

**Short Title:** AGEs and chronic renal transplant dysfunction

**Title:** Current insights in the role of advanced glycation end-products in the development of chronic renal transplant dysfunction.

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## **Abstract**

Short-term survival in kidney transplant recipients has improved dramatically over the past decades. However, a large group of transplant recipients still develop chronic renal transplant dysfunction in the long run after transplantation. Whereas risk factors of chronic renal transplant dysfunction have been thoroughly documented, the actual pathophysiological background remains largely unknown. Recently, we hypothesized that advanced glycation end-products (AGEs) might be playing an important role in the development of chronic renal transplant dysfunction. The current review summarizes the present research supporting a role for AGEs in the development of chronic renal transplant dysfunction. From this it can be concluded that, although current studies are suggestive for a role of AGEs in the development of chronic renal transplant dysfunction, prospective and intervention studies are warranted to confirm this hypothesis.

## **Introduction**

Although short term renal allograft function has become excellent, long-term allograft survival has lagged behind markedly.(1;2) Approximately 60% of the patients receiving cadaveric donor kidneys will develop graft failure within 10 years after transplantation.(1) Chronic renal transplant dysfunction, also known as chronic allograft nephropathy, is one of the leading causes of graft failure after the first year of transplantation. Chronic renal transplant dysfunction is characterized clinically by a slow but steady decline in function of the transplanted kidney, associated with the development of hypertension and proteinuria.(3) The histopathology of chronic renal transplant dysfunction includes arteriosclerosis of the intrarenal vasculature, glomerulosclerosis and interstitial fibrosis with tubular atrophy.(4) A complex interplay of both alloantigen-dependent and alloantigen-independent risk factors is thought to underlie the development of chronic renal transplant dysfunction.(3) However, the

precise pathophysiology of chronic renal transplant dysfunction remains largely unknown. Recently, we hypothesized that advanced glycation end-products play an important role in the development of chronic renal transplant dysfunction.(5) Based upon various in vitro studies we proposed a cascade of events which might be involved (figure 1). It refers to the cell types that express AGE-receptors, to the mediators that are released in response to activation of these receptors, and to the tissue damage that has resulted from those mediators in various in vitro experiments. Further support for our hypothesis comes from experimental studies in which AGE-induced renal tissue damage is well established in both diabetic and non-diabetic animal models(6-12). The purpose of this review is to summarize the research that studied the role of AGE accumulation in the development of chronic renal transplant dysfunction.

### **The influence of kidney transplantation on AGE accumulation**

Almost all kidney transplant recipients have had a long period of impaired renal function prior to transplantation. AGEs accumulate both during the period of gradual renal function loss prior to the start of dialysis, and during dialysis-treatment.(13) Thus, kidney transplant patients most often have high AGE-levels prior to transplantation. AGE-levels in transplant donors are unknown. Presumably, there is a wide variability in donor kidney AGE-levels, because of the heterogeneity of the donors. However, it is reasonable to assume that on average donors will probably have lower tissue AGE-levels than transplant recipients. Thus, a kidney with presumably low AGE-levels is transplanted in an AGE-rich environment. Kidney transplantation aims to restore renal function, and is thereby thought to lower AGE-levels. Questions are, however, to what extent AGE-accumulation will resolve after kidney transplantation, and how the transplanted kidney behaves in an AGE-rich environment. Several research groups have investigated the influence of kidney transplantation on AGE-levels in tissue and blood. Unfortunately, only data on extra-renal AGE-levels have been

published. No data are available on AGE-levels in kidneys of transplant patients, either with or without CRTD. Thus we do not know how the transplanted kidney handles the AGE rich environment it is placed in. Recently, we summarized all studies on the influence of kidney transplantation on AGEs in tissue as well as in blood of transplant recipients.(5) We concluded that, although transplantation reduces levels of blood AGEs, AGE levels generally remain above normal. Results of studies on the influence of kidney transplantation on accumulation of AGEs in tissue are inconclusive. Although there is reason to believe that a decrease in blood AGEs is eventually reflected in a decrease in tissue AGE-accumulation, studies on tissue AGEs are limited in number, size, and duration after transplantation.

