Improved adrenocortical PET imaging

ISABELLA SILINS
Introduction: Adrenal tumours can either be benign or malignant, hormone secreting or not, and they can be discovered through clinical examination of the patient or by pure chance. Increased knowledge in the area, plus the widespread use of imaging techniques, have resulted in a rising number of patients with adrenal tumours that subsequently need to be diagnosed. Improved imaging is needed for primary aldosteronism (PA) and adrenocortical carcinoma (ACC) but the positron emission tomography (PET) tracer currently in use, [11C]metomidate (MTO), has many important limitations. This thesis aims to improve adrenocortical PET imaging.

Methods: Paper 1 investigated the pre-clinical properties of Para-Chloro-2-[18F]fluoroethyl-etomidate (CETO), by autoradiography, binding studies, ex vivo biodistribution on rats and in vivo imaging using mice and one non-human primate (NHP). Paper II investigated the clinical properties of [18F]CETO and included patients with various kinds of adrenocortical tumours, and healthy volunteers. Metabolic and kinetic analyses were performed and three out of five healthy volunteers also underwent [15O]water PET/CT to measure adrenal blood flow. Test-retest was performed on all healthy volunteers. Paper III assessed the in vivo and in-human radiation dosimetry of [18F]CETO. Ex vivo uptake data from rats and in vivo PET/CT from NHP and humans were used to calculate residence times. Paper IV evaluated the use of the block-sequential regularized expectation maximization (BSREM) reconstruction algorithm (Q.Clear, GE Healthcare, Milwaukee, USA) for [11C]MTO PET/CT in patients with PA.

Results: Papers I and II demonstrated that [18F]CETO is highly specific to the adrenal cortex both in vitro and in vivo. The non-specific binding of [18F]CETO in the liver was significantly lower than that of [11C]MTO. [18F]CETO metabolizes rapidly and the single tissue irreversible (1T1k) kinetic model provided the best fit. [15O]water PET/CT results indicated that the adrenal [18F]CETO uptake was flow limited. Several retest values, including adrenal blood flow, were lower than the test values. Paper III found that the effective dose based on human data was 18.2 μSv/MBq and that the adrenal glands were the limiting organ regardless of species used. Paper IV showed that the BSREM reconstruction algorithm improves image quality, without compromising SUVmax quantification, and a β-value between 70 and 130 was found optimal.

Conclusion: [18F]CETO PET/CT is a promising method for adrenocortical imaging and is safe for clinical imaging in terms of radiation dose. [18F]CETO PET/CT should be further investigated in patients with PA or ACC, preferably in conjunction with BSREM reconstruction.

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Dedicated to the memory of my maternal grandparents, who taught me the value of higher education, and of my late father who took immense pride in my accomplishments.

And to my wife who have always been my greatest cheerleader, and whose support made this thesis possible.
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Abbreviations

1T1k  single tissue irreversible
2T3k  two-tissue irreversible
2T4k  two-tissue reversible
ACC  adrenocortical carcinoma
ACTH  adrenocorticotrophic hormone
APA  aldosterone-producing adenoma
ARR  aldosterone to renin ratio
AVS  adrenal vein sampling
APCC  aldosterone-producing cell cluster
BAH  bilateral adrenal hyperplasia
BSIF  blood sample derived input function
BSREM  block-sequential regularized expectation maximization
CPA  cortisol-producing adenoma
CT  computed tomography
CYP11B1  11-β-hydroxylase
CYP11B2  aldosterone synthase
GMP  good manufacturing practice
IDIF  image derived input function
MRI  magnetic resonance imaging
NHP  non-human primate
OSEM  ordered subsets maximization
PA  primary aldosteronism
PCC  pheochromocytoma
PET  positron emission tomography
ROIs  regions of interest
SBR  signal-to-background ratio
SNR  signal-to-noise ratio
TAC  time-activity-curve
TOF  time of flight
VOIs  volumes of interest
ZF  zona fasciculata
ZG  zona glomerulosa
ZR  zona reticularis
\[^{18}\text{F}]\text{CETO}\  \text{para-chloro-2-[^{18}\text{F}]fluoroethyl-etomidate}\n\[^{18}\text{F}]\text{FETO}\  \text{2-[^{18}\text{F}]fluoroethyletomidate}\n\[^{11}\text{C}]\text{MTO}\  \[^{11}\text{C}]\text{metomidate}\
Introduction

For 45 years, the diagnostics and treatment of endocrine tumours have been a special area of interest for Uppsala University Hospital. Today the Uppsala Centre of Excellence for Endocrine Tumours, certified by the European Neuroendocrine Tumour Society (ENETS), is considered as one of the world’s leading centres for the diagnosis and treatment of endocrine tumours. The endocrine oncology and endocrine surgery units have achieved this degree of excellence through collaboration with various medical specialties (e.g., radiology, nuclear medicine, pathology and the PET centre) as well as through the continuous development and utilization of new treatments and diagnostic methods [1].

This thesis focuses on improving adrenocortical positron emission tomography (PET) imaging, which admittedly, is a medical niche, considering the vastness of the medical field. At its heart though it is simply a continuation of a long tradition of (and dedication to) providing new and improved treatment options to our patients.

The adrenal glands

The adrenal glands are located cranially and ventrally to the kidneys. They are small hormone-producing organs approximately 6-7 mm wide when measured on CT [1]. They consist of an outer adrenal cortex and an inner adrenal medulla.

