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Analysis of Tissue Bioimpedance as a Measurement of Liver Steatosis: Experimental Model in Large Animals

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ABSTRACT

Background. Electrical bioimpedance (BI) has been used to indirectly measure steatosis. This method has not yet been established in the clinics thus experimental studies are needed in big animals. We assessed BI to measure liver steatosis in porcine animals.

Methods. Twelve large-white × Landrace pigs weighing 35 kg were allocated to a study ($n = 9$) and a control group ($n = 3$). A special diet was used to promote steatosis among the study group: methionine deficient and choline-restricted diet that contains supplements of cholesterol, collate and excess of saturated fat. Control group animals were fed a normal diet. A new tetrapolar electrode model was used for BI measurement, which were performed during open laparotomy by inserting a probe into one of the lobes. Measurements were done in the third and fourth segments of the pig liver, placing the probe either on the surface or inserted into the parenchyma of the liver. Open biopsies were obtained at the end of the measurements. Histological samples were processed and stained with hematoxylin-eosin to estimate macrosteatosis. Pearson correlation coefficient between BI and percentage steatosis were calculated at different frequencies.

Results. After 4 months of the special diet all the animals in the study group developed steatosis (90% to 20%), whereas none of the control group was affected. Pearson correlation coefficients between BI and percentage of steatosis were significant (0.877–0.878) with the best correlations obtained with a probe placed on the fourth segment of the liver surface and the best frequency to perform the measurements being 50 and 75 kHz.

Conclusions. BI is an accurate, fast method for steatosis measurements, that is easier and cheaper than either open or needle biopsy.

FATTY LIVER (or liver steatosis) is defined as the proportion of hepatocytes containing fat droplets. In the absence of other liver lesions, it is by itself a relatively benign condition. It acquires relevance in three circumstances: liver transplantation, major liver resections and nonalcoholic steatohepatitis, which may progress to cirrhosis.[1,2] In liver transplantation, macrovesicular steatosis in the graft is a major determinant of outcome.[3] Steatosis of >30% increases the risk of primary nonfunction.[4] It is therefore important to accurately diagnose the grade of donor hepatic steatosis to estimate liver functional reserve.

In Spain in 2009, 380 grafts (26.6% of all potential livers) were rejected for transplantation an increasing trend over the past 5 years. The main cause of liver graft discard has

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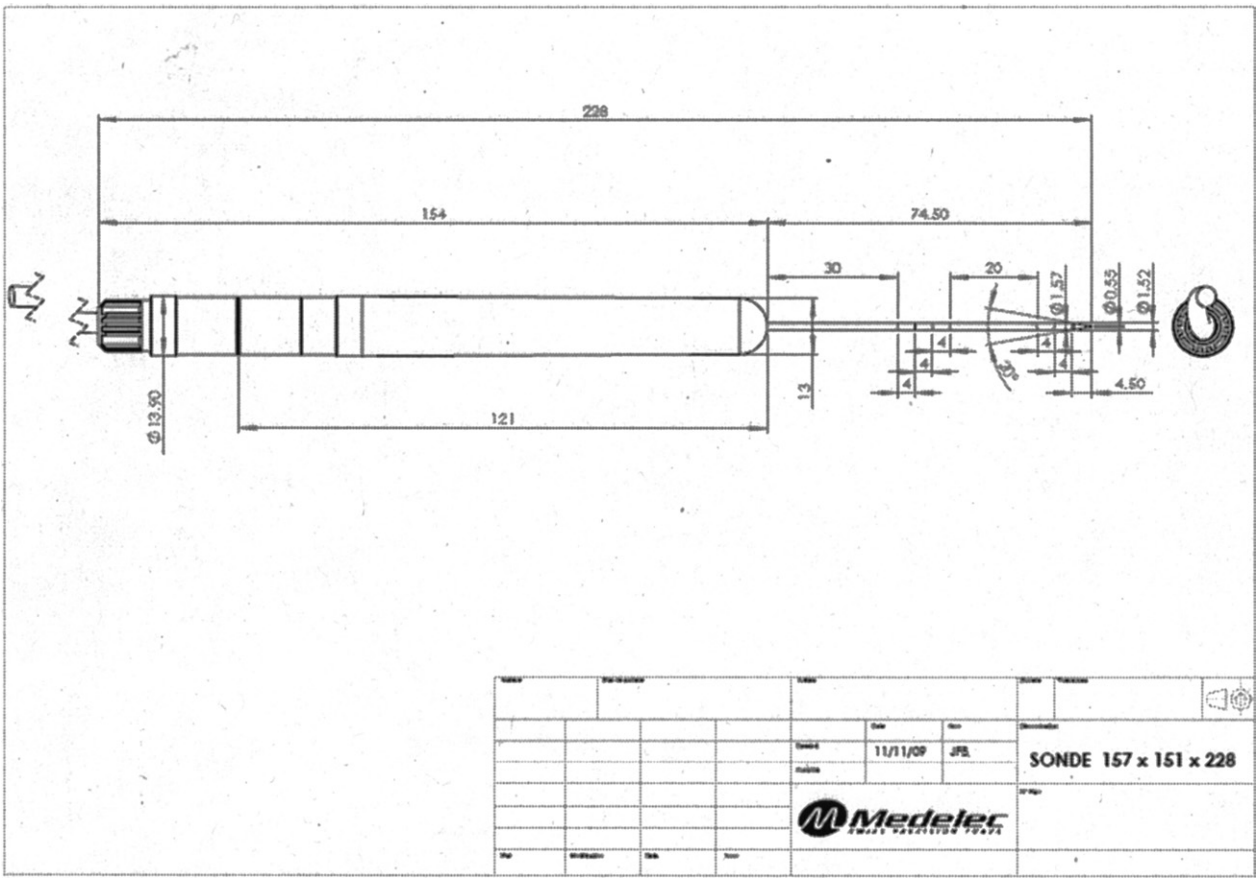

