

Kidney hyperfiltration and mitochondrial changes are associated with eGFR decline in young people with type 1 diabetes

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ABSTRACT

Objectives: To examine the relationship between kidney hyperfiltration during adolescence and subsequent changes in estimated glomerular filtration rate (eGFR) and urinary albumin creatinine ratio (UACR) in a young cohort of participants with type 1 diabetes. Additionally, to explore urinary mitochondrial DNA:nuclear DNA ratio (mtDNA:nDNA) as a marker of metabolic stress and its association with early changes in kidney function.

Methods: Eighty adolescents were studied at baseline [mean (SD) age 14.2 (1.5) years; mean diabetes duration 6.7 (3.0) years] and followed up 9.2 (1.3) years later. Blood pressure, HbA1c, lipids, eGFR, UACR and heart rate variability were assessed at each visit. Urinary mtDNA:nDNA was measured by quantitative PCR (qPCR).

Results: Overall, 4.2% of participants had diabetic kidney disease (DKD) at follow-up. Hyperfiltration at baseline (>135 mL/min/1.73m²) was seen in 31% of adolescents and was associated with a decline in eGFR at follow-up when adjusted for sex, diabetes duration and HbA1c [hyperfiltration -1.46 (3.07) mL/min/1.73 m²/year vs non-hyperfiltration -0.51 (2.48) mL/min/1.73m²/year, $P=0.02$]. Participants with hyperfiltration also had higher odds of undergoing rapid eGFR decline (>3 mL/min/1.73m²/year) compared to those without hyperfiltration [OR 14.11, 95% CI (2.30-86.60), $P=0.004$]. Baseline urinary mtDNA:nDNA was

significantly associated with both greater annual rate of eGFR decline and rapid eGFR decline in univariable but not multivariable modelling.

Conclusion: Hyperfiltration during adolescence is significantly associated with greater reduction in eGFR and higher risk of rapid eGFR decline after ~9 years, following transition into young adulthood in type 1 diabetes. Urinary mtDNA:nDNA measured during adolescence may be a novel predictor of early changes in kidney function.

INTRODUCTION

Improving health care outcomes for young people with type 1 diabetes remains a significant challenge in clinical practice. Diagnosis at a young age and hence longer diabetes duration increases the risk of developing diabetes-related complications ¹, with diabetic kidney (DKD) and cardiovascular disease being major drivers of morbidity and mortality ².

Historically, the conventional view held was that DKD primarily affects older individuals with longstanding, suboptimal glycaemic control. However, despite ongoing advances in diabetes care, emerging data from more contemporary studies challenge our understanding of risk factors for DKD. A large cross-sectional examination of the SEARCH for Diabetes in Youth study showed that approximately one third of young individuals with type 1 diabetes had evidence of at least one complication, with the prevalence of DKD being 5.8% following a mean of only 7.9 years of diabetes ³. Indeed, the first signs of complications, including DKD, may present during childhood or adolescence, making this an important period in the lifetime risk of complications ⁴. Further, histological changes characteristic of DKD have been observed in kidney biopsies performed in adults and children less than 5 years after diabetes onset ⁵. This is particularly concerning given that the presence of DKD accounts for the majority of the excess risks in all-cause and

cardiovascular mortality in individuals with diabetes ^{6,7}. Improved strategies for the early detection, prevention and treatment of DKD are therefore urgently needed. This is especially pertinent for type 1 diabetes, given the relative paucity of targeted clinical trials and recent call for action for new therapies for this group ⁸.

Hyperfiltration is thought to be an early sign of kidney dysfunction and is frequently observed during adolescence ⁹. Whether it plays a role in promoting kidney disease progression or represents an adaptive response to the underlying metabolic derangements present in diabetes remains incompletely understood. While observational data have demonstrated associations between kidney hyperfiltration and markers of disease progression including albuminuria ¹⁰ and decline in glomerular filtration rate (GFR) ¹¹, its clinical relevance remains a subject of debate ¹². Careful assessment of temporal changes in GFR after diagnosis may provide valuable insight into how kidney disease develops and progresses in the early stages especially in individuals without traditional albuminuria. Therefore, we aimed to examine the relationship between kidney hyperfiltration during adolescence and subsequent changes in markers of kidney function in a cohort of young individuals with type 1 diabetes.

Furthermore, novel biomarkers of kidney disease remain an important unmet need. Elevated urinary mtDNA has been detected in the context of both acute and chronic forms of kidney disease and aligns with markers of interstitial fibrosis in biopsy-proven DKD ¹³. Given that DKD is by nature a heterogenous condition underpinned by metabolic derangements and alterations in mitochondrial bioenergetics, we also sought to explore the relationship between urinary mitochondrial DNA:nuclear DNA ratio (mtDNA:nDNA) and early changes in kidney function.

METHODS

Study design and participants

The study population consisted of adolescents and young adults with type 1 diabetes from two tertiary diabetes centres in Australia, the Mater Hospital, Brisbane and the Children's Hospital at Westmead, Sydney. Participants were identified through a screening phase for entry into the Adolescent Type 1 Diabetes Cardio-Renal Intervention Trial (AddIT) which included adolescents aged 10 to 16 years with type 1 diabetes diagnosed for ≥ 1 year or C-peptide negative. However, most (87.5%) did not proceed to randomisation into AddIT with seven randomised to placebo for a median of 2.6 years ¹⁴. Exclusion criteria at baseline