Atrial fibrillation is associated with the fibrotic remodelling of adipose tissue in the subepicardium of human and sheep atria

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Aims

Accumulation of atrial adipose tissue is associated with atrial fibrillation (AF). However, the underlying mechanisms remain poorly understood. We examined the relationship between the characteristics of fatty infiltrates of the atrial myocardium and the history of AF.

Methods and results

Atrial samples, collected in 92 patients during cardiac surgery and in a sheep model of persistent AF, were subjected to a detailed histological analysis. In sections of human right atrial samples, subepicardial fatty infiltrations were commonly observed in the majority of patients. A clear difference in the appearance and fibrotic content of these fatty infiltrations was observed. Fibro-fatty infiltrates predominated in patients with permanent AF (no AF: 37 ± 24% vs. paroxysmal AF: 50 ± 21% vs. permanent AF: 64 ± 23%, P < 0.001). An inverse correlation between fibrotic remodelling and the amount of subepicardial adipose tissue suggested the progressive fibrosis of fatty infiltrates with permanent AF. This hypothesis was tested in a sheep model of AF. In AF sheep, an increased accumulation of peri-atrial fat depot was observed on cardiac magnetic resonance imaging and dense fibro-fatty infiltrations predominated in the left atria of AF sheep. Cellular inflammation, mainly consisting of functional cytotoxic T lymphocytes, was observed together with adipocyte cell death in human atri.

Conclusion

Atrial fibrillation is associated with the fibrosis of subepicardial fatty infiltrates, a process in which cytotoxic lymphocytes might be involved. This remodelling of the atrial subepicardium could contribute to structural remodelling forming a substrate for AF.

Keywords

Atrial fibrillation • Adipose tissue • Fibrosis • Atrial myocardium

Translational perspective

The amount of adipose tissue that accumulates around the atria is considered to be a determinant of the risk and persistence of atrial fibrillation (AF). We hypothesize that AF results in fibrotic remodelling of the adipose tissue which is present in the subepicardium of the atrial myocardium. This fibro-fatty remodelling could contribute to the structural remodelling underlying the progressive nature of AF. Advances in cardiac imaging with an improved ability to visualize myocardial fat depostions together with the development of new circulating biomarkers could aid in the detection of an advanced substrate for irreversible AF and guide clinical decision making between rate vs. rhythm control. The prevention of adipose tissue accumulation into the atrial myocardium and its transformation in fibro-fatty infiltrates could also represent targets for upstream therapy of AF.

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Introduction

A relationship between the amount of adipose tissue that accumulates around the atria and both the risk and persistence of AF has been reported by several studies. The Framingham Heart study showed that increased atrial fat volume was associated with a high risk of AF, and outcomes after AF radiofrequency ablation can also be predicted by the amount of atrial adipose tissue. However, the underlying mechanisms linking AF to adipose tissue are still largely unknown.

Cardiac adipose tissue is composed of the paracardial fat outside the visceral pericardium and the epicardial adipose tissue (EAT) adjacent to the epicardium. In addition to its role in energetic and lipid metabolism, EAT produces a number of adipokines that can freely diffuse into the neighbouring myocardium. For instance, the secretome of human EAT can induce atrial fibrosis, an effect mediated by Activin A. Cross talk between adipose and myocardial tissue has been demonstrated by the observation that rapid atrial pacing or AF induces the expression of several genes able to regulate adipose tissue accumulation. Adipose tissue can also infiltrate the myocardium and contribute to its functional disorganization as described for the right ventricle.

The present study was undertaken to investigate the pathophysiological significance of fatty infiltrates, which are commonly observed in the atrial myocardium. The relationship between the histological characteristics of atrial fatty infiltrates and the patient’s clinical history was retrospectively addressed in human right atrial specimens collected during cardiac surgery. Atrial adipose tissue accumulations and characteristics, and their association with the progressive nature of AF, were also prospectively studied in a sheep model of persistent AF. We provide compelling evidence that fatty infiltrates contribute to the progressive fibrosis of subepicardial areas of the atrial myocardium.

Methods

Human study populations

In accordance with the declaration of Helsinki and with the ethical committees from the different institutions involved (Paris, Bordeaux, and Greifswald), atrial samples were obtained from 92 patients who underwent cardiac surgery after obtaining an informed consent; their clinical information is indicated in Table 1. Specimens were collected from 2011 to 2014, and only right atrial samples of sufficient histological quality and at least 5 mm in epicardial length were included for analysis. On average, the epicardial length was 19 ± 8 mm.

