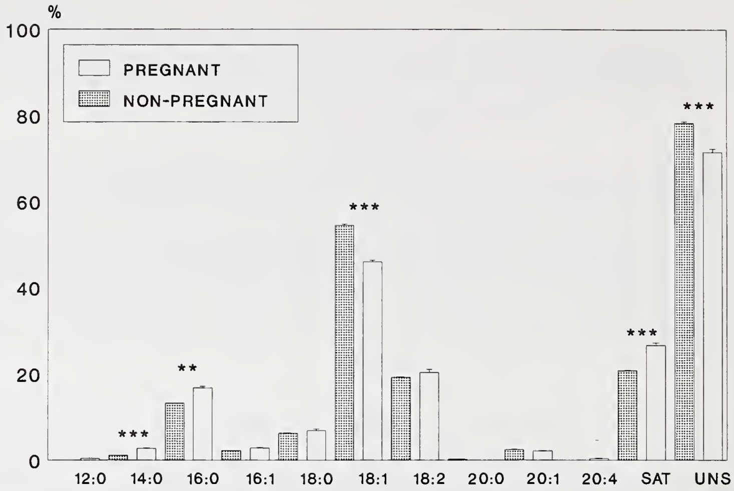

Fig. 1. Fatty acid composition of subcutaneous white adipose tissue in *P. pipistrellus*. All assays were performed in duplicate. The values given as % total fatty acids are means ± SE. Asterisks show statistical comparisons between pregnant and non-pregnant bats $^{**}p < 0.01$, $^{***}p < 0.001$

white adipose tissue (WAT) weight relative to body weight does not show significant differences between pregnant and non-pregnant animals. The relative lipid content (g. lipid/g wet tissue) was higher in pregnant than in non-pregnant bats ($p < 0.01$). Interscapular brown adipose tissue (BAT) weight and relative lipid content was not modified during pregnancy. Relative phospholipids from WAT were unmodified during pregnancy although BAT relative phospholipids increased significantly ($p < 0.001$) during pregnancy (Tab. 1).

Distribution of fatty acids in the total lipids from WAT tissue for non-pregnant and pregnant bats is shown in figure 1. The proportion of myristic acid (14:0) and palmitic acid (16:0) in the WAT of pregnant bats is higher ($p < 0.001$ and $p < 0.01$ respectively) than that observed in non-pregnant bats. However, lauryl acid (12:0) was only present in WAT from pregnant bats and arachidonic acid (20:4) only appeared in non-pregnant bats.

Pregnancy did not significantly affect the percentages of unsaturated fatty acid in WAT, with the exception of oleic acid (18:1), which decreased ($p < 0.001$) in pregnant bats.

The fatty acid in total lipids of BAT (Fig. 2) shows an increase in oleic acid (18:1) monounsaturated fatty acid and a decrease in linoleic acid (18:2) polyunsaturated fatty acid that were both statistically significant ($p < 0.001$). The fatty acid composition of the total lipids in WAT and BAT (Figs. 1, 2) shows few differences between pregnant and non-pregnant bats. Unsaturated fatty acids are the most abundant (71–78 %) in both kinds of tissues and in both pregnant and non-pregnant bats. However, the level of saturated fatty acids increased during pregnancy from 21 to 27 % ($p < 0.001$) in WAT, but not in BAT, where the level remained constant (21 %).

Discussion

Although copulation by temperate-zone insectivorous bats normally occurs in autumn, fertilization does not occur until early spring when the bats arouse from hibernation and enter maternity colonies (Racey 1982). The length of gestation in heterothermic bats may

