MR AXEL TJÖRNSTRAND (Orcid ID : 0000-0002-5622-7744)
DR OSKAR RAGNARSSON (Orcid ID : 0000-0003-0204-9492)

Article type : Original Article - UK, Europe

Lower ^{68}Ga-DOTATOC Uptake in Non-Functioning Pituitary Neuroendocrine Tumors Compared to Normal Pituitary Gland – a Proof-of-Concept Study

Running title: ^{68}Ga-DOTATOC PET in non-functioning PitNET

Authors: Axel Tjörnstrand[1,2] | Olivera Casar-Borota[3,4] | Kerstin Heurling[5,6] | Michael Schöll[5,7,8] | Peter Gjertsson[9,10] | Jakob Himmelman[11] | Oleksiy Itsenko[11] | Oskar Ragnarsson[1,12] | Helena Filipsson Nyström[1,12]

Affiliations:

[1]Department of Internal Medicine and Clinical Nutrition, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden

[2]Department of Radiology, Sahlgrenska University Hospital, Göteborg, Sweden

[3]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

[4]Department of Clinical Pathology, Uppsala University Hospital, Uppsala, Sweden

[5]Wallenberg Centre for Molecular and Translational Medicine, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, University of Gothenburg, Göteborg, Sweden

[6]Antaros Medical, Mölndal, Sweden

[7]Clinical Memory Research Unit, Lund University, Sweden

[8]Dementia Research Centre, Institute of Neurology, University College London, UK

[9]Department of Clinical Physiology, Sahlgrenska University Hospital, Göteborg, Sweden

[10]Deptartment of Molecular and Clinical Medicine, Institute of Medicine, at Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden
[11]Department of Medical Physics and Biomedical Engineering, Sahlgrenska University Hospital, Göteborg, Sweden
[12]Department of Endocrinology, Sahlgrenska University Hospital, Göteborg, Sweden

Correspondence: Axel Tjörnstrand, Department of Radiology Sahlgrenska University Hospital, Bruna Stråket 11 B, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden; Email: axel.tjornstrand@vgregion.se; Tel: +46-313426861

Article type: Original article
Abstract length: 247 words
Text length: 3372 words
No. of graphics: 5 (2 tables, 3 figures)
No. of references: 27

Separate Appendix file supplied

Author contributions: All authors contributed significantly to the work, meet the criteria for authorship, provided critical review of the manuscript, and approved the final version for publication.

Acknowledgements: We thank Professor Anders Sundin, Uppsala University, for the initial design of the research protocol. We also thank pathologist Thomas Olsson Bontell and biomedicine technician Dorotea Berkowicz at the Department of Clinical Pathology and Genetics at Sahlgrenska University Hospital, who performed the primary diagnostics of the pituitary tumors. We thank biomedicine analysts Dijana Cerjan and Tor Halle, Uppsala University Hospital, for technical assistance with IHC analysis. We also thank; Lena Kullin, specialist nurse, Department of Endocrinology, for help with patient recruitment and Ann-Christin Berg, nurse at Department of Nuclear Medicine, for managing the PET scan logistics, both working at Sahlgrenska University Hospital.

Conflict of interest: The authors declare that they have no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding information: The study was financed by grants from the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (ALFGBG-717311), the Healthcare Board, Region Västra Götaland (Hälso- och sjukvårdsstyrelsen), Novo Nordisk foundation, Wilhelm and Martina Lundgren Fund, and the Swedish Medical Society.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Objectives: ^{68}Ga-DOTATOC PET targets somatostatin receptors (SSTRs) and is well established for the detection of SSTR-expressing tumors, such as gastrointestinal neuroendocrine tumors. Pituitary adenomas, recently designated as pituitary neuroendocrine tumors (PitNETs), also express SSTRs, but there has been no previous evaluations of ^{68}Ga-DOTATOC PET in PitNET patients. The aim of this pilot study was to evaluate the diagnostic properties of ^{68}Ga-DOTATOC PET in the most common PitNET, i.e. non-functioning (NF)-PitNET.

Design/Methods: NF-PitNET patients (n = 9) and controls (n = 13) were examined preoperatively with ^{68}Ga-DOTATOC PET for 45 min after tracer injection in dynamic list mode. Tumor specimens were collected during surgery in patients. MRI and PET images were co-registered using PMOD software. The maximum standard uptake value (SUV_{max}) was analyzed in manually outlined regions of interest (ROC) around the tumor in patients and around the pituitary gland in controls. Immunohistochemical analyses were conducted on tumor specimens for assessment of tumor cell type and SSTR expression.

Results: Median SUV_{max} (IQR) was lower in patients than in controls (3.9 [3.4-8.5] *vs* 14.1 [12.5-15.9]; $P < .01$]. In ROC analysis, the area under the curve was 0.87 ($P < .01$) for SUV_{max}, with 78% sensitivity and 92% specificity. Immunohistochemical analysis showed NF-PitNETs were of gonadotroph (n = 7) and corticotroph (n = 2) origin. SSTR expression was high for SSTR3, low-to-moderate for SSTR2, and low for SSTR1 and SSTR5.

Conclusions: This proof-of-concept study shows that ^{68}Ga-DOTATOC PET can be used to differentiate between normal pituitary tissue and NF-PitNET.

KEYWORDS: DOTATOC-PET, non-functioning pituitary adenoma, pituitary adenoma, pituitary neuroendocrine tumor localization, somatostatin receptor imaging

1 | INTRODUCTION

The incidence of pituitary adenomas or, as recently proposed, pituitary neuroendocrine tumors (PitNETs),[1] is 3.9 per 100,000 person-years,[2] making it one of the most common intracranial tumors.[3] PitNETs usually appear in the anterior pituitary and can be either functioning, i.e. hormone-producing, or non-functioning (NF).[4] The second group is the most common form of all PitNETs.[2,5] Histologically, NF-PitNETs are composed of tumor cells that do not secrete active anterior pituitary hormones, but may produce biologically inactive hormones. According to the 2017 WHO classification, NF-PitNETs are divided into eight subtypes based on the immunohistochemical expression of adenohypophyseal hormones and pituitary-specific transcription factors.[3] The most common NF-PitNET subtypes are gonadotroph and corticotroph tumors, comprising about 80% and 15% of all NF-PitNETs, respectively.[6,7] Other subtypes account for only a few percent.

