Study of body composition in small animals by a multifrequency impedancemeter

E RIBBE\textsuperscript{1}, N KHIDER\textsuperscript{2} and M V MORENO\textsuperscript{1}

\textsuperscript{1} Research Department, BioparHom, Savoie Technolac, BP238, 73374 Le bourget du Lac Cedex, France
\textsuperscript{2} Research Department, Bio2M, Parc Technologique des Rives de l'Oise, BP50149, 60201 Compiègne Cedex, France

E-mail: eva.ribbe@gmail.com

Abstract. Bioimpedance is essentially used today to study the body composition in the human body but not really in small animals. The aim of this paper is to develop a model for body composition in rats to help pharmaceutical labs assessing effects of medicine on rats. We propose a non invasive, rapid and scientific method. With a multifrequency impedancemeter, Z-Métrix\textsuperscript{®} (BioparHom© Company France), resistances and reactances are measured at 55 frequencies for a population of 40 rats (males and females). With our model, derived from Cole-Cole model, resistances of extracellular (Re) and total body (Rinf) compartment are extrapolated. Three methods were applied: posterior to posterior leg, anterior to posterior leg on the left and on the right side. Measurements by CT imaging were performed on the anesthetized population to determine Fat Mass (FM), Lean Body Mass (LBM) and Bone Mineral Content (BMC), as our reference measurements. With electrical data, age, sex and weight, equations are created to calculate FM, LBM and BMC with the three methods. Graphs of correlation, between tissue masses calculated by bioimpedance and obtained with scanner, indicate that measurements with posterior to posterior leg are better. Moreover, there is no significantly difference between tissue masses measured by bioimpedance and with the scanner.

1. Introduction

Bioimpedance is a common technique for accessing body composition in human. However, it isn’t a case in small animals, which are usually sacrificed. Moreover, many laboratories need to know the effects of drugs, hormones, vaccines on body composition, or to model the physiology of human or other animals.\cite{1} To do so, they use small animals included rats. Body composition analysis by bioimpedance is a convenient diagnostic tool as it is non invasive, rapid and can be repeated frequently. Using this method can avoid sacrificing the animal and permit monitoring data during a study and not only at the end and at the beginning. The aim of this paper is to develop a model for body composition in rats.

2. Materials and methods

This study has been carried on a 40 rats ‘population: 10 males aged 63 days, 10 males aged 70 days, 10 females aged 63 days and 10 females aged 70 days. Males weigh $363.52 \pm 17.11$ grams and females weigh $224.62 \pm 7.13$ grams. Rats have been anesthetized with isoflurane and placed in dorsal decubitus.
In order to measure fat mass (FM), lean body mass (LBM) and bone mineral content (BMC) as reference measurements, we used a scanner GE HiSpeed NX/I Pro. After we have taken the images, we also have to quantify the volume of the tissues. We have converted volumes in masses with density of BMC, LBM and FM which are respectively 1.6667 g.cm$^{-3}$, 1.1364 g.cm$^{-3}$ and 0.9375 g.cm$^{-3}$. These densities have been calculated by samplings.

We used a multifrequency impedancemeter Z-Métrix® (BioparHom®, France) measuring electrical characteristics of the whole body with two electrodes placed on the right hand and two placed on the right foot. This device operates on six 1.5 volt batteries. Injection current is low with 77 µA. Resistances and reactances have been measured at 55 frequencies between 1 and 1000 kHz. The device uses patented equations to calculate the resistance of extracellular compartment (Re) and the resistance of total body compartment (Rinf) using a new model derived from Cole-Cole model. [2]

We chose three methods to collect electrical data and to calculate tissue mass: posterior to posterior leg ("posterior" method), anterior to posterior legs on the right side ("right side" method) and on the left side ("left side" method). Table 1 gives number of animals in the three methods.

### Table 1: Number of animals in the three methods: posterior, right side and left side.

|       | Right side | Left side | Posterior |
|-------|------------|-----------|-----------|
| Male  | 16         | 15        | 14        |
| Female| 13         | 14        | 16        |

Using electrical data and rats’ age and weight, we developed multivariate equations in order to calculate tissue mass with bioimpedance. We based our model on the whole population.

Then FM, LBM and BMC have been calculated, using these equations, in order to compare with reference measurements in a sample of 10 rats from the population.

We used paired Student T-test in order to compare tissue masses obtained by bioimpedance and our reference.

### 3. Results and discussion

Mean values and standard deviations (SD) for FM, LBM and BMC calculated by bioimpedance in population (male and female) with the three methods and measured with the scanner, are summarized in tables 2 to 4.

#### Table 2: FM, LBM, BMC measured by scanner and by bioimpedance in male and female population with method “right side” (in grams).

|       | Scanner Mean ± SD | Bioimpedance Mean ± SD | P. T test |
|-------|-------------------|------------------------|-----------|
| **Male** |                   |                        |           |
| FM    | 41.26 ± 8.54      | 41.27 ± 9.27           | 0.997     |
| LBM   | 222.01 ± 10.25    | 222.05 ± 11.59         | 0.966     |
| BMC   | 36.09 ± 1.35      | 36.11 ± 1.56           | 0.929     |
| **Female** |                  |                        |           |
| FM    | 29.23 ± 3.48      | 29.17 ± 3.92           | 0.910     |
| LBM   | 121.90 ± 7.27     | 121.87 ± 7.80          | 0.971     |
| BMC   | 27.52 ± 0.69      | 27.57 ± 0.69           | 0.597     |
Means and SD of FM, LBM and BMC obtained by bioimpedance and with the scanners are substantially identical whichever population and method. Tissue Mass are not significantly different with P T-test from 0.597 to 0.998.