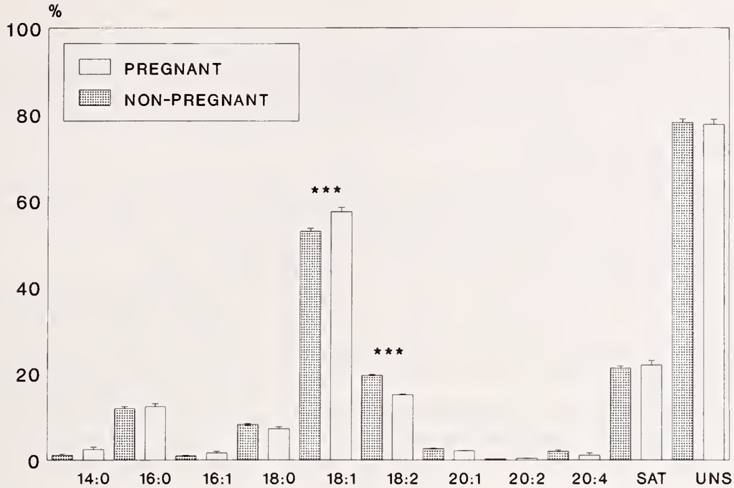

Fig. 2. Fatty acid composition of interscapular brown adipose tissue in *P. pipistrellus*. The same symbols as in figure 1 were used

vary with the external conditions of food supply and temperature. The pregnant *P. pipistrellus* bats studied here carried only one pup, and, according to date of capture (last week of June or first of July), size of the fetuses, and the results reported by RACEY and SWIFT (1981), gestation was probably far advanced.

The increase of body weight in these *P. pipistrellus* at late gestation is not due only to the presence of the conceptus since the excess weight persists after calculating the conceptus free maternal body weight. Such weight increase in gestating females has also been observed in other bat species (STEBBINGS 1976; STUDIER and O'FARRELL 1976; SPEAKMAN and RACEY 1987). The mass increase in *P. pipistrellus* is partially explained by the enlargement of the mammary glands.

In our study the weight of subcutaneous WAT in pregnant bats showed no significant difference compared with that in non-pregnant individuals. However, although increased fat during pregnancy has been detected in *M. lucifugus, P. auritus* (SPEAKMAN and RACEY 1987) and *M. grisescens* (KRULIN and SEALANDER 1972), it cannot be completely confirmed since STUDIER and O'FARRELL (1976) found no evidence of fat increase during pregnancy in *M. lucifugus* and *M. thysanodes*.

Our results for WAT weight and lipid content suggest that *P. pipistrellus* responds to the increased energy requirements of pregnancy by increasing the caloric content of the fat rather than by increasing the amount of fat stores, as PISTOLE (1989) has also suggested in pregnant *Eptesicus fuscus*.

The main alteration in the pattern of total lipid fatty acid composition in WAT of *P. pipistrellus* caused by pregnancy could indicate increased lipogenesis in this tissue during gestation as also occurs in pregnant rats (PALACIN et al. 1991).

Brown adipose tissue, traditionally associated with thermogenesis, plays an important role in maintaining the energy balance in small mammals (ROTHWELL and STOCK 1983; HIMMS-HAGEN 1983; TRAYHURN 1984). It has been previously shown that there is an increase of BAT during late pregnancy in the rat (AGIUS and WILLIAMSON 1980; LOPEZ LUNA et al. 1991). The present results show that BAT tissue weight and total lipids remain unmodified in *P. pipistrellus* during pregnancy.

Similarly, the fatty acid profile of BAT from pregnant bats was similar to non-pregnant ones; however the levels of the 18:2 and 20:4 fatty acids were decreased, which could be related to their importance in the prostaglandin production involved in the normal parturition process (Arahuetes et al. 1982).

Although BAT wheight, lipid content, and fatty acid do not differ between pregnant and non-pregnant bats, the phospholipid levels are higher in pregnant than in non-pregnant bats. The scarce bibliography does not help explaining the rise we found in BAT phospholipids from pregnant bats.

Cold and diet have also been reported to modify BAT phospholipid composition and/or content (Giradier 1983; Ide and Sugano 1988; Arévalo et al. 1990). Masoro (1968) suggests that muscle phospholipids may serve as an energy source, but we cannot affirm whether the phospholipids of brown fat are an energy source or not.

Several factors, like quantity and nature of food, temperature, or physiological state, are known to affect the fat composition of mammals (Pearce 1983). Present findings show that in *P. pipistrellus* gestation may produce changes in the length and/or unsaturation of some of the fatty acid chains as well as in their fat composition. In this sense Lockwood et al. (1970) have shown that sex hormones are involved in controlling the specific activities of lipogenic enzymes in both male and female rats. In fact, and as Pistole (1989) suggests, bats alter their fat composition in response to a particular energy need such as hibernation, pregnancy or lactation.