Sheep model of atrial fibrillation

Atrial fibrillation was induced in sheep by long-term rapid atrial pacing (n = 26), as previously described (Supplementary material online). Instrumented non-paced sheep served as sham animals (n = 5), and non-instrumented sheep served as control animals (n = 11).

Cardiac magnetic resonance imaging in sheep

Cardiac magnetic resonance imaging (cMR) was performed to assess cardiac adipose tissue volume over time (Supplementary material online).

Table 1 Patient characteristics (n = 92)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean ± SD)</td>
<td>68 ± 10</td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>63 (68)</td>
</tr>
<tr>
<td>Female</td>
<td>29 (32)</td>
</tr>
<tr>
<td>BMI, kg/m² (mean ± SD)</td>
<td>26.7 ± 5.9</td>
</tr>
<tr>
<td>Surgical procedure (%)</td>
<td></td>
</tr>
<tr>
<td>CABG</td>
<td>54 (59)</td>
</tr>
<tr>
<td>Valve replacement/repair</td>
<td>26 (29)</td>
</tr>
<tr>
<td>Combined (CABG and valve)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>Transplantation</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Myxoma resection</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Closure patent foramen ovale</td>
<td>1 (1)</td>
</tr>
<tr>
<td>History of atrial fibrillation (%)</td>
<td></td>
</tr>
<tr>
<td>No AF</td>
<td>60 (65)</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td>18 (20)</td>
</tr>
<tr>
<td>Persistent/permanent AF</td>
<td>14 (15)</td>
</tr>
<tr>
<td>Ejection fraction, % (mean ± SD)</td>
<td>55 ± 15</td>
</tr>
<tr>
<td>Risk factors (%)</td>
<td></td>
</tr>
<tr>
<td>Hypertension (n = 86)</td>
<td>65 (71)</td>
</tr>
<tr>
<td>Diabetes (n = 84)</td>
<td>25 (27)</td>
</tr>
</tbody>
</table>

Data are presented as n (%).

Histological study of human and sheep samples

Atrial appendage samples, fixed in 4% paraformaldehyde and embedded in paraffin, were used for the quantification of fibrosis, fatty infiltrates, and inflammatory cells (Supplementary material online).

Immunohistochemistry

Immunohistochemistry was performed in 12 patient samples (Supplementary material online).

Statistics

Normally distributed continuous data are reported as mean ± standard deviation. A Student’s t-test was used for comparison between groups if data was normally distributed. A Mann–Whitney test was used for non-parametric test. Correlation was assessed using a Spearman correlation coefficient (r). A two-way repeated-measure analysis of variance (ANOVA) was conducted to assess a difference in change of adipose tissue volume over time between the AF and sham groups. Agreement on the histological scoring system (intra- and inter-rater agreement) was evaluated using weighted κ statistics. The χ² test was used to compare the distribution of fatty infiltration grades between AF and control sheep. A Fisher’s exact test was conducted to compare the presence of inflammatory infiltrates between AF and control sheep. One-way ANOVA was used to assess significant differences between three or more different groups. If significant, Tukey’s post hoc test or Bonferroni’s post hoc test was used to detect the level of significant differences. A multivariable analysis, with a backward-elimination approach, was run to predict the extent of fibrotic epicardial remodelling. A P-value of <0.05 was considered statistically significant. All tests were performed with either
Results

Fibrotic remodelling of the epicardium in human atrial samples

Samples of right atrial appendage were used for the histological study as (i) a sample could be obtained during routine cardiac surgery from a large number of patients and (ii) samples were taken from a fixed area of the right atria and were of quite regular anatomic morphology allowing a comparison between samples.

Infiltration of the subepicardial area by adipose tissue was observed in the vast majority of the right human atrial specimens studied (91% of samples). The extent of this fatty infiltration varied markedly, from 0 to 76% across atrial sections without a relationship between the extent of fatty infiltrations and AF (percentage adipose tissue—no AF: 14 ± 17% vs. paroxysmal AF: 11 ± 12% vs. permanent AF: 12 ± 9%, P = 0.711).

However, clear differences in fatty infiltrate characteristics were observed. Some infiltrates were characterized by discrete fibrosis, smooth interfaces with the neighbouring myocardium, and a thin epicardium. These we considered as normal infiltrates (Figure 1A). Others were markedly fibrotic with intermingled adipocytes, fibrosis, and myocytes, as well as a thick and irregular epicardium, which we considered as remodelled infiltrates (Figure 1B).

