CuATSM PET to diagnose age-related diseases: a systematic literature review

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Abstract

Purpose Cu(II)-diacetyl-bis(*N*4-methylthiosemicarbazone) positron emission tomography (CuATSM PET) is a non-invasive imaging technique that can be used to detect hypoxia and inform prognosis in cancer. Hypoxia and oxidative stress are also hallmarks of various age-related diseases. Whether CuATSM PET has a role in the evaluation of hypoxia and oxidative stress in age-related diseases has yet to be established. The aim of this systematic review is to evaluate the utility of CuATSM PET in the diagnosis and management of age-related diseases.

Methods EMBASE, Medline, Scopus, Web of Science and Psychinfo were systematically searched for articles published between January 1st 1997 and February 13th 2020. We included articles published in English reporting the use of CuATSM PET in the diagnosis and management of age-related diseases in humans or animals.

Results Nine articles were included describing CuATSM PET measures in neurological and cardiovascular disease. There was higher CuATSM uptake in diseased compared to control subjects in Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), myocardial ischemia (MI), cardiac dysautonomia and atherosclerosis. Higher CuATSM uptake was seen in diseased compared to control anatomical areas in PD, cerebrovascular disease (CVD), MI and atherosclerosis. CuATSM uptake was associated with disease severity in PD, ALS, CVD and atherosclerosis. An association between CuATSM uptake and disease duration was shown in atherosclerosis.

Conclusion CuATSM uptake is higher in neurological and cardiovascular diseases and associated with disease severity and duration. Further investigations using CuATSM PET in other age-related diseases are needed.

Keywords Positron-emission tomography · Hypoxia · Ferroptosis · Aged · Nervous system disease · Cardiovascular disease · Morbidity

Introduction

Aging is associated with an increased risk of developing age-related disease [1]. Age-related diseases, such as neurological and cardiovascular diseases, represent the leading

cause of death worldwide [2, 3]. Many of these diseases share important hallmarks of aging including accumulation of DNA damage [4], mitochondrial dysfunction [5], decreased autophagy [6], and production of reactive oxygen species (ROS) [7]. The latter can lead to oxidative stress and hypoxia [7, 8], which in turn can induce ferroptosis [9, 10]. This iron-dependent programmed cell death is seen in age-related diseases such as Parkinson's disease (PD) [11], Alzheimer's disease (AD) [12, 13] and cardiovascular diseases [14].

Cu(II)-diacetyl-bis(*N*4-methylthiosemicarbazone) (CuATSM), first developed as a positron emission tomography (PET) hypoxia marker [15], has been used to evaluate tumor hypoxia, predict disease prognosis and tailor therapy in various types of cancer [16–19]. More recently, increased CuATSM PET uptake has been seen in PD in the absence of hypoxia [20]. This is thought to be due to CuATSM's ability

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to detect redox changes [21]. Hypoxia and redox changes such as oxidative stress [22] also play an important role in age-related diseases [23–25]. Whether CuATSM PET has a role in the diagnosis and management of age-related human diseases has yet to be established.

The aim of this systematic review is to evaluate the utility of CuATSM PET, as a non-invasive imaging technique, in the diagnosis and management of age-related diseases.

Material and methods

Protocol registration

The protocol of the systematic review was registered with the International prospective register of systematic reviews (PROSPERO): CDR/code CRD42020167009. The systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards [26].

Search strategy

A systematic search was performed with an academic senior liaison librarian (P.C.) in five electronic databases: EMBASE, Medline, Scopus, Web of Science and Psycinfo. The search included keywords: “CuATSM”, “PET” and other synonyms of CuATSM (“diacetyl-bis(*N*4-methylthiosemicarbazone) copper (II)”, “diacetyl-di (*N*4-methylthiosemicarbazone) copper”) and all radioactive isotopes of CuATSM (60 , 61 , 62 , 64 CuATSM). The first investigation of CuATSM as a hypoxia marker was conducted in 1997 [15]. Therefore, articles published between January 1st 1997 and February 13th 2020 were included in this search. The full search strategy is available in Appendix 1. The reference section of each included article was also used to identify additional related articles.

Study selection

The articles obtained using the search strategy were assessed for eligibility against predefined inclusion and exclusion criteria independently by two authors (J.J.Y. and N.M.) by screening titles and abstracts. The same reviewers then screened the full text of resulting articles. A third reviewer (A.B.M.) resolved any disagreements between the authors regarding eligibility. Inclusion criteria were: articles using CuATSM PET in the diagnosis and management of age-related diseases in humans or animals, published in English: Opinion articles, conference abstracts, editorials, letters to the editor, and case reports were excluded.

Data extraction and quality assessment

The following variables were extracted independently by two reviewers (J.J.Y. and N.M.) from the included studies: first author, year of publication, title, study design, study population, sample size, age, proportion of females, country, age-related disease studied, intervention, primary purpose of the scan, type of PET tracer, duration of the scan, scan results, clinical outcomes measured, other reported outcomes, adverse events related to intervention and statistical analysis used.

Articles were assessed for their risk of bias independently by two reviewers (J.J.Y. and N.M.) using the Newcastle Ottawa Scale (NOS) for cohort studies [27, 28]. A system of points was given to the following categories: (i) selection of the study population, (ii) comparability, and (iii) description of the outcome (Appendix 2). A study was given a maximum of one point in each item within the “selection” and “outcome” categories and a maximum of two points in the “comparability” category. The scores ranged from 0 to 9. A score of seven or more was classified as high quality.

Analysis

We summarized the difference between CuATSM uptake in diseased and control subjects, and between diseased and control anatomical areas. Additionally, the association between CuATSM uptake and severity and duration of age-related disease was summarized.

Results

Search strategy

The search retrieved 9633 articles. After exclusion of duplicates ($n=5774$), articles were screened for title and abstracts of which 244 were screened for full text and nine articles were included in this review (Fig. 1).