Acknowledgements

We wish to thank Dr. Pérez-Suarez for his field assistance and Carol F. Warren of the translation service at the Instituto de Ciencias de la Educación (ICE) of the University of Alcalá de Henares. This investigation was supported in part by the Comisión Asesora de Investigación Científica y Técnica, Project: PB85--0242, Spain.

Zusammenfassung

Fettspeicher in trächtigen und nicht trächtigen Fledermäusen (Pipistrellus pipistrellus)

Weißes und braunes Fettgewebe von graviden und nicht graviden Fledermaus-Weibchen (*Pipistrellus pipistrellus*) wurde analysiert, um trächtigkeitsbedingte Änderungen im Gesamtgehalt von Lipiden, Fettsäuren und Phospholipiden festzustellen. Der Gesamtanteil an Lipiden war in weißem Fettgewebe bei trächtigen höher als bei nicht trächtigen Fledermäusen. Das gleiche traf für die Phospholipide im braunen Fettgewebe zu. Die Trächtigkeit bewirkt bei *P. pipistrellus* in beiden Typen von Fettgeweben Änderungen in der Länge der Fettsäuren und deren Anteil an ungesättigten Bindungen.

References

Agius, L.; Williamson, D. H. (1980): Lipogenesis in interscapular brown adipose tissue of virgin, pregnant and lactating rats. The effects of intragastric feeding. Biochem. J. **190**, 477–480.

Arahuetes, R. M.; Fraile, A.; Suárez, A. (1982): Lipid metabolism in pregnancy. Perfusion of liver in pregnant rats. Rev. Esp. Fisol. **38**, 201–208.

Arévalo, F.; Burgos, M. J.; del Hoyo, N.; López-Luna, P. (1990): Seasonal variations in the lipid composition of white and brown tissues in the bat *Pipistrellus pipistrellus*. Comp. Biochem. Physiol. **95**, 535–539.

Beaton, G. H.; Beare, J.; Ryv, M. H.; McHewry, E. W. (1954): Protein metabolism in the pregnant rat. J. Nutr. **54**, 291–313.

Folch, J.; Lees, M.; Stanley, G. H. S. (1957): A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226**, 497–509.

Gittleman, J. L.; Thompson, S. D. (1988): Energy allocation in mammalian reproduction. Amer. Zool. **28**, 863–875.

Girardier, L. (1983): Brown fat. An energy dissipating tissue. In: Mammalian thermogenesis Ed. by L. Girardier and M. J. Stock. London: Chapman and Hall. Pp. 50–98.

Herrera, E.; Lasunción, M. A.; Gómez-Coronado, D.; Aranda, P.; López-Luna, P. (1988): Role of lipoprotein lipase activity on lipoprotein metabolism and the fate of circulating triglycerides in pregnancy. Am. J. Obstet. Gynecol. **158**, 1575–1583.

Himms-Hagen, J. (1983): Brown adipose tissue thermogenesis in obese animals. Nutr. Rev. **41**, 261–267.

Hytten, R. E.; Leitch, I. (1971): The physiology of human pregnancy. 2nd ed. Oxford: Blackwell.

Hytten, R. E.; Thompson, A. M.; Taggart, N. (1966): Total body water in normal pregnancy. Obstet. Gynecol. Br. Commonw. **73**, 553–561.

Ide, T.; Sugano, M. (1988): Effects of dietary fat types on the thermogenesis of brown adipocites isolated from rat. Agric. Biol. Chem. **52**, 511–518.

Krulin, G. S.; Sealander, J. A. (1972): Annual cycle of the gray bat, *Myotis grisescens*. Comp. Biochem. Physiol. **42A**, 537–549.

Kurta, A. (1986): Factors affecting the resting and postflight body temperature of little brown bats, *Myotis lucifigus*. Physiol. Zool. **59**, 429–438.

Lockwood, E. A.; Bailey, E.; Taylor, C. B. (1970): Factors involved in changes in hepatic lipogenesis during development of the rat. Biochem. J. **118**, 155–162.

López-Luna, P.; Muñoz, T.; Herrera, E. (1986): Body fat in pregnant rats at mid and late gestation. Life Science **39**, 1389–1393.