Nowadays, magnetic resonance imaging (MRI) is the "gold standard" for PitNET detection.[8] However, MRI has some limitation in that it mostly provides morphological information. To fully determine PitNET functionality, advanced endocrine evaluation is needed, sometimes including invasive procedures. Further, it is challenging after pituitary surgery to discriminate tumor tissue from postoperative changes with MRI and often requires repeated MRI evaluations. An imaging modality for both the functional and anatomical properties of PitNETs is therefore needed.

PitNETs, as well as normal pituitary tissue, have somatostatin receptors (SSTRs), of which five subtypes have been characterized (SSTR1-5). NF-PitNETs demonstrate variable expression of SSTRs depending on the histological tumor type.[9-11] SSTR3 is the most abundantly expressed in gonadotroph tumors followed by much lower expression of SSTR2.[10,11] The SSTR family is a potential target for functional imaging.

Functional imaging with somatostatin receptor scintigraphy (SRS), using indium-labeled octreotide, has previously been evaluated in patients with PitNET with varying results.[12-15] Generally, SRS has poor ability to discriminate PitNET from normal pituitary tissue and, even though some functioning- and NF-PitNETs can be visualized, its clinical usefulness is limited.[14-16] In contrast to SRS, positron emission tomography (PET) has higher spatial resolution and increased tumor-to-background contrast. ^{68}Ga-DOTATOC PET mainly targets SSTR2 and to some extent SSTR5,[17] and has been successfully integrated in the evaluation of gastrointestinal neuroendocrine tumors (GI-NETs).[18,19] Although PitNETs, like GI-NETs, express SSTRs, only a

few studies have evaluated the diagnostic proprieties SSTR-directed PET. A recent study demonstrated a high detection rate for hormone-producing pituitary microadenomas when combining ^{68}Ga-DOTATATE PET and ^{18}F-FDG PET.[20] For non-functioning PitNETs, however, the field still lacks studies evaluating the use of the SSTR-directed PET for tumor detection.

In this proof-of-concept study, our aim was to evaluate the ability of ^{68}Ga-DOTATOC PET to discriminate tumor from normal pituitary tissue in patients with the most common form of PitNET, NF-PitNET. More specifically, we aimed to explore the kinetic properties of the radiotracer, to compare relative radiotracer uptake in NF-PitNET and normal pituitary, and to relate the radiotracer-binding in NF-PitNETs to immunohistochemical expression of SSTRs in tumor cells.

2 | MATERIALS AND METHODS

2.1 | Study design

This observational, prospective, case-control, proof-of-concept study of adult NF-PitNET patients and matched controls was conducted at the Department of Endocrinology and the Department of Nuclear Medicine at the Sahlgrenska University Hospital, Gothenburg, Sweden, between December 2015 and March 2018. Patients were evaluated before pituitary surgery. Height and weight were measured. Pregnancy testing was performed to exclude pregnancy in premenopausal women. ^{68}Ga-DOTATOC PET was then performed. All patients completed a standardized report form 24 hr after tracer administration to record any possible side effects. Controls underwent the same procedures.

The Regional Ethical Review Board in Gothenburg, Sweden, approved the study, which was conducted in accordance with the Declaration of Helsinki. Written consent was obtained from each subject after full explanation of the purpose and nature of all procedures used.

2.2 | Subjects

Ten patients (44-78 years of age; 8 men), with a NF-PitNET were included in this study. The diagnoses were based on MRI evidence of a pituitary tumor without clinical or biochemical signs of pituitary hormone overproduction. Patients were recruited from the waiting list for pituitary surgery at Sahlgrenska University Hospital, Gothenburg, Sweden, and were eligible if they were >18 years of age and had a treatment-naive (including surgery, somatostatin analogues, or dopamine agonists) NF-PitNET. None of the patients had metastatic disease. The demographics and clinical characteristics of the patients are presented in Table 1. One patient who had a panic attack in the PET scanner withdrew from further participation. This pilot study therefore reports data from nine patients, all with macroadenomas (tumor size >10 mm).

Thirteen controls (38-79 years of age; 7 men) were included (Table 1) comprising two groups. The first group included 10 healthy volunteers who were age- and gender-matched to the patients and were randomly selected from the population registry in Gothenburg. Exclusion criteria included any pituitary disease and/or ongoing treatment with somatostatin analogues or dopamine

agonists. The second group included three controls with thyroid-associated ophthalmopathy (TAO) who were participating in another study (http://www.clinicaltrials.gov; identifier NCT02378298), where ^{68}Ga-DOTATOC PET was performed using the same protocol as in our patients to evaluate eye-muscle inflammation. Inclusion criteria for that study were euthyroid men or women ages 18-70 years with TAO necessitating intravenous glucocorticoid treatment. ^{68}Ga-DOTATOC PET was undertaken before any glucocorticoid treatment was administered. Exclusion criteria for TAO controls were the same as those for healthy controls.

2.3 | MRI

MRI was performed according to clinical routine at the Department of Radiology, Sahlgrenska University Hospital, Gothenburg, Sweden, including 3D rendering, gadolinium contrast enhancement, and T1- and T2-weighted sequences. MRI scans were performed within 3 months of the ^{68}Ga-DOTATOC PET. MRI scans for the controls were performed according to the same protocol as for the patients, but without any contrast agent.