To validate the distinct patterns of epicardial area and fatty infiltrates, we applied a scoring system to 20 selected samples (Figure 1D). This clearly confirmed that higher fibrotic content and lower levels of adipose tissue were observed in the epicardial region of those samples scored as highly remodelled (Figure 1E). Noteworthy, in 10 left atrial samples, fatty and fibro-fatty infiltrations were also observed (fibo-fatty infiltrate in 80% of samples; 20 ± 18% adipose tissue).

The extent of remodelled epicardium was determined based on the degree of fibrosis of the adipose tissue, and the presence of infiltrating fibrosis (Figure 1C). An inverse correlation between the amount of adipose tissue and the extent of remodelled epicardium was observed (Figure 1G), whereas a positive correlation was noted between myocardial fibrosis and remodelled epicardium (Figure 1H). Several samples demonstrated both normal and fibrotically remodelled fatty infiltrates with an apparent transition zone in between (Figure 1I, Supplementary material online, Figure S1).

Advanced age and a patient history of AF were associated with higher degrees of fibrotically remodelled epicardium. Following multivariable analysis, only a history of AF remained as a significant clinical predictor (Table 2). Furthermore, the degree of fibrotically remodelled epicardium was related to the duration of AF (no AF 37 ± 24% vs. paroxysmal AF 50 ± 21% vs. permanent AF 64 ± 23%, P = 0.0004) (Figure 1F). These results indicate that AF could be a predictor of the fibrotic remodelling of subepicardial fatty infiltrates.

Fibrosis of fatty infiltrates is associated with localized inflammatory processes

In 47% of the human atrial samples, small lymphoid aggregates could be observed in the subepicardial adipose tissue (Figure 2A, Supplementary material online, Figure S6) in both no AF and AF patients (no AF: 46% of samples vs. history of AF: 48%, P = 0.817). These clusters of inflammatory cells were mainly located at the epicardial site and often in the transition zone between adipocytes and fibrosis in human atria (Supplementary material online, Figure S6). Immunohistochemistry in 12 patient samples, identifying a total of 17 inflammatory infiltrates, demonstrated a predominance of lymphocytes, and a few evenly distributed CD14+ monocytes and CD15+ neutrophils (Figure 2C). The majority of these lymphocytes were CD3+ T lymphocytes, the vast majority of them were CD8+ cytotoxic T cells, with fewer CD20+ B lymphocytes. Crown-like structures were frequently encountered, suggesting adipocyte cell death (Supplementary material online, Figure S6). Indeed, CD8+ T cells in the lymphoid aggregates displayed functional cytotoxic activity as indicated by their staining with the cytolytic enzyme granzyme. In addition, some adipocytes in contact with the lymphoid aggregates were terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) positive, suggesting cell death. A number of TUNEL-positive cells were also detected inside the lymphoid aggregates (Figure 2D). These results indicate that an immune response mediated by cytotoxic CD8+ T lymphocytes is activated in fibro-fatty infiltrates.

Persistent atrial fibrillation is associated with fibro-fatty infiltrations of the sheep atria

In order to determine whether AF causes fibrosis of atrial fatty infiltrates independently of co-morbidity factors such as ischaemic or valvular heart disease, we studied a sheep model of persistent AF. First, we assessed changes in the amount of peri-atrial fat deposits using cMR. In AF sheep, total atrial and left atrial adipose tissue volume increased significantly after AF induction (total atrial adipose tissue—0 week: 33.8 ± 7.3 mL vs. 16 weeks: 41.2 ± 7.4 mL, P = 0.0149; LA adipose tissue—0 week: 17.4 ± 3.4 mL vs. 16 weeks: 22.2 ± 3.4 mL, P = 0.0009; LA posterior fat thickness—0 week: 0.88 ± 0.25 cm vs. 16 weeks: 1.03 ± 0.18 cm, P = 0.036; Figure 3). The ventricular adipose tissue volume remained stable (97.8 ± 24.5 mL vs. 102 ± 12.0 mL, P = 0.5890).