Fig 1. New model of tetrapolar electrode.

been a pathological liver (91%), with the more frequent causes being steatosis ($n = 158$) or macroscopic appearance ($n = 78$).[5] In deceased donor liver transplantation, visual assessment of steatosis performed by the surgeon is inaccurate as well as neither being sensitive nor specific. Liver biopsy is time-consuming; a pathologist must be on call. In living donor liver transplantation, some groups use a biopsy to evaluate the liver prior to transplantation.[6] Liver biopsy remains the "gold standard" to evaluate histological abnormalities in most forms of liver diseases.[7] Laboratory analysis can only indicate to this pathology. Ultrasound is probe-dependent and computed tomography or magnetic resonance imaging are less precise than biopsy as they only provide a qualitative diagnosis.[8]

Since the beginning of the last century, impedance to an electrical current (BI) through living tissues has been used to monitor a range of physiological events.[9,10] Resistivities or BI of different tissues depend on composition and intrinsic tissue characteristics of water content, pH, temperature, vascularization, and so on. Adipose tissue is a poor conductor of current compared with other tissues. Fat infiltration promotes differences in BI measurements.[11] Animal models have been used to assess these data but most of the previous models have been designed for small animals,[12] due to the difficulty to produce liver steatosis in large animals.[13,14]

METHODS

Animals

We used a model of steatosis in large animals, 12 pigs Large White × Landrace that weighed 25 kg: Nine in the study group were fed a special steatotic diet that was methionine-deficient and choline-restricted containing supplements of cholesterol, collate and excess saturated fat. Three animals in the control group were fed a conventional diet. An ultrasound-guided needle biopsy was performed to confirm the presence of steatosis. Thereafter BI measurements and an open biopsy were performed sequentially.

Experimental Procedures

General anesthesia was induced and maintained with propofol. The abdomen was opened through a middle line laparotomy. BI measurements were obtained in the third and fourth segments of the pig's liver[15] by placing a probe on the surface of every segment as well as inserted into the parenchyma. Open biopsies were obtained at the end of the procedure. All the procedures were performed under the European Union guidelines for the handling and care of laboratory animals and with the approval of our Ethics Animals Committee.