2.4 | ^{68}Ga-DOTATOC PET

2.4.1 | Radiotracer synthesis and quality control

^{68}Ga-labeled DOTATOC (N-[[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]acetyl]-D-phenylalanyl-L-cysteinyl-L-tyrosyl-D-tryptophyl-L-Iysyl-L-threonyl-N-[(1R,2R)-2-hydroxy-1-(hydroxymethyl)propyl]-L-cysteinamide cyclic (2→7)-disulfide) was prepared using a cassette-based, automated synthesis system (Modular-Lab PharmTracer; Eckert & Ziegler GmbH, Berlin, Germany) according to the manufacturer's instructions. Cassettes were obtained from the same manufacturer and auxiliary chemicals kits were from Eckert & Ziegler GmbH or Rotem GmbH (Leipzig, Germany). The synthesis precursor was purchased from ABX GmbH (Radeberg, Germany). ^{68}Ge/^{68}Ga generators were obtained from Eckert & Ziegler Eurotope GmbH (Berlin, Germany).

^{68}Ga was eluted from the ^{68}Ge/^{68}Ga radionuclide generator with 100 mM HCl solution. Eluted $^{68}Ga^{3+}$ was collected on a strong cation exchange, solid-phase extraction (SPE) cartridge. Further, $^{68}Ga^{3+}$ was selectively removed from the cartridge with 20 mM HCl in 98% acetone into the reaction vial containing an acetate buffer solution of the precursor peptide.[21] Later in the study, the $^{68}Ga^{3+}$ pre-purification step was accomplished with 5M NaCl solution acidified with small amount of HCl.[22] This change was caused by irregularities in material supply and was evaluated as having negligible influence on the overall preparation process. The reaction mixture containing $^{68}Ga^{3+}$ and the precursor peptide was heated to 95°C over 300 sec. When the reaction was finished, the reaction mixture was diluted with saline solution and transferred to C18 SPE cartridge, which was then washed with saline. The purified product was eluted with 1 mL of ethanol-water mixture (1:1 v/v) and further formulated by diluting it with 8.5 mL saline. Sterilization was achieved during the last stage by filtration through a 0.22-μm membrane filter into a sterile vial.

The quality of the product was checked through analytical procedures described in the European Pharmacopoeia and met specifications stated therein. The gas chromatography method for residual solvents test was developed in-house and validated according to International Conference on Harmonisation guidelines.

2.4.2 | PET scanning procedures

^{68}Ga-DOTATOC solution was administered to participants as an intravenous bolus. Target injected activity was 2 MBq/kg. PET/CT examinations were performed using the same protocol for every participant on a Biograph mCT Flow Edge PET/CT scanner (Siemens Medical Solutions USA, Tarrytown, NY). A pre-injection, non-contrast-enhanced CT scan of the head was acquired with the following parameters: 120 kV, pitch 0.55, and CARE Dose4D Quality Reference mAs 340 (CTDIvol 50 mGy). A list mode PET acquisition starting at the time of injection was used to collect emission data over a 45-min period. The list mode data were reconstructed into 14 frames (5×60, 5×180, 3×300, 1×600 sec). The first eight patients also underwent later static 300-sec scans starting at 60, 90, and 120 min after tracer injection. These additional static scans were later not undertaken for the remaining subjects since there was no additional uptake after 45 min. All PET images were iteratively reconstructed (MLEM/OSEM) into five iterations and 21 subsets with time-of-flight, resolution recovery (TrueX), CT-based attenuation and scatter corrections, and a 3-mm Gaussian post-processing filter. Examples of representative PET images are shown in Figure 1.

2.4.3 | Image analysis

PMOD software v3.8 (PMOD Technologies, Ltd., Zürich, Switzerland) was used for image analysis. MRI and PET images were co-registered using the "Fuse it" toolkit. Abnormal findings that visually matched the characteristics of a tumor were outlined in transverse slices and automatically adapted to three-dimensional volume, creating a volume of interest (VOI) around the tumor area. VOIs for the control group was created in same manner, delineating the pituitary gland. ^{68}Ga-DOTATOC uptake was analyzed in the VOI with regard to maximum standardized uptake value (SUV_{max}). Data from the 35- to 45-min frame were chosen for the statistical analysis.

2.5 | Tumor classification

Tumor tissues samples were collected during surgery from the nine NF-PitNET patients. Representative tumor tissue was confirmed in routinely hematoxylin/eosin-stained sections from formalin-fixed, paraffin-embedded tissue blocks. Pituitary tumors were classified into histological subtypes according to the 2017 WHO classification based on the immunohistochemical expression of the anterior pituitary hormones and pituitary-specific transcription factors.[3]

2.6 | Immunohistochemical analyses

2.6.1 | Anterior pituitary hormones

The following antibodies were used: anti-FSH (monoclonal, clone C10, DAKO, catalogue number M3504, dilution 1:300), anti-LH (monoclonal, clone 93C, DAKO, catalogue number M3502, dilution 1:400), anti-TSH (monoclonal, clone 0042, DAKO catalogue number M3503, dilution 1:100) , anti-GH (polyclonal, catalogue number A0570, dilution 1:3000), anti-ACTH (monoclonal, clone 02A3, DAKO, catalogue number M3501, dilution 1:1200), anti-Pit-1 (Novus Biologicals, polyclonal, code no. NBP1-92273, dilution 1:500), anti-SF-1 (ThermoFisher Scientific, monoclonal, clone N1665, dilution 1:100), and anti-T-Pit (TBX19) (monoclonal, clone CL6251, Atlas Antibodies, dilution 1:1000). Normal pituitary gland served as a positive control for immunohistochemical analyses with the antibodies towards anterior pituitary hormones and pituitary-specific transcription factors

2.6.2 | Somatostatin receptors

Immunohistochemical analyses of somatostatin receptors were performed as previously described,[23] with the following monoclonal antibodies: anti-SSTR1 (clone UMB-7, 1:100), anti-SSTR2a (clone UMB-1, 1:1000), anti-SSTR3 (clone UMB-5, 1:4000), and anti-SSTR5 (clone UMB-4, 1:750) [Abcam, Cambridge, UK]. Normal pancreatic tissue demonstrating SSTR expression in Langerhans islet endocrine cells was used as a positive control. Negative control was obtained by omitting the primary antibody. All controls gave satisfactory results.

