FATS OF BATS

DIPLOMA THESIS

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Submitted by

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# INDEX OF CONTENTS

1. **INTRODUCTION** ............................................................................................................. 1
   1.1. Biological Membranes ............................................................................................. 1
   1.2. What are fatty acids? ............................................................................................... 4
   1.3. Why are PUFAs so important? .................................................................................. 5
   1.4. Oxidative stress ........................................................................................................ 6
   1.5. Maximum life span ................................................................................................... 8
   1.6. Bats in general .......................................................................................................... 9
   1.7. Animal ecology ......................................................................................................... 11
      1.7.1. *Nyctalus noctula* (Common noctule) ............................................................... 12
      1.7.2. *Pipistrellus pipistrellus* (Common pipistrelle) .................................................. 13
      1.7.3. *Hypsugo savii* syn. *Pipistrellus savii* (Savi’s pipistrelle) ............................... 14
      1.7.4. *Vespertilio murinus* (Partie-coloured bat) ......................................................... 15

2. **MATERIALS AND METHODS** ................................................................................... 16
   2.1. Materials .................................................................................................................. 16
   2.2. Methods .................................................................................................................... 16
      2.2.1. Preparation of mitochondrial and ER membranes ................................................ 16
      2.2.2. Phospholipid preparation .................................................................................... 17
      2.2.3. GC analysis ........................................................................................................ 18
      2.2.4. Data and statistical analysis ................................................................................ 19

3. **RESULTS** .................................................................................................................. 20
   3.1. Phospholipid fatty acid composition ....................................................................... 20
   3.2. Genders vs. fatty acid composition ......................................................................... 23
   3.3. Species vs. fatty acid composition .......................................................................... 24
   3.4. Tissues vs. fatty acid composition .......................................................................... 26
   3.5. Maximum lifespan vs. fatty acid composition ........................................................ 28

4. **DISCUSSION** ............................................................................................................ 30
1. INTRODUCTION

1.1. Biological Membranes

Every creature consists of cells surrounded by a membrane (cell membrane), containing also an intracellular membrane, which serves a lot of important biological functions including biochemical processes. The inner membrane consists of a lipid bilayer that is selectively permeable. There are three classes of amphipathic lipids (containing both polar and nonpolar domains) located within membranes: phospholipids, glycolipids and steroids. A phospholipid molecule is constructed from four components: fatty acids, a core to which the fatty acids are attached (usually glycerol), a phosphate group, and an alcohol attached to the phosphate (Fig.1).

![Fig.1: Structure of a phospholipid molecule](Reece JB 2011)

The fatty acid tails provide a hydrophobic barrier, whereas the other moieties possess hydrophilic properties to enable interaction with the aqueous environment. The movement of substances into and out of the cell is controlled by the membrane structure, which effectively separates the cell interior of a cell from the external environment. There are two main components comprising a cell membrane: first is the lipid constituent that forms the permeability barrier, and second is the protein component which serves numerous functions, including transport of substances across the membrane (Berg et al. 2002). Fatty acids found in membranes consist of saturated (no double bonds) or unsaturated (includes double bonds)
hydrocarbon chains. The fatty acyl chains within phospholipids and glycolipids contain variable numbers of carbon atoms, usually between 16 and 22. The 16- and 18-carbon fatty acids are the most abundant in membranes (Berg et al. 2002). Molecules can cross a membrane actively or passively. The two main important features for net movement across a membrane are that the molecule must be able to cross the hydrophobic barrier, and that the movement must be powered by an energy source. Lipophilic molecules move down their concentration gradients and therefore pass through a membrane by simple diffusion. Polar or charged molecules however, require specific channels in order to move across membranes. These molecules that move against a concentration gradient require an energy source such as adenosine triphosphate (ATP) (Berg et al. 2002). Mitochondria (Fig. 2) are cell organelles found in most eukaryotic cells and are referred to as the power stations of the cells. Contrary to most other cell organelles (except plastids of plants), they have their own independent genome: the mitochondrial DNA (mtDNA), which is located in the cellular matrix. Mitochondria have two membranes: 1) The outer membrane for protection against the external environment and 2) the inner membrane that consists of folds (cristae, tubules or sacs) in order to increase surface area. The region between the outer and inner membrane is entitled the “intermembrane space”, and the compartment enclosed by the inner membrane is called the “matrix”. The most important function of mitochondria is in the production of ATP via oxidative respiration, which occurs via the citric acid cycle in the organelles matrix, and through a series of enzymes (electron transfer chain) located in the inner membrane (Davidson 2010).

Fig. 2: The mitochondrial structure showing the inner and outer membrane and the matrix where the ATP production takes place (DAVIDSON 2010)
Another very important organelle in all eukaryotic cells (with the exception of erythrocytes) is the endoplasmic reticulum (ER; Fig. 3). The ER consists of a single continuous membrane which connects a network of flattened sacs, vesicles and branching tubules, forming one large and complexly arranged lumen (internal space). The endoplasmic reticulum often takes up more than 10% of the total volume of a cell. The ER membrane is directly connected to the membrane of the nucleus. Some parts of the ER presents ribosomes on its surface, and are therefore called rough endoplasmic reticulum (rER). Other areas which are free of ribosomes are called smooth endoplasmic reticulum (sER). These types of ER possess different functions. For example, the sER plays an important role in some metabolic processes such as the synthesis of lipids (mainly phospholipids), fatty acids and steroids (hormones). However, the rER is instead relied upon for protein biosynthesis and the production of functional membranes. A specialized form of ER, known as the sarcoplasmic reticulum (SR) is found within muscle cells. SR is a smooth endoplasmic reticulum which stores calcium ions used to trigger contraction of the muscle (Davidson 2010).

**Fig.3:** The structure of the endoplasmic reticulum including the rER and sER where the phospholipid synthesis takes place (DAVIDSON 2010)
1.2. What are fatty acids?