The adrenal cortex is divided into three distinct cellular layers (or “zones”): zona glumerolusa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) [2]. The adrenal cortex secretes corticosteroids, such as cortisol, and mineralocorticoids, particularly aldosterone, as well as smaller amounts of androgens (Figure 1).

The enzyme CYP11B1/11β-hydroxylase is expressed in the ZF and is responsible for converting 11-deoxycortisol to cortisol. It is regulated by adrenocorticotropic hormone (ACTH) from the pituitary. The enzyme CYP11B2/aldosterone synthase is expressed in the ZG and (through a series of steps) converts
deoxycorticosterone to aldosterone. Aldosterone synthase is regulated by Angiotensin II [2].

Adrenal pathologies

Adrenal pathologies often cause an overproduction of the adrenal hormones, aldosterone or cortisol or both, and in certain cases may be clinically suspected even after the first examination of the patient. In other cases, it is not initially suspected, or the diseased adrenal is accidentally discovered (and subsequently diagnosed) when the patient is being investigated for something else entirely.

Primary aldosteronism

In Sweden it is estimated that 20% of the population above the age of 20 suffer from hypertension [4]. Between 3% and 13% of these cases are caused by primary aldosteronism (PA) [5-8]. PA is usually caused by bilateral adrenal hyperplasia (BAH) or by an aldosterone-producing adrenal adenoma (APA), and more rarely by an adrenocortical carcinoma (ACC) [9] or familial hyperaldosteronism [10].

Adrenocortical carcinoma

Adrenocortical carcinoma is a rare malignant tumour of adrenocortical origin, with a bimodal age distribution [11]. Common sites of metastases include lymph nodes, lungs, bone as well as the liver [12]. In 66% to 77% of cases ACC leads to hormone hypersecretion. As mentioned in the preceding paragraph, even though ACC may secrete aldosterone, it most commonly secretes only cortisol or mostly cortisol and androgens [13, 14]. Solely androgen secreting ACC accounts for 10% of cases. In those very rare cases, patients may present with symptoms of hirsutism and cessation of menses [15].
Cushing’s syndrome
Normally, Cushing’s syndrome (CS) is caused by the overproduction of adrenocorticotropic hormone (ACTH) in the pituitary gland, which in turn stimulates an excess production of adrenal cortisol. However, CS can, in some cases, be ACTH-independent. ACTH-independent CS is usually caused by a cortisol-producing adrenal adenoma, or occasionally an ACC [16].

There are also adrenal adenomas that produce both aldosterone and cortisol [17].

Pheochromocytoma
All the above-mentioned adrenal pathologies are adrenocortical in origin, whereas pheochromocytomas may originate from the adrenal medulla or a paraganglion [18]. As seen in Figure 1, the adrenal medulla secretes epinephrine and norepinephrine. Unsurprisingly, pheochromocytomas usually secrete said hormones. After being secreted, the hormones undergo several steps of enzymatic modification rendering metabolites, e.g., sulphated metanephrines with a longer half-life in plasma and urinary secretion. For practical reasons these metabolites are measured in plasma or urine for diagnostic purposes [19].

Adrenal imaging
Due to the widespread use of high-resolution imaging techniques, mainly CT and MRI, incidentally discovered adrenal masses have become a frequent finding in clinical practice. Incidentally discovered masses over 1 cm are classified as “adrenal incidentalomas” with a prevalence of about 4% in patients undergoing abdominal anatomic imaging [20] and require radiological and biochemical characterisation.

Among incidentalomas, adrenocortical adenomas are the most frequent benign finding. Adrenal myelolipomas are the second most common benign tumour in the adrenals. Their origin is, however, not adrenal but a result of extra medullar haematopoiesis [21].

Even though most incidentalomas are benign [22], it is of great importance to safely distinguish any malignant findings. One such distinguishing feature is tumour size. Most benign adrenal adenomas are smaller (<4 cm) than the adrenocortical carcinomas (usually >4 cm) [23]. Another distinguishing feature is attenuation on a non-contrast CT scan. When a morphologically homogenous adenoma on a non-contrast CT shows an attenuation of ≤10 Hounsfield units (HU), it can be diagnosed as a lipid-rich adrenocortical adenoma, with high
specificity [24]. However, recent findings suggest that a higher HU cut-off value (<20 HU in conjunction with size <3 cm or <15 HU and size <4 cm) would increase specificity for benign adrenal adenomas in patients with normal metanephrines [25]. Of all adrenocortical adenomas, 30% are categorized as lipid-poor adenomas, and cannot be properly characterized on CT or MRI [26].

Additionally, ACCs on CT imaging usually present as tumours with irregular borders, inhomogeneity, calcifications, lymph node enlargement or invasion of surrounding tissues [23]. However, the use of only CT-derived characteristics, such as irregular borders, is not sufficient to identifying ACC [27]. Calcifications also occur in adrenal adenomas [24]. In cancer patients, adrenal metastasis may be suspected and require work-up accordingly. Especially small malignancies tend to mimic adenomas with a homogenous density [27]. Hence, CT imaging must always be combined with clinical and biochemical investigations such as tests for plasma aldosterone and renin, plasma metanephrines or 24-hour urinary metanephrines, and dexamethasone suppression tests [9, 28, 29]. Figure 2 shows healthy adrenals depicted with CT.

![CT scan of healthy adrenals](image)

Figure 2 Healthy adrenals (white arrows) on CT.

