Management of adreno-cortical adenomas using microwave ablation: study of the effects of the fat tissue

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ABSTRACT

Background and objectives: Adrenocortical neoplasms are the main causes of secondary hypertension and related comorbidities including hypokalemia and cardiovascular diseases. Conventional techniques for the management of this condition are often invasive and not resolutive. Recent studies proposed microwave thermal ablation (MWA) to eradicate adrenocortical adenomas arising in proximity to sensitive structures. This study explores a new MWA approach to selectively direct the electromagnetic energy into the target and shield the surrounding tissues. The new solution relies on the anatomical and dielectric characteristics of the adrenal gland and the surrounding fat capsule.

Methods: A 3D model of the adrenal gland is developed, and a cooled microwave applicator is placed parallel to the interface between the fat and adrenal tissue. Numerical simulations are conducted at 2.45 GHz accounting for two energy delivery settings, two orientations of the applicator and blood perfusion of the tissues. Ex vivo and in vivo ablation procedures are conducted on ovine adrenal glands. Histology analysis completes the experimental studies.

Results: Numerical results show asymmetric ablation profiles in ex vivo and in vivo conditions. The asymmetry ratio is influenced by the procedure settings and orientation of the applicator. Ablation zones obtained experimentally agree with those predicted by the numerical simulations. Histology analysis confirms irreversible cellular changes only in the adrenal tissue close to the applicator.

Conclusions: The outcomes show that the dielectric contrast between the fat layer and tissue target can be a tool in MWA to shape ablation zones to protect the surrounding structures from excessive temperature increases.

1. Introduction

Abnormalities arising from adrenal glands, such as unilateral adenomas and bilateral hyperplasia are the primary causes of Primary Aldosteronism (PA). PA is responsible for secondary hypertension, which is currently recognized in approximately 11% of the hypertensive population worldwide [1–3]. Besides hypertension, PA is responsible for other comorbidities including hypokalemia, ventricular hypertrophy, and cerebrovascular ischemic events [4–6].

Adrenalectomy and pharmacotherapy are the gold standard techniques typically used to treat unilateral adenoma and bilateral hyperplasia, respectively [7–12]. Both techniques present limitations. Adrenalectomy is generally an invasive and challenging procedure given the position of the adrenal glands in the abdominal cavity. Figure 1 shows that adrenal glands are surrounded by sensitive structures, e.g., inferior vena cava, abdominal artery, renal arteries, diaphragm, kidneys, and esophagus. As a result, adrenalectomy is a procedure at high risk of damaging those structures and causing hemorrhage [10,13]. Pharmacotherapy is a life-long therapy whose side effects, such as gynecomastia, menstrual disorders, mastodynia, and obesity-related insulin resistance [14] are poorly tolerated.

Microwave thermal ablation (MWA) has been recently investigated to address some of the above-mentioned limitations [15,16]. MWA is an electromagnetic-based thermal technique designed to work at selected ISM frequencies in the microwave frequency range (500 MHz–10 GHz). The technique relies on the interaction between the electromagnetic field (EMF) and the biological tissues to induce cytotoxic temperatures (>55 °C) in the target tissue [17,18]. In the last decades, MWA has been used for treating relatively large tumors (diameter > 30 mm) [19–22]. This is because of the capability of the microwaves to pass through biological tissues regardless of the increase of the tissue’s impedance during the procedure [18,23,24].

Recently, MWA at 2.45 GHz has been also investigated to treat small critical targets such as adrenal neoplasms (<20 mm diameter) in patients who refuse surgical procedures or are not surgical candidates [25–27]. Promising performances have been observed in recent clinical studies. However, further investigations are required to have a comprehensive understanding of the feasibility of MWA for managing PA.
The risk of damaging organs surrounding the adrenal glands remains a concern (Figure 1).

To face this challenge, most recent studies have combined the MWA technique with the infusion of a saline solution to create artificial ascites between the tumor target and the surrounding structures [25,27]. Successful results are observed only where separation of at least 5 mm is ensured during the MWA procedure. Also, the necessity to use a further catheter for the injection of the liquid enhances the invasiveness of the treatment [15,16,25]. Another alternative technique recently explored is based on the use of reflector-based antennas to create directional heating patterns [28]. It should be mentioned that the diameter of this type of applicator must be increased in order to accommodate the reflector. Also, reflector-based antennas need an accurate orientation to achieve satisfying results. This requirement could be challenging to apply in a typical clinical scenario with 2D-image guidance.

The dielectric properties characterized in animal adrenal models [29,30] combined with the well-known anatomy of the adrenal glands suggest that a natural dielectric contrast exists between the fat capsule of the gland and its functional tissues. This contrast in the biophysical properties of the tissues provides the substrate for directing the electromagnetic field toward a preferred direction, as demonstrated in [31–34].

In this study, the main goal is to explore how the geometrical characteristics of the adrenal gland, the orientation of the antenna and the blood perfusion may influence the capability of the fat layer to limit the temperature increase in the surrounding healthy tissues. To this end, numerical and experimental studies have been conducted considering both ex vivo and in vivo conditions. In the following sections, the methods and results for the ex vivo and in vivo studies are described separately in Section Materials and methods and Section Results. For both studies, histology analysis has been performed to evaluate the changes in the cellular structure of the tissue target and the integrity of the surrounding structures tissue after the MWA procedure.