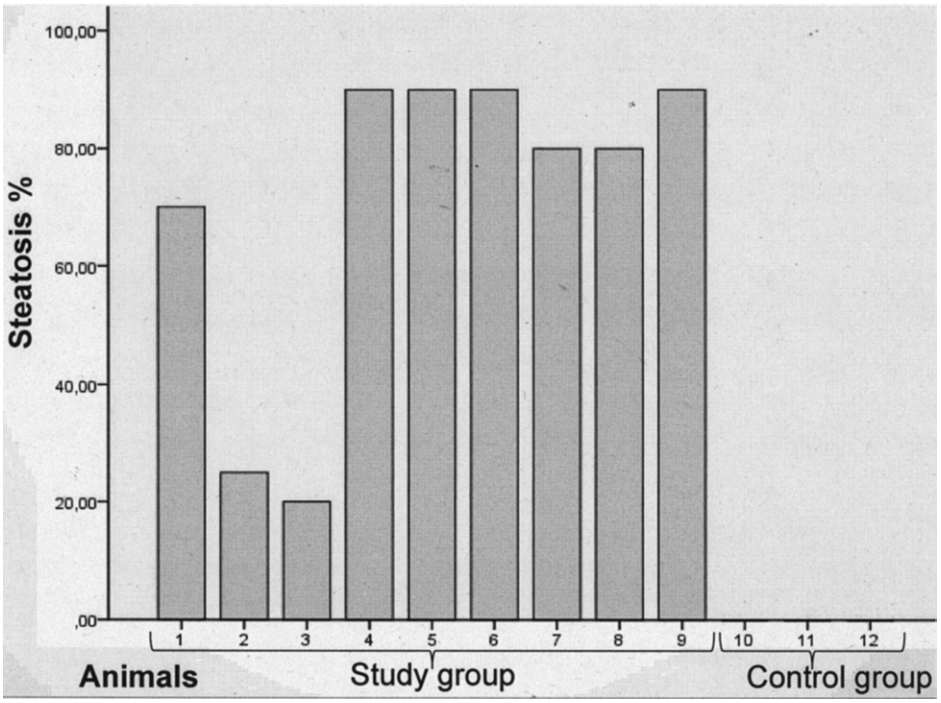

Fig 2. Steatosis data in study and control groups.

Histological samples were processed by fixing tissue in formaldehyde and embedding in paraffin. Hematoxylin-eosin staining was used to assess the degree of steatosis as a percentage by an experienced liver pathologist, who was blinded with regard to the BI readings.

Bioelectrical Impedance

A new tetrapolar electrode model (Fig 1) was used for BI measurements. The probe consists of a linear array of four electrodes in a double circuit coupled along a needle. The needle tip is round. A microscope and laser techniques were used to solder small wires to the electrodes placed inside the needle (1.52 mm diameter). The outer circuit applies the current and the inner measures the tension. The inner circuit spans 2 cm, long enough to measure a significant portion of the parenchyma. The probe was built by Medelec Minimeca S.A. (Puidoux-Gare, Suisse 1070, Switzerland). The electrode is connected to a multifrequency analyzer Imp SFB7 (Impedimed, 50 Parker Court, Pinkenba, QLD 4008 Australia). The Impedimed SFB7 analyzer measures BI among a broad spectrum of 256 current frequencies. We analyzed only the 10% of frequencies that we employed for the measurements.

Statistical Analysis

Results for weight and steatosis were presented as mean values and standard deviations. Bivariate correlations were analyzed using Pearson correlation coefficients (PCC). A pearson correlation coefficient was calculated for each one of the 21 studied frequencies. All statistical analyses were performed using. The Statistical Package for the Social Sciences version 17.0 (SPSS Inc. Chicago, Ill, USA).

RESULTS

The mean initial average weights of the animals were 29.14 ± 5.97 kg in the study and 21.33 ± 3.59 kg in the control group. The final weights after 4 months of feeding were 37.69 ± 8.4 kg in the study and 111.66 ± 7.63 kg in the control group.

The percentage of steatosis at laparotomy after 4 months ranged from 20% to 90%, (mean = 64.16 ± 36.15) in the study versus none in the control group (Fig 2).