Magnetic resonance imaging (MRI) has the main advantage of not exposing the patient to radiation and can be used to depict the adrenal gland. Adrenal
adenomas contain fatty tissue, and protons resonate in different frequencies depending on tissue type (fat or water) [30]. It is possible to register these differences ("the chemical shift") in the MRI signal by applying conventional in- and out-of-phase MRI sequences [31].

Whereas both MRI and CT imaging are used to outline anatomical structures, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are nuclear medicine imaging technique that provide functional and metabolic information. In PET and SPECT, a molecule is labelled with a radionuclide and the preparation, the tracer, is administered to the patient, usually by i.v. injection. For PET, positron emitting radionuclides are utilized whereas SPECT instead use gamma emitting radionuclides Both PET and SPECT produce three-dimensional images. PET is superior to SPECT in terms of contrast and spatial resolution. SPECT and PET are usually performed together with CT (SPECT/CT, PET/CT)) and PET can also be performed together with MRI (PET/MRI).

Several different radiotracers can be used in adrenal imaging. An overview of adrenal radiotracers, and their usages, is found in Figure 3.

Figure 3 Depicting a schematic display of radiopharmaceuticals for imaging of adrenal cortical and medullary tumours. Dexamethasone (DXM) is used as premedication before [11C]MTO imaging in primary aldosteronism. SSR = somatostatin receptors. This is a modification of a figure originally published in JNM [32]
[18F]fluorodeoxyglucose (FDG) and [11C]metomidate (MTO)

[18F]-fluorodeoxyglucose (FDG)-PET/CT is well established for imaging in general oncology whereas [11C]-metomidate (MTO) PET is used in clinically complex scenarios to distinguish lesions of adrenocortical origin, for example an ACC, from non-cortical tumours, and additionally for postoperative ACC surveillance [33]. [11C]MTO-PET/CT has shown superior to CT for identifying non-necrotic adrenocortical carcinomas [34]. Recently, [11C]MTO-PET/CT has also been explored as an adjunct to adrenal vein catheterisation for lateralisation in primary aldosteronism. In the MATCH study [35] preprint (currently under review), the authors concluded that pre-treatment with dexamethasone and [11C]MTO PET/CT is not inferior to adrenal vein sampling, when used for lateralization of PA [36].

[11C]MTO-PET/CT images of healthy adrenals are shown in Figure 4 (A and D).

Other radiotracers

Several different PET tracers have been suggested for adrenocortical imaging. Most of the suggestions are based on promising preclinical findings. The following PET tracers use CYP11B as a binding ligand: [18F]AldoView [37], [18F]FETO [38], [18F]CDP2230 [39] and [18F]FAMTO [40], as well as the tracer that is evaluated in this thesis (Papers I-III), [18F]CETO.

In Figure 4, b and c, healthy adrenals are seen with [18F]CETO.

[68Ga]-pentixafor instead uses the C-X-C chemokine receptor type 4 (CXCR4) as an alternative binding ligand and has been suggested for the laterization of PA patients [41]. Another trial with [68Ga]-pentixafor showed low CXCR4 staining in 29% of the investigated APA’s [37].

SPECT tracers

SPECT of the adrenal cortex can be performed with [123I]iometomidate ([123I]IMTO). However, as mentioned before, PET/CT is superior in terms of spatial resolution. Additionally, [11C]-MTO PET has exhibited higher specificity than [123I]IMTO SPECT for identifying tumours with adrenocortical origin [42].

Adrenomedullary imaging

There are several radiotracers for adrenomedullary imaging that target different aspects of the catecholamine synthesis, e.g. the dopamine analogue 6-[18F]fluorodopamine ([18F]FDA) [43], iodine-123-metaiodobenzylguanidine ([123I]MIBG) scintigraphy, and [11C]hydroxyephedrine ([11C]HED). The use of [11C]HED is limited due to its carbon-11 coupling, even though it has a high specificity for pheochromocytomas [44]. As has been known since the 1990s, pheochromocytomas may overexpress somatostatin-receptors. Today 68Ga-
labelled somatostatin analogues, such as $[^{68}\text{Ga}]\text{DOTATE}$, are commonly used for PET imaging of pheochromocytomas. $[^{68}\text{Ga}]\text{DOTATE}$ has been shown superior to $[^{18}\text{F}]\text{FDG}$ in several cohorts of patients with metastatic pheochromocytoma[45].

Figure 4 Healthy adrenals (white arrows) pictured with PET A: $[^{11}\text{C}]\text{MTO}$ transaxial and D coronal; B $[^{18}\text{F}]\text{CETO}$ transaxial and C coronal

Figure 5 A PET isotope like $^{11}\text{C}$ will decay and emit positrons in the patient. A positron will move a short distance in the tissue before it interacts with an electron and annihilates. Every annihilation creates two gamma ray photons, which are emitted in opposite directions. The detectors in the PET scanner detects photons. Illustration from van der Veldt et al. [46] published under creative commons license CC BY 3.0 (https://creativecommons.org/licenses/by/3.0/).
PET and PET image reconstruction

For an annihilation to be detected by the PET scanner’s detectors (see Figure 5) and regarded as a true event, three different conditions must be met [47]:

1. The two photons need to be registered within a pre-defined time window, called the coincidence window.
2. The line between the two activated detectors ($d_a, d_b$) where the annihilation took place, and which is called the line of response (LOR), should be within the acceptance angle of the PET scanner.
3. The energy of each detected photon needs to be within a pre-set energy window.