2. Materials and methods

2.1. Computational models and numerical simulations

2.1.1. Without blood perfusion

Figure 2 shows a three-dimensional (3D) model representing a pyramidal-shaped adrenal gland and the position of the gland on the top of the kidney. First, a pyramidal shell is designed within a three dimensional (3D) full-wave electromagnetic software (CST MWS Suite 2018, Darmstadt, Germany). The shell is scaled to reproduce the dimensions of the medulla (inner shell) and the cortex (outer shell) [35]. The geometries representing the medulla and cortex are 13 mm and 24 mm in height, 40 mm and 60 mm in width, 20 mm and 30 mm in thickness. A fat capsule envelope the modeled adrenal tissues. The dimensions of the fat capsule are 45 mm in height, 108 mm in width and 30 mm in thickness. A fat capsule envelope the modeled adrenal tissues. The dimensions of the fat capsule are 45 mm in height, 108 mm in width and 30 mm in thickness. A fat capsule envelope the modeled adrenal tissues.
and 33 mm in thickness) in agreement with [31]. Table 1 lists dielectric and thermal properties assigned to each tissue of the model. For the cortex and medulla, the dielectric properties at the operating frequency of 2.45 GHz reported in [30] are loaded into the material library of the CST MW Studio software. The dielectric properties of fat and kidney as well as the values related to the specific heat capacity, thermal conductivity and density of each material are acquired from the literature [36] and manually loaded into the material settings of the simulation software.

The cooled coaxial MW applicator (outer diameter = 1.2 mm, inner diameter = 0.3 mm) described in [32] is modeled and positioned at the boundary between fat and cortex. Two different orientations of the MW applicator are considered: Orientation #1 and Orientation #2 (Figure 2). In the case of Orientation #1, the angle between the longitudinal axis of the applicator and the y-axis of the adrenal model approximates 90°. For Orientation #2, the longitudinal axis of the applicator is approximately parallel to the y-axis; for this configuration, the geometry was rotated at −45° with reference to the z-axis.

The entire geometry is Discretized in order to have a finer mesh in the regions characterized by the smallest size, (i.e., within the antenna) and a coarser mesh in the homogeneous tissue. First, a minimum meshing size is considered (i.e., ten cells per wavelength in the tissue model). The number of cells per wavelength is then increased until two consecutive simulations show a discrepancy in the amplitude of the reflection coefficient lower than 0.1% (Cauchy convergence test) [37]. A total of 11,856,000 tetrahedral cells ranging between 0.04 mm and 0.4 mm discretise the entire geometry. Because of the excessive computational load, only half of the three-dimensional model is used to compute the electromagnetic and thermal simulations (Figure 2). In this configuration, the number of meshing cells is reduced to 6,278,052 and the computational time decreases accordingly. CST MW studio software is used to complete coupled electromagnetic and thermal simulations. First, the software solves the Helmholtz electromagnetic wave equation reported by Equation (1).

$$\nabla^2 \mathbf{E} - k_0^2 \left( \varepsilon_r - \frac{j\sigma}{\varepsilon_0} \right) \mathbf{E} = 0$$

(1)

$\mathbf{E}$ is the electric field vector, $k_0$ ($m^{-1}$) is the propagation constant in the free space, $\omega$ (rad s$^{-1}$) is the angular frequency, $\mu_0$ is the permeability of free space, $\varepsilon_0$ is the relative permittivity in free space, $\varepsilon_r$ and $\sigma$ ($S m^{-1}$) are the relative permittivity and the effective conductivity of the tissue. For the

| Table 1. Tissue dielectric and thermal properties were employed in the numerical simulations. |
|---------------------------------------------------------------|
| **Parameter** | **Fat** | **Cortex** | **Medulla** | **Kidney** |
| Relative permittivity, $\varepsilon_r$ | 8.7 | 45.2 | 52.0 | 52.7 |
| Effective conductivity, $\sigma_{\text{eff}}$ (S m$^{-1}$) | 0.1 | 1.6 | 1.8 | 2.43 |
| Specific heat capacity, $c$ (J kg$^{-1}$ K$^{-1}$) | 2065 | 3425 | 3425 | 3763 |
| Density, $\rho$ (kg m$^{-3}$) | 909 | 1030 | 1030 | 1049 |
| Thermal conductivity, $k$ (W m$^{-1}$ K$^{-1}$) | 0.2 | 0.4 | 0.4 | 0.5 |
| Frequency (GHz) | 2.45 | | | |

Figure 2. Three-dimensional geometry for modeling each functional tissue (i.e., cortex and medulla) of the adrenal gland and the surrounding fat layer. Symmetry planes ($z = 0$) are used for Orientation #1 and Orientation #2 to reduce the computational load for the electromagnetic and thermal simulations. Along with the global coordinates system (x, y, z), the local coordinates system (u, v, w) is reported to better visualize the orientations (Orientation #1, Orientation #2) of the MW applicator with respect to the adrenal gland model.
electromagnetic simulation, the specific absorption rate (SAR) distribution is calculated according to Equation (2). SAR defines the power transferred in an infinitesimal volume of tissue divided by the mass of the object.