Fatty acids consist of both a carboxyl group (-COOH) and a hydrocarbon chain of varying length. Fatty acids differ in the number of carbon atoms (chain length) they contain, as well as the number and position of their double bonds. Natural fatty acids normally contain an even number of carbon atoms, with chains consisting of at least 4 carbon atoms such as butyric acid (C₄H₈O₂). The synthesis of fatty acids is located within the cytosol of eukaryotic and prokaryotic cells and utilises the major enzyme complex, fatty acid synthase. Beta-oxidation causes decomposition of fatty acids into acetyl coenzyme A (acetyl-CoA), which is located in the mitochondria. Acetyl-CoA can then be incorporated into the citric acid cycle and subsequent respiratory chain to produce ATP. Based on the occurrence of double bonds, there are three classes of fatty acids: Saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). SFAs normally consist of between 12 and 24 carbons, and have no double bonds between their carbon atoms. This results in all additional carbon bonds being saturated by hydrogen atoms. The relevant SFAs featured in this study were: 14:0 (myristic acid; indicates that it has 14 carbons and no double bond), 15:0 (pentadecanoic acid), 16:0 (palmitic acid), 17:0 (margaric acid) and 18:0 (stearic acid). MUFAs contain only one double bond (mono), and the remainder of their carbon atoms are connected through single bonds (Berg et al. 2002). Two MUFAs were analysed in this study: 16:1 (palmitoleic acid, double bond between C9 and C10) and 18:1 (oleic acid, double bond on C9). PUFAs however, possess a minimum of two double bonds between their carbon atoms. Some PUFAs are essential for mammals including humans, due to their metabolic inability to synthesize such fatty acids (Berg et al. 2002). Within PUFA’s the location of the first double bond is identified by the n-number, counted from the methyl end of the fatty acid. For example docosahexaenoic acid, which is an important component of cell membranes and because of its high amount of double bonds highly susceptible to peroxidation, (DHA, 22:6 n-3) has 22 carbon atoms and six double bonds, with the first double bond being located on the 3rd carbon (Berg et al. 2002). Due to the large quantity of double bonds found within PUFAs, they are often prone to lipid peroxidation. As a result PUFAs are more likely to become damaged, whilst SFAs and MUFAs are substantially resistant to peroxidation (Hulbert et al.
Lipid peroxidation is a chemical conversion or rather damage, of lipids through oxygen bonding. The process of peroxidation mostly begins with the generation of a carbon radical between two double bonds of a fatty acid chain. This process runs as a chain reaction until it gets interrupted from a scavenger (antioxidant) and produces very reactive hydro peroxide which is degraded to key precursors and final products (Pamplona et al. 1999). Within organisms lipid peroxidation is toxic due to the ability of lipid peroxides to seriously influence or damage membrane functions. Key precursors can attack enzymes, carbohydrates, proteins and nucleic acids (Hulbert et al. 2007).

1.3. Why are PUFAs so important?

Fatty acid composition influences membrane fluidity as well as the synthesis and function of enzymes, receptors, carrier proteins and ion channels within membranes. PUFAs produce energy required by the body, and also form an integral part of membranes and are therefore a very important phospholipid component (Berg et al. 2002). PUFAs are the precursors of eicosanoids, which influence a large amount of important procedures in cells: They are essential for cell structure compositions as well as transport units, are regulating the lipid metabolism and are also involved in the synthesis of molecular cues like prostaglandin. Within the heart, liver and neurons, n-6 fatty acids are the most dominant whereas more n-3 fatty acids are found in the retina, synapses, mitochondria and microsomes of the brain (Whelan 2008). Maximum lifespan (MLS) and membrane fatty acid composition are correlated with the MLS of mammals and birds (Hulbert et al. 2007). Membrane compositions of remarkably long living mammals such as naked mole rats and birds are more peroxidation resistant and more enriched with MUFAs compared to other similar sized mammals with a shorter MLS (Hulbert 2008). As PUFAs are more prone to oxidative damage due to their amount of double bonds, lower membrane polyunsaturation often leads to longer MLS (Hulbert et al. 2007). This study investigates the fatty acid profile, with particular emphasis on the DHA (22:6 n-3) amount in the mitochondrial and endoplasmic reticulum
membranes of selected organs from five European bat species in relation to their maximum lifespan. Mitochondrial membranes were analysed because they are the site of the respiratory chain and hence particularly prone to oxidative damage, thus their fatty acid profile might show a better correlation with lifespan than any other cellular membranes. To my knowledge no work has yet been published on bat membrane composition related to MLS. Therefore, for the first time we were able to correlate bat fatty acid (FA) composition with data from other similar sized mammals, such as the naked mole rat and mice.

1.4. Oxidative stress

Reactive oxygen species (ROS) are toxic forms of oxygen which cause lipid peroxidation. The most important ROS within lipids are the peroxyl radical (ROO\(^\cdot\)) and the alkoxyl radical (RO\(^\cdot\)). Most ROS are produced within the inner membrane of mitochondria (Fig. 4), and cause oxidative stress when the concentration of ROS exceeds the detoxification and repairing abilities of the cells, resulting in damage (Schmidt and Lang 2007). If the physiological balance gets disturbed, cellular and extracellular macromolecules can be damaged.

![Fig.4: ROS production in mitochondria. ROS produced by the respiratory chain can lead to lipid peroxidation, mutations and deletions and furthermore to mitochondrial dysfunction, apoptosis and necrosis which are triggers of diseases and ageing (MURPHY, 2009).](image)

Mitochondria also play a very important role in this thesis because they are an important source of ROS (Andreyev et al. 2005). As for MLS, a lot of interesting research concerning reactive oxygen species and oxidative stress is available. A current review describes the production of ROS in mitochondria, their effects on the cell and how they can be measured (Murphy 2008). Another notable review discusses how ROS damage mitochondrial DNA (mtDNA) and how they relate to animal longevity. It refers to comparative studies in mammals and birds which show that mitochondrial ROS generation in long lived species is lower than in short lived species (Pamplona 2011). Based on the oxidative stress theory, Hulbert et al. (2007) explains in detail the importance of mitochondrial free radical production and the role of ROS from mitochondria. In addition, an extension of this theory suggests that the vulnerability of membranes to oxidative attack is particularly important and may determine rates of ageing. The membranes most exposed to ROS are located in the mitochondria, where respiration takes place. Fig.5 presents the importance of membrane fatty acid composition (low and high membrane poly-unsaturation) and ROS in determining low or high oxidative stress, respectively leading to longer or shorter maximum lifespans (Hulbert et al. 2007). However, there is dispute about reviews to the oxidative stress theory of aging: One study discusses possible effects of manipulating antioxidant genes on lifespan (Salmon et al. 2010). Two possible explanations of contradictory findings were given: Oxidative stress might be not directly correlated to aging but might play a role in lifespan or healthy aging; finally, the environment might play a very important role for oxidative stress (Salmon et al. 2010).