### **AGE accumulation long-term after transplantation**

In our studies we did not examine the direct effect of kidney transplantation on AGE accumulation. Recently, we have however determined AGE accumulation in the long run after transplantation. We analyzed AGE accumulation in 285 kidney transplant recipients (163 male; 122 female) and 231 control patients (92 male, 139 female). Transplant recipients were seen 73 [32-143] months after transplantation. Patients were aged 52 [41-60] years. AGE accumulation was measured as autofluorescence of the skin using the AGE Reader as described in detail previously.(14) Controls were submitted to the hospital for different surgical interventions, unrelated to cardiovascular, and/or inflammatory disorders and had no prior history of diabetes and/or renal failure. Controls were aged 51 [40-65] years. Transplant recipients and controls were matched for age by dividing data in decades of age. Autofluorescence of the lower arm was significantly higher in transplant recipients compared with the control patients ( $2.6 \pm 0.7$  v.s.  $2.1 \pm 0.6$  a.u.;  $p < 0.0001$ ). In all subgroups autofluorescence was significantly increased in transplant recipients as well (figure 2).

## **AGE accumulation in patients with chronic renal transplant dysfunction**

Just recently, Raj et al.(15) analyzed oxidative stress and AGE accumulation in 11 postrenal transplant recipients with normal renal function, and 10 patients with biopsy proven chronic renal transplant dysfunction at various time points after transplantation. Data were also obtained in 16 controls and 13 patients with chronic renal failure. As results they found that, serum creatinine, malonyldialdehyde, carbonyl protein, pentosidine, and argpyrimidine levels decreased during follow-up in transplant recipients with normal renal function, whereas these values progressively increased in patients who developed chronic renal transplant dysfunction. Markers of oxidative stress and AGEs measured at 18 to 24 months post-transplant in patients with chronic renal transplant dysfunction were higher than in kidney transplant recipients with normal renal function, controls, and patients with chronic renal failure. Thus the authors concluded that the increased levels of oxidative stress and AGEs in patients with chronic renal transplant dysfunction, could not be explained by the decline in renal function alone.

## **AGE accumulation and risk factors for chronic renal transplant dysfunction**

Several risk factors for chronic renal transplant dysfunction have been documented. Alloantigen-dependent factors include episodes of acute rejection, inadequate immunosuppression and increased human leukocyte antigen mismatching.(3) Alloantigen-independent factors include recipient and donor age, impaired renal function, hypertension, the presence of diabetes, proteinuria, hyperlipidemia, obesity, transplant ischemia, and the use of calcineurin inhibitors.(3) The extent of their contributions is largely unknown. Interestingly, alloantigen-independent risk factors for the development of chronic renal transplant dysfunction overlap to a certain extent with risk factors for the formation and accumulation of AGEs. This overlap is well established for age, renal function impairment,

and diabetes.(16-19) Although less conclusive, evidence does exist that associates hypertension, proteinuria, and hyperlipidemia with enhanced AGE-accumulation.(20-23)

In the cohort of 285 kidney transplant recipients described above we determined which risk factors for chronic renal transplant dysfunction were related to AGE accumulation as determined by the AGE-reader. Increased autofluorescence was associated with creatinine clearance at day of measurement (index;  $P<0.0001$ ), creatinine clearance at one year posttransplant (baseline;  $P<0.0001$ ) and delta creatinine clearance (day of measurement minus one year posttransplant;  $P=0.01$ ). Univariate associations with increased autofluorescence were also found with recipient female sex ( $p=0.02$ ), recipient age ( $P<0.0001$ ), systolic blood pressure ( $P<0.0001$ ), HbA1c ( $P<0.0001$ ), smoking ( $P=0.017$ ), history of cardiovascular disease ( $P=0.045$ ), duration of pre-transplant dialysis ( $p=0.012$ ) and donor age ( $P=0.037$ ). No effect of the types or cumulative dosages of immunosuppressive treatment was found in the univariate analysis with autofluorescence.