Study characteristics

The included articles were classified according to the type of age-related disease: neurological (four articles) and cardiovascular disease (five articles) (Table 1). A total of 46 humans with neurological disease (mean age 62.7 ± 6.58 years, $n=19$ female) and 22 controls (mean age 43.6 ± 8.33 years, $n=3$ female) as well as seven humans with cardiovascular disease (aged 63–80 years, $n=3$ female (no controls)) were included. A range of animal models including dog, macaque, rabbit and mouse served to study cardiovascular disease (total $n=40$ diseased subjects and $n=7$ controls). The aim of all included articles was to use CuATSM PET to diagnose or prognosticate

Fig. 1 Flow chart of the study selection process **Table 1** Characteristics of included articles assessing the utility of CuATSM PET in age-related diseases

Author (year)	Species	Diseased subjects				Control subjects				CuATSM PET scan		
		Type	N	♀	Age, years	Type	N	♀	Age, years	*Cu	Type (duration, min)	Anatomical location
Neurological disease												
Ikawa (2011)	Human	PD	15	8	72.2±9.4	HC	6	0	34.7±9.0	62	Dynamic (20)	Brain-striatum
Ikawa (2015)	Human	ALS	12	5	65.2±9.4	HC	9	3	61.2±8.0	62	Dynamic (20)	Whole-brain
Isozaki (2011)	Human	CVD	10	3	66±7	HC	7	0	35±8	62	Dynamic (20)	Whole-brain
Neishi (2017)	Human	PD	9	3	67.3±7.1	NA	0	NA	NA	62	Dynamic (20)	Brain-striatum
Cardiovascular disease												
Lewis (2002)	Dog	MI	16	0	NR	NA	0	NA	NA	64	Dynamic (60)	Myocardium
Metzger (2018)	Macaque	CD	10	0	Adult	NA	0	NA	NA	61	Dynamic (45)	Heart
Nie (Dec 2016)	Rabbit	ATH	5	NR	NR	NA	0	NR	NA	64	Dynamic (60)	Femoral artery
Nie (Sep 2016)	Mouse	ATH	9	NR	1	WT	7	NR	0.83	64	Dynamic (30)	Aortic arch
Takahashi (2001)	Human	CAD	7	3	[63–80]	NA	0	NA	NA	62	Static (10)	Myocardium

ALS amyotrophic lateral sclerosis, ATH atherosclerosis, CAD coronary artery disease, CD cardiac dysautonomia, CVD cerebrovascular disease, *Cu type of Cu copper isotope, HC healthy controls, MI myocardial ischemia, N sample size, NA not applicable, NR not reported, PD Parkinson's disease WT wild type. Mean ± SD, [range]

age-related diseases. The Cu isotope, type, duration and anatomical location of the scan differed according to the disease and type of subjects studied. All articles describing neurological diseases used a 20 min dynamic $^{62}\text{CuATSM}$ PET scan and measured standard uptake value (SUV) in the brain: striatum for PD [20, 29], whole-brain for ALS [30] (Fig. 2a) and cerebrovascular disease [31] (Fig. 2b). All cardiovascular disease articles used dynamic 30–60 min PET scan except one which used static 10 min PET scan data [32] (Fig. 2c). The ^{64}Cu isotope was used to image atherosclerosis in rabbit and mouse [33, 34] and myocardial ischemia (MI) in dogs [35]. The ^{61}Cu isotope was used to image cardiac dysautonomia in macaques [36] and ^{62}Cu for coronary artery disease (CAD) in humans [32].

Quality assessment

The total NOS scores are shown in Appendix 3. Three articles [30, 33, 35] were scored as high quality and the remaining as medium quality.

Qualitative description of CuATSM PET uptake and age-related diseases

Table 2 shows the difference in CuATSM uptake in diseased compared to control subjects, and diseased compared to control anatomical areas, as well as the association of CuATSM uptake with disease severity and duration. Compared to healthy controls, CuATSM uptake was higher in the

Fig. 2 Distribution patterns of CuATSM uptake in age-related diseases. Distribution patterns of PET CuATSM uptake seen in included studies. **a** Human Parkinson's disease and amyotrophic lateral sclerosis studies [20, 29, 30]; **b** human cerebrovascular diseases studies [31]; **c** human, dog, macaque, mouse and rabbit cardiovascular disease studies [32–36]

striatum of PD patients and the bilateral cortices around the central sulcus, including the motor cortex and right superior parietal lobule, of ALS patients [20, 30]. Higher CuATSM uptake was seen in affected compared to non-affected hemispheres in PD patients [29]. CuATSM uptake in the brain was significantly associated with disease severity expressed as the total and motor unified Parkinson's disease rating scale (UPDRS) in PD patients [20, 29] and revised amyotrophic lateral sclerosis functional rating scale (ALSSFR-S) in ALS patients [30]. No association was observed between CuATSM uptake and ALS disease duration [30], while a positive trend was observed between CuATSM uptake and PD duration [20].

Compared to controls, higher CuATSM uptake was detected in the myocardium of dogs after MI, the anterior ventricle of macaques with cardiac dysautonomia and in the aortic arch of mice with atherosclerosis [34–36]. CuATSM uptake was greater in affected compared to non-affected hemispheres in patients with unilateral major cerebral artery occlusion [31], in ischemic compared to normal tissue in dog myocardium and in injured (modelling atherosclerosis) compared to the non-injured femoral artery of rabbits [33, 35]. CuATSM uptake was significantly associated with disease severity expressed as the oxygen extraction fraction (OEF) in patients with unilateral major cerebral artery occlusion [31] and with the degree of thickening of the vessel wall in rabbits with atherosclerosis [33]. CuATSM uptake increased significantly from 14 to 23 weeks after atherosclerosis induction in mice [34] suggesting an association between CuATSM uptake and duration of atherosclerosis. Finally, CuATSM uptake was enhanced in one patient with unstable angina compared to patients with stable coronary artery disease [32]. The heterogeneity of study designs and outcome measures precluded a meta-analysis.