LORs represent two-dimensional PET data which are often saved by parametrization into sinograms. It is also possible to store the coordinates ($d_a, d_b$) for each recorded true event, usually together with the time and the energy of every registered photon, in a consecutive data list called list mode data. The saved data are then used to reconstruct dynamic or static PET images. Commonly used reconstruction methods are iterative reconstructions, for example, Maximum-Likelihood Expectation Maximization (ML-EM) and Ordered Subset Expectation Maximization (OSEM) which is a faster method. With ML-EM, a theoretical sinogram based on an initial guess image is compared to the true sinogram. The suggested image will then be gradually updated, based on identified differences between the expected and measured data. With more iterations, the suggested image gradually converges to the true image. An increasing number of iterations will, however, also increase the level of noise in the image. With OSEM, the LOR data are divided into separate subsets which speeds up convergence and results in faster image reconstruction [48]. The drawback of ML-EM and OSEM is that the final image quality is a compromise between convergence, and correspondingly correct activity values, and noise [49]. Full convergence will lead to noisy images as shown in Figure 6. Block Sequential Regularized Expectation Maximization (BSREM) has recently been suggested as an alternative allowing for full convergence without noise amplification. This method is mathematically similar to OSEM but with the addition of a term that penalizes noise, the strength of which can be controlled by the term $\beta$ [50].
Figure 6 Simulated PET images of two large ellipses and two identical small spheres. OSEM with two iterations (A) results in a large underestimation of the activity concentration in the small sphere between the two ellipses. OSEM with 25 iterations (B) converges fully, showing the two small spheres correctly but leading to a lot of noise in the large ellipses. BSREM (C) converges fully without the excessive noise seen in the OSEM image (B). Adapted from Ross, S, Q.Clear white paper [50], GE Healthcare Chicago, Illinois, USA. © 2014 General Electric Company. Published with written permission from Ola Sorli, GE Healthcare.

Why kinetic modelling is needed

In clinical practice, quantitative PET image evaluation often equals the use of standard uptake values (SUVs) since it only requires static PET imaging after assumed tracer equilibrium and does not require the patient to undergo arterial blood sampling of the patient. SUV equals activity concentration normalised to injected activity per body weights. For example, when $^{11}$C]MTO PET is used for lateralization in PA patients, the maximal SUV ($SUV_{max}$) of the adrenal lesion is divided by the $SUV_{max}$ of the normal adrenal tissue. An $SUV_{max}$ ratio >1.25 represents lateralization, with the clinical implication that the PA patient will be offered surgical treatment [51, 52].

SUV equals the tissue activity in the region of interest (ROI)/adrenal lesion when corrected for the patient’s body weight and the injected dose of $^{11}$C]MTO. However, SUV is impacted by many factors, such as type of PET scanner used, correct registration of patient weight and injected activity, type of reconstruction algorithm used, and the length of time between tracer injection and start of PET-scanning [53].

Kinetic modelling is regarded as the most accurate PET quantification method, as it provides rate constants and macro-parameters quantifying the underlying biology. It requires dynamic PET scanning and preferably continuous arterial blood sampling, in both cases from the time of tracer injection, and correction for radiolabelled metabolites in blood. The arterial blood samples can be used together with the image-derived, whole blood time-activity curve, as the input function. In kinetic modelling the tracer is assumed to move...
between different compartments such as the plasma and tissue compartments, which are different states of the tracer (free, bound, metabolised, etc.) rather than physical compartments. The rate of the tracer’s movement between compartments is expressed as first order differential equations, from which rate constants can be calculated [54, 55].

When evaluating a new PET tracer, such as $^{18}$FCETO, it is important to perform a full quantitative analysis to validate SUV and simplified image-derived methods that do not require arterial blood sampling.

Why improved diagnostics are needed
Currently, many diagnostical challenges remain in the field of adrenal pathologies.

Primary aldosteronism
Adrenal vein sampling (AVS) is the gold standard for lateralization of PA. However, AVS is both difficult and invasive, and requires a high degree of technical proficiency. Furthermore, the method often fails to produce conclusive results [56]. $^{11}$C-MTO PET/CT with dexamethasone (DXM) pre-treatment has been presented as a non-inferior alternative to AVS. However, there are serious limitations to the usefulness of the tracer [57-60].

Adrenocortical carcinoma
As with other malignant diseases, it is essential to swiftly and correctly diagnose and stage a patient with ACC, as well as to be able to evaluate treatment response. Current issues with ACC imaging include the fact that small ACCs with low fat content, may mimic benign adrenocortical adenomas on CT [61]. As complete surgical resection remains the main treatment option in terms of curatively intended therapy, early detection is of great importance. $^{11}$C-MTO PET/CT is a valuable tool for detection of distant ACC metastasis in patients with verified ACC [12, 62].