Second, macroscopic and histological examination of explanted hearts revealed abundant atrial adipose tissue, together with a number of fatty infiltrates in the various regions of the left and right atria in both groups (Supplementary material online, Figure S7). Quantification of subepicardial fatty infiltrates was performed on left atrial appendages (fixed anatomy in all animals). We could not show a difference in the proportion of fatty infiltrates vs. total area between AF and control sheep (left atrium—AF sheep: 3.1 ± 2.6% vs. control sheep: 3.3 ± 3.4%; P = 0.8256). However, as in human, distinct types of fatty infiltrations could be distinguished based on the degree of fibrosis, epicardial thickness, adipocyte size, and inflammation (Figure 4A and Table 3). After verifying intra- and inter-rater agreement (κ index, respectively, 0.818; P < 0.0001 and 0.487; P < 0.0001), a total of 314 infiltrations were graded (211, 14 ± 4 per sheep and 103, 9 ± 4 per sheep in AF and control group, respectively). A significant difference in distribution pattern of the four histological grades was observed between the two groups, with a higher average grade per AF sheep compared with sham (2.0 ± 0.4 AF group vs. 0.7 ± 0.4 control group,
Figure 1 Fibrotic remodelling of the epicardium in human atrial samples. The extent of fibrotic was assessed on Sirius Red stained sections. (A) Example of non-fibrotic remodelled epicardium, either without subepicardial adipose tissue (a) or with subepicardial adipose tissue (b). (B) Fibrotic remodelled epicardium, in the presence of subepicardial adipose tissue (a) or without adipose tissue (b). (C) The complete length of the epicardium was assessed and the extent of fibrotic remodelling was expressed as a percentage of total length (illustrative example with 82% of fibrotic remodelled epicardium; in green, non-fibrotic epicardium, and in red, fibrotic remodelled epicardium). (D) Semi-automated histological quantification of the epicardial and endocardial region composition, to validate the visually assessed extent of fibrotic remodelling. (A) Regions of interest are shown in orange lines. Epicardial total tissue area (b), adipose tissue area (c), and fibrosis (d) after digital processing. (E) Epicardial and endocardial composition was assessed in 20 samples, with either the lowest or highest percentage of visually assessed fibrotic remodelling [X represents the sample group with the lowest percentage (4 ± 3%, n = 10); Y represents the sample group with the highest percentage (89 ± 8%, n = 10)]. The epicardial region of Group Y clearly demonstrates a higher percentage of fibrosis and less adipose tissue. (F) The extent of epicardial fibrotic remodelling was significantly higher in patients with a history of permanent atrial fibrillation (Par AF, paroxysmal atrial fibrillation; Per AF, permanent atrial fibrillation). (G) Negative correlation between the amount of adipose tissue and the extent of fibrotic remodelled epicardium. (H) Positive correlation between the amount of myocardial fibrosis and the extent of fibrotic remodelled epicardium. (I) Most samples contained both non-fibrotic (*) and fibrotic (†) remodelled epicardium, often with a transition zone in between. *P ≤ 0.05, **P ≤ 0.01 and ***P ≤ 0.001.
Table 2 Univariable and multivariable linear regression analysis of clinical and histological variables predictive for the extend of fibrotic remodeled epicardium on 92 human atrial samples

<table>
<thead>
<tr>
<th>Clinical and histological variables</th>
<th>Univariable analysis</th>
<th>Multivariable analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman r</td>
<td>P-value</td>
</tr>
<tr>
<td>Age</td>
<td>0.251</td>
<td>0.016</td>
</tr>
<tr>
<td>Gender</td>
<td>NA</td>
<td>0.164</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.130</td>
<td>0.264</td>
</tr>
<tr>
<td>History of AF</td>
<td>NA</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ejection fraction</td>
<td>−0.045</td>
<td>0.679</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>NA</td>
<td>0.934</td>
</tr>
<tr>
<td>Hypertension</td>
<td>NA</td>
<td>0.985</td>
</tr>
<tr>
<td>Myocardial fibrosis (histology)</td>
<td>0.477</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fat percentage (histology)</td>
<td>−0.254</td>
<td>0.015</td>
</tr>
</tbody>
</table>

P < 0.0001 (Figure 4). Noteworthy, small lymphoid aggregates were also observed in the fatty infiltrations of sheep atria (Figure 2B) and were more frequently present in AF than control sheep [AF sheep: 6 out of 15 sheep or 7% (14/211) of fatty infiltrations vs. control sheep: 1 out of 11 sheep or 1% (1/103) of fatty infiltrations, P = 0.0433]. These results indicate that persistent AF might induce fibrosis of fatty infiltrates in sheep atria.