## **Discussion**

Our data showed that tissue autofluorescence, as a validated marker of AGE accumulation, is increased in kidney transplant patients as compared to controls. Moreover in the kidney transplant group a clear relation exists not only with baseline (1-year) creatinine clearance and with the fall in creatinine clearance after 1 year since transplantation, but also with several of the known risk factors for chronic renal transplant dysfunction. The relation between AGE accumulation and renal function is probably two-sided: renal failure and loss of renal function may be responsible for AGE accumulation, both because of disturbed clearance of AGEs and intermediate products, and due to increased oxidative stress. On the other hand AGEs may well have a central role in the development of chronic renal transplant dysfunction. Although our findings and those others support the hypothesis that AGEs are involved in the

development of chronic renal transplant dysfunction, by far they do not deliver proof for the hypothesis. Currently, no studies have analyzed the accumulation of AGEs in the deteriorating transplanted kidney itself. The problem is that in our, like in most transplant centers, no routine transplant biopsies are performed in the period of development of, or in established chronic allograft nephropathy. Furthermore, as no results of prospective and intervention studies aimed at lowering AGE accumulation in transplant recipients have been published so far, the causality of the associations found cannot be inferred. However, the serial assessments of AGEs by Raj et al(15) do provide strong support that AGE levels rise more strongly in the kidney transplant patients with evolving chronic renal transplant dysfunction.

In our studies AGE accumulation was determined using our newly developed and validated AFR. This tool is based upon the principle of the fluorescent properties of several (but not all) AGEs. Collagen linked fluorescence (CLF) has long been used as a single standard for measuring AGE accumulation.(24) Not all AGEs exhibit fluorescent properties, and fluorescence is a group reactivity, which fails to provide quantitative information on concentrations of individual compounds. We cannot exclude the interference of other fluorophores in our AFR measurements: changes in skin fluorescence may also occur as a consequence of light absorption by chromophores such as melanin and hemoglobin.(25) However, our previous validation studies have shown that the AFR can serve as a reliable and clinically useful marker for the tissue AGE pool, even for non-fluorescent AGE.(14)

## **Conclusion and future perspectives**

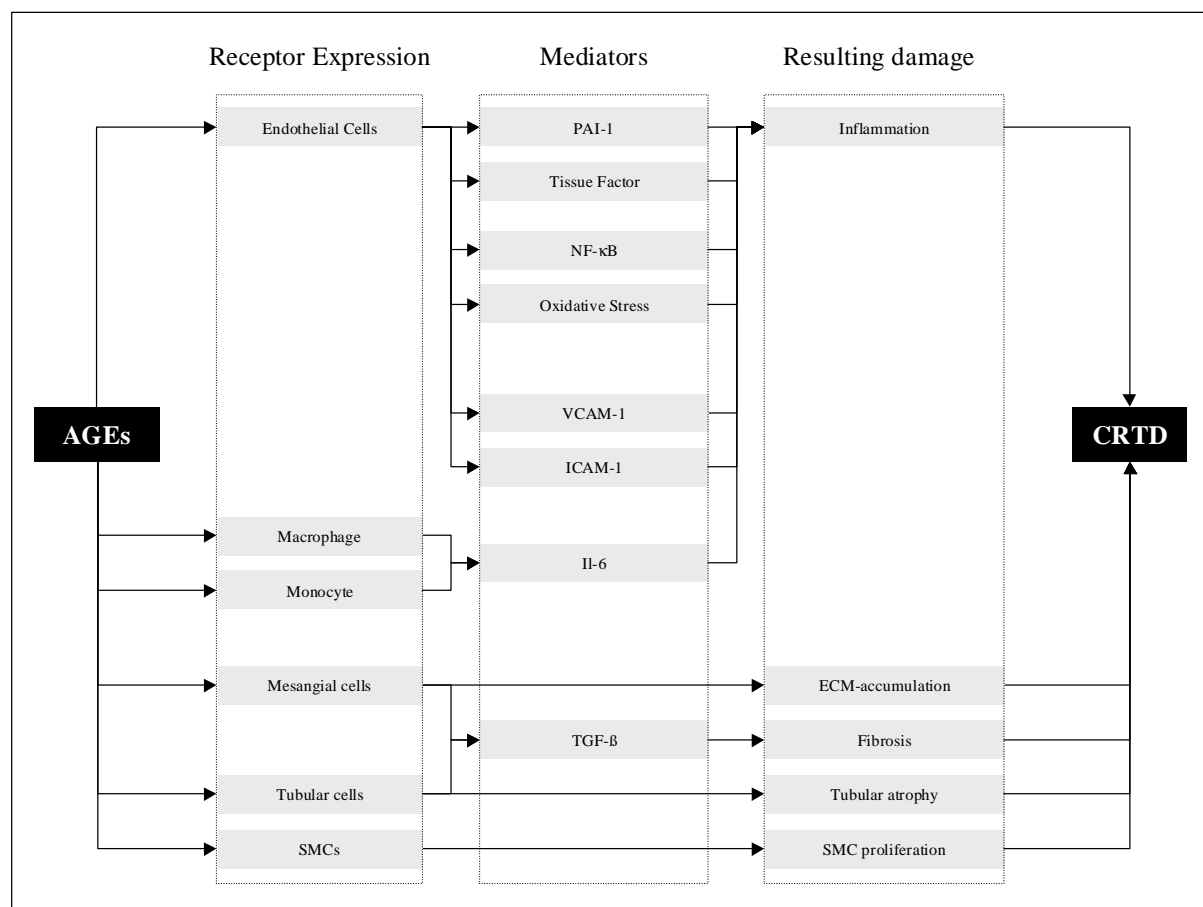
Increased accumulation of advanced glycation end-products measured as autofluorescence in vivo is associated with several risk factors for chronic renal transplant dysfunction. Furthermore, enhanced oxidative stress and AGE accumulation has been found