Then, we are able to measure FM, LBM and BMC in rats by bioimpedance equally as with a scanner. But, we are aware of having validated our calculation model on the population which is used to develop equations.

However, these results aren’t sufficient because we can’t see which method is better in order to estimate tissue mass in small animals.

Table 3: FM, LBM, BMC measured by scanner and by bioimpedance in male and female population with method “left side” (in grams).

|       | Scanner | Bioimpedance | P. T-Test |
|-------|---------|--------------|-----------|
|       | Mean ± SD | Mean ± SD    |           |
| Male  |          |              |           |
| FM    | 40.63 ± 7.32 | 40.63 ± 7.49 | 0.998     |
| LBM   | 212.31 ± 9.14 | 212.30 ± 8.75 | 0.992     |
| BMC   | 35.32 ± 0.98  | 35.30 ± 1.11  | 0.909     |
| Female| Scanner | Bioimpedance | P. T-Test |
|       | Mean ± SD | Mean ± SD    |           |
| FM    | 28.09 ± 3.41 | 27.84 ± 3.56 | 0.829     |
| LBM   | 119.88 ± 2.89 | 120.14 ± 1.77 | 0.857     |
| BMC   | 26.77 ± 0.41  | 26.77 ± 0.48  | 0.788     |

Table 4: FM, LBM, BMC measured by scanner and by bioimpedance in male and female population with method “posterior” (in grams).

|       | Scanner | Bioimpedance | P. T-Test |
|-------|---------|--------------|-----------|
|       | Mean ± SD | Mean ± SD    |           |
| Male  |          |              |           |
| FM    | 42.46 ± 8.67 | 42.35 ± 9.02 | 0.930     |
| LBM   | 212.55 ± 11.32 | 212.69 ± 11.30 | 0.900     |
| BMC   | 35.33 ± 1.41  | 35.41 ± 1.60  | 0.616     |
| Female| Scanner | Bioimpedance | P. T-Test |
|       | Mean ± SD | Mean ± SD    |           |
| FM    | 28.94 ± 3.83 | 28.81 ± 4.15 | 0.809     |
| LBM   | 120.85 ± 7.05 | 120.85 ± 7.33 | 0.989     |
| BMC   | 27.38 ± 0.62  | 27.43 ± 0.67  | 0.577     |

Means and SD of FM, LBM and BMC obtained by bioimpedance and with the scanners are substantially identical whichever population and method. Tissue Mass are not significantly different with P T-test from 0.597 to 0.998.

Then, we are able to measure FM, LBM and BMC in rats by bioimpedance equally as with a scanner. But, we are aware of having validated our calculation model on the population which is used to develop equations.

However, these results aren’t sufficient because we can’t see which method is better in order to estimate tissue mass in small animals.

Figure 1: Example of obtaining error rate for the BMC male, right size with the relation between BMC measured by bioimpedance and BMC estimated by the scanner.
Figure 1 shows that bioimpedance underestimate BMC about 0.02% by scanner with a coefficient of linear regression ($R^2$) of 0.8596. Error rate has been calculated from the difference of director coefficients between the bisector and the linear regression of the population.

Table 5: Error rates (%) and $R^2$ for the two populations calculated with graphics representations, with the three methods.

| Method          | Error rate Male | Error rate Female | $R^2$ Male | $R^2$ Female |
|-----------------|-----------------|-------------------|------------|--------------|
| Right side FM   | 3.29            | 0.29              | 0.601      | 0.739        |
| LBM             | 0.03            | 0.03              | 0.894      | 0.879        |
| BMC             | 0.02            | 0.01              | 0.860      | 0.802        |
| Left side FM    | 0.5             | 0.6               | 0.814      | 0.445        |
| LBM             | 0.01            | 0.03              | 0.959      | 0.729        |
| BMC             | 0.02            | 0.01              | 0.882      | 0.869        |
| Posterior FM    | 0.09            | 0.45              | 0.699      | 0.620        |
| LBM             | 0.03            | 0.02              | 0.903      | 0.704        |
| BMC             | 0.02            | 0.1               | 0.812      | 0.728        |

Table 5 shows error rates and $R^2$, for FM, LBM and BMC, using the same approach of figure 1, for the three methods of measurements. Method “right side” shows error rates between 3.29% for the male FM and 0.01% for the female BMC. Method “left side” lists error rates between 0.6% for the female FM and 0.01% for the male LBM and female BMC. Method “posterior measure” gives error rates between 0.45% for female FM and 0.02% for male BMC and female LBM.

For the three methods, coefficients of linear regression are between 0.445 and 0.903.

With these results, we can see that error rates are lower with the “posterior” method. We can say that this method of measurement is better than right and left side. Moreover, calculation method shows no significantly difference between tissue masses obtained by the reference and assessed by bioimpedance.

Moreover, we are aware that a second population would be interesting to validate clearly our model for assessing BMC, FM and LBM in rats.

4. Conclusions
This study shows that bioimpedance with theses equations can be relevant to assess tissue masses in small animals. Using this model could avoid the animal being sacrificed. Moreover, it’s a quick, non invasive and low cost method in front of the scanner and it can be used in clinical routine by laboratories for example. This study focus on tissue masses but the next step is validation of metabolic and fluids characteristics with the same device.

5. References
[1] Eisen E J, October 1989 Livestock Production Science 23 17-32
[2] Moreno M V; May 2008 tel-00282690 52