SSTR expression was quantified using the Immunoreactive Score (IRS).[23,24] IRS (range, 0-12) is the product of the proportion of immunoreactive cells (0 = 0%, 1 = <10%, 2 = 10–50%, 3 = 51-80%, and 4 = >80%) and staining intensity (0 = no staining, 1 = weak, 2 = moderate, and 3 = strong) [see Figure 1 for examples]. In cases with heterogeneous staining intensity throughout the tumor specimen, the most prevalent intensity was used. IRS scoring was undertaken by an experienced pathologist (O.C.-B.), who was blind to clinical data.

2.6.3 | Ki67 proliferation index

For each tumor, Ki67 proliferation index was analyzed (monoclonal antibody, clone MIB1, DAKO, catalogue number IR626/GA626, ready to use) and assessed by counting the percentage of Ki67-immunolabeled cells per 2000 tumor cells in hotspot regions. Normal lymph gland served as a positive control for Ki67 immunostaining. All immunohistochemical staining was performed with a DAKO EnVision FLEX system and DAKO Autostainer.

2.7 | Statistical analysis

Continuous variables were reported as median and interquartile range (IQR). The Mann-Whitney U-test was used to compare ^{68}Ga-DOTATOC uptake in patients and controls. Diagnostic accuracy was evaluated using receiver operating characteristic (ROC) analysis. Linear regression was used to analyze the linear relationship between the radiotracer and SSTR and Ki67 expression. Spearman's rank-order correlation were used for correlation analyses between Ki67 expression and SUV_{max}. All statistical analyses were performed using Prism 8.0 (GraphPad Software, Inc., San Diego, CA). For all tests, $P < .05$ was considered as statistically significant.

3 | RESULTS

3.1 | SUV_{max} in patients and controls

Median (IQR) DOTATOC SUV_{max} was significantly lower in patients compared to controls (3.9 [3.4-8.5] *vs* 14.1 [12.5-15.9]; $P < .01$) [Figure 2]. In ROC analysis, the area under the curve was 0.87 ($P < .01$), with a sensitivity of 78% and specificity of 92% for SUV_{max}. Uptake kinetics demonstrated clear differences between the two groups (Figure 3). All but two of the NF-PitNET patients showed a low-grade uptake that did not increase after reaching peak at 5 min. The two exceptions (#NF1 and #NF3) showed tracer uptake that was comparable to that of the normal pituitary in controls. All controls had highly homogenous early uptake with a peak intensity at 45 min. No further increase in SUV_{max} was observed in patients (n = 8) and controls (n = 3) studied for up to 120 min.

3.2 | Histopathological characterization of NF-PitNET

Seven patients had silent gonadotroph tumors and two patients had silent corticotroph tumors. SSTR3 was expressed with a high IRS (6-12) in all tumors. SSTR expression was high for SSTR3, low-to-moderate for SSTR2, and low for SSTR1 and SSTR5. Four patients had a moderately high IRS (3-6) for SSTR2 (Table 2).

3.3 | ^{68}Ga-DOTATOC in relation to the proliferation index Ki67

Ki67 was <3% for all tumor samples, i.e. levels compatible with low proliferation (Table 2). Linear regression between Ki67 level and SUV_{max} was –2.97 (95% confidence interval, –10.1 to 4.17) and Spearman's rank correlation coefficient was –0.37 (P = .33).

3.4 | Adverse events and incidental findings

There were no serious or non-serious adverse events during the study. No incidental findings were revealed in controls.

4 | DISCUSSION

The use of PET in oncology has increased rapidly with a variety of new tracers for tumor detection. To our knowledge, ^{68}Ga-DOTATOC PET has not previously been studied in patients with NF-PitNET. This proof-of-concept study revealed a lower functional ^{68}Ga-DOTATOC uptake in NF-PitNET as compared to normal pituitary, with a high sensitivity (78%) and specificity (92%), and ROC analysis presenting an area under the curve of 0.87.

The field of functional imaging for the detection of pituitary tumors is limited and there are few studies evaluating SSTR tracers.[20] In the late 1990s, some studies of pituitary tumors were conducted that evaluated a scintigraphic method using indium-labeled octreotide. Results from these studies were highly variable, at best showing positive scans in 60-70% of patients.[12-15] Also, scintigraphy was deemed impractical since the procedure required a long time, up to 24 hr, between administration of the radiotracer and the scan as well as a long scanning time for image acquisition. Compared to scintigraphy, PET scanning provides higher spatial resolution, shorter scanning time, and a semi-quantitative uptake measurement. Although PET has many advantages over scintigraphy, it has not been sufficiently evaluated for pituitary tumors. Recently, a study evaluated two different radiotracers, ^{18}F-FDG and ^{68}Ga-DOTATATE, in hormone-producing (mainly ACTH-producing) PitNETs. In concordance with our study, the authors demonstrated a significantly lower uptake in tumor tissue compared to normal pituitary.[20] Despite similarities between our study and the ^{68}Ga-DOTATATE-study, further comparison is problematic since

different types of tumors were examined, i.e. hormone-producing tumors of mostly corticotroph origin in the ^{68}Ga-DOTATATE-study and non-hormone-producing tumors of mostly gonadotroph origin in our study. Two tumors were corticotroph cell type in our study but, since no histopathological data were presented in the ^{68}Ga-DOTATATE-study, we could not perform further comparison of SSTR expression in hormone- *versus* non-hormone-producing tumors. An interesting hypothesis for future research is whether SSTR expression is the same in the same cell type whether or not it produces active hormones – a hypothesis we hope to assess and subsequently report.