Discussion

Our study reveals that AF is associated with the fibrosis of the adipose tissue, which is present in the subepicardium of the atrial myocardium in human and sheep. An immune response could be involved in this remodelling process. We hypothesize that these fibro-fatty infiltrates from the subepicardial area could be an important pathogenic mechanism for structural remodelling leading to the progressive nature of AF.

We report that fatty infiltration of the myocardium is a common observation in the right atrium, as reported for the right ventricle.16,17 We show that this fat can become fibrotic. In some patients, only a dense subepicardial fibrosis was observed and only little fatty infiltrate. The fibrotic remodelling of fat is also supported by an inverse correlation between amounts of fat and fibrosis. Permanent AF is a strong predictor for the presence of fibro-fatty infiltrates but which can be observed also in a number of patients in sinus rhythm, indicating that various clinical conditions can favour the fibrosis of atrial adipose tissue in addition to AF. It is difficult to assess the exact contribution of AF vs. underlying comorbid conditions to the development of atrial fibro-fatty infiltration in patients; therefore, experimental models were necessary. Therefore, we used the rapid atrial pacing sheep model, a pure model of persistent AF without significant left ventricle dysfunction.18 As previously described by others in humans, we found that AF in this model was associated with the accumulation of pericardial adipose tissue around the atria notably the posterior wall39 and not the ventricle. Moreover, whereas fatty infiltrations of the subepicardium were equally observed in atria of control and AF sheep, fibro-fatty infiltrations with small adipocytes and thick epicardium predominated in the latter. Taken together, the histological study of human and sheep atria both indicate that AF might be an important pathogenic trigger for fibrotic remodelling of the subepicaldial adipose tissue.

The observation of atrial fibro-fatty infiltrations during AF is reminiscent of the histology of arrhythmogenic right ventricular cardiomyopathy, characterized by replacement of the common fatty infiltration with a mixture of fibrous tissue and fat.19 Moreover, the propensity of adipose tissue to become fibrotic is well known and has been described in other tissue types.20–22 For instance, after chronic injury, skeletal muscle is replaced by a mix of fibrous tissue and white adipocytes.21,22 In obese patients, fibrosis of the adipose tissue is a hallmark of disease progression.23 Most often, inflammation plays a pivotal role in fibrotic remodelling of adipose tissue20 also observed in human and sheep atria. Inflammatory infiltrates were mainly composed of CD8+ cytotoxic effector T cells in human. CD8+ effector T cells are involved in the inflammation of adipose tissue in obese patients.24 Whether the infiltrating cytotoxic CD8+ T cells are clonal and recognize a specific antigen will require additional studies.

Our observation that subepicardial fatty infiltrates seen in most atria are fibrotic, inflammatory, and infiltrative during AF indicates that this adipose tissue component of the atrial myocardium is not an innocent bystander. Good and bad functions have already been described for EAT, i.e. energy supplier, protective adipokines, cell regeneration vs. pro-inflammatory, pro-fibrotic, and pro-atherosclerotic.8,9,26 If this holds true for the atrial adipose tissue notably the fat infiltrating the myocardium, then AF, but also heart failure, diabetes, or obesity,27,28 could modify the biological properties of fat tissue, resulting in its proliferation and fibrosis.

Potential clinical significance

AF is characterized by wavebreaks and rotors that predominate in the subepicardium of the atrial wall.29,30 This is a consequence of electrical dissociation between epicardial layers and the endocardial bundle network, favouring disturbances in electrical conduction.
This electrical dissociation is aggravated by the accumulation of interstitial fibrosis that spreads from the epicardial layer to the neighbouring subepicardial myocardium. Fibro-fatty infiltrates of the atrial subepicardium might contribute to such an epicardial/endocardial electrical dissociation.

In addition, fibro-fatty infiltrates could favour functional disorganization and local conduction defects. Moreover, it can secrete several cytokines or adipokines such as Activine A, which are known to regulate cardiac ion channels.

**Limitations**

Only right atrial myocardium specimens could be routinely obtained from a large number of patients. Therefore, we can only speculate that in other atrial areas, notably the left atrium, fibro-fatty infiltration of the subepicardium could also accumulate. In support of this assumption, we also obtained 10 left atrial samples, revealing fibro-fatty infiltrate in the majority of them. In addition, fibro-fatty infiltrates as observed in human predominate in the left atria of sheep in AF.
Figure 4  Atrial fibrillation is associated with fibrosis of fatty infiltrates in the atrial fibrillation sheep model. Subepicardial infiltrations were digitized and scored in a blinded fashion according to a four grade system (grades 0–3; see Table 3). The atrial fibrillation sheep demonstrated a shift to higher grades, either expressed as average grade or as (C) distribution pattern of all fibro-fatty infiltrations (atrial fibrillation sheep, n = 211; control sheep, n = 103). (magnification ×100, scale bar of 500 μm).