Limitations of $^{11}$C-MTO use
Firstly, $^{11}$C-MTO binds to both CYP11B1 and CYP11B2, hence the need of dexamethasone pre-treatment to suppress the CYP11B1 expression. Concerns have been raised, however, that DXM pre-treatment may considerably impact CYP11B2 expression [63]. This is could potentially muddle the results when using $^{11}$C-MTO PET to lateralize PA. Secondly, in vivo, $^{11}$C-MTO metabolism breaks down into radioactive metabolites, which may have an impact on image quality [40, 64, 65]. Thirdly, there is a high degree of $^{11}$C-MTO accumulation in the liver. A previous clinical $^{11}$C-MTO study suggests that
liver uptake is mediated by targets other than CYP11B and that another P450 enzyme in the liver might be involved [62]. Because of this, combined with the liver’s anatomical proximity to the right adrenal gland, adrenal pathologies may be obscured – rendering the PET measurements unreliable. Fourthly, an increase in $[^{11}\text{C}]$MTO uptake has been discovered in a variety of other liver lesions, e.g., in hepatocellular carcinoma (HCC) and focal nodular hyperplasia (FNH), increasing the risk of false positive results [66]. The high liver uptake of $[^{11}\text{C}]$MTO may hinder detection of small ACC liver metastasis and the lack of specificity regarding other liver lesions could possibly lead to misinterpretations of liver findings. And lastly, carbon-11 has a very short half-life (T1/2 = 20.4 minutes). This fact, in and of itself, restricts the compound’s clinical applicability, as it confines its use to highly specialized health care facilities with an in-house cyclotron and radio-pharmacy [67].
Aims

The overarching aim of the study was, as previously stated, to improve adrenocortical PET imaging, while the four papers included in this study each had their own specific aims.

1. To further evaluate the previously published fluorine-18 etomidate analogue, para-chloro-2-[\textsuperscript{18}F]CETO, as an adrenal PET tracer. \textit{(Paper I, pre-clinical)}

2. To perform a first evaluation of para-chloro-2-[\textsuperscript{18}F]fluoroethyletomidate positron emission computed tomography ([\textsuperscript{18}F]CETO-PET/CT) in patients with adrenal tumours and in healthy volunteers. \textit{(Paper II, clinical)}

3. To assess in vivo and in-human radiation dosimetry of ([\textsuperscript{18}F]CETO. \textit{(Paper III, dosimetry)}

4. To assess the image quality of a block-sequential regularized expectation maximization (BSREM) reconstruction algorithm (Q. Clear, GE Healthcare, Chicago, Illinois, USA), and to determine the optimal penalization factor (expressed as $\beta$-values) for clinical [\textsuperscript{11}C]metomidate-PET/CT \textit{(Paper IV, optimizing reconstruction)}
Materials and methods

Radio synthesis of $[^{18}\text{F}]\text{CETO}$ (Papers I-III)

Synthesis of $[^{18}\text{F}]\text{CETO}$ was performed using a precursor (Pharmasynt AS, Tartu, Estonia) with a chemical purity above 97% according to high performance liquid chromatography (HPLC) results. $[^{18}\text{F}]\text{fluoride}$ was diluted in a QMA SPE cartridge and dried at 120°C. The precursor (pCETO) was allowed to react with the $[^{18}\text{F}]\text{fluoride}$ at 110°C for 10 minutes. After cooling and dilution, $[^{18}\text{F}]\text{CETO}$ was purified by HPLC.

Ethics (Papers I-IV)

The study in Papers I and IV was approved by the Local Ethics Committee. The animal studies in Papers I and III were approved by the Animal Ethics Committee. Tissues used in Paper I were obtained from the Uppsala Biobank. All patients provided written informed consent. Studies in Papers II and III were approved by the Swedish Medical Product Agency and the Swedish Ethical Review Authority.

Animals (Papers I & III)

In Papers I and III, eight female C57BL/6 mice, 15 male and 15 female Sprague Dawly rats as well as one female non-human primate (NHP), Macaca fascicularis, were used. Mice and rats were housed in the animal room at Rudbeck Laboratory and in the animal room at the pre-clinical PET centre at Uppsala University. The NHP female was obtained from, and housed at the Astrid Fagræus Laboratory, Karolinska Institute.

Biomaterials (Paper I)

Human tissue-samples were obtained from the Uppsala Biobank containing many snap-frozen endocrine tumour-samples as well as samples of healthy endocrine tissue. NHP tissue samples were obtained from the pre-clinical PET centre at Uppsala University.
Immunohistochemistry (Paper I)

Tissues samples from chosen healthy organs and a variety of adrenal tumours were cut with a cryostat. The 6 μm thick tissue slices were incubated with CYP11B1 or CYP11B2 primary antibodies. Afterwards a VECTASTAIN® ABC kit was used for staining.

In vitro binding studies (Paper I)

A saturation study was performed twice, using homogenate of NHP normal adrenal tissue and different concentrations of $^{18}$F[CETO with or without metomidate as competing ligand. Results were used to calculate the total target density for binding of $^{18}$F[CETO ($B_{\text{max}}$) and the equilibrium dissociation constant ($K_d$).

A competing assay was performed, also using homogenate of NHP normal adrenal tissue with $^{18}$F[CETO incubated with MTO, FETO and CETO as competing ligands. GraphPad Prism 8.2 (San Diego, California, USA) was used to calculate $B_{\text{max}}$, $IC_{50}$ and $K_d$.

An in vitro kinetic study was performed for $^{18}$F[CETO association and dissociation to homogenate of NHP normal adrenal tissue, using LigandTracer™ yellow (Ridgeview Instruments AB, Uppsala, Sweden). Tracedrawer software (Ridgeview Instruments AB, Uppsala, Sweden) and a 1:1 kinetic binding model were used for calculations of the association rate constant ($K_{\text{on}}$), the dissociation rate constant ($K_{\text{OFF}}$) and the equilibrium dissociation constant ($K_D$).

Autoradiography (Paper I)

Duplicated tissue slices (20 μm) were incubated with $^{18}$F[CETO or $^{18}$F[CETO and metomidate. Incubation was followed by washing and drying, before being exposed to Super Resolution Storage Phosphor Screens (PerkinElmer, Downers Grove, IL, USA). After at least 240 minutes the screens were scanned in a Cyclone Plus Phosphor Imager (PerkinElmer, Model C431200, Downers Grove, IL, USA).