$$\text{SAR} = \frac{\sigma |E|^2}{2\rho}$$  \hspace{1cm} (2)

$|E|^2$ is the intensity of the electric field (V m$^{-1}$) and $\rho$ (kg m$^{-3}$) is the density of the tissue. Then, Pennes’ bioheat equation described by Equation (3) is used to calculate the resulting temperature distribution in the tissue.

$$\rho c \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + \rho Q + \frac{m_b p_b c_b}{C_1} (T - T_b),$$  \hspace{1cm} (3)

$T$ (°C) is the temperature, $t$ (s) is the time, $k$ (W m$^{-1}$ °C$^{-1}$) is the thermal conductivity of the biological tissue, $Q$ (W kg$^{-1}$) indicates the metabolic heat generation rate, $m_b$ (m$^3$ kg$^{-1}$ s$^{-1}$) is the blood mass perfusion rate, $c_b$ (J kg$^{-1}$ °C$^{-1}$) is the specific heat capacity of the blood, $p_b$ (kg m$^{-3}$) is the density of blood, $T_b$ (°C) is the temperature of the blood. Equation (3) shows the factors that determine the increment of temperature other than the heat induced by the external sources (SAR). Those factors include the heat transfer through the tissues ($\nabla \cdot (k \nabla T)$), the heat developed by the metabolic processes ($\rho Q$), and the heat dissipated because of the blood flow through the tissues ($\frac{m_b p_b c_b}{C_1} (T - T_b)$) [38]. For the ex vivo condition, the contribution of the metabolic heat and blood perfusion are neglected.

The initial temperature of the tissue model is set at 25 °C for consistency with ambient temperature in the case of ex vivo conditions. Boundary conditions described by Equation (4) are used to model the convection mechanism between the tissue model and the surrounding environment.

$$-k \frac{\partial T(r)}{\partial n} \bigg|_{S_n} = h \left(T(r) - T_e\right)$$  \hspace{1cm} (4)

$n$ is the vector perpendicular to the boundary of the surface $S$, $r$ is the vector indicating the position in the space domain, $h$ (W m$^{-2}$ °C$^{-1}$) is the convection coefficient and $T_e$ (°C) is the temperature of the surrounding environment. The convection coefficient in the boundary conditions is set equal to 20 (W m$^{-2}$ °C$^{-1}$) to account for the air-free convection between the tissue model and the surrounding medium [39]. The dielectric properties of the air ($\varepsilon_a = 1, \sigma = 0$) is set as the background material, which is representative of the ex vivo conditions of the experimental scenario.

Convective boundary conditions are also applied between the external surface of the applicator and the tissue. Here, the convection coefficient $h$ is set equal to 1000 (W m$^{-2}$ °C$^{-1}$) to account for the forced convection induced by the cooling system [39].

Two different input power levels are used in the electromagnetic-thermal simulations: 30 W and 60 W both for 60 s. The values obtained from each simulation were exported and analyzed in MATLAB (R2017a, The MathWorks, Inc., Natick, MA, US).

### 2.1.2. With blood perfusion

The 3D numerical model used in the previous study (Figure 2) is adopted in the numerical study accounting for the in vivo conditions. The same thermal and dielectric properties reported in Table 1 characterize each tissue. Blood instead of air is assigned as the background material to better represent the in vivo scenario. The values of the thermal and dielectric properties for blood are acquired from [36].

Rectangular functions are used to model the blood perfusion both in the adrenal gland and in the fat tissue against the temperature. In particular, when the temperature exceeds 55 °C, the values of blood perfusion drop to zero in order to account for the collapse of the blood supply in the area of the tissue under treatment [40]. Two different volumetric blood flow rates are assigned to each tissue according to [41]: 5·10$^{-7}$ m$^3$ kg$^{-1}$ s$^{-1}$ for the fat tissue and to 4·10$^{-5}$ m$^3$ kg$^{-1}$ s$^{-1}$ for adrenal tissues.

The cooled triaxial MW applicator (outer diameter = 0.584 mm, inner diameter = 0.127 mm) described in [33] is modeled and positioned at the boundary between fat and cortex. The triaxial applicator allows for more efficient delivery of the EM power in the tissue, reducing the return of the current flow along the cable. This current could cause the applicator to overheat, resulting in cable failure. The third metallic cable also provides the inbound water flow of the cooling system. An external polyamide tube is modeled to account for the outward water flow.

The entire geometry is Discretized by a total number of tetrahedral meshing cells equal to 5,085,045 ranging from 0.1 mm to 6.1 mm in the case of Orientation #1. The total number of meshing cells is equal to 3,876,880 ranging from 0.1 mm to 6.4 mm, for Orientation #2. Convergence criteria consistent with the ex vivo study are adopted.

Electromagnetic and thermal simulations are performed using CST MW Studio to obtain the distributions of SAR and temperature. Boundary conditions are modeled as described for the ex vivo numerical scenario. The initial temperature of the tissue model is set equal to 37 °C to reproduce the initial body conditions of the in vivo scenario. Consistent with the previous study, two different input power levels are supplied for 60 s for each orientation of the MW applicator (30 W and 60 W).