Discussion

CuATSM uptake was higher in human and animal subjects with neurological and cardiovascular disease compared to controls and in diseased compared to non-diseased anatomical areas. CuATSM uptake was positively associated with disease severity and duration.

CuATSM PET and neurological diseases

Currently, neurodegenerative diseases like PD and ALS can only be diagnosed when symptoms manifest, while biomarkers allowing earlier detection remain elusive [37–39]. Hypoxia and oxidative stress are associated with cell death mechanisms involved in ALS and PD [40, 41]. Thus, using CuATSM as a redox change biomarker could lead to earlier detection of neurodegenerative diseases [42]. Due to its

sensitivity to disease severity in ALS, PD and cerebrovascular disease [20, 29–31], CuATSM PET could also be used as a tool for monitoring therapy. Additionally, vascular cognitive impairment, in part caused by chronic cerebral hypoperfusion [43], is generally not detected until patients present with cognitive decline [44]. Therefore, using CuATSM PET to detect chronic hypoperfusion may result in earlier diagnosis and intervention in cognitive impairment and dementia [45].

CuATSM PET and cardiovascular diseases

CuATSM PET provides a quantitative assessment of myocardial ischemia in research [35] and clinical settings [32] and shows promise in diagnosing systemic atherosclerosis in research settings [33, 34, 46], but its utility has not yet been translated into clinical practice. Animal and human studies show the presence of hypoxia in atherosclerotic plaques and its link to plaque rupture [47, 48]. Thus, using CuATSM PET to image hypoxia in atherosclerosis may provide important pathophysiological insight and help identify vulnerable atherosclerotic plaques, which could leave to improved patient management [49].

CuATSM PET and other age-related diseases

No evidence of the use of CuTASM PET in the detection of hypoxia and oxidative stress in age-related diseases was found other than in neurological and cardiovascular diseases. Other age-related diseases associated with redox changes include chronic kidney disease [50], urinary tract infections [51], type 2 diabetes mellitus [52] and sarcopenia [53]. Hypoxia in tubulointerstitium and glomerular oxidative stress play a role in chronic kidney disease progression [54, 55], although blood oxygen level-dependent magnetic resonance imaging (BOLD-MRI) has failed to demonstrate this association in the past [55–57]. Given the ability of CuATSM PET to detect much lower concentrations of molecules than MRI [58], it may be useful in predicting the progression of chronic kidney disease before any clinical manifestations and hence identify patients who require more intense primary prevention [59, 60]. Urinary tract infections are associated with oxidative stress [61] and the accumulation of hypoxia-inducible factor-1 α subunit (HIF-1 α) [62]. HIF-1 α is also induced by hyperglycemia in diabetes [63, 64], where inhibition of HIF-1 α leads to decreased insulin resistance [65]. Thus, CuATSM PET may also be useful in research into the pathogenesis of recurrent urinary tract infections [66, 67] and insulin resistance [68]. Recent evidence suggests that oxidative stress and hypoxia play a role in the development of sarcopenia [53, 69]. Given the current need for new imaging tools for sarcopenia [70], the potential

Table 2 Standard uptake values of CuATSM and its association with severity and duration of age-related disease stratified by species

Author (year)	SUV area	Subjects		Anatomical areas			Disease severity		Disease duration	
		Diseased	Controls	p	Diseased	Control	p	Outcome	r, p	r, p
Neurological disease										
Human										
Ikawa (2011)	B-S/C	1.15 ± 0.10	1.08 ± 0.02	p < 0.05	NA	NA	NA	tUPDRS	r = 0.52, p < 0.05	r = 0.48, p = 0.07
	C-S/C	NR	NR	NR	NA	NA	NA	tUPDRS	r = 0.62, p < 0.05	r = 0.49, p = 0.06
	C/I	1.20 ± 0.05	1.00 ± 0.08	NS	NA	NA	NA	mUPDRS	r = 0.49, p = 0.07	
Ikawa (2015)	RC-CS	Mean nSUV in ALS patients was greater than that of controls		p < 0.001	NA	NA	NA	tUPDRS	r = 0.53, p < 0.05	NA
								mUPDRS	r = 0.54, p < 0.05	
								ALSFRS-R	r = - 0.64, p < 0.05	NS (NR)
	LC-CS		p < 0.005	NA	NA	NA	ALSFRS-R	r = - 0.58, p < 0.05		
	BC-CS		p < 0.001	NA	NA	NA	ALSFRS-R	r = - 0.69, p < 0.05		
	RSPL		p < 0.001	NA	NA	NA	ALSFRS-R	r = - 0.62, p < 0.05		
Isozaki (2011)	H D/E	1.07 ± 0.07	1.01 ± 0.02	NS	52.6 ± 5.0	49.0 ± 2.8	p < 0.05	OEf	r = 0.73, p < 0.0005	NA
Neishi (2017)	C/I/SBR	NA	NA	NA	9.60 ± 6.2	6.8 ± 3.02	p < 0.05	tUPDRS	r = 0.85, p < 0.005	NA
								mUPDRS	r = 0.83, p < 0.01	
Cardiovascular disease										
Animal										
Lewis (2002)	MMR	4.94 ± 3.00	0.70 ± 0.42	p < 0.005	40.7 ± 39.0	26.7 ± 22.3	p < 0.05	NA	NA	NA
Metzger (2018)	ALV	[1.681]	[0.829]	p < 0.044	NA	NA	NA	NA	NA	NA
Nie (Dec 2016)	IF/BM	NA	NA	NA	2.30 ± 0.26	1.75 ± 0.21	p < 0.001	VWCS	r ² = 0.74, p = 0.0002	NA
Nie (Sep 2016)	A/M	5.66{0.23}	3.87{0.22}	p < 0.0001	NA	NA	NA	NA	NA	p < 0.001
Human										
Takahashi (2001)	MMR	NA	NA	NA	NA	NA	NA	Enhanced in one patient with unstable angina		

A/M average aortic arch/muscle, ALSFRS-R revised amyotrophic lateral sclerosis functional rating scale, ALV, anterior left ventricle, B-SC bilateral striatum/cortex, C-I contra/ipsilateral striatum, C/I SUV/SBR contra/ipsilateral hemisphere divided by specific binding ratio, H D/E hemisphere delayed to early phase, IF/BM injured femoral artery/background muscle, MMR mean myocardium retention, NA not applicable, NR not reported, NS not significant, NSM standard uptake value normalized by the global mean, OEF oxygen extraction fraction, R/L/BC-CS right/left/bilateral cortex around the central sulcus, RSPL right superior parietal lobule, SUV standard uptake value, t/mUPDRS total/motor unified Parkinson disease rating scale, VWCS vessel wall cross-section. Mean ± SD or [SEM], or median [IQR]

of CuATSM PET as a non-invasive diagnostic tool for identifying sarcopenia in older adults should be explored.