![Fig.5](image)

**Fig.5:** Contrary to low membrane polyunsaturation, high membrane polyunsaturation produces more ROS which leads to high oxidative stress and furthermore causes damage to membrane fatty acids, DNA and proteins, leading to fast ageing and therefore a short MLS (HULBERT et al., 2008).
1.5. Maximum life span

Maximum life span (MLS) is the age of the oldest known member of a species (www.medical-dictionary.com). Mammalian species differ dramatically in their MLS. For example, *Sorex araneus* (Eurasian shrew; MLS ~ two years) compared to *Loxodonta africana* (African elephant; MLS ~ 70 years) (Hulbert et al. 2007). A lot of scientists argue in their reviews that MLS is positively related to body mass. The bigger (and heavier) an animal, the longer its MLS (Pamplona et al. 1998). This mostly indicates the lack of predators of big animals contrary to small species. Aside from this there are exceptions, such as some birds (for example *Fringilla coelebs* = chaffinch; MLS ~ 29 years; adult body weight ~ 20.7 g), the naked mole rat (*Heterocephalus glaber*; MLS >28.3 years; adult body weight ~ 35 g) and some bat species (for example *Myotis brandti* = Brandt’s bat; MLS ~ 41 years; adult body weight ~ 7 g) (De Magalhaes and Costa 2009). A notable and still up to date study from 1991 compares a huge number of species (580 species data-base) with respect to their body size, MLS, BMR and ecology, especially focused on two groups: bats and marsupials (Austad and Fischer 1991). In this study the authors could not find a clear relationship between body mass (< 1 kg) and MLS within bats or marsupials (Austad and Fischer 1991). The correlation between the high basal metabolic rate of bats and birds (Racey and Speakman 1987) and their MLS and mitochondrial oxidative damage was reviewed in a study (Munshi-South and Wilkinson 2009). High metabolic rates of bats vary seasonally within species, and are also regulated by hibernation and daily torpor (Lyman 1970, Bourliere 1958, Sacher 1977). A study of Brunet-Rossinni in 2004 on the little brown bat (*Myotis lucifugus*; MLS = 34 years) compared to *Blarina brevicauda* (short-tailed shrew; MLS = two years) and *Peromyscus leucopus* (white-footed mouse; MLS = eight years) support the free radical theory of aging as a possible explanation of the longer MLS of little brown bats with low levels of free radicals when compared to *P. leucopus* and *B. brevicauda*. As this study was the first report of oxygen radical generation in bat mitochondria, *Myotis lucifugus* was compared to similar sized non-flying mammals with different MLS (Brunet-Rossinni 2004). Hulbert provided an extensive study about how metabolic rate, membrane fatty acid composition (PUFA, Unsaturation index (UI), Peroxidation index (PI)) and lipid peroxidation is related to MLS in mammals, birds,
ectothermic vertebrates and invertebrates (Hulbert et al. 2007). In 2007, Valencak and Ruf presented a study where their results argue that the n-3/n-6 ratio in mammals is more relevant for longevity than PUFA content on membrane unsaturation as such. In addition, relationships between DHA and basal metabolic rate (BMR) and MLS (Speakman 2005) could not be confirmed (Valencak and Ruf 2007). The fatty acid composition may be an important factor in the relationships between body size, weight and age (maximum lifespan). This study aims at showing why the maximum lifespan of bats differs so much from other mammals with similar sizes and weight such as mice and naked mole rats by investigating the fatty acid composition of four bat species (*Nyctalus noctula, Vespertilio murinus, Hypsugo savii, Pipistrellus pipistrellus*).

### 1.6. Bats in general

Bats are small mammals belonging to the order *Chiroptera* (Burda et al. 2015). Media, as well as very old traditional stories and tales, cast a damning light on bats and still nowadays associate them with vampires and bloodsuckers. The oldest and most famous story is about Dracula from Bram Stoker (1897). In fact, there are just three living bat species in the world that feed on blood: the white winged vampire bat (*Diaemus youngi*), the hairy legged vampire bat (*Diphylla ecaudata*) and the common vampire bat (*Desmodus rotundus*). The distributions of all three species encompass Northern Mexico to Chile, Argentina, Brazil and Uruguay. Only in rare cases do these bats suck human blood (Nowak 1999). Due to their size, bats cannot be dangerous to people. However, a large number of bat species carry transmissive diseases such as rabies. Bats are the only mammals - and next to the birds the only living vertebrates – with the ability of sustained (uninterrupted) flight. Their forelimbs have developed into wings and their body size can vary from that of the large flying fox (*Pteropus vampyrus*) with a body weight of 1200 g, to a very small bat such as the bumblebee bat (*Craseonycteris thonglongyai*) with a body weight of 2 g. Bats are distributed all over the world from deserts to forests, and even in big cities there are large numbers of different bat
species. The only exception to this being Antarctica (Brunet-Rossini and Austad 2004). The preferred roosts quarters of bats in summer are found in buildings, underneath bridges and within trees. Local bats hibernate in winter because of insufficiencies in food supply such as insects, which they need for survival and their preferred hibernation spots include caves, galleries, buildings and trees (Dietz and Kiefer 2014). There are ~ 1100 different species of bats in the world, with around 38 of them living in Europe, and approximately 26 or more are living in Austria (www.fledermausschutz.at). The average age of a bat is between two and five years, but some of them have very long maximum life spans (an indicator of their rate of ageing) for their size and reach an age of over 40 years (Wilkinson and South 2002). Hence, bats clearly age at a slower rate than most other similar-sized mammals, therefore making bats such interesting subjects in which to investigate the mechanism related to ageing. Results of a review showed that the MLS of female bats that produce multiple pups per year is lower when compared to those who give birth to just one pup per year (Wilkinson and South 2002). On the other hand, this review shows that hibernating bats can live up to six years longer compared to those who do not hibernate (Wilkinson and South 2002). Also, cave roosting bats tend to have a longer MLS values compared to those which do not roost in caves (Wilkinson and South 2002). A great overview of aging studies on bats was given by Brunet-Rossini and Austad (2004). Some authors believe that the reduction in metabolism during hibernation causes the longer MLS, compared to their body size (Bourliere 1958, Sacher 1977) while others show that both (hibernating and non-hibernating) bats have a longer MLS compared to similar sized mammals (Wilkinson and South 2002). Others discuss that the production of ROS during mitochondrial respiration might be an important factor (Harman 1956, Sohal 1986). This study investigates the fatty acid profile, especially the DHA (22:6) proportion within the mitochondrial and endoplasmic reticulum membranes of selected organs, from four European bat species in correlation to their maximum lifespans. The mitochondrial membranes were analysed separately as a result of them being the site of the respiratory chain and hence particularly prone to oxidative damage. Therefore, their fatty acid profile might show a better correlation with lifespan than that of other cellular membranes.
1.7. Animal ecology

For this study I obtained bats from the Austrian agency for health and food safety (AGES). The AGES obtained the bats from veterinarians, the Wiener Tierschutzhaus (Vienna society for the prevention of cruelty to animals), the emergency unit of the University of Veterinary Medicine Vienna and local people who found them dead or alive. In the latter case the bats were euthanized for ethical reasons at the institute for veterinary analysis in Mödling.