in relation to the development of chronic renal transplant dysfunction. Although these relations are suggestive for a causative role of AGE in chronic renal transplant dysfunction, prospective and intervention studies are warranted to more definitely determine the relative role of AGE accumulation in the development of chronic renal transplant dysfunction. The availability of a simple, noninvasive method (AGE-reader) to measure AGE accumulation in kidney transplant recipients may be useful in identifying and monitoring patients at risk for AGE accumulation.

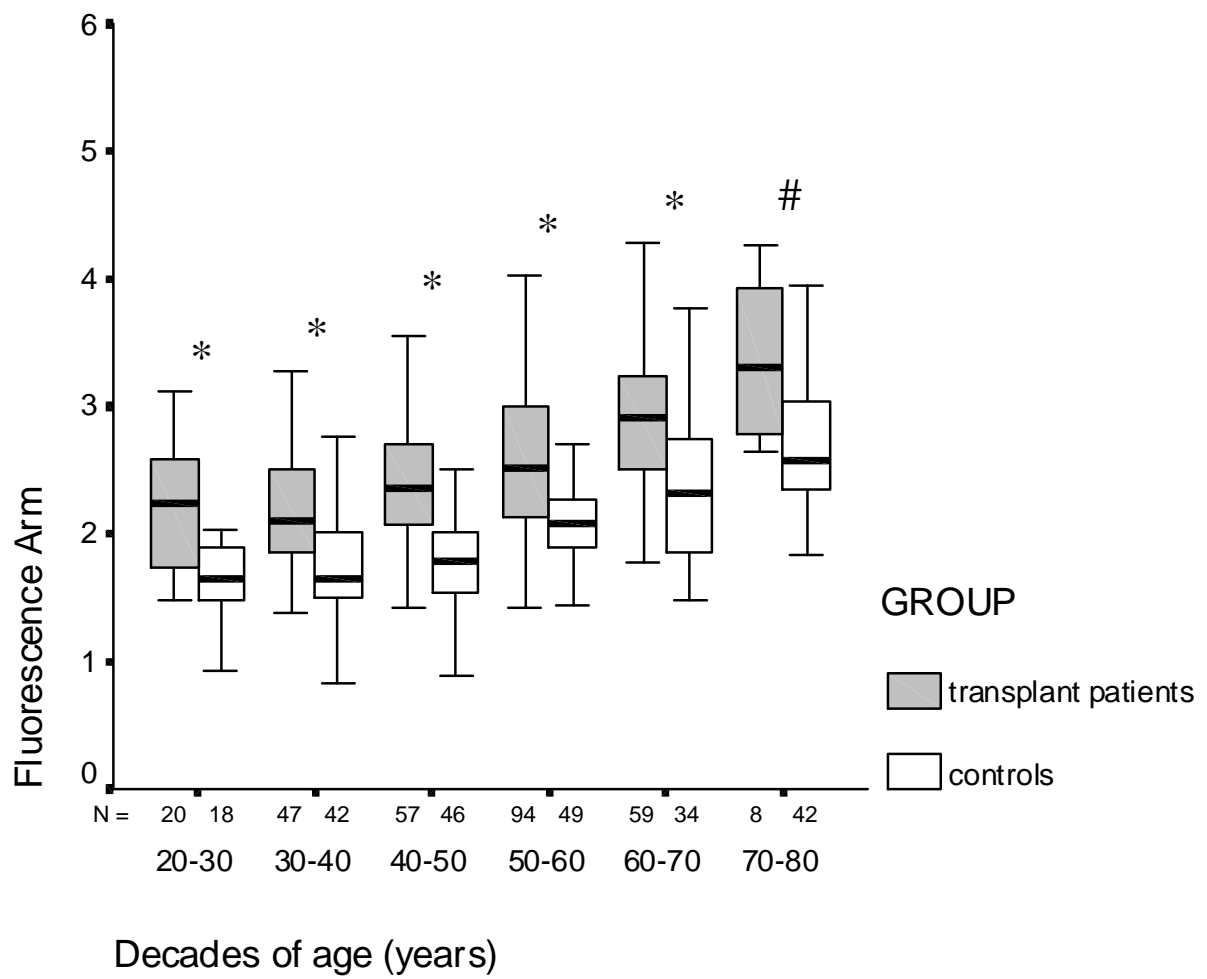
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**Figure 1: Effect of AGEs on different cell-types involved in the development of chronic renal transplant dysfunction.** Abbreviations: AGEs, advanced glycation end-products; CRTD, chronic renal transplant dysfunction; PAI-1, plasminogen activator inhibitor 1; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; Il-6, interleukine 6; NF- $\kappa$ B, nuclear factor- $\kappa$ B; ECM, extra-cellular matrix; TGF- $\beta$ , transforming growth factor- $\beta$ ; SMC, smooth muscle cell.



**Figure 2: Autofluorescence in transplant recipients compared with normal controls separated by decades of age. Annotations: \*:  $P < 0.0001$ ; #:  $P < 0.001$ .**

## Reference List

- (1) Cecka JM. The UNOS Scientific Renal Transplant Registry-2000. In: Cecka JM, Terasaki PI, editors. *Clinical Transplant*. Los Angeles: UCLA Immunogenetics, 2000: 1-18.
- (2) Hariharan S, Johnson CP, Bresnahan BA, Taranto SE, McIntosh MJ, Stablein D. Improved graft survival after renal transplantation in the United States, 1988 to 1996. *N Engl J Med* 2000; 342(9):605-612.
- (3) Womer KL, Vella JP, Sayegh MH. Chronic allograft dysfunction: mechanisms and new approaches to therapy. *Semin Nephrol* 2000; 20(2):126-147.