Figure 3  Expansion of peri-atrial adipose tissue deposits by atrial fibrillation. Adipose tissue volume was assessed by cardiac magnetic resonance imaging. Significant expansion of the total (A) and left (B) atrial adipose tissue volume was observed in the atrial fibrillation sheep (n = 11) at 16 weeks. Ventricular (C) and sham (n = 5) adipose tissue remained stable (*paired t-test; †two-way repeated-measure analysis of variance).
**Table 3** Histological features of the scoring system classifying fatty infiltrations in the sheep model

<table>
<thead>
<tr>
<th>Grade</th>
<th>Histological feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Limited fibrosis without myocardial infiltration, large adipocytes, no inflammation, thin epicardium</td>
</tr>
<tr>
<td>1</td>
<td>Increased fibrosis, no or limited infiltration of fibrosis in the myocardium, no inflammation, no or moderate thickened epicardium</td>
</tr>
<tr>
<td>2</td>
<td>Increased fibrosis with infiltration into the myocardium, small adipocytes, ± inflammatory infiltrates, thickened epicardium</td>
</tr>
<tr>
<td>3</td>
<td>Extensive scarring, limited or no remaining adipocytes, ± inflammatory infiltrates, thickened epicardium</td>
</tr>
</tbody>
</table>

**Conclusion**

Infiltration of the atrial subepicardium by adipose tissue is a common component of atrial histology. However, in some clinical circumstances, these fatty infiltrates can become fibrotic. For instance, AF is an independent predictor of the presence of fibro-fatty infiltrations. This remodelling process of the atrial subepicardium could be favoured by an immune and inflammatory response and might contribute to the formation of the substrate of AF.

**Authors’ contributions**

P.H. performed statistical analysis; R.W. and S.N.H. handled funding and supervision; P.H., H.H., K.G., N.S., N.P., and S.N.H. acquired the data; P.H., P.C., R.W., and S.N.H. conceived and designed the research; P.H., R.W., and S.N.H. drafted the manuscript; and P.H., R.W., S.N.H., A.N., J.J., U.L., and P.J. made critical revision of the manuscript for key intellectual content.

**Supplementary material**

Supplementary material is available at European Heart Journal online.

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**Conflict of interest:** none declared.

**References**

17. Sons HJ, Hoffmann V. Epicardial fat cell size, fat distribution and fat infiltration of the right and left ventricles of the heart. Anatomostrischer Anzeiger 1986;161:335–373.
Deep brain stimulator-induced flutter-like artefact on Holter recording

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Flashlight
A 12-year-old patient with an implanted deep brain neurostimulator (Activa® RC, Medtronic Inc) for severe dystonia was admitted for dystonic attacks. Because of suspected concurrent tachycardia a 3-channel Holter ECG (SEER® Light Extend; GE Medical Systems) was made. This showed a slow ventricular rate with an atrial rate of 130 bpm. After turning off the stimulator immediately the atrial flutter-like pattern (Panel A) disappeared (Panel B). The regular 12-lead ECG showed neurostimulator-induced high-frequency interference (Panel C).

The appearance of the flutter-like pattern on Holter ECG can be explained by aliasing, a phenomenon that occurs if the sampling frequency is less than twice the source frequency. Given the 130 Hz neurostimulator source frequency, and the 125 Hz Holter ECG sampling frequency, a 5 Hz aliasing frequency occurs.

The regular 12-lead ECG has a sampling frequency of 500 Hz which is well above twice the 130 Hz source frequency and therefore does not display the flutter-like aliasing artefact. Since the 12-lead ECG has a low-pass filter with a cut-off frequency of 140 Hz, it can show the neurostimulator-induced interference of 130 Hz.

In conclusion, the atrial flutter pattern was caused by a 5 Hz difference between Holter ECG sampling frequency and neurostimulator frequency, causing aliasing. If possible, Holter sampling frequency should be at least twice the neurostimulator’s frequency to avoid aliasing. Otherwise, possible interference should be taken into account when assessing Holter ECG’s in patients with a neurostimulator.