SSTR-PET has also been evaluated for tumor detection in patients with multiple endocrine neoplasia (MEN), where SSTR-tracers seems to provide a higher tumor detection rate compared to FDG-PET.[25] Although pituitary tumors are common in these patients, only few studies have presented PET-data. In a recent study, including18 patients with MEN-1 syndrome, ^{68}Ga-DOTATATE positive scans were present in 9 out of 12 patients with tumor manifestations in the pituitary gland on MRI.[26] However, in contrast to our study, only positive PET-scans were determined as a tumor finding. Since all these positive scans illustrated a prolactin producing PitNETs, one could speculate that some NF-PitNETs may have been missed if photopenic zones are overlooked as negative findings. Analogous to discordant uptake patterns in adrenal nodules, low uptake lesions could provide equally important clinical information.[27]

Histopathological data were rarely presented in published scintigraphic studies. Among the major studies discussed earlier,[12-15] only minor material of six tumors was assessed for SSTR profile. Using an autoradiographic *in vitro* method, four tumor specimens were determined as SSTR-positive, but no further information regarding receptor subtype or expression intensity was reported.[13]

The high level of SSTR3 expression in all of the NF-PitNET patients may potentially have important diagnostic and therapeutic implications. In theory, these tumors should be better visualized by SSTR-imaging tracers with higher affinity for the SSTR3-receptor, e.g. ^{68}Ga-DOTANOC (IC_{50} = 40 nM for SSTR3).[17] Furthermore, a better treatment response to somatostatin analogues, such as pasireotide that targets SSTR3[28,29] is also an interesting topic for future research. At the time when the study was designed, SSTR expression in NF-PitNET was not sufficiently explored, and the possible advantage with ^{68}Ga-DOTANOC was not known. DOTATOC was also the SSTR-tracer with the best pharmaceutical documentation at that time. Before 2012, SSTR analyses were performed using polyclonal antibodies that had a higher degree

of cross-binding, thereby yielding inconsistent results of SSTR expression in NF-PitNETs. More recently, larger and well-conducted studies[10,11], using monoclonal antibodies, have demonstrated more consistent result of high SSTR3 expression in NF-PitNETs. In our study, specific monoclonal antibodies towards SSTR1-3 and SSTR5 were used, further demonstrating the pattern of SSTR expression in NF-PitNETs.

Ki67 is a nuclear antigen expressed by proliferating cells and previous studies have found an association between elevated Ki67 index and tumor invasiveness or recurrence.[3] In the current study, all tumors had an Ki67 index below 3% and we detected no correlation between Ki67 level and radiotracer. In the future, it will be interesting to see if there is a possible association between Ki67 index and radiotracer in repeated tumor evaluation at 6-9 months following surgery in our patient cohort.

Two tumors in our cohort with the gonadotroph immunohistochemistry profile had moderate SSTR2 expression (mean IRS 6 compared to 1.4 in the other tumors). This is consistent with previous findings using histopathological material where moderate SSTR2 expression has been described in approximately 25% of gonadotrophic tumors.[10] This heterogeneity among gonadotrophic tumors is probably a limitation to using ^{68}Ga-DOTATOC PET to distinguish NF-PitNET from normal pituitary. Also, the proportion of these tumors in our study (2 of 9) seems to correspond well with the attained sensitivity of 78%.

Our study did not make any correction for partial volume effect. The primary rationale for this was that all the studied tumors were large macroadenomas and that the partial volume effect plays a more important role in smaller lesions. Additionally, there was very low uptake in the tissue adjacent to the pituitary, resulting in minimal spillover effect to the tumors. The outcome variable was SUV_{max}, which was typically located in the center of the tumor, further minimizing partial volume effects.

In our cohort, the majority of patients had hypogonadotropic hypogonadism. However, SSTR expression pattern is not known to be affected by the functionality of the pituitary. Therefore, we consider that pituitary insufficiency has not influenced the ^{68}Ga-DOTATOC uptake.

The small sample size is a major limitation in our study, which was not sufficient to detect differences in uptake between gonadotroph and corticotroph tumors, despite differences in IRS score for SSTR2. Additionally, a dedicated threshold level could not be calculated to clinically distinguish tumor from the normal pituitary with this sample size. However, the nature of the study, being an exploratory proof-of-concept study, the number of patients and controls was

sufficient to meet the primary endpoint, to discriminate tumor from normal pituitary. Threshold level as well as uptake differences in different subtypes of NF-PitNETs will be interesting topics for a future, larger study.

Our study indicates that ^{68}Ga-DOTATOC PET can improve the diagnostic workup of PitNETs. Large NF-PitNETs are less problematic with respect to diagnosis in current clinical practice. However, classifying small PitNETs as functional or non-functional is a challenge, as accidental findings of small NF-PitNETs in the pituitary are detected with an increasing frequency.[30] Additionally, postoperative evaluations can be challenging when post-surgical changes can be hard to discriminate from a remaining tumor tissue. 68Ga-DOTATOC-PET/CT, providing a spatial resolution of 4.33-7.8mm[31], has a comparable spatial resolution to a 3T MRI. Nevertheless, 68Ga-DOTATOC-PET/CT, can add important functional information that reflects histopathological differences between tumor tissue and post-surgical changes. In this study, we found a low-grade uptake in NF-PitNETs with low levels of proliferation marker Ki67. In a future study, hormone producing PitNETs will be assessed with the same protocol, possibly generating different uptake profiles depending on hormone production or higher proliferation potential. In a further perspective, these results could have important clinical implications in other SSTR-expressing tumors, e.g. for patients with MEN-1 syndrome, where early tumor detection and the determination of metastatic development are crucial for an effective treatment.

Furthermore, with increasing use of SSTR PET, incidental findings of abnormal uptake in the pituitary region are likely to become common. Therefore, uptake profiles reflecting the different histopathological natures of these findings are important to be described for future evaluation. These results could also provide important use in screening for patients with MEN, where PitNETs are a common finding. The study provides important data for NF-PitNETs, which could easily be missed in tumor screening if only positive scans are considered compatible with PitNETs.