### 2.2. Experimental validations

#### 2.2.1. Ex vivo setup and procedure

Microwave ablation experiments are conducted on a total of six ($N = 6$) ex vivo ovine adrenal glands with the objective of assessing the validity of the computational models. The glands are obtained from a local abattoir together with the corresponding kidneys and the surrounding fat. Each adrenal gland is then dissected in our laboratories, within one hour of excision. Figure 3 provides an overview of the experimental setup and the related schematization of each component included in the setup.

The temperature of the material under test is measured using an infrared thermometer (Fluke 62 Max IR Thermometer, −30°C − 500°C temperature range, 1.5°C accuracies at temperature ≥ 0°C). The fully cooled monopole applicator is placed between the surface of the gland and the periadrenal fat. In agreement with the numerical study, two different power
settings are supplied for a duration of 60 s: 30 W and 60 W. A total of six ablations are performed (one for each adrenal gland): three applying 60 W for 60 s and three applying 30 W for 60 s. The input power is supplied at 2.45 GHz by a microwave generator (Sairem, SAS, France) connected to the MW applicator through a low-loss coaxial cable. A peristaltic dispensing pump (DP2000, Thermo-Fisher Scientific Inc., Waltham, Massachusetts, US) is connected to the inner and outer tube of the applicator. A continuous water flow at a flow rate of 50 ml/min is ensured for the duration of the microwave heating. A temperature of approximately 10°C was maintained in the thermal bath to ensure a temperature of the cooling water within 16–18.5°C during the procedure [42].

In five out of six experiments, two fiber optic sensors (Neoptix Inc., Québec, CA) are placed in the adrenal and fat tissues along the radial direction from the antenna feed of the applicator. The thermal sensors system is connected to a laptop equipped with a dedicated MATLAB code for automatic temperature recording.

Immediately after the MWA procedure, each tissue is processed to conduct hematoxylin and eosin staining (H&E). The tissue embedded into the paraffin block is cut into sections of 5 μm thickness. A digital scanner (Olympus VS120-55, Hamburg, DE) and the integrated software (Olympus VS-ASW-S6) are used for the visualization of the histology images. At the end of each scanning, the images are exported and then examined with a dedicated program (OlyVIA v3.2.1, 64 bit Installer, Olympus, Tokyo, JP). In this study, the histology analysis helped to qualitatively assess the thermal injury of the tissue after the ablation procedure and verify the integrity of the structures surrounding the ablation site, i.e., blood vessels.

2.2.2. In vivo setup and procedure
An acute animal study is conducted at the Hospital Virtual Valdecilla (HvV), Santander, Cantabria, Spain, according to the approved protocol (National Regulatory Body approval and HvV Ethical Committee authorisation). An adult 20 kg sheep is used to test the ablation hypothesis in vivo on the left adrenal gland. The animal is positioned on dorsal recumbency and anesthetised with Ketamine (15–35 mg/kg) and xylazine (5 mg/kg). The anesthesia is maintained by halothane or isofluorane for the duration of the procedure. To access the adrenal gland, a ventral midline celiotomy is executed and the triaxial MW applicator is placed in direct contact with the surface of the adrenal gland beneath the adrenal layer. The use of a triaxial antenna makes access to the adrenal gland easier and less invasive due to the smaller diameter compared with the monopole antenna used in the ex vivo study. Figure 3 shows the positioning of the MW applicator adjacent to the adrenal gland and the surrounding fat; the figure also shows a schematization of the experimental setup.

The vital parameters of the animal are monitored to confirm the status of the animal prior to the procedure. Input power set at 30 W is supplied for 60 s from a microwave generator (Sairem, SAS, France). The generator is connected to the applicator through a low-loss coaxial cable and an SMA connector. A cooling system including a peristaltic pump (DP2000, Thermo-Fisher Scientific Inc., Waltham, Massachusetts, US) is connected to the MW applicator and ensures continuous water at 16–18 °C via a flow rate of 50 ml/min. During the procedure, analgesics (fentanyl citrate 0.05 mg/kg or nalbuphine hydrochloride 2 mg/kg) and tranquilizer (diazepam or midazolam at 2 mg/kg) are administered. The animal is euthanised after the procedure with intravenous pentobarbital (65–100 mg/kg), and the adrenal gland is removed to be visually inspected. A tissue section of 40 mm in length, 32 mm in width and 17 mm in thickness is histologically analyzed within 48 h after the procedure. The H&E staining is used to enable the histology analysis through the light microscope (Zeiss Axio Observer, Oberkochen, DE).

3. Results
3.1. Numerical simulations
3.1.1. Without blood perfusion
Figures 4 and 5 maps the distributions of SAR (A and B) and temperature (C and D) obtained in the 3D model at
Figure 4. Maps of SAR (A and B) and of temperature (C and D) were obtained in the 3D model at 30 W and 60 W considering the applicator aligned at the interface between fat and adrenal gland as depicted in Figure 1 (Orientation #1). Profiles of SAR and temperature are presented both in the frontal plane (xy-plane) and in the coronal plane (xz-plane) with reference to the feed of the antenna.