The potential clinical use of CuATSM PET in age-related diseases

This review has demonstrated the ability of CuATSM PET to delineate hypoxic tissue and showed the association between CuATSM uptake with disease severity in neurologic and cardiac diseases in clinical and pre-clinical models. Given these results, potential future clinical applications of CuATSM PET include disease detection prior to symptom manifestation allowing improved risk factor management, earlier disease diagnosis and sensitive monitoring of disease activity. To achieve clinical translation, future research should focus on studies in humans with larger sample sizes and robust study designs in the diseases already studied, as well as examining a broader range of age-related diseases in which hypoxia and oxidative stress are implicated.

Ionic Cu(II) salts as imaging tracers for age-related diseases

PET CuATSM uptake in cancer and age-related diseases has been demonstrated in preclinical and clinical studies. Interestingly, PET uptake of ionic copper Cu(II) salts, such as $[^{64}\text{Cu}]\text{CuCl}_2$, has also been seen in various cancer xenografts [71] and was found to be comparable to CuATSM in imaging and therapeutic cancer studies [72]. The mechanism of tissue uptake of CuATSM and ionic Cu(II) salts, however, remain controversial [73]. Given the redox changes common to both cancer and age-related diseases, future studies focusing on these mechanisms may clarify to whether Cu(II) salts could also be used in research into age-related diseases [73].

Strengths and limitations

To our knowledge, this is the first systematic review examining the utility of CuATSM PET in the diagnosis and management of age-related diseases. So far, there are only a few studies in this area with heterogeneous study design and small number of study subjects which precluded a meta-analysis. All included articles reported on the diagnostic capacity of CuATSM PET but not on its utility in the management of age-related disease. Given the specificity of CuATSM as an imaging biomarker of hypoxia has been challenged previously [74], we would have liked to report on the sensitivity and specificity of the CuATSM PET scans used in the articles included in this review. However, only one of these articles reported these parameters [31]. This

limits the translational potential of the results to clinical and other research settings. Finally, healthy controls in many of these studies were not matched for age and sex.

Conclusions

Hypoxia, oxidative stress and ferroptosis are important hallmarks of various age-related diseases [11–14, 23, 75, 76]. CuATSM PET is a non-invasive imaging technique that detects hypoxia and oxidative stress in neurological and cardiovascular diseases [23–25, 77, 78]. The clinical potential of CuATSM PET in early diagnosis, disease prognostication and monitoring disease activity [79, 80] and its utility in research settings in other age-related diseases, such as chronic kidney disease, recurrent urinary tract infections, diabetes and sarcopenia, should be investigated further.

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Author contributions All authors made substantial contributions to the design of the review, the acquisition, analysis, and interpretation of data; drafted the work or revised it critically for important intellectual content; approved the version to be published; and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability Not applicable.

Compliance with ethical standards

Conflict of interest None of the authors reported any conflict of interest.

Consent for publication All authors have read and approved all versions of the manuscript, its contents, and submission.

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Appendix 1 Full search strategy

1. (ferroptosis or ferroptotic or ((iron or ferrous or ferritin or ferric) adj5 (cell death or apoptosis))).mp.
2. (((((oxidative or oxidation) adj2 glutamate) or ((oxidative or oxidation) adj2 glutamic)) adj4 (toxic* or death))).mp.
3. 1 or 2
4. (CuATSM or ATSM or methylthiosemicarbazonato or methylthiosemicarbazone or Cu62ATSM or Cu61ATSM or Cu63ATSM or Cu64ATSM or Cu60ATSM or 62CuATSM or 61CuATSM or 63CuATSM or 64CuATSM or 60CuATSM).mp.
5. 3 or 4

Appendix 2 Newcastle–Ottawa quality assessment scale

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability.

Selection

1) Representativeness of the exposed cohort

- a) truly representative of the average age-related disease in human or animal subjects*
- b) somewhat representative of the average age-related disease in human or animal subject *
- c) selected group of users eg nurses, volunteers
- d) no description of the derivation of the cohort

2) Selection of the non exposed cohort

- a) drawn from the same community as the exposed cohort*
- b) drawn from a different source
- c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure

- a) secure record (eg surgical records) *
- b) structured interview*
- c) written self report
- d) no description

4) Demonstration that outcome of interest was not present at start of study

- a) yes*
- b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis

- a) study controls for age or sex*
- b) study controls for age and sex*

Outcome

1) Assessment of outcome

- a) independent blind assessment*
- b) record linkage*
- c) self report
- d) no description

2) Was follow-up long enough for outcomes to occur

- a) yes (^{60}Cu : 23.4 min half-life, ^{61}Cu : 3.4 h half-life, ^{62}Cu : 9.7 min half-life, ^{64}Cu : 12.7 h half-life) *
- b) no

3) Adequacy of follow up of cohorts

- a) complete follow up - all subjects accounted for*
- b) subjects lost to follow up unlikely to introduce bias - small number lost (>20 % follow up, or description provided of those lost) *
- c) follow up rate <20% and no description of those lost
- d) no statement

Appendix 3 Quality assessment of included articles using the Newcastle–Ottawa Scale