Table 1. Correlations Between Bioelectrical Impedance and Steatosis at 21 Different Frequencies of the Current (Probe Placed on the Liver Surface, Fourth Segment)

Frequency	Pearson Correlation	*P*
3.084	0.848	.000
4.028	0.851	.000
5.067	0.855	.000
6.074	0.856	.000
7.113	0.859	.000
8.151	0.859	.000
9.127	0.860	.000
10.23	0.861	.000
15.01	0.866	.000
25.33	0.872	.000
50.01	0.877	.000
75.25	0.878	.000
101.1	0.873	.000
152.1	0.854	.000
204.2	0.832	.001
300.4	0.791	.002
403.5	0.752	.005
506.2	0.716	.009
711.5	0.627	.029
913.2	0.524	.08
1000	0.491	.105

Best correlations were at 50.01 and 75.25 kHz.

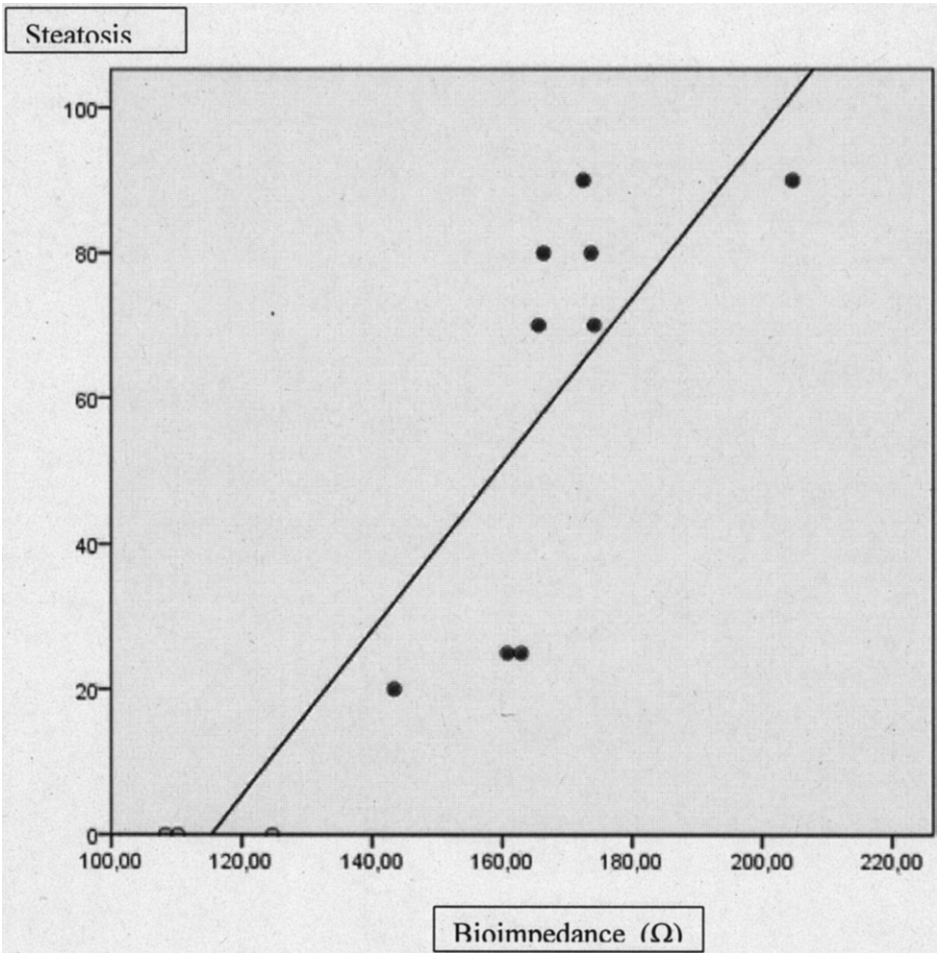

Fig 3. Correlation of bioimpedance and steatosis (probe placed on the liver surface, fourth segment, at 50.01 kHz).

All the correlations were significant at low frequencies (Table 1), the most significant at 75.25 kHz. The best correlations of steatosis and BI were obtained when the measurements of BI were performed in the fourth segment by placing the probe over the liver surface (Table 1). We observed good correlations with the probe on the liver surface of the fourth segment at 50 and 75 kHz (Figs 3 and 4). Pearson coefficient was close to 0.9 ($P < .000$).

DISCUSSION

Experimental liver steatosis is difficult to achieve in large animals without the use of direct cellular toxins such as alcohol. Large-animal models allow us to test new instruments and techniques that can be used in a clinical setting; thus, this model may be useful for clinical studies. The method we designed to promote steatosis was effective, promoting a high degree of steatosis in the porcine liver. Only two cases showed less than 20% fat infiltration, but they were due to initial troubles with diet accommodation in the initial animals.