The areas where they were found are: Mödling, Vienna, St. Pölten, Gänserndorf, Bad Vöslau, Mattersburg, Krems, Klosterneuburg, Baden, Vösendorf, Wolkersdorf and Hollabrunn. Species I took for my study were: *Hypsugo savii* (males and females), *Nyctalus noctula* (*N.*noctula, males and females), *Pipistrellus pipistrellus* (*P.*pipistrellus, males and females) and *Vespertilio murinus* (*V.*murinus, males and females).
1.7.1. *Nyctalus noctula* (Common noctule)

**Physical description:** *N. noctula* is one of the largest native bats in Austria and Europe. Colours range from golden to dark brown above and usually pale brown below. Head and body length is 50-70 mm, tail length is 35-65 mm, and forearm length is 40-70 mm.

**Weight:** 15-40 g

**Maximum lifespan:** twelve years

**Appearance:** Europe, most of temperate Asia to Japan and Burma, Oman, Vietnam, Taiwan, Algeria and possibly Mozambique and Singapore.

**Classification:** Family *Vespertilionidae*, subfamily *Vespertilioninae*, genus *Nyctalus*, species *Nyctalus noctula*

(Ballenger 2010)

**Sample size:** four (two males, two females)

*Fig. 6: Nyctalus noctula*  
(Unep/Eurobats 2015)
1.7.2. *Pipistrellus pipistrellus* (Common pipistrelle)

**Physical description:** The Common *Pipistrellus pipistrellus* (*P.pipistrellus*) is one of the smallest bats. It has a wingspan between 180-240 mm, and its wings are narrow. Like other bats in the Vespertilionidae family, *P.pipistrellus* has a tragus, and in this species, the tragus is rounded at the top and quite long. The pelage is usually brown, but other colours, such as chestnut and dark brown have also been observed. Their wing and tail membranes are dark brown, and they do not have any fur on them. This species is very common in towns, cities, parks and forests.

**Weight:** 5-10 g

**Maximum lifespan:** 16.5 years

**Appearance:** Europe, including the northern countries, such as England, Ireland and even reaching Southern Scandinavia. Its range extends eastward through Asia to China and perhaps Korea, Japan, and Taiwan.

**Classification:** Family *Vespertilionidae*, subfamily *Vespertilioninae*, genus *Pipistrellus*, species *Pipistrellus pipistrellus*

(Kuester 2010)

**Sample size:** two (one male, one female)

![Fig.7: Pipistrellus pipistrellus](Unep/Eurobats 2015)
1.7.3. *Hypsugo savii* syn. *Pipistrellus savii* (Savi’s pipistrelle)

**Physical description:** *Hypsugo savii* (*H.savii*) is a small and for bats a very colourful bat: The ears are short and widish and mostly gleamy black. The fur of the back can have a lot of different brown colours (sometimes with gold and yellow too) and the underside is mostly yellow or white. The face and wings are dark brown to black. *H.savii* is about 40-54 mm, its wingspan can range from 220-225 mm and it prefers to stay mainly in caves and buildings.

**Weight:** 5-9 g

**Maximum lifespan:** 14 years

**Appearance:** From the Iberian Peninsula, over the whole European Mediterranean area to the Balkan, little Asia and the Middle East, Mediterranean islands, France, Switzerland, southern Germany, Austria, Czech Republic, Hungary and Bulgaria.

**Classification:** Family *Vespertilionidae*, subfamily *Vespertilioninae*, genus *Hypsugo*, species *Hypsugo savii* syn. *Pipistrellus savii*

(Fledermausschutz 2009)

**Sample size:** three (two males and one female pooled)

![Fig.8: Hypsugo savii](Unep/Eurobats 2015)
1.7.4. *Vespertilio murinus* (Partie-coloured bat)

Physical description: *Vespertilio murinus* (*V.murinus*) is robust and of medium size, has short, broad ears which barely reach the tip of the snout when laid forward and may be found in almost every landscape. The tragus is small, short, blunt and rounded. Wing membranes, ears, and face are nearly black. The dorsal fur is dense and distinctively bicoloured and the ventral side may be creamy white in sharp contrast to the dorsal side, or beige or greyish and less contrasting. Measurements are: head and body, 48-64 mm and tail, 37-44.5 mm.

Weight: 10-15 g

Maximum lifespan: twelve years

Appearance: This species occurs over an extensive area, from eastern France and Switzerland northward to southern Scandinavia, eastward through central Europe, Belorussia and Ukraine, Azerbaijan, northern Iran, Afghanistan and southern Russia. *V.murinus* is rare or absent over much of western and southern Europe.

Classification: Family *Vespertilionidae*, subfamily *Vespertilioninae*, genus *Vespertilio*, species *Vespertilio murinus*

(Rydell 1994)

Sample size: four (two males, two females)

*Fig.9: Vespertilio Murinus*
(Unep/Eurobats 2015)
2. MATERIALS AND METHODS

2.1. Materials

The bat species of this study were: *Nyctalus noctula* (n = 4), *Vespertilio murinus* (n = 4), *Hypsugo savii* (n = 3) and *Pipistrellus pipistrellus* (n = 4), determined by AGES. All bats were tested negative for rabies (AGES). Bats were found either dead or they were euthanized for ethical reasons. Liver, heart and skeletal muscle samples of seven males and eight females were analysed. As bats were used for multiple scientific studies, tissue samples were provided from AGES already packed in small bags, labelled with numbers, tissue type, species and gender. Therefore, skeletal muscle determination was not possible. The tissue of all *H.savii* individuals were pooled because of their small body size (small tissue content) as well as the males and females of *P.pipistrellus*.