As a major strength in this study, PET data were accompanied by histopathological data and quantified data of SSTR expression. Furthermore, this study presented both maximum tracer uptake and tracer kinetics. Our study therefore provides important data both for normal pituitary uptake and the uptake profile of the most commonly occurring pituitary tumors, i.e. NF-PitNETs. In agreement with previous studies, high expression of SSTR3 in gonadotroph[10,11] and corticotroph tumors[11] was confirmed, and can be used as a novel target for diagnostic imaging and direction of medical treatment for these tumors.

In conclusion, this study demonstrates that ^{68}Ga-DOTATOC PET can differentiate between normal pituitary tissue and NF-PitNETs with a high sensitivity and specificity. As indicated by a recent study using a similar tracer,[20] future research will determine whether this method will be applicable to all hormone-producing PitNETs.

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TABLE 1 Demographic and clinical data for patients and controls

	Patients (N = 9)	**Controls (N = 13)**
Sex, n (%)		
Male	7 (78)	7 (54)
Female	2 (22)	6 (46)
Median age, years (IQR)	64 (58-71)	65 (58-72)
Median body weight, kg (IQR)	92 (76.5-103)	70 (69.5-78)
Pituitary insufficiencies, n (%)	7 (78)	0
GH	Not evaluated	
FSH/LH	7 (78)	
TSH	4 (44)	
ACTH	3 (33)	
ADH	0	
Tumor size, mm[a]		
#NF1	30×30×28	
#NF2	16×17×19	
#NF3	27×20×27	
#NF4	25×27×37	
#NF5	24×15×22	
#NF6	15×18×12	
#NF7	20×15×20	
#NF8	20×25×35	
#NF9	38×34×28	
Radiolabel dose administered, MBq/kg	2	2

Abbreviations: ACTH, adrenocorticotropic hormone; ADH, antidiuretic hormone; FSH, follicle-stimulating hormone; IQR, interquartile range; LH, luteinizing hormone; NF, non-functioning; TSH, thyroid-stimulating hormone.

[a]Length, width, and height dimensions, respectively.

Table 2. PET and histopathological data for nine patients with non-functioning pituitary neuroendocrine tumors

Patient no.	SUV_{max}[a]	IRS[b]				Tumor type[c]	Ki67 (%)[b]
		SSTR1	SSTR2	SSTR3	SSTR5		
#NF1	14.4	0×0=0	2×3=6	2×3=6	0×0=0	Gonadotroph	2.3
#NF2	4.1	2×3=6	0×0=0	3×2=6	0×0=0	Corticotroph	2.4
#NF3	16.6	0×0=0	2×3=6	4×3=12	0×0=0	Gonadotroph	1.0
#NF4	1.3	0×0=0	0×0=0	4×3=12	2×2=4	Corticotroph	1.7
#NF5	3.5	0×0=0	1×1=1	4×3=12	0×0=0	Gonadotroph	2.0
#NF6	8.0	0×0=0	1×1=1	4×3=12	0×0=0	Gonadotroph	1.2
#NF7	3.5	0×0=0	1×1=1	4×2=8	0×0=0	Gonadotroph	2.3
#NF8	3.8	0×0=0	2×2=4	3×3=9	0×0=0	Gonadotroph	1.2
#NF9	3.3	0×0=0	1×3=3	3×3=9	1×1=1	Gonadotroph	2.8

Abbreviations: IRS, immunoreactive score; SSTR, somatostatin receptor; SUV_{max}, maximum standard uptake value.

[a]PET data from the region of interest containing the tumor with SUV_{max} measured over the 35- to 45-min frame.

[b]IRS determined as the product of the proportion of immunoreactive cells (0 = 0%, 1 = <10%; 2 = 10-50%, 3 = 51-80%, and 4 = >80%) and the staining intensity (0 = no staining, 1 = weak; 2 = moderate, and 3 = strong).

[c]Determined by immunohistochemistry.

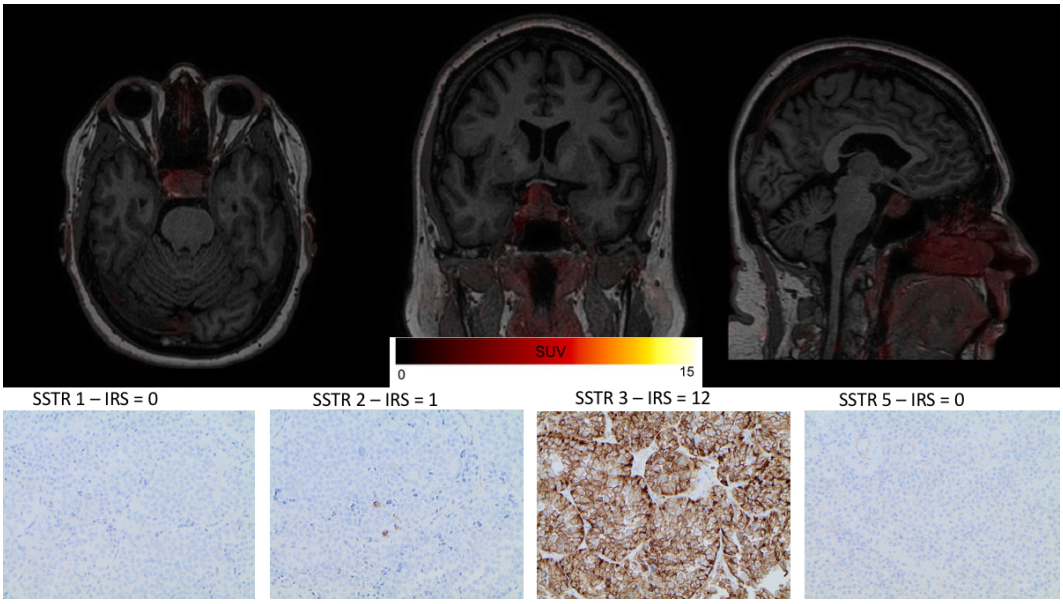
[d]Number of Ki67-positive cells per 2000 tumor cells in hotspots. Positive staining >3% is compatible to high proliferation.