BI has been used for fat quantification in the liver. Many probes have been described; almost every author has his own design but none of the probes has been designed for use in large animals nor in the human setting. Our instrument is capable of both things; furthermore, it is cheap and portable. Using our method, we obtained a significant correlation between BI and steatosis that was more significant that reported by other authors.[16] BI measurements seemed to be a precise method to measure steatosis.

Few studies have analyzed the characteristics of current frequency for the measurements, they have only suggested that low frequencies were best. Our study sought to precisely define the frequency.

The setting for the measurement itself is an important issue. Our needle electrode measures BI of significant portions of the liver, although proper placement of the probe (mainly far from large vessels) is mandatory. We observed that the most significant measurements were obtained by placing the electrode over the liver surface. This method was affected by the thickness of the lobe and the anatomy of the porcine liver hepatic veins that flow along the axis of the lobe, suggesting that our needle electrode can be used in the wider human liver.

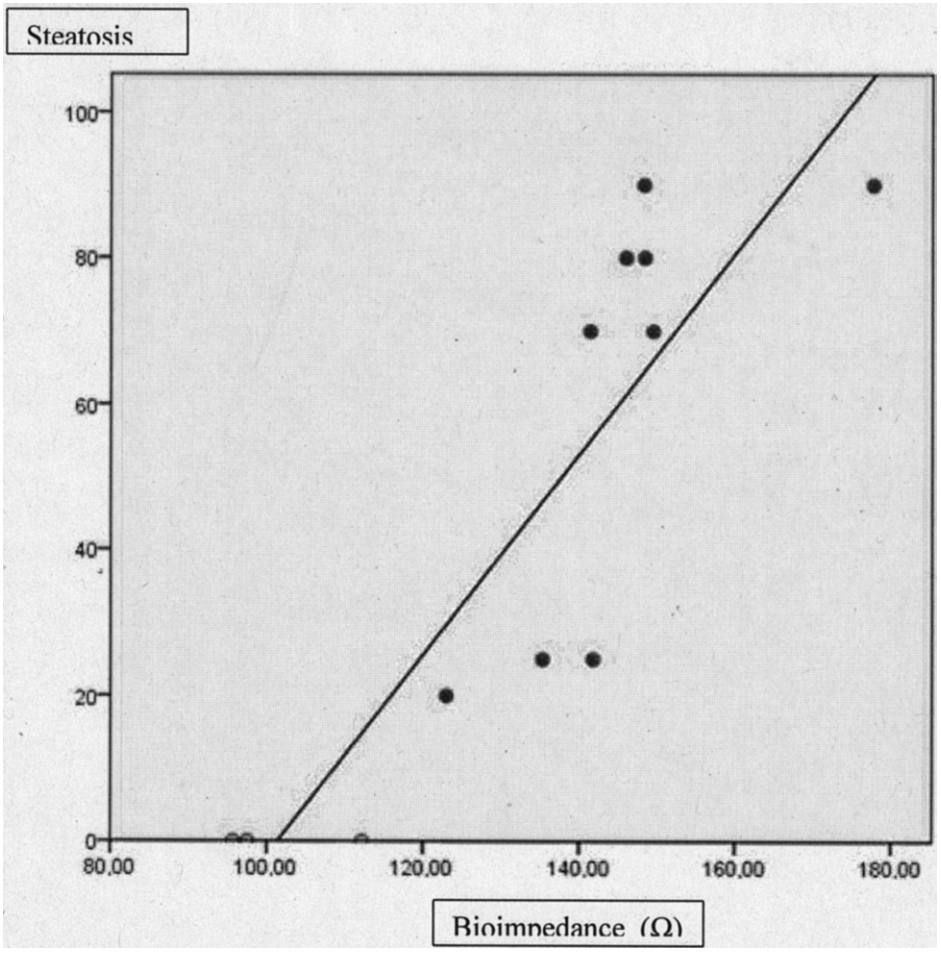

Fig 4. Correlation of bioimpedance and steatosis (probe placed on the liver surface, fourth segment, at 75.25 kHz).

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