Figure 5. Maps of SAR (A and B) and of temperature (C and D) were obtained in the 3D model at 30 W and 60 W considering the applicator aligned at the interface between fat and adrenal gland as depicted in Figure 1 (Orientation #2). Profiles of SAR and temperature are presented both in the frontal plane (xy-plane) and in the coronal plane (xz-plane) with reference to the feed of the antenna.
30 W–60 s and 60 W–60 s without considering the influence of tissue blood perfusion. The maps refer to Orientation #1 (Figure 4) and Orientation #2 (Figure 5) of the MW applicator with respect to the boundary between fat and the adrenal cortex. SAR, normalized to the maximum and expressed in decibel (dB), and thermal profiles at 60 s are depicted in the frontal (A and C) and coronal (B and D) planes. The isothermal contour at 55 °C indicates the temperature threshold suitable to achieve instantaneous coagulation (i.e., ablation zone). The isothermal contour at 90 °C indicates the temperature at which vaporization in the tissue may occur.

In the adrenal gland values of SAR exceeding −20 dB extend along the radial distance from the axis of the applicator up to 5 mm at 30 W and up to 7 mm at 60 W. In the fat tissue, values of SAR higher than −20 dB extend no further than 3 mm independent of the input power.

Table 2 lists the radial (R) and longitudinal (L) dimensions of the area marked by 55 °C isothermal contours for the two different orientations of the applicator. This area will be indicated as ‘ablation zone’. The figures shown in Table 2 are details zoomed from Figures 4 and 5. The values reported in Table 2 refer to the portions of these ablation zones that include only the adrenal tissues and not the surrounding fat.

At 30 W, results show a relatively small and spherical ablation zone in the fat layer for both orientations of the MW applicator. In the adrenal tissue, the ablation zone is bigger than in fat as expected. This is because of the higher electrical conductivity of the adrenal tissues compared to fat which determines the higher deposition of SAR (Equation (2)). At 60 W, the ablation zones in the fat and adrenal tissues become visibly larger and more elongated compared with the case of low power. Thus, the ability of the fat layer to shield the electromagnetic field weakens with the increase of the input power. It is worth noting that the 3D model has an impact on the longitudinal dimension of the ablation zone more than on the radial dimension. In particular, a percentage difference of up to 53% is observed in the longitudinal dimension of the ablation zone between Orientation #2 and Orientation #1. Finally, it can be noted that the kidney is unaffected by the increase in temperature for both antenna orientations and both input power settings adopted.

### Table 2. Radial and longitudinal extents of the ablation zone were indicated by the isothermal contour at 55 °C and evaluated at 30 W (first line) and at 60 W (second line) both delivered for 60 s in the 3D model for each orientation of the MW applicator.

| Input power | Orientation #1 | Orientation #2 |
|-------------|----------------|----------------|
|             | Radial dimension (mm) | Longitudinal dimension (mm) | Radial dimension (mm) | Longitudinal dimension (mm) |
| 30 W        | Adrenal 5 Fat 2        | Adrenal 15 Fat 4        | Adrenal 5 Fat 3        | Adrenal 7 Fat 3        |
| 60 W        | Adrenal 7 Fat 5        | Adrenal 17 Fat 21       | Adrenal 8 Fat 5        | Adrenal 20 Fat 13      |

*For Orientation #1, the final longitudinal dimensions of the ablation zones in the region from z = 0 to the central axis of the applicator (see Figure 4 and Figure 5) are 21 mm (30 W) and 27 mm (at 60 W). The values reported in Table 2 refer to the portions of these ablation zones that include only the adrenal tissues and not the surrounding fat.
perfusion is considered both for fat and adrenal tissues to better represent in vivo conditions. The numerical results show that the extent of the values of SAR higher than 
-20 dB is comparable to those observed in the "ex vivo" numerical scenario both in the adrenal layers and fat.

Table 3 lists the radial and longitudinal dimensions of the ablation zones achieved in the adrenal tissues and in the fat layer for the two different orientations of the MW applicator both at 30 W and 60 W. As well as Table 2, also Table 3 shows the details of the ablation zones from Figures 6 and 7. It can be observed that in the fat layer the radial dimensions of the ablation zone remain unvaried with the change in the orientation of the MW applicator. In the adrenal gland Orientation #1 and Orientation #2 differ by 63% at 30 W and 31% at 60 W. As a result, the ablation zones obtained in the case of Orientation #1 are shorter compared to those observed in the case of Orientation #2. In addition, at a lower input power, the ablation zone in fat is more spherical than that observed at a higher power level. In particular, the ablation zone increases in the longitudinal dimension by 183% in the case of Orientation #1 and 280% for Orientation #2, increasing the input power from 30 W to 60 W.

### 3.2. Experimental validations

#### 3.2.1. Ex vivo scenario

The shielding effect caused by the presence of the fat layer at the interface between the adrenal and cortical tissues is investigated experimentally on N = 6 ex vivo ovine adrenal glands surrounded by the adrenal fat capsule. Figure 8 shows an example of an ablation zone achieved ex vivo in an ovine adrenal gland. A shallow coagulation area is marked by the whitening effect of the tissue resulting from the MWA procedure. The figure also shows that the applicator is placed at the interface between the adreno-cortical tissue and the surrounding fat. It should be noted that the 3D model provides a reliable representation of the rounded shape characterizing ovine adrenal glands [31].