Author (year)	Selection				Comp	Outcome			Score	Quality
	Representa- tiveness	Selection	Ascertain- ment	Outcome was not present at start of study		Ascertain- ment	Duration of follow- up	Com- plete-ness of follow- up		
Neurological										
Ikawa (2011)	*	—	*	—	—	*	*	*	5	M
Ikawa (2015)	*	—	*	—	**	*	*	*	7	H
Isozaki (2011)	*	—	*	—	—	*	*	*	5	M
Neishi (2007)	*	—	*	—	—	*	*	*	5	M
Cardiovascular										
Lewis (2002)	—	*	*	*	**	*	*	*	8	H
Metzger (2018)	—	—	*	*	—	*	*	*	5	M
Nie (Dec 2016)	*	*	*	—	**	*	*	*	8	H
Nie (Sep 2016)	*	—	*	—	*	*	*	*	6	M
Takahashi (2001)	*	—	*	—	—	*	*	*	5	M

Number of stars (*): 1–3, low quality (L); 4–6, medium quality (M); 7–9, high quality (H). Comp: comparability.

References

- Marengoni A, Angleman S, Melis R, Mangialasche F, Karp A, Garmen A et al (2011) Aging with multimorbidity: a systematic review of the literature. *Ageing Res Rev* 10(4):430–439. <https://doi.org/10.1016/j.arr.2011.03.003>
- Feigin VL, Nichols E, Alam T, Bannick MS, Beghi E, Blake N et al (2019) Global, regional, and national burden of neurological disorders, 1990–2016: a systematic analysis for the Global Burden of disease study 2016. *Lancet Neurol* 18(5):459–480. [https://doi.org/10.1016/S1474-4422\(18\)30499-X](https://doi.org/10.1016/S1474-4422(18)30499-X)
- World Health Organization. Global health estimates 2018: disease burden by cause, sex, by country and region, 2000–2016. World Health Organization. 2018. https://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html. Accessed 13 Apr 2020.
- Chen X, Xie C, Tian W, Sun L, Wang Z, Hawes S et al (2020) Parkinson's disease-related Leucine-rich repeat kinase 2 modulates nuclear morphology and genomic stability in striatal projection neurons during aging. *Mol Neurodegener* 15(1):12. <https://doi.org/10.1186/s13024-020-00360-0>
- Alexeyev M, Shokolenko I, Wilson G, LeDoux S (2013) The maintenance of mitochondrial DNA integrity—critical analysis and update. *Cold Spring Harb Perspect Biol* 5(5):a012641. <https://doi.org/10.1101/cshperspect.a012641>
- Gaziev AI, Abdullaev S, Podlitsky A (2014) Mitochondrial function and mitochondrial DNA maintenance with advancing age. *Biogerontology* 15(5):417–438. <https://doi.org/10.1007/s10522-014-9515-2>
- Hoeijmakers JHJ (2009) DNA damage, aging, and cancer. *N Engl J Med* 361(15):1475–1485. <https://doi.org/10.1056/NEJMr0804615>
- Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D (2016) ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxid Med Cell Longev* 2016:3565127. <https://doi.org/10.1155/2016/3565127>
- Hirschhorn T, Stockwell BR (2019) The development of the concept of ferroptosis. *Free Radic Biol Med* 133:130–143. <https://doi.org/10.1016/j.freeradbiomed.2018.09.043>
- Guiney SJ, Adlard PA, Bush AI, Finkelstein DI, Ayton S (2017) Ferroptosis and cell death mechanisms in Parkinson's disease. *Neurochem Int* 104:34–48. <https://doi.org/10.1016/j.neuint.2017.01.004>
- Van Do B, Gouel F, Jonneaux A, Timmerman K, Gelé P, Pétrault M et al (2016) Ferroptosis, a newly characterized form of cell death in Parkinson's disease that is regulated by PKC. *Neurobiol Dis* 94:169–178. <https://doi.org/10.1016/j.nbd.2016.05.011>
- Raven EP, Lu PH, Tishler TA, Heydari P, Bartzokis G (2013) Increased iron levels and decreased tissue integrity in hippocampus of Alzheimer's disease detected in vivo with magnetic resonance imaging. *J Alzheimers Dis* 37(1):127–136. <https://doi.org/10.3233/JAD-130209>
- da Rocha TJ, Silva Alves M, Guisso CC, de Andrade FM, Camozzato A, de Oliveira AA et al (2018) Association of GPX1 and GPX4 polymorphisms with episodic memory and Alzheimer's disease. *Neurosci Lett* 666:32–37. <https://doi.org/10.1016/j.neulet.2017.12.026>
- Fang X, Wang H, Han D, Xie E, Yang X, Wei J et al (2019) Ferroptosis as a target for protection against cardiomyopathy. *Proc Natl Acad Sci USA* 116(7):2672–2680. <https://doi.org/10.1073/pnas.1821022116>
- Fujibayashi Y, Taniuchi H, Yonekura Y, Ohtani H, Konishi J, Yokoyama A (1997) Copper-62-ATSM: A new hypoxia imaging agent with high membrane permeability and low redox potential. *J Nucl Med* 38(7):1155–1160 **notavailable**
- Takahashi N, Fujibayashi Y, Yonekura Y, Welch MJ, Waki A, Tsuchida T et al (2000) Evaluation of 62Cu labeled diacetyl-bis(N4-methylthiosemicarbazone) as a hypoxic tissue tracer in