FIGURE LEGENDS

FIGURE 1 Co-registered ^{68}Ga-DOTATOC PET and MRI images (upper panels), and SSTR expression and IRS (lower panels) in two representative patients. PET scale shows SUV from 0 to 15. (A) Patient #NF5, a 64-year-old female with a non-functioning pituitary neuroendocrine tumor measuring 24×15×22 mm. Hormonally, the patient had low estradiol but had normal levels of the other pituitary hormones. The tumor did not evoke visual impairment. Histopathological images show low-grade staining for SSTR2 and, consequently, low radiotracer intensity (SUV 3.9) on PET. (B) Patient #NF3, 71-year-old male with a gonadotroph non-functioning pituitary neuroendocrine tumor measuring 27×20×27 mm. Hormonally, the patient had low testosterone and cortisol levels, but was sufficient in the other pituitary hormones. The tumor did not evoke any visual impairment. Histopathological images show a moderately high SSTR2 staining and, consequently, high radiotracer intensity (SUV 16.6) on PET. SSTR, somatostatin receptor; SUV, standard uptake value

FIGURE 2 Scatterplot of ^{68}Ga-DOTATOC uptake in patients with non-functioning pituitary neuroendocrine tumors (NF-PitNETs) and controls. Bars show median and interquartile ranges. SUV_{max}, maximum standard uptake value

FIGURE 3 Tracer kinetics in patients with non-functioning pituitary neuroendocrine tumors (NF-PitNETs) and controls showing median value as line and interquartile range as error-bars. SUV, standard uptake value



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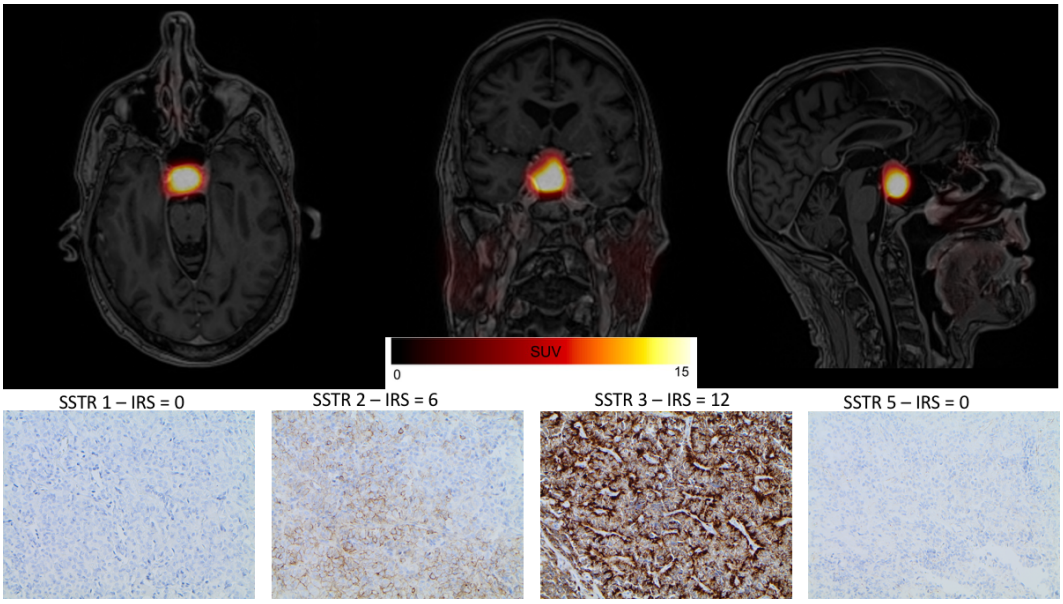