MWA procedures conducted on ex vivo adrenal glands yielded ablation zones of 4 ± 2 mm in radial dimension and 14 ± 2 mm in longitudinal dimensions at 30 W and of 8 ± 2 mm and 16 ± 2 mm in radial and longitudinal dimensions respectively at 60 W.

Figure 9 shows the temperature values acquired by the fiber optic sensors. The data refer to 30 W (A) and 60 W (B) input power and two time-steps (30 s and 60 s). The data is presented as a function of the radial distance from the longitudinal axis of the MW applicator. A temperature threshold equal to 55 °C is highlighted to assess the extension of the coagulation necrosis [18,43]. The experimental values are compared with the values achieved in the numerical model, considering the same settings. Figure 9 also provides a comparison between the two different orientations of the MW applicator with respect to the interface between the fat and the adrenal gland.

#### 3.2.2. In vivo scenario

The findings of the numerical study inform the experimental protocol designed for the in vivo study. The low input power...
Maps of SAR (A and B) and of temperature (C and D) were obtained in the 3D model considering the “in vivo” numerical scenario at 30 W and 60 W. Consistent with the ex vivo scenario, the applicator is aligned at the interface between fat and adrenal gland as described for Orientation #2. Profiles of SAR and temperature are presented both in the frontal plane (xy-plane) and in the coronal plane (xz-plane) with reference to the feed of the antenna.

Table 3. Radial and longitudinal extents were indicated by the isothermal contour at 55 °C and evaluated at 30 W (first line) and at 60 W (second line) in the “in vivo” numerical study considering the presence of blood perfusion both in the fat tissue (5·10⁻⁷ m³ kg⁻¹ s⁻¹) and in the adrenal gland (4·10⁻⁵ m³ kg⁻¹ s⁻¹).

*For Orientation #1, the final longitudinal dimensions of the ablation zones in the region from z = 0 to the central axis of the applicator (see Figure 6 and Figure 7) are 9 mm (30 W) and 20 mm (at 60 W). The values reported in Table 3 refer to the portions of these ablation zones that include only the adrenal tissues and not the surrounding fat.
(30 W) is selected and applied for 60 s, to spare the surrounding anatomical structures from the excessive increase of temperature and to preserve the functionalities of the adrenal tissues.

Figure 10 shows the shallow ablation zone achieved in the ovine adrenal cortex after the in vivo MWA procedure conducted at 30 W for 60 s. The radial and longitudinal dimensions are measured on the plane of maximum exposure of the ablation zone by cutting the adrenal gland along the longitudinal tract of the applicator. The dimensions achieved in the adrenal cortex are approximately 6 mm in the radial dimension and 9 mm in the longitudinal dimension. Thus, the prediction errors of the numerical simulations mimicking in vivo conditions are The extent of the ablation zone measured after the ablation procedure lies within the radial and longitudinal values predicted by the numerical simulations accounting for the two different orientations of the MW applicator.

3.3. Histology analysis

3.3.1. After ex vivo MWA procedures

Figures 11 and 12 show the histological results obtained in the adrenal sample after MWA procedures conducted at 30 W (Figure 11) and 60 W (Figure 12) for 60 s. Figures 13 and 14 show the histological findings related to the periadrenal fat (Figure 13) and the adjacent blood vessel (Figure 14) after an MWA procedure conducted at 60 W for 60 s.

The changes in the cellular structure due to the thermal effect are visible to a certain extent in the adrenal glands. Three different zones are defined with respect to distance from the applicator: Zone 1 (proximal zone), Zone 2 (transition zone) and Zone 3 (distal zone).

Zone 1 is a more eosinophilic region of the adrenal sample, due to the absence of the cellular nuclei. This characteristic indicates that complete cell death occurs both at high and low input powers. The main difference between the two power
settings is determined by the presence of brown-colored spots visible only at 60 W. These spots indicate charred areas in the tissue in contact with the applicator. Zone 2 and Zone 3 show similar histological features for both input power settings. Zone 2 presents a mixture of intact areas of tissue and areas injured by thermal insult. In Zone 3, cells appear with rounded blue-coloured nuclei and well-defined cytological architecture. The rounded shapes of the cellular nuclei indicate that the tissue remained intact after the MWA procedure.

The effects of the heating on the microstructure of the peri-adrenal fat and the adjacent blood vessel are studied only in the case of the highest input power (i.e., 60 W). At 60 W, temperatures above 55 °C are observed numerically up to 5 mm from the axis of the applicator in fat (Figures 4, 5 and 9). Thus, 60 W shows a higher potential to cause unintended damages to the structures surrounding the gland (e.g., blood vessels).

Two different zones are identified in the fat layer, by reference to the distance from the applicator (Figure 13).
Zone 1 is the proximal region, thus more susceptible to heating due to MWA. Vascular congestion is visible within adipocytes (i.e., fat cells) in this zone. Zone 2 is the area far from the applicator and closer to the blood vessel reported in Figure 13. This region shows no changes in the microvasculature within adipocytes. This result suggests that the area of the fat abutting the blood vessel was not affected by the increase in temperature.