2.2. Methods

2.2.1. Preparation of mitochondrial and ER membranes

Mitochondrial and ER membranes were prepared from heart, liver and skeletal muscle. All steps were performed on ice (4 °C). The homogenization buffer (pH 7.4) for skeletal muscles and hearts contained 10 mM EDTA, 100 mM Tris (hydroxymethyl) aminomethane (Tris), 100 mM sucrose and 100 mM KCl. For livers, the buffer (pH 7.4) contained 10 mM EDTA, 100 mM Tris and 200 mM sucrose. Tissues were cut and rinsed well with homogenization buffer and transferred to a 2 ml potter tube. 5 mg butylated hydroxytoluene (BHT) were
added to the tissue and pottedter about ten to 15 times with a Teflon pestle. After centrifugation at 3600 rpm (1000 x g) for 10 min, the supernatant was transferred to a new tube. The pellet was mixed with 300 μl homogenization buffer, vortexed for 30 sec and centrifuged at 3600 rpm (1000 x g) for 10 min again. The combined supernatants were centrifuged at 1200 rpm (11000 x g) for 10 min. The supernatant (ER/SR fraction) was stored at -80 °C. The pellet was washed with 2 ml homogenization buffer by vortexing and centrifuged at 12000 rpm for 10 min. The pellet (mitochondria) was resuspended in 500 μl homogenization buffer and stored at -80 °C.

2.2.2. Phospholipid preparation

The ER/SR fractions and mitochondria were transferred into a tube and mixed with 2 ml of chloroform/methanol (2:1 v/v). Lipids were extracted by vortexing for 4 min. After adding 100 μl NaCl (180 mg/ml), the extraction was repeated for 2 min. The phases were separated by 10 min centrifugation at 4000 rpm and the lower phase (chloroform) was pipetted in a conical flask. Another 2 ml chloroform were added to the samples and vortexed for 4 min, centrifuged (10 min) and the lower phases were combined. The blank was prepared by vortexing 2 ml chloroform/methanol + 2 ml chloroform + 100 μl NaCl + 2 ml distilled H₂O for 30 sec. The conical flasks were transferred to a rotavapor until the solvent evaporated. The lipids were recovered with 100 μl chloroform/methanol by brief vortexing and loaded on a thin layer chromatography (TLC) plate with a capillary. The flasks were rinsed with another 50 μl of chloroform/methanol and loaded on the TLC plate. The plate was transferred to a TLC chamber until the mobile phase almost reached the upper edge. After drying, the start band (phospholipids) was labelled under UV with a pencil (blank: same area). The layer was scratched off with a spatula and transferred into tubes with 1 ml methanol/sulphuric acid (2.2 % v/v). After transesterification (conversion of phospholipid fatty acids to fatty acid methyl esters) for 30 min in a boiling water bath with briefly vortexing every 10 min, the samples were cooled down and 500 μl deionized H₂O was added. Finally, FAME (fatty acid
methyl esters) were extracted two times with 400 μl hexane by vortexing for 1 min each. The upper phases were transferred into a gas chromatography (GC) vial which was sealed with a Viton septum and stored at -20° C until GC analysis (Christie 2003). Chloroform, methanol, sodium chloride (NaCl), diethyl ether, sulphuric acid and formic acid were from Sigma (Sigma-Aldrich Handels GmbH, Austria), hexane from LGC Standards (LGC Standards, UK), silica gel GF plates (not predried) from Analtech (Silica gel GF Uniplates, Analtech Inc., USA) and the thin layer chromatography (TLC) chamber from VWR (TLC Developing Chamber, VWR, USA).

2.2.3. GC analysis

Gas chromatography (Autosystem XL with autosampler and FID, Perkin Elmer, Norwalk, USA) was used to analyse the samples with a capillary column (HP INNOWax, 30 m x 0.25 mm x 0.25 μm, Hewlett Packard, USA). Parameters were: injector 240 °C, column 130-180 °C at 4 °C/min, 180-200 °C at 3 °C/min, 200-240 °C at 15 °C/min, 240 °C for 8 min. For peak identification external FAME standards (Supelco) were used and also for the response factor calculation. Turbochrom 4.1 software (Turchochrom 4.1, Perkin Elmer, Norwalk, USA) was used to calculate the percentages of the integrated peak areas.
2.2.4. Data and statistical analysis

The data are given in weight % (w/w %). Based on the unsaturation degree, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA and saturated fatty acids (SFA) were identified. Monounsaturated fatty acids were calculated as MUFA = \( \sum \% (16:1 + 18:1) \), polyunsaturated fatty acids as PUFA = \( \sum \% (12:2 + 18:3 + 20:4 + 20:5 + 22:5 + 22:6) \) and saturated fatty acids as SFA = \( \sum \% (14:0 + 15:0 + 16:0 + 17:0 + 18:0) \). In addition, I calculated the peroxidation index PI = 0.025 * (% MUFA) + 1 * (% dienoic acids) + 2 * (% trienoic acids) + 4 * (% tetraenoic acids) + 6 * (% pentaenoic acids) + 8 * (% hexaenoic acids), the unsaturation index UI = \( \sum \% \) (number of double bonds) and the n-6/n-3 ratio (\( \Sigma n-6/\Sigma n-3 \)) of PUFA (Armitage et al. 2002). Bat phospholipids were compared to other mammals. An average value was calculated for each species when data were available from multiple studies. The maximum lifespan was obtained from the AnAge database (http://genomics.senescence.info/species/). Statistical analysis were performed with SPSS (SPSS Statistics, 20.0, IBM, 2012, Ehringen, Germany) and Excel (Microsoft Excel 2013, Microsoft Corporation, USA).
3. RESULTS

3.1. Phospholipid fatty acid composition

Investigated fatty acids in phospholipids in this study of four bat species (*N. noctula*, *V. murinus*, *H. savii*, *P. pipistrellus*) were: 14:0 (Myristic acid), 15:0 (Pentadecanoic acid), 16:0 (Palmitic acid), 16:1 (Palmitoleic acid), 17:0 (Heptadecanoic acid), 18:0 (Stearic acid), 18:1 (Oleic acid), 18:2 (Linoleic acid), 18:3 (α-Linolenic acid), 20:4 (Arachidonic acid), 20:5 (Eicosapentaenoic acid), 22:5 (Docosapentaenoic acid) and 22:6 (Docosahexaenoic acid). All mean single fatty acids of phospholipids from the livers, hearts and skeletal muscles from bats are presented in Tab. 1 and 2.

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Tab.1: Phospholipid fatty acid composition of bat tissues. Data are presented as weight percent of total fatty acids. Males and females were combined. There is no SD for *Hypsugo savii* because the organs had to be pooled.