López-Luna, P.; Maier, I.; Herrera, E. (1991): Carcass and tissue fat content in the pregnant rat. Biology Neonate **60**, 29–38.

Masoro, E. J. (1968): Physiological chemistry of lipids in mammals. Philadelphia: Saunders.

Naismith, D. J.; Richardson, D. P.; Pritchard, A. E. (1982): The utilization of protein and energy during lactation in the rat, with particular regard to the use of fat accumulated in pregnancy. Br. J. Nutr. **48**, 433–441.

Palacín, M.; Lasunción, M. A.; Asuncion, M.; Herrera, E. (1991): Circulating metabolite utilization by periuterine adipose tissue "in situ" in the pregnant rat. Metabolism **40**, 534–539.

Pearce, J. (1983): Fatty acid synthesis in liver and adipose tissue. Symposium on comparative aspects of fatty acid metabolism. Proc. Nutr. Soc. **42**, 263–271.

Pistole, D. H. (1989): Sexual differences in the annual lipid cycle of the big brown bat *Eptesicus fuscus*. Can. J. Zool. **67**, 1891–1894.

Pulido, J. A.; del Hoyo, N.; Pérez-Albarsanz, M. A. (1986): Composition and fatty acid content of rat ventral prostate phospholipids. Biochim. Biophys. Acta **879**, 51–55.

Racey, P. A. (1973): Environmental factors affecting the length of gestation in bats. J. Reproduct. Fertil. Suppl. **19**, 175–189.

— (1982): Ecology of bat reproduction. In: Ecology of bats. Ed. by H. T. Kunz. New York: Plenum Press. Pp. 57–104.

Racey, P. A.; Speakman, J. R. (1987): The energy cost of pregnancy and lactation in heterothermic bats. In: Reproductive energetics in mammals. Ed. by A. S. I. London and P. A. Racey, New York: Oxford University Press. P. 371.

Racey, P. A.; Swift, S. M. (1981): Variations in gestations length in a colony of pipistrellus bats (*Pipistrellus pipistrellus*) from year to year. J. Reprod. Fertil. **61**, 123–129.

Rothwell, N. J.; Stock, M. J. (1983): Diet-induced thermogenesis. In: Mammalian thermogenesis. Ed. by L. Giradier and M. J. Stock. London: Chapman and Hall. Pp. 208–233.

Rouser, G.; Siakotos, A. N.; Fleischer, S. (1966): Quantitative analysis of phospholipids by thin layer chromatography and phosphorus analysis of spots. Lipids **1**, 85–87.

Sadleir, R. M. F. S. (1984): Ecological consequences of lactation. Acta Zool. Fennica **171**, 179–182.

Schneider, J. S.; Wade, G. N. (1987): Body composition, food intake, and brown fat thermogenesis in pregnant Djungarian hamsters. Am. J. Physiol. **253**, R314–R320.

Stebbings, R. E. (1976): Studies on the population ecology of British bats. Ph. D. Thesis, Univ. East Anglia.

Speakman, J. R.; Racey, P. A. (1987): The energetics of pregnancy and lactation in the brown long-eared bat *Plecotus auritus*. In: Recent advances in the study of bats. Ed. by M. B. Fenton, P. Racey, and J. M. V. Rayner. Cambridge: Cambridge University Press. Pp. 367–394.

Studier, E. H.; O'Farrell, M. J. (1976): Biology of *Myotis thysanodes* an *M. lucifugus* (Chiroptera: Vespertilionidae). III. Metabolism heart rat and general energetics. Comp. Biochem. Physiol. **54A**, 423–432.

Towsend, C. R.; Callow, P. (1981): Physiological Ecology: An evolutionary approch to resource use. Oxford: Blackwell.

Trayhurn, P. (1984): The development of obesity in animals: the role of genetic susceptibility. Clin. Endocrinol. Metab. **13**, 451–474.

Authors' addresses: Prof. P. López Luna, Departamento de Fisiología y Farmacología; Dr. F. Arévalo, Departamento de Biología Animal; Dr. N. del Hoyo, Departamento de Bioquímica y Biología Molecular; M. J. Burgos, Departamento de Fisiología y Farmacología, Universidad de Alcalá, E-28871 Alcalá de Henares, Madrid, Spain