Figure 14 shows the integrity of the three main structures which typically compose the wall of the blood vessel [44]. The tunica adventitia is the external component of the vasculature wall. This layer consists mainly of connective tissue (Figure 14(C)). The tunica media is the middle layer including smooth muscle cells, which appear as elastic lamellae. These cells appear in vessels of at least 2 mm in diameter and regulate blood pressure through vasodilation and vasoconstriction.
3.3.2. After an in vivo MWA procedure
Within 48 h of the procedure, a section of the tissue including the ablation zone and a margin of the adjacent fat layer is resected and processed for the following histology analysis. Figure 15 shows the cellular morphology of the adrenal tissue and the margin of the adjacent fat. The power level used (30 W) induces limited tissue damage and coagulative necrosis, as predicted by the numerical simulations.

The zone adjacent to the axis of the MW applicator (Zone 1) includes part of the adrenal cortex and a rim of the surrounding fat. This zone shows hyalinization both of the adrenal tissue and the adjacent fat. Hyalinization indicates the degradation of the tissue due to heating, after which the tissue becomes translucent (i.e., hyalinised) [40]. The hyalinised tissue generally appears pinkish and the shape of the cells appears squamous and elongated in the zone closest to the MW applicator. A detail of this zone shows the difference in shape between normal round-shaped nuclei and the nuclei affected by the thermal insult, i.e., pyknosis nuclei.

The distal zone with respect to the applicator (Zone 2) shows neutrophil infiltration (i.e., immune infiltration) due to the thermal insult. The infiltration of immune cells (such as neutrophil) provides evidence of an acute inflammation reaction and blood extravasation.

4. Discussion
The numerical study shows consistent asymmetrical SAR patterns: Figure 4 and Figure 5 refer to ex vivo mimicking conditions and Figure 6 and Figure 7 refer to in vivo mimicking conditions. As a consequence, the thermal maps reflect the asymmetric profiles obtained from the deposition of SAR in the tissues. High variability in the ablation profiles can be observed between the two orientations of the MWA with reference to the tissue interface. This is particularly visible in the longitudinal dimension of the ablation zone in the adrenal tissue. A possible reason for this effect is that the pronounced curvature of the surfaces of the 3D model shapes the space around the applicator forcing the ablation zone to grow toward the longitudinal direction. A similar effect has been also observed in previous studies [32] and [45]. In [45] MWA procedures in thin samples (i.e., smaller in width than the reference samples) yields an ablation zone 23% more elongated than the ablation zones observed in reference samples (i.e., samples whose size is bigger than the achievable size of the ablation zones). Orientation #1 is the scenario exacerbating the described detrimental effect of the geometry on the growth of the ablation that has been described. Orientation #2 is a scenario that better stresses the shielding effect of the fat layer and the resulting asymmetric ablation profiles. This result might be linked to a better alignment of the MW applicator at the interface between the fat and adrenal gland guaranteed with Orientation #2 compared with Orientation #1. The two orientations of the MW applicator are also responsible for two different radiating conditions. In the case of Orientation #2, the force lines of the electric field are parallel to the interface between the fat and adrenal gland. In the case of Orientation #1 the force lines of the electric field approximate more the perpendicular condition with respect to the interface between the two tissues. The first condition (Orientation #2) likely induces a greater absorption of the electromagnetic power in the tissue characterized by higher electrical conductivity compared with the second condition [46–48].

Introducing the tissue blood perfusion in the numerical model, slightly (i.e., <2 mm of difference) larger ablation zones are observed in the radial dimension both in the fat layer and in the adrenal gland (Table 3) compared with the “ex vivo” numerical condition (Table 2). This difference is likely due to the higher initial temperature (37°C) that is considered compared with the ex vivo scenario (25°C). The most visible effect in the shaping of the ablation zone can be observed along the longitudinal direction. In particular,
Orientation #1 shows more spherical ablation profiles compared to the scenario without blood perfusion. From the numerical simulations, it can be also observed that the presence of blood perfusion appears to lower the reachable peak of temperatures. Indeed, the extent of the area delineated by the isothermal contour set at 90°C (Figures 6(C–D) and 7(C–D)), in the case of high input power (i.e., 60 W) is visibly smaller compared to the non-perfused numerical scenario (Figures 4(C–D) and 5(C–D)).

The slower increase in temperature in presence of blood perfusion likely contributes to reaching more spherical ablation profiles in the case of Orientation #1 compared to the “ex vivo” scenario. This result is confirmed by the ablation profiles obtained experimentally (Figures 7 and 10). It is worth mentioning that the triaxial MW applicator adopted for the in vivo study helps to reduce the backward current, thus improving the sphericity of the ablation zones. The elongated ablation zones confirmed from the experimental validations on ex vivo adrenal tissues (Figure 7) may be caused by the redistribution of the electromagnetic field along the applicator, as also observed in [32] This phenomenon occurs because of the mismatch in dielectric properties between fat and adrenal gland at the boundary along which the MW applicator is placed. Part of the electromagnetic field reflected from the fat toward the adrenal gland redistributes along the MW applicator contributing to increasing the longitudinal dimension of the ablation zone. Compared to the ex vivo, the acute animal test shows a more spherical ablation zone in the target tissue.