Sp. = species, N.n. = *N. noctula*, V.m. = *V. murinus*, H.s. = *H. savii*, P.p. = *P. pipistrellus*, er = endoplasmic reticulum, sr = sarcoplasmic reticulum, m. = mitochondria, s.m. = skeletal muscle, SD = standard deviation
## Tab. 1 (continued)

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Table 2: Descriptive statistics summarising the phospholipid composition of bat tissues. Data are presented as weight percent of total fatty acids. Males and females were combined. There is no SD for *Hypsugo savii* because the organs had to be pooled from three animals, as there was not enough sample size amount for each. Sp. = species, N.n. = *N.noctula*, V.m. = *V.murinus*, H.s. = *H.savii*, P.p. = *P.pipistrellus*, l.er = liver endoplasmic reticulum, l.m. = liver mitochondria, h.sr = heart sarcoplasmic reticulum, h.m. = heart mitochondria, s.m.sr = skeletal muscle sarcoplasmic reticulum, s.m.m. = skeletal muscle mitochondria, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, UI = unsaturation index, PI = peroxidation index

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3.2. Genders vs. fatty acid composition

Before starting with other comparisons, correlations between peroxidation index (PI) of mitochondria and ER/SR- fractions from male and female bats were assessed (Fig. 10). PI values of the liver ER- fractions and mitochondria were on average 150 while those of heart and skeletal muscle were in the range 250-300 and 200-230, respectively. No gender differences of the PI of all organs was found (Tab. 3), thus males and females were combined.

![Fig. 10: PI of bat mitochondria and ER/SR- fractions of all organs from males and females. All species were obtained. Data are presented as weight percent of total fatty acids. Error bars represent ± SD.]

Tab.3: Results of the statistical analysis for a comparison of the PI between males and females of liver, heart and skeletal muscle in mitochondria and ER/SR-fraction. H = ER/SR-fraction, M = mitochondria, PI = peroxidation index.

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3.3. Species vs. fatty acid composition

A comparison between MUFA, PUFA and SFA contents of total fatty acid composition of *N. noctula, V. murinus, H. savii* and *P. pipistrellus* indicated a similarity between all four bat species (Fig. 11). MUFA values ranged from 17.6 % (*V. murinus*) to 24.5 % (*N. noctula*) while those of PUFAs were much higher with values from 41.6 % for *H. savii* to 45.8 % for *V. murinus*. DHA (22:6 n-3) values of skeletal muscle SR- fraction differ remarkably within the two species of *N. noctula* with 13.5 % compared to *P. pipistrellus* with 22.8 %. The same applies for Linoleic acid (18:2) liver ER- fraction with a value of 8.9 % from *H. savii* compared to 19.9 % from *P. pipistrellus*.

![Fig. 11: MUFA, PUFA, SFA and DHA amount of skeletal muscle SR-fraction from Nyctalus noctula, Vespertilio murinus, Hypsugo savii and Pipistrellus pipistrellus. Males and females were combined. Data are presented as weight percent of total fatty acids. MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids, DHA = Docosahexaenoic acid.](image-url)
Results of the peroxidation index (PI) and unsaturation index (UI) of all four bat species are presented in Fig. 12. PI values ranged from 215.3 (H. savii) to 336.6 (P. pipistrellus) and UI values ranged from 201.8 (H. savii) to 282.4 (P. pipistrellus).

![Fig. 12: PI and UI of heart ER- fraction of N. noctula, V. murinus, H. savii and P. pipistrellus. Males and females were combined. Data are presented as weight percent of total fatty acids. Error bars represent ± SD. There is no SD for H. savii because samples had to be pooled.](image)

Compared to other similar sized mammals, a similarity of PUFA contents of total fatty acid composition between bats and H. glaber (naked mole rat) could also be confirmed (Fig. 13). There is a significant difference of MUFA amounts in liver mitochondria (t = 3.641, p = 0.002) of H. glaber compared to bats.

![Fig. 13: MUFA, PUFA and DHA amount of liver mitochondria from naked mole rats (H. glaber) (Hulbert et al. 2006) and bats. Males and females were combined. to be pooled.](image)
3.4. Tissues vs. fatty acid composition

**Fig. 14** represents a comparison between mitochondria and ER/SR- fractions of mean PI of all four species of bat studied. Differences between PI values of liver mitochondria vs. liver SR-fraction and skeletal muscle mitochondria vs. skeletal muscle ER- fraction were found not to be significant (**Tab. 4**).

![Graph](image)

**Fig. 14**: Comparison of mean PI values of bat mitochondria and ER/SR- fractions from all organs. Males and females were combined. Data are presented as weight percent of total fatty acids. Error bars represent ± SD. S.m. = skeletal muscle.

**Tab. 4**: Results of the statistical analysis for a comparison of the PI and DHA (22:6 n-3) between ER-/SR-fraction and mitochondria. PI = peroxidation index, 226 = DHA (22:6 n-3)
**Fig. 15** shows that PUFAs in heart and skeletal muscle SR-fraction from all four bat species were higher than both their SFAs and MUFAs. Only in liver ER-fractions were SFA abundances seen to be slightly greater and therefore comparable to PUFA abundances. In all three organs studied, MUFA concentration was seen to be the lowest.

![Bat fatty acid composition of ER/SR-fractions from all organs.](image)

**Fig. 15**: bat fatty acid composition of ER/SR-fractions from all organs. All species and males and females were combined. Data are presented as weight percent of total fatty acids. Error bars represent ± SD. MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids, SFA = saturated fatty acids.

All four bat species were compared with respect to their DHA (22:6 n-3) concentrations within their livers, hearts and skeletal muscles, presented in **Fig. 16**. Notably, results show both a high heart and a high skeletal muscle DHA (22:6 n-3) value within *P. pipistrellus*. The heart sarcoplasmic reticulum fraction (SR-fraction) from *N. noctula* contained an average of 26.6 % n-3 (DHA), while the liver endoplasmic reticulum fraction (ER-fraction) had only 7.7 % DHA (22:6 n-3). The heart SR-fraction also contained a higher percentage of 18:0 (stearic acid), 22:5 (docosapentaenoic acid), PUFA, n-3 and larger values of UI, PI and the n-3/n-6 ratio in comparison to the liver ER-fraction. For *V. murinus, H. savii* and *P. pipistrellus* the same parameters were also higher for the heart SR-fraction than for that of the liver ER-fraction, except stearic acid (18:0) of *V. murinus*. Values for the skeletal muscle SR-fraction were mostly intermediary of that of the heart and liver fractions. The same was found for heart, liver and skeletal muscle mitochondria.
3.5. Maximum lifespan vs. fatty acid composition

A significant relationship between PI and MLS within the four bat species was not confirmed (t = 1.997, p = 3.532) (Fig. 17). In contrast to PI and UI, MLS did significantly decrease with increasing DHA (22:6 n-3) amounts (t = 1.994, p = 0.021). However, comparisons between bats, naked mole rats (H. glaber) and mice (M. musculus) generally confirm the existence of a negative correlation between PI and MLS values (-0.7121), although for P. pipistrellus such values are higher than expected (Fig. 18). The same applies for DHA (22:6 n-3) (-0.7549).
Fig. 17: PI values of all four bat species compared to their MLS. Males and females were combined. Data are presented as weight percent of total fatty acids. ER = endoplasmic reticulum, SR = sarcoplasmic reticulum.