Biodistribution (Paper I)

Thirty Sprague Dawley rats of both genders were used. Three rats of each gender, and for each timepoint, were injected with a bolus of $^{18}$F[CETO. At each timepoint (10, 30, 60, 120 and 240 minutes post injection) the rats were
euthanized for organ harvesting. Radioactivity in the organs was measured in a well counter.

Selection of patients (Papers II-IV)

Patients treated at the endocrine surgery clinic at Uppsala University hospital were asked to enrol in the clinical study resulting in Papers II and III. All 15 enrolled patients provided written consent and each patient had an established diagnosis of either primary aldosteronism, cortisol-producing adrenocortical adenoma, non-functioning adrenocortical adenoma, myelolipoma or adrenocortical carcinoma, all according to the inclusion criteria. Exclusion criteria for patients were claustrophobia, pregnancy/breastfeeding or assessed as unwilling or unable to comply with the requirements of the study protocol.

For Paper IV, patients were selected based on whether (1) they had undergone an $^{11}$Cmetomidate PET/CT scan at Uppsala University Hospital due to hyperaldosteronism and had provided given written consent, and (2) that appropriate raw data were still available for reconstructions.

Healthy volunteers (Paper II)

A non-associated external provider (Clinical Trials Consultant, Uppsala, Sweden) was used to recruit five healthy volunteers. Inclusion criteria were age $\geq 18$ and the provision of written informed consent. The exclusion criteria were the same as for patients, with the addition of no known adrenal disease in the healthy controls.

Clinical information (Papers II-IV)

Clinical information was obtained from electronic patient records.

MRI (Paper I)

A Nanoscan PET-3TMRI scanner (Mediso Mediso, Medical Imaging Systems, Budapest, Hungary) was used for $^{18}$FCETO and $^{18}$FETOT PET/MRI-imaging of rats, and for $^{18}$FCETO PET/MRI-imaging of mice.
PET/CT (Papers I-IV)

In all papers, a Discovery MI 4- or 5-ring PET/CT scanner (General Electric, Milwaukee, Wisconsin, USA) was used for $^{18}$F[CETO and $^{11}$C]MTO PET/CT of NHP; $^{18}$F[CETO PET/CT of patients, as well as $^{18}$F[CETO PET/CT of healthy volunteers. Some healthy volunteers also underwent $^{15}$O]water PET/CT for measurements of adrenal blood flow.

In Paper I-III, PET images were reconstructed using Time-of-Flight (TOF) Ordered Subset Expectation Maximization (OSEM) (VPFX-S). In Paper IV PET images were reconstructed using TOF OSEM and block-sequential regularized expectation maximization including TOF (BSREM) (Q.Clear, GE Healthcare, Milwaukee, USA).

Dosimetry (Paper III)

By dosimetry [68], the radiation doses in the various tissues in animals and humans were calculated. In Paper III, uptake data from rats was based on ex vivo data from the biodistribution study. Uptake data from NHP and humans were based on in vivo $^{18}$F[CETO PET. Organ dose and effective dose were calculated with OLINDA 1.1.

Radio metabolite analysis (Papers I & II)

NHP venous blood was used in Paper I. Blood and plasma were measured for radioactivity. Proteins were separated from plasma, before high-performance liquid chromatography (HPLC) separation and fraction collection, where a CET参考er was used for identification of the parent compound. A reverse phase (RO) HPLC method was used to separate radio metabolites from the parent compound.

In Paper II, arterial blood from patients was drawn using a Veenstra automatic sampling system for continuous whole blood measurements. Discreet arterial samples were also taken for determination of per cent intact $^{18}$F[CETO in plasma. Metabolite analyses were performed using the same method as in Paper I.

Kinetic modelling (Papers I & II)

NHP venous blood was used in Paper I. Plasma time-activity curves (TACs) were calculated by multiplying the aorta TAC with the mean plasma-to-whole blood ratio based on analyzed blood samples. The plasma TAC was multiplied
with a sigmoid fit to the measured parent fraction, to achieve a metabolite-corrected input function representing intact $[^{18}\text{F}]$CETO in plasma. Adrenal TACs were fitted with several models including reversible/irreversible, singe-tissue/two-tissue compartment models. Additionally, graphical models Patlak (irreversible kinetics) & Logan (reversible kinetics) were used, and parametric uptake rate images were calculated using the Patlak method.

Human arterial blood from patients was used in Paper III. Aorta TACs, sample- or image-based, were multiplied as described above, to derive blood sampler-derived (BSIF) and image-derived (IDIF) input functions. Additionally, IDIFs based on individual whole-blood TACs and population-averaged data for plasma-to-whole-blood ratio and parent fraction were calculated (IDIF-PA). In-house developed Matlab software (The Mathworks, Natick, MA, USA) was used for all tracer kinetics modelling, including the construction of parametric images.

In both Papers I and II the Akaike information criterion [69] was used to determine the optimal model to describe data.

**Adrenal blood flow (Paper III)**

The Hermes hybrid viewer was used to transfer already drawn VOIs from the dynamic $[^{18}\text{F}]$CETO scans to the $[^{15}\text{O}]$water images. A single-tissue compartment model was used to calculate adrenal blood flow with the aorta TAC as input function. A regression analysis was used to assess the correlation between the adrenal blood flow and the $[^{18}\text{F}]$CETO adrenal uptake.