Overall the results show that the role of the fat layer in shaping asymmetric thermal patterns remains almost unchanged independent of the type of background material and the presence of blood perfusion. For both ex vivo and in vivo scenarios, the radial extent of the ablation zone in the fat layer does not exceed 5 mm (Table 2 and Table 3). A radial extent of the ablation zone within 5 mm is desirable to preserve the surrounding sensitive structures, such as blood vessels [49]. Consistent with the ex vivo scenario, in vivo numerical models show that a low input power may provide better control of the increase of temperature in the space surrounding the gland, compared with high power. Thus, a cautious approach using low power (30 W) has been adopted for the acute animal study.

Nonetheless, histology results confirm the capability of the fat layer to preserve the integrity of the blood vessel abutting the adrenal gland even at the highest input power (60 W). In detail, the histology analysis shows the endothelial lamina along with the other structures of the blood vessel remains intact after the MWA procedure (Figure 14(B)). The endothelium acts as an anti-thrombogenic barrier and ensures the tone of the vasculature wall [50]. When the barrier of endothelial cells is damaged, the entire structure of the blood vessel collapses, and a cascade of platelet aggregations obstructs the normal blood flow. This phenomenon results in the formation of intravascular clots or thrombi, which are potentially life-threatening conditions [50]. Blood vessels abutting ablation targets are exposed to higher risks of thrombotic events induced by coagulation necrosis.

The histology analysis also shows that the coagulation necrosis of the tissue is reached only in ex vivo adrenal tissues (Figures 11 and 12). At both input powers used, the region proximal to the applicator appears eosinophilic indicating the absence of the cellular nuclei. The adrenal sample analyzed after in vivo MWA procedure (Figure 15) shows only a change in the morphology of the cellular nuclei from rounded-shaped to squamous-shaped in the area in proximity to the applicator (Figure 15). Instead, squamous and elongated cells are visible in the transition zone of the ex vivo samples (Figures 11(B) and 12(B)). Such a difference in the histological findings between the in vivo and ex vivo scenarios confirms that lower peak temperature values are achieved in the in vivo study due to the effect of blood perfusion. A further difference is in the immune infiltration delineating the transition zone between the innermost part of the cortex (reticularis zona) and the non-ablated medulla (Figure 15, zone 2). It indicates the immediate reaction of the immune system to the thermal insult typically observed in the in vivo scenarios [51–53] This result agrees with the findings presented in [52] for lower MWA dose tested (i.e., 45 W). The delineating immune infiltration which separates the ablated and non-ablated zones is visible only after the in vivo MWA procedure while it is absent after ex vivo procedures.

It is worth noting that the time (60 s) at which MWA procedures are conducted helps to spare the medulla both in vivo and ex vivo even in the case of the highest power. Relatively short times may contribute to preserving the functionalities of the adrenal tissues, which are crucial to the release of hormones. In addition, short durations help to minimize the stress induced by the adrenal medulla. The heating of the gland during the MWA induces the medulla to release a higher amount of catecholamines which contribute to the increase of the cardiac rate of the patient.

5. Conclusion
This study proposes a novel ‘side firing’ MWA approach for the treatment of cortical adenomas arising from the adrenal glands. This new approach exploits the natural presence of the fat surrounding the gland to create asymmetric ablation profiles. The primary goal is to eradicate the target tissue protecting the surrounding healthy structures from excessive increases in temperature. Also, the positioning of the applicator parallel to the target aims to avoid puncturing it and, in turn, reduce the risk of damaging the functional adrenal tissues.

Asymmetric distributions of SAR and temperature are obtained numerically in a 3D model representing the adrenal gland and the surrounding fat capsule. The numerical results show variations in the shape and size of the ablation zones depending on the MW applicator orientation with respect to the interface between the two tissues. The more the antenna is aligned with the interface between the fat and target tissue, the better the fat is capable of shielding the EM energy and inducing asymmetric heating patterns. This is particularly true when low input power is delivered.
for a relatively short time (i.e., 60 s). At low input power (30 W) the ablation zone in fat is negligible and the ablation zones in the tissue target are more spherical compared with those obtained at high input power (60 W). Consistent results are obtained by simulating ex vivo and in vivo conditions. In the in vivo scenario, the blood perfusion has a small impact on the ultimate dimensions of the ablation zones.

The ablation zones in the adrenal gland predicted by the numerical simulation are validated experimentally in ex vivo and in vivo ovine adrenal glands. In addition, the histology analysis conducted on the tissues after MWA proves the ability of the fat layer to protect close anatomical structures, e.g., artery.

Overall, the outcomes of this study confirm the capability of the fat layer to act as a shield for electromagnetic energy when the ‘side firing’ MWA approach is adopted. The shallow ablation zones achievable at 30 W with the proposed method may help to effectively treat adrenal adenomas. This method also helps to preserve the functionalities of the adrenal tissues responsible for regulating blood pressure and heart rhythm. Preserving the integrity of most of the gland and its functionalities is a crucial aspect to avoid complications during the procedure and lifelong pharmacotherapy after the procedure.

Future studies should be devoted to exploring the impact of different energy delivery settings (i.e., input power, time, frequency) on the growth of the ablation zone and shaping of asymmetric heating patterns. Moreover, patient-specific models based on diagnostic imaging data (e.g., PET/CT) would help to position the MW applicator at the interface between the tissue target and the fat layer in order to maximize the shielding effect of the fat layer.

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