- (4) Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; 55(2):713-723.
- (5) Hartog JW, Smit AJ, van Son WJ, Navis G, Gans RO, Wolffenbuttel BH et al. Advanced glycation end products in kidney transplant patients: a putative role in the development of chronic renal transplant dysfunction. *Am J Kidney Dis* 2004; 43(6):966-975.
- (6) Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation end products induce glomerular sclerosis and albuminuria in normal rats. *Proc Natl Acad Sci U S A* 1994; 91(24):11704-11708.
- (7) Li YM, Steffes M, Donnelly T, Liu C, Fuh H, Basgen J et al. Prevention of cardiovascular and renal pathology of aging by the advanced glycation inhibitor aminoguanidine. *Proc Natl Acad Sci U S A* 1996; 93(9):3902-3907.
- (8) Zheng F, He C, Cai W, Hattori M, Steffes M, Vlassara H. Prevention of diabetic nephropathy in mice by a diet low in glycoxidation products. *Diabetes Metab Res Rev* 2002; 18(3):224-237.
- (9) Soulis-Liparota T, Cooper M, Papazoglou D, Clarke B, Jerums G. Retardation by aminoguanidine of development of albuminuria, mesangial expansion, and tissue fluorescence in streptozocin-induced diabetic rat. *Diabetes* 1991; 40(10):1328-1334.
- (10) Wilkinson-Berka JL, Kelly DJ, Koerner SM, Jaworski K, Davis B, Thallas V et al. ALT-946 and aminoguanidine, inhibitors of advanced glycation, improve severe nephropathy in the diabetic transgenic (mREN-2)27 rat. *Diabetes* 2002; 51(11):3283-3289.
- (11) Nakamura S, Makita Z, Ishikawa S, Yasumura K, Fujii W, Yanagisawa K et al. Progression of nephropathy in spontaneous diabetic rats is prevented by OPB-9195, a novel inhibitor of advanced glycation. *Diabetes* 1997; 46(5):895-899.
- (12) Degenhardt TP, Alderson NL, Arrington DD, Beattie RJ, Basgen JM, Steffes MW et al. Pyridoxamine inhibits early renal disease and dyslipidemia in the streptozotocin-diabetic rat. *Kidney Int* 2002; 61(3):939-950.