**Test retest (Paper III)**

Bland-Altman analysis was used for assessing the agreement between test and retest outcome parameters.

**Image analysis (Paper IV)**

VOIs were drawn on the TOF OSEM reconstruction and then propagated to all other reconstructions, using Volume Viewer 13.0 ext. 2 (GE Healthcare, Chicago, Illinois) in the Advantage Workstation Server. Signal-to-noise ratio (SNR) and signal-to-background ratio (SBR) were calculated. TOF OSEM was compared to BSREM reconstructions with $\beta$-values 70, 130, 400 and 800.

*Further details on materials and methods may be found in the respective papers.*
Summary of included papers

Paper I
Doi:10.7150/ijms.51206
*Contributed equally as last authors.

Paper I investigated the pre-clinical properties of [18F]CETO using autoradiography on human tissue (ACC, cortisol-producing adenoma, pheochromocytoma, aldosterone-producing adenoma, adrenal gland, liver, small intestine, spleen and kidney) and non-human primate (NHP) tissue (adrenal gland and spleen), binding studies on NHP adrenal tissue, ex vivo biodistribution on rats (n=30) as well as in vivo imaging using mice (n=8), rats (n=2) and one NHP. We found that [18F]CETO specifically binds to normal adrenal cortex both in humans (in vitro) and in NHP and rats (in vivo). [18F]CETO also exhibits specific binding to adrenocortical adenomas and adrenocortical carcinomas on human tissue (in vitro). Importantly, [18F]CETO does not bind to pheochromocytomas (in vitro), a different kind of endocrine adrenal tumour that originates from the adrenal medulla.

Paper II
Doi: 10.1007/s00259-022-05957-9
*Contributed equally as last authors.

The first clinical study of [18F]CETO included patients (n=15) with various kinds of adrenocortical tumours (aldosterone producing adenoma, adrenocortical hyperplasia, cortisol producing adenoma, non-functioning adenoma, myelolipoma and adrenocortical carcinoma) and five healthy volunteers. All
subjects underwent $[^{18}\text{F}]$CETO-PET/CT. Test retest was performed on the healthy volunteers.

Three out of five healthy volunteers also underwent $[^{15}\text{O}]$water PET/CT to measure adrenal blood flow.

Arterial blood samples were used for tracer metabolite analysis. Tracer kinetic outcome measures were used for validation by comparison to simplified quantitative methods.

$[^{18}\text{F}]$CETO was found to have a rapid initial metabolism. The uptake of $[^{18}\text{F}]$CETO was high in the adrenal glands and low in the liver. An irreversible single-tissue compartment model could best describe $[^{18}\text{F}]$CETO kinetics in healthy adrenals as well as in all adrenal tumours except for adrenocortical carcinoma.

Standardized uptake values (SUVs) and the uptake rate constant $K_i$ correlated highly to adrenal blood flow in healthy volunteers. Adrenal SUV at 1 hour post injection correlated well with $K_i$. The results, in combination, suggest that $[^{18}\text{F}]$CETO is a suitable tracer for adrenal imaging.

**Paper III**


*Contributed equally as last authors.

The aim of this study was to assess in vivo and in-human radiation dosimetry of $[^{18}\text{F}]$CETO. Uptake data from rats (n=30), biodistribution study with ex vivo measurements) as well as in vivo PET/CT in cynomolgus (n=1) and humans (n=9) was used to calculate residence times.

OLINDA 1.1 was used to ascertain absorbed doses in human organs (mGy/MBq) and effective dose (mSv/MBq). The adrenal glands were confirmed as the dose-limiting organ regardless of species used. Based on human data the effective dose was 18.2 $\mu$Sv/MBq. $[^{18}\text{F}]$CETO has a favourable biodistribution in humans for adrenal imaging. A typical clinical PET/CT examination with 200 MBq $[^{18}\text{F}]$CETO corresponds to an effective dose of 3.6 mSv. Based on our findings we confirm that $[^{18}\text{F}]$CETO is safe for clinical use with respect to radiation dose.
PET raw data, in the form of a sinogram, must be reconstructed to form what we recognize as a PET image. In this retrospective study, we evaluated the use of block-sequential regularized expectation maximization (BSREM) reconstruction algorithm (Q.Clear, GE Healthcare, Milwaukee, USA) for $^{[11]}$C-metomidate-PET/CT in patients with primary aldosteronism (PA). The reconstruction algorithm that is currently used in clinical practice, time-of-flight coupled ordered subset expectation maximization (TOF OSEM), was used for comparison.

Seven PA-patients who had previously undergone $^{[11]}$C-metomidate PET/CT at Uppsala University Hospital were selected.

Raw data were reconstructed applying BSREM, with β-values 70-800, or TOF OSEM. Tumour SUV$_{\text{max}}$ in the aldosterone producing adenomas (APAs) were also measured. Image quality was assessed regarding signal-to-noise ratio (SNR) and signal-to-background ratio (SBR) in comparison with TOF OSEM, applying a two-tailed Wilcoxon matched pairs signed-rank test. The effect of BSREM on lateralization was also addressed.

With the BSREM algorithm the average lesion SUV$_{\text{max}}$ increased for β-values 70 and 130 (p=0.0156) compared to the TOF OSEM reconstruction.