Fig. 18: Mean DHA values of liver mitochondria of all four bat species, *Heterocephalus glaber* (Hulbert et al. 2006) and *Mus musculus* (Hulbert et al. 2006) compared to their MLS. Males and females were combined. Data are represented as weight percent of total fatty acids.
4. DISCUSSION

4.1. Maximum lifespan and body weight

Aging is a very complex mechanism, regulated by various factors (both extrinsic and intrinsic), which are not fully decoded. Due to continued scientific progress and development of new technologies, it has become possible to investigate innovative research fields. Previous studies refer to the hypothesis that body mass is positively related to MLS (Speakman et al. 2005). Although it is true that in most cases large animals possess higher MLS compared to smaller species, this hypothesis does not apply to all individuals making organisms such as bats an interesting model. Based on a large number of scientific studies, it is possible to predict MLS of an organism using body mass and the following equation: \[ \text{MLS} = 10.2M^{0.22} \] (Hulbert et al. 2007). Using this calculation would predict that for an 80 kg human their MLS value would equate to 27 years. However, the maximum recorded lifespan of human is 122.5 years (Jones et al. 2009). When using this same equation to predict the MLS value of bats for example \textit{N. noctula} (M = 0.04 kg), the expected MLS would be 5 years but in reality as mentioned previously (section 1.8), the reported MLS for this species is 12 years. The same applies for \textit{V. murinus} (M = 0.019 kg, MLS = 14 years) with a predicted MLS of 4.2 years, \textit{H. savii} (M = 0.009 kg, MLS = 14 years) with a prediction of 3.6 years, and \textit{P. pipistrellus} (M = 0.01 kg, MLS = 16 years) with a previous 3.7 year MLS prediction. This therefore shows that in the case of estimating MLS values for bat species, body mass on its own is not a reliable factor.
4.2. Maximum lifespan and Fatty acid composition

It is currently known that fatty acid composition influences a large number of membrane functions and membrane fluidity (Hulbert 2005) and represent an important intrinsic factor for aging (Hulbert et al. 2007, Hulbert et al. 2006, Berg et al. 2002). A large number of studies focus particularly on PUFAs (polyunsaturated fatty acids), especially DHA (22:6 n-3), based on their higher susceptibility to lipid peroxidation, which results in membrane damage (Hulbert et al. 2007). Bats, with their exceptional MLS compared to their body size, are therefore an interesting model for further investigation. Due to the absence of literature regarding bat fatty acid composition and their correlation to MLS, this study is the first to investigate if there is any accordance with other similar sized mammals. The main focus was given to *Heterocephalus glaber* (*H.glaber*, naked mole rat) because of its similarity to the four bat species, with a body weight of ~35 g and an exceptional MLS of >28.3 years (Hulbert et al. 2006) and *Mus musculus* (*M.musculus*, common house mouse) with a body weight of ~40 g and a MLS of 4 years (Hulbert et al. 2006). As mentioned previously, the distribution of membrane lipids may be reportedly an important factor of MLS (Hulbert et al. 2006). A comparison between MUFA and PUFA contents of total fatty acid composition of liver mitochondria indicated a similarity between bats and *H.glaber*. In 2006 results of a study demonstrated that long-lived naked mole rats have a remarkable lower amount of DHA (22:6 n-3) compared to short-lived mice (Hulbert et al. 2006). This study confirmed the oxidative stress theory as well as the membrane peacemaker theory, which suggested that membranes with low n-3 PUFA amounts (especially DHA (22.6 n-3)) are more resistant to lipid peroxidation, and therefore influence MLS (Hulbert et al. 2006). This may lead to the assumption that bats which have an MLS value lying between that of naked mole rats and mice, should in turn have an approximately intermediate concentration of DHA (22:6 n-3) which could be confirmed for *N.noctula, V.murinus* and *H.savii* (MLS). However, DHA (22:6 n-3) values from *P.pipistrellus* (MLS 16 years), especially in heart SR- fraction, were higher than expected with no plausible explanation.
Because PI is a calculated value which indicates the lipid peroxidation of membrane phospholipids, it is assumed that PI levels should decline with rising MLS (Hulbert et al. 2007). Following this postulation, bats would have lower PI values compared to similar sized short-lived mammals like mice (*M. musculus*) which also could be confirmed. However, calculations within the four bat species did not show a relationship between PI and MLS. This might be a result of the fact that there are very few records of MLS values for the bat species investigated in this study. In addition, bat lifespan data are generally inaccurate and are also most commonly underestimations.

In summary, the higher amount of MUFA together with a lower amount of PUFA (especially DHA) in bats when compared to short-lived mice (*M. musculus*) (**Fig. 19**), as well as their intermediate DHA and PI amounts compared to other similar sized mammals with various MLS, confirm to predictions stated in the oxidative stress theory (Hulbert et al. 2006). A possible explanation for the remarkable lifespan in bats could be their high quantities of MUFAs, which are in turn to PUFAs more resistant to oxidative damage (section 1.2). However, phospholipid composition should not be deemed as the only, or even the most important factor in determining lifespan as aging is a complex process about which further research is needed. Investigations of brain tissue phospholipid fatty acid composition in bats could also give a better explanation of their exceptional MLS, as neuron cells contain a large number of DHA (Walczewska et al. 2011). As bats are actively flying mammals, comparisons to birds might give further explanations too.