- patients with lung cancer. *Ann Nucl Med* 14(5):323–328. <https://doi.org/10.1007/BF02988690>
17. Tsujikawa T, Asahi S, Oh M, Sato Y, Narita N, Makino A et al (2016) Assessment of the tumor redox status in head and neck cancer by ^{62}Cu -ATSM PET. *PLoS ONE* 11(5):e0155635. <https://doi.org/10.1371/journal.pone.0155635>
 18. Lewis JS, Laforest R, Dehdashti F, Grigsby PW, Welch MJ, Siegel BA (2008) An imaging comparison of ^{64}Cu -ATSM and ^{60}Cu -ATSM in cancer of the uterine cervix. *J Nucl Med* 49(7):1177–1182. <https://doi.org/10.2967/jnumed.108.051326>
 19. Toriihara A, Ohtake M, Tateishi K, Hino-Shishikura A, Yoneyama T, Kitazume Y et al (2018) Prognostic implications of ^{62}Cu -diacetyl-bis (N 4-methylthiosemicarbazone) PET/CT in patients with glioma. *Ann Nucl Med* 32(4):264–271. <https://doi.org/10.1007/s12149-018-1241-4>
 20. Ikawa M, Okazawa H, Kudo T, Kuriyama M, Fujibayashi Y, Yoneda M (2011) Evaluation of striatal oxidative stress in patients with Parkinson's disease using [^{62}Cu]ATSM PET. *Nucl Med Biol* 38(7):945–951. <https://doi.org/10.1016/j.nucmedbio.2011.02.016>
 21. Okazawa H, Ikawa M, Tsujikawa T, Kiyono Y, Yoneda M (2014) Brain imaging for oxidative stress and mitochondrial dysfunction in neurodegenerative diseases. *Q J Nucl Med Mol Imaging Off Publ Ital Assoc Nucl Med* 58(4):387–397 **notavailable**
 22. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D et al (2018) Oxidative stress, aging, and diseases. *Clin Interv Aging* 13:757–772. <https://doi.org/10.2147/CIA.S158513>
 23. Singh A, Kukreti R, Saso L, Kukreti S (2019) Oxidative stress: a key modulator in neurodegenerative diseases. *Molecules* 24(8):1583. <https://doi.org/10.3390/molecules24081583>
 24. Chen Y, Qin C, Huang J, Tang X, Liu C, Huang K et al (2020) The role of astrocytes in oxidative stress of central nervous system: a mixed blessing. *Cell Prolif* 53(3):e12781. <https://doi.org/10.1111/cpr.12781>
 25. Daiber A, Chlopicki S (2020) Revisiting pharmacology of oxidative stress and endothelial dysfunction in cardiovascular disease: evidence for redox-based therapies. *Free Radic Biol Med* S0891–5849(19):31702–31712. <https://doi.org/10.1016/j.freeradbiomed.2020.02.026>
 26. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Rev Esp*. 2015;4(1):1. <https://doi.org/10.1186/2046-4053-4-1>
 27. Lo KL, Mertz D, Loeb M (2014) Newcastle-Ottawa scale: comparing reviewers' to authors' assessments. *BMC Med Res Methodol* 14:45. <https://doi.org/10.1186/1471-2288-14-45>
 28. Wells G, Shea B, O'Connell D, Peterson J. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa, ON: Ottawa Hospital Research Institute. 2000. https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 13 Apr 2020.
 29. Neishi H, Ikawa M, Okazawa H, Tsujikawa T, Arishima H, Kikuta KI et al (2017) Precise evaluation of striatal oxidative stress corrected for severity of dopaminergic neuronal degeneration in patients with Parkinson's disease: a study with ^{62}Cu -ATSM PET and ^{123}I -FP-CIT SPECT. *Eur Neurol* 78(3–4):161–168. <https://doi.org/10.1159/000479627>
 30. Ikawa M, Okazawa H, Tsujikawa T, Matsunaga A, Yamamura O, Mori T et al (2015) Increased oxidative stress is related to disease severity in the ALS motor cortex. *Neurology* 84(20):2033–2039. <https://doi.org/10.1212/WNL.0000000000001588>
 31. Isozaki M, Kiyono Y, Arai Y, Kudo T, Mori T, Maruyama R et al (2011) Feasibility of ^{62}Cu -ATSM PET for evaluation of brain ischemia and misery perfusion in patients with cerebrovascular disease. *Eur J Nucl Med Mol Imaging* 38(6):1075–1082. <https://doi.org/10.1007/s00259-011-1734-z>
 32. Takahashi N, Fujibayashi Y, Yonekura Y, Welch MJ, Waki A, Tsuchida T et al (2001) Copper-62 ATSM as a hypoxic tissue tracer in myocardial ischemia. *Ann Nucl Med* 15(3):293–296. <https://doi.org/10.1007/BF02987849>
 33. Nie X, Laforest R, Elvington A, Randolph GJ, Zheng J, Voller T et al (2016) PET/MRI of hypoxic atherosclerosis using ^{64}Cu -ATSM in a rabbit model. *J Nucl Med* 57(12):2006–2011. <https://doi.org/10.2967/jnumed.116.172544>
 34. Nie X, Randolph GJ, Elvington A, Bandara N, Zheleznyak A, Gropler RJ et al (2016) Imaging of hypoxia in mouse atherosclerotic plaques with ^{64}Cu -ATSM. *Nucl Med Biol* 43(9):534–542. <https://doi.org/10.1016/j.nucmedbio.2016.05.011>
 35. Lewis JS, Herrero P, Sharp TL, Engelbach JA, Fujibayashi Y, Laforest R et al (2002) Delineation of hypoxia in canine myocardium using PET and copper(II)-diacetyl-bis(N4-methylthiosemicarbazone). *J Nucl Med* 43(11):1557–1569 **notavailable**
 36. Metzger JM, Moore CF, Boettcher CA, Brunner KG, Fleddermann RA, Matsoff HN, et al. (2018) In vivo imaging of inflammation and oxidative stress in a nonhuman primate model of cardiac sympathetic neurodegeneration. *npj Parkinsons Dis*. 4:22. doi: 10.1038/s41531-018-0057-1.
 37. DeMarshall CA, Han M, Nagele EP, Sarkar A, Acharya NK, Godsey G et al (2015) Potential utility of autoantibodies as blood-based biomarkers for early detection and diagnosis of Parkinson's disease. *Immunol Lett* 168(1):80–88. <https://doi.org/10.1016/j.imlet.2015.09.010>
 38. Sharma S, Moon CS, Khogali A, Haidous A, Chabenne A, Ojo C et al (2013) Biomarkers in Parkinson's disease (recent update). *Neurochem Int* 63(3):201–229. <https://doi.org/10.1016/j.neuint.2013.06.005>
 39. Blasco H, Garçon G, Patin F, Veyrat-Durebex C, Boyer J, Devos D et al (2017) Panel of oxidative stress and inflammatory biomarkers in ALS: a pilot study. *Can J Neurol Sci* 44(1):90–95. <https://doi.org/10.1017/cjn.2016.284>
 40. Xu R, Wu C, Zhang X, Zhang Q, Yang Y, Yi J et al (2011) Linking hypoxic and oxidative insults to cell death mechanisms in models of ALS. *Brain Res* 1372:133–144. <https://doi.org/10.1016/j.brainres.2010.11.056>
 41. Zhang Z, Yan J, Chang Y, ShiDu Yan S, Shi H (2011) Hypoxia inducible factor-1 as a target for neurodegenerative diseases. *Curr Med Chem* 18(28):4335–4343. <https://doi.org/10.2174/092986711797200426>
 42. Sedgwick AC, Brewster JT, Harvey P, Iovan DA, Smith G, He X-P et al (2020) Metal-based imaging agents: progress towards interrogating neurodegenerative disease. *Chem Soc Rev* 49(10):2886–2915. <https://doi.org/10.1039/c8cs00986d>
 43. Duncombe J, Kitamura A, Hase Y, Ihara M, Kalaria RN, Horsburgh K (2017) Chronic cerebral hypoperfusion: a key mechanism leading to vascular cognitive impairment and dementia. Closing the translational gap between rodent models and human vascular cognitive impairment and dementia. *Clin Sci*. 131(19):2451–2468. <https://doi.org/10.1042/CS20160727>
 44. Rodríguez García PL, Rodríguez GD (2015) Diagnosis of vascular cognitive impairment and its main categories. *Neurologia* 30(4):223–239. <https://doi.org/10.1016/j.nrl.2011.12.014>
 45. De La Torre JC (2012) Cardiovascular risk factors promote brain hypoperfusion leading to cognitive decline and dementia. *Cardiovasc Psychiatry Neurol* 2012:367516. <https://doi.org/10.1155/2012/367516>
 46. Ikawa M, Okazawa H, Arakawa K, Kudo T, Kimura H, Fujibayashi Y et al (2009) PET imaging of redox and energy states in stroke-like episodes of MELAS. *Mitochondrion* 9(2):144–148. <https://doi.org/10.1016/j.mito.2009.01.011>
 47. Björnheden T, Levin M, Evaldsson M, Wiklund O (1999) Evidence of hypoxic areas within the arterial wall in vivo.