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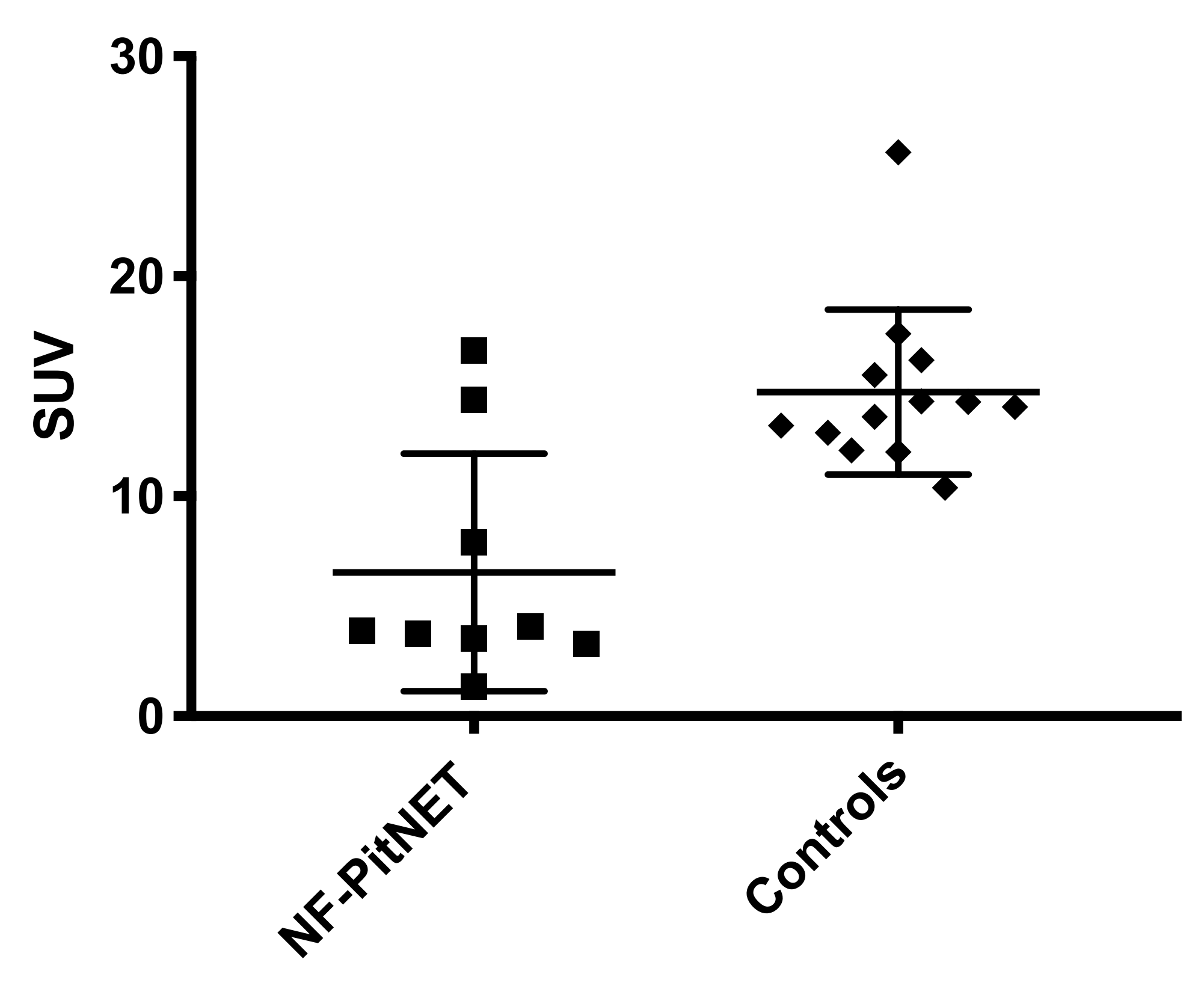
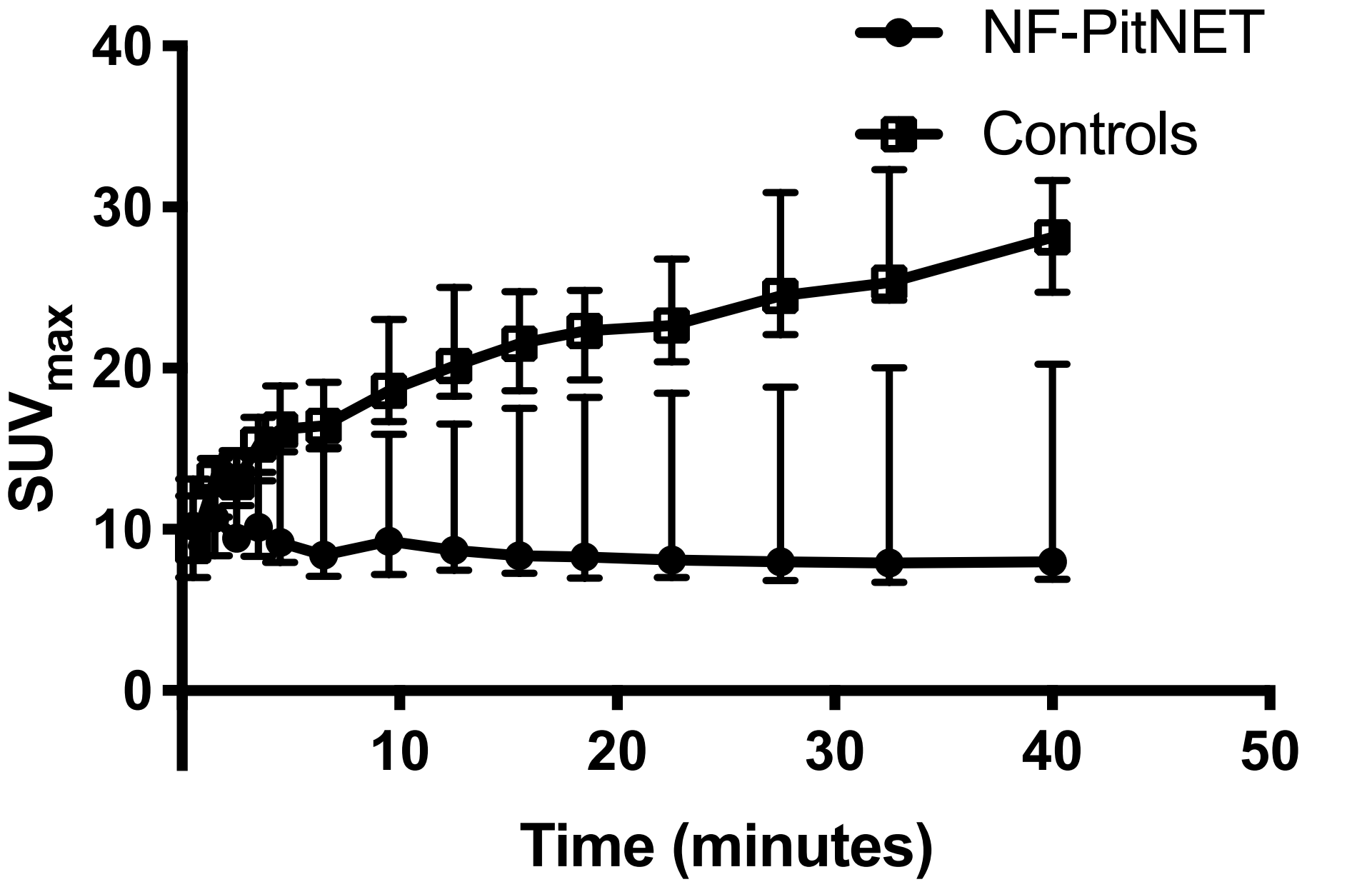

30
20
10
0
SUV
NF-PitNET
Controls


NF-PitNET
Controls
40
30
20
10
0
SUV max
10
20
30
40
50
Time (minutes)