- (13) Yamada K, Miyahara Y, Hamaguchi K, Nakayama M, Nakano H, Nozaki O et al. Immunohistochemical study of human advanced glycosylation end-products (AGE) in chronic renal failure. *Clin Nephrol* 1994; 42(6):354-361.
- (14) Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004; 47(7):1324-1330.
- (15) Raj DS, Lim G, Levi M, Qualls C, Jain SK. Advanced glycation end products and oxidative stress are increased in chronic allograft nephropathy. *Am J Kidney Dis* 2004; 43(1):154-160.
- (16) Dyer DG, Dunn JA, Thorpe SR, Bailie KE, Lyons TJ, McCance DR et al. Accumulation of Maillard reaction products in skin collagen in diabetes and aging. *J Clin Invest* 1993; 91(6):2463-2469.
- (17) Hricik DE, Schulak JA, Sell DR, Fogarty JF, Monnier VM. Effects of kidney or kidney-pancreas transplantation on plasma pentosidine. *Kidney Int* 1993; 43(2):398-403.
- (18) Misselwitz J, Franke S, Kauf E, John U, Stein G. Advanced glycation end products in children with chronic renal failure and type 1 diabetes. *Pediatr Nephrol* 2002; 17(5):316-321.
- (19) Nishino T, Horii Y, Shiiki H, Yamamoto H, Makita Z, Bucala R et al. Immunohistochemical detection of advanced glycosylation end products within the vascular lesions and glomeruli in diabetic nephropathy. *Hum Pathol* 1995; 26(3):308-313.
- (20) Wu L, Juurlink BH. Increased methylglyoxal and oxidative stress in hypertensive rat vascular smooth muscle cells. *Hypertension* 2002; 39(3):809-814.
- (21) Sugiyama S, Miyata T, Ueda Y, Tanaka H, Maeda K, Kawashima S et al. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. *J Am Soc Nephrol* 1998; 9(9):1681-1688.
- (22) Aso Y, Inukai T, Tayama K, Takemura Y. Serum concentrations of advanced glycation endproducts are associated with the development of atherosclerosis as well as diabetic microangiopathy in patients with type 2 diabetes. *Acta Diabetol* 2000; 37(2):87-92.
- (23) Bucala R, Makita Z, Vega G, Grundy S, Koschinsky T, Cerami A et al. Modification of low density lipoprotein by advanced glycation end products contributes to the dyslipidemia of diabetes and renal insufficiency. *Proc Natl Acad Sci U S A* 1994; 91(20):9441-9445.
- (24) Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Kohn RR. Relation between complications of type I diabetes mellitus and collagen- linked fluorescence. *N Engl J Med* 1986; 314(7):403-408.

- (25) Na R, Stender IM, Henriksen M, Wulf HC. Autofluorescence of human skin is age-related after correction for skin pigmentation and redness. *J Invest Dermatol* 2001; 116(4):536-540.