- Arterioscler Thromb Vasc Biol 19(4):870–876. <https://doi.org/10.1161/01.atv.19.4.870>
48. Parathath S, Mick SL, Feig JE, Joaquin V, Grauer L, Habel DM et al (2011) Hypoxia is present in murine atherosclerotic plaques and has multiple adverse effects on macrophage lipid metabolism. *Circ Res* 109(10):1141–1152. <https://doi.org/10.1161/CIRCRESAHA.111.246363>
 49. Dweck MR, Aikawa E, Newby DE, Tarkin JM, Rudd JHF, Narula J et al (2016) Noninvasive molecular imaging of disease activity in atherosclerosis. *Cir Res* 119(2):330–340. <https://doi.org/10.1161/CIRCRESAHA.116.307971>
 50. Hill NR, Fatoba ST, Oke JL, Hirst JA, O'Callaghan CA, Lasserson DS et al (2016) Global prevalence of chronic kidney disease—A systematic review and meta-analysis. *PLoS ONE* 11(7):e0158765. <https://doi.org/10.1371/journal.pone.0158765>
 51. Matthews SJ, Lancaster JW (2011) Urinary tract infections in the elderly population. *Am J Geriatric Pharmacotherapy* 9(5):286–309. <https://doi.org/10.1016/j.amjopharm.2011.07.002>
 52. Risk NCD, Collaboration F (2016) Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. *Lancet* 387(10027):1513–1530. [https://doi.org/10.1016/S0140-6736\(16\)00618-8](https://doi.org/10.1016/S0140-6736(16)00618-8)
 53. Wandrag L, Siervo M, Riley HL, Khosravi M, Fernandez BO, Leckstrom CA et al (2017) Does hypoxia play a role in the development of sarcopenia in humans? Mechanistic insights from the Caudwell Xtreme Everest expedition. *Redox Biol* 13:60–68. <https://doi.org/10.1016/j.redox.2017.05.004>
 54. Balasubramanian S (2013) Progression of chronic kidney disease: mechanisms and interventions in retardation. *Apollo Med* 10(1):19–28. <https://doi.org/10.1016/j.apme.2013.01.009>
 55. Mimura I, Nangaku M (2010) The suffocating kidney: tubulointerstitial hypoxia in end-stage renal disease. *Nat Rev Nephrol* 6(11):667–678. <https://doi.org/10.1038/nrneph.2010.124>
 56. Neugarten J (2012) Renal BOLD-MRI and assessment for renal hypoxia. *Kidney Int* 81(7):613–614. <https://doi.org/10.1038/ki.2011.462>
 57. Inoue T, Kozawa E, Okada H, Inukai K, Watanabe S, Kikuta T et al (2011) Noninvasive evaluation of kidney hypoxia and fibrosis using magnetic resonance imaging. *J Am Soc Nephrol* 22(8):1429–1434. <https://doi.org/10.1681/ASN.2010111143>
 58. Catana C, Guimaraes AR, Rosen BR (2013) PET and MR imaging: the odd couple or a match made in heaven? *J Nuc Med* 54(5):815–824. <https://doi.org/10.2967/jnumed.112.112771>
 59. Li LP, Halter S, Prasad PV (2008) Blood oxygen level-dependent mr imaging of the kidneys. *Mag Reson Imaging Clin N Am* 16(4):613–625. <https://doi.org/10.1016/j.mric.2008.07.008>
 60. Thomas R, Kanso A, Sedor JR (2008) Chronic kidney disease and Its complications. *Prim Care* 35(2):329–344. <https://doi.org/10.1016/j.pop.2008.01.008>
 61. Kurutas EB, Ciragil P, Gul M, Kilinc M (2005) The effects of oxidative stress in urinary tract infection. *Mediators Inflamm* 4:242–244. <https://doi.org/10.1155/MI.2005.242>
 62. Lin AE, Beasley FC, Olson J, Keller N, Shalwitz RA, Hannan TJ et al (2015) Role of hypoxia inducible factor-1 α (HIF-1 α) in innate defense against uropathogenic escherichia coli infection. *PLoS Pathog* 11(4):e1004818. <https://doi.org/10.1371/journal.ppat.1004818>
 63. Sada K, Nishikawa T, Kukidome D, Yoshinaga T, Kajihara N, Sonoda K et al (2016) Hyperglycemia induces cellular hypoxia through production of mitochondrial ROS followed by suppression of aquaporin-1. *PLoS ONE* 11(7):e0158619. <https://doi.org/10.1371/journal.pone.0158619>