SNR increased only for β-value 130 (p=0.0156) and SBR increased for β-values 130 or higher (p=0.0156). An adenoma-to-normal-adrenal gland ratio of 1.25 was used as the cut-off, rendering one additional lateralized patient by $^{[11]}$C-metomidate PET reconstructed with the BSREM (β 70 and 130, p=0.0156 and p=0.0312) as compared to TOF OSEM.
Discussion

Combined results from Papers I-III have demonstrated that \[^{18}\text{F}]\text{CETO}\) is highly specific to the adrenal cortex both in vitro and in vivo, and safe for clinical imaging in terms of radiation dose.

Furthermore, the in vivo binding properties of \[^{18}\text{F}]\text{CETO}\) qualitatively surpass those of \[^{11}\text{C}]\text{MTO}\). For example, non-specific binding to the liver was significantly lower than that of \[^{11}\text{C}]\text{MTO}\), making the right adrenal gland easier to visualize. Paper III additionally confirmed that the effective dose differed less than 15%, when results were compared between species (one NHP, nine humans and 30 rats), even though methodical differences between the studies. Paper IV demonstrated that using the BSREM reconstruction algorithm improves image quality, without compromising \(\text{SUV}_{\text{max}}\) quantification, and a \(\beta\)-value between 70 and 130 was found optimal. BSREM may therefore improve lateralization by \[^{11}\text{C}]\text{metomidate PET/CT}\) in primary aldosteronism. It is highly likely that BSREM reconstruction of \[^{18}\text{F}]\text{CETO PET/CT}\) in primary aldosteronism would show similar benefits.

Moreover, the relatively longer half-life of \[^{18}\text{F}]\text{CETO}\) means that a higher number of health care facilities can implement it into clinical practice and labelling of one batch of \[^{18}\text{F}]\text{CETO}\) may be used the same day in multiple patients.

The included studies (Papers I-IV) have several limitations. In Papers I and III that NHP data was based solely on one individual. In Paper II, the adrenal blood flow measurements were not performed on any patients and complete arterial data were only obtained in four out of ten patients. In Paper IV, the study group was small and histopathological conformation was available in just two out of the total of seven patients.

The low liver uptake of \[^{18}\text{F}]\text{CETO}\), compared to that of \[^{11}\text{C}]\text{MTO}\), is expected to improve imaging of ACC liver metastasis and right-sided adrenal lesions in general. With CETO and MTO being structurally quite similar, it is unlikely that intact \[^{11}\text{C}]\text{MTO}\) binds in the liver when \[^{18}\text{F}]\text{CETO}\) does not: this is most likely due to non-identified radioactive metabolites. While both \[^{11}\text{C}]\text{MTO}[62]\) and \[^{18}\text{F}]\text{CETO}\) metabolize rapidly, it seems that different non-
identified metabolites are formed. Whether this impacts visualization of focal nodular hyperplasia, liver adenoma or HCC needs to be further investigated.

$[^{18}F]CETO$ is not exclusively specific to CYP11B2, meaning that dexamethasone pre-treatment will most likely still be needed if used for lateralization of PA. For PA imaging, a better option would be a CYP11B2-specific tracer. Preclinically, $[^{18}F]AldoView$ has been shown to have high selectivity for CYP11B2 compared to CYP11B1 [37] but this has still to be verified in clinical studies.

Thus, improved imaging for PA would offer a more reliable algorithm in the work-up of hypertensive patients suffering from PA than today, when AVS is used only in selective cases. This may also lead to increased awareness of this disease, and better treatment for more hypertensive patients resulting in reduced morbidity and mortality in cardiovascular disease.

Imaging of ACC might be more suitable with a tracer like $[^{18}F]CETO$ due to a variable steroid expression. The adrenal uptake of $[^{11}C]MTO$ or $[^{18}F]CETO$ per se cannot distinguish between benign or malignant adrenal lesions. However, both tracers may be used in unclear adrenal pathologies where ACC may be one differential diagnosis. Specifically, findings of unsuspected uptake in metastases will reveal the malignant nature of the lesion and at the same time diagnose the ACC.
Future directions

Diagnostics and treatment options are becoming more and more personalized, and it is highly likely that PET scans and radiotracers will become increasingly important to the medicine of the future.

Our area of study has focused on evaluating $^{18}$F-CETO. However, further studies are needed. Studies are ongoing to explore $^{18}$F-CETO with dexamethasone pre-treatment as a less invasive option to AVS for lateralization of PA [42], as well as a comparison between $^{18}$F-CETO and $^{11}$C-MTO when used for lateralization of PA [42].

Further studies should also explore the potential usefulness of $^{18}$F-CETO in the staging of ACC.

New types of reconstruction methods are being tested and used to improve PET image quality. Recently, a study on respiratory gating on $^{68}$Ga-DOTATOC PET/CT found higher SUVmax and SUVmean values and smaller tumour values [42]. As adrenal tumours, especially aldosterone-producing adenomas, are small [43], respiratory gating should be explored for $^{18}$F-CETO PET/CT.

With BSREM reconstruction and $^{11}$C-MTO potentially changing the number of lateralized PA patients, it is important to verify this finding in a study where all patients receive surgical treatment. The use of BSREM and respiratory gating may greatly improve adrenal imaging when used with adrenal tracers as $^{11}$C-MTO or $^{18}$F-CETO.

Further, as a future field of investigation image-related genetic derangements may also be studied regarding $^{18}$F-CETO. This area, denoted imiomics, is important in the field of personalized precision medicine, where certain genetic derangements may be visualized and thus governing treatment. The genetic landscape of ACC is being currently investigated thoroughly, and relation to image appearance is most certainly a future perspective.
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