**Fig. 19**: Comparison of MUFA, PUFA and DHA (22:6) of liver mitochondria from bats and *M. musculus* (Hulbert et al. 2006). Males and females were combined. Data are presented as weight percent of total fatty acids.
5. SUMMARY

Bats possess an exceptional maximum lifespan in relation to their body size when compared to other similar sized mammals. Consequently, bats are excellent models in which to investigate the mechanism that underlies the process of ageing. The oxidative stress theory proposes that species with a high maximum lifespan possess a larger quantity of membrane lipids resistant to peroxidation, compared to species with a lower maximum lifespan. As fatty acid composition of livers, hearts and skeletal muscles may potentially offer a better explanation for the long maximum lifespan of bats, monounsaturated fatty acids, polyunsaturated fatty acids and saturated fatty acids of four bat species (Nyctalus noctula, Vespertilio murinus, Hypsugo savii and Pipistrellus pipistrellus) were measured. When compared to other similar sized mammals with lower maximum lifespans such as Mus musculus (normal house mouse), bat membranes were seen to contain a higher amount of monounsaturated fatty acids and a lower amount of polyunsaturated fatty acids, thus conforming to the membrane pacemaker hypothesis. Docosahexaenoic acid (22:6 n-3) is a frequently investigated polyunsaturated fatty acid in literature and is supposedly prone to oxidation, so it is an important fatty acid to compare with. This study confirms the expected correlation of monounsaturated fatty acids with maximum lifespan of four bat species (Nyctalus noctula, Vespertilio murinus, Hypsugo savii, Pipistrellus pipistrellus) and other similar sized mammals. For example, docosahexaenoic acid has got a negative impact on maximum lifespan, whilst monounsaturated fatty acids instead show a positive influence. Within Pipistrellus pipistrellus, docosahexaenoic acid (22:6 n-3) values together with the Peroxidation Index (PI) were higher than expected with no plausible explanation. Due to the fact that Heterocephalus glaber (naked mole rat), a similar sized mammal (~35 g), also possesses an exceptional maximum lifespan (> 28.3 years), a special focus was given to comparing these two species. Although docosahexaenoic acid (22:6 n-3) liver mitochondria levels within Heterocephalus glaber were remarkably lower when compared to those of bats, comparisons still confirm the oxidative stress theory of declining polyunsaturated fatty acid values with increasing maximum lifespan. The same hypothesis applies for the peroxidation index. Overall, membrane composition is undoubtedly a determining factor influencing maximum lifespan.
6. ZUSAMMENFASSUNG

7. LIST OF ABBREVIATIONS

AGES................. Austrian agency for health and food safety

Acetyl-CoA........ Acetyl-Coenzyme-A

ATP.................. Adenosine triphosphate

BMR.................. Basal metabolic rate

BHT................... Butylated hydroxytoluene

*B.brevicauda*...... *Blarina brevicauda*

DHA.................. Docosahexaenoic acid

DNA.................. Desoxyribonucleic acid

EDTA................ Ethylenediaminetetraacetic acid

ER..................... Endoplasmic reticulum

FA.................... Fatty acid

FAME................ fatty acid methyl ester

GC.................... Gas chromatography

g...................... Gram

h..................... heart

*H.glaber*.......... *Heterocephalus glaber*

H.s................... *Hypsugo savii*

*H.savii*........... *Hypsugo savii*
I. liver

m. mitochondria

mM milli mol

\textit{M.\textit{musculus}} \ldots \textit{Mus \textit{musculus}}

min. Minute

ml. Millilitre

mg milligram

MLS Maximum lifespan

mtDNA Mitochondrial DNA

MUFA Monounsaturated fatty acids

\textit{M.\textit{lucifugus}} \ldots \textit{Myotis \textit{lucifugus}}

n. Number of samples

N.n. \textit{Nyctalus noctula}

\textit{N.\textit{noctula}} \ldots \textit{Nyctalus noctula}

NaCl Sodium chloride

P.p. \textit{Pipistrellus pipistrellus}

\textit{P.\textit{pipistrellus}} \ldots \textit{Pipistrellus pipistrellus}

PI Peroxidation index

PUFA Polyunsaturated fatty acids

\textit{P.\textit{leucopus}} \ldots \textit{Peromyscus \textit{leucopus}}
rER ....................... rough endoplasmic reticulum
RO ....................... alkoxy radical
ROO ..................... peroxyl radical
ROS ................. Reactive oxygen species
Rpm ................... revolutions per minute
Sec. ..................... seconds
sER ................. smooth endoplasmic reticulum
s.m. ............. skeletal muscle
sp .................... species
SD ................... Standard deviation
SFA ................... Saturated fatty acids
SR ................... Sarcoplasmic reticulum
TLC ................... Thin layer chromatography
Tris ................... Tris (hydroxymethyl) aminomethane
UI .................. Unsaturation index
UV .................... ultraviolet
V.m. ............. *Vespertilio murinus*
*V.murinus* ..... *Vespertilio murinus*
w.t ................... whole tissue
μl ...................... micro litre
8. LIST OF REFERENCES


Davidson MW, Florida State University. 


Fledermausschutz, Koordinationsstelle für Fledermausschutz- und forschung in Österreich. 


9. LIST OF FIGURES

Figure 1: Phospholipid structure ............................................................................................... 1
Figure 2: The mitochondrial structure ....................................................................................... 2
Figure 3: The endoplasmic reticulum structure ......................................................................... 3
Figure 4: ROS production in mitochondria ............................................................................... 6
Figure 5: Schematic diagram of the oxidative stress theory ...................................................... 7
Figure 6: Nyctalus noctula ....................................................................................................... 12
Figure 7: Pipistrellus pipistrellus ............................................................................................ 13
Figure 8: Hypsugo savii ........................................................................................................... 14
Figure 9: Vespertilio murinus .................................................................................................. 15
Figure 10: PI of bat mitochondria and ER/SR-fraction of males and females ........................ 23
Figure 11: MUFA, PUFA, SFA and DHA (22:6 n-3) amounts of bats ........................................... 24
Figure 12: PI and UI of heart ER-fraction of bats ..................................................................... 25
Figure 13: MUFA, PUFA and DHA (22:6 n-3) amounts of bats and H.glaber ......................... 25
Figure 14: PI of bat mitochondria and ER/SR-fraction from all organs ...................................... 26
Figure 15: MUFA, PUFA and SFA amounts in bats ................................................................. 27
Figure 16: DHA (22:6 n-3) amount of all four bat species ......................................................... 28
Figure 17: PI values of bats compared to MLS .......................................................................... 29
Figure 18: DHA (22:6 n-3) values of of bats, H.glaber and M.musculus compared to MLS . 29
Figure 19: MUFA, PUFA and DHA amount of bats compared to M.musculus ......................... 32
10. LIST OF TABLES

Table 1: Fatty acid composition of bat tissues.................................20-21

Table 2: Fatty acid sum parameters of bat tissues .........................22

Table 3: Statistical analysis of the PI of bats between males and females..........................23

Table 4: Statistical analysis of the PI compared to DHA (22:6 n-3).............................26