 64. Biswas S, Mukherjee R, Tapryal N, Singh AK, Mukhopadhyay CK (2013) Insulin regulates hypoxia-inducible factor-1 α transcription by reactive oxygen species sensitive activation of Sp1 in 3T3-L1 preadipocyte. *PLoS ONE* 8(4):e62128. <https://doi.org/10.1371/journal.pone.0062128>
 65. Lee YS, Kim JW, Osborne O, Oh DY, Sasik R, Schenk S et al (2014) Increased adipocyte O₂ consumption triggers HIF-1 α , causing inflammation and insulin resistance in obesity. *Cell* 157(6):1339–1352. <https://doi.org/10.1016/j.cell.2014.05.012>
 66. Hooton TM (2000) Pathogenesis of urinary tract infections: an update. *J Antimicrob Chemother*. 46(Suppl A):1–7 **doi not available**
 67. Rowe TA, Juthani-Mehta M. Urinary tract infection in older adults. *Aging Health*. 2013;9(5) DOI: 10.2217/ahe.13.38
 68. Barsanti C (2015) Diagnostic and prognostic utility of non-invasive imaging in diabetes management. *World J Diabetes* 6(6):792–806. <https://doi.org/10.4239/wjcd.v6.i6.792>
 69. Bellanti F, Romano AD, Lo Buglio A, Castriotta V, Guglielmi G, Greco A et al (2018) Oxidative stress is increased in sarcopenia and associated with cardiovascular disease risk in sarcopenic obesity. *Maturitas* 109:6–12. <https://doi.org/10.1016/j.maturitas.2017.12.002>
 70. Lee K, Shin Y, Huh J, Sung YS, Lee IS, Yoon KH et al (2019) Recent issues on body composition imaging for sarcopenia evaluation. *Korean J Radiol* 20(2):205–217. <https://doi.org/10.3348/kjr.2018.0479>
 71. Jørgensen JT, Persson M, Madsen J, Kjær A (2013) High tumor uptake of ⁶⁴Cu: implications for molecular imaging of tumor characteristics with copper-based PET tracers. *Nucl Med Biol* 40(3):345–350. <https://doi.org/10.1016/j.nucmedbio.2013.01.002>
 72. Hueting R, Kersemans V, Cornelissen B, Tredwell M, Hussien K, Christlieb M et al (2014) A comparison of the behavior of ⁶⁴Cu-acetate and ⁶⁴Cu-ATSM in vitro and in vivo. *J Nucl Med* 55(1):128–134. <https://doi.org/10.2967/jnumed.113.119917>
 73. Liu T, Karlens M, Karlberg AM, Redalen KR (2020) Hypoxia imaging and theranostic potential of [⁶⁴Cu][Cu(ATSM)] and ionic Cu(II) salts: a review of current evidence and discussion of the retention mechanisms. *EJNMMI Res* 10(1):33. <https://doi.org/10.1186/s13550-020-00621-5>
 74. Fleming IN, Manavaki R, Blower PJ, West C, Williams KJ, Harris AL et al (2015) Imaging tumour hypoxia with positron emission tomography. *Br J Cancer* 112(2):238–250. <https://doi.org/10.1038/bjc.2014.610>
 75. Jha NK, Jha SK, Sharma R, Kumar D, Ambasta RK, Kumar P (2018) Hypoxia-induced signaling activation in neurodegenerative diseases: targets for new therapeutic strategies. *J Alzheimers Dis* 62(1):15–38. <https://doi.org/10.3233/JAD-170589>
 76. Abe H, Semba H, Takeda N (2017) The roles of hypoxia signaling in the pathogenesis of cardiovascular diseases. *J Atheroscler Thromb* 24(9):884–894. <https://doi.org/10.5551/jat.RV17009>
 77. Imam SK (2010) Review of positron emission tomography tracers for imaging of tumor hypoxia. *Cancer Biother Radiopharm* 25(3):365–374. <https://doi.org/10.1089/cbr.2009.0740>
 78. Fujibayashi Y, Taniuchi H, Yonekura Y, Ohtani H, Konishi J, Yokoyama A. r f Copper-62-ATSM : A new hypoxia imaging agent with high membrane permeability and low redox potential. *J Nucl Med*. 2014;1155–60 **doi not available**.
 79. Southworth R, Martin T, de Rosales R, Meszaros LK, Ma MT, Mullen GED, Fruhwirth G et al (2016) Opportunities and challenges for metal chemistry in molecular imaging. *Adv Inorg Chem* 68:1–41. <https://doi.org/10.1016/bs.adioch.2015.09.001>
 80. Lapi SE, Lewis JS, Dehdashti F (2015) Evaluation of hypoxia with Cu-ATSM. *Semin Nucl Med* 45(2):177–185. <https://doi.org/10.1053/j.semnuclmed.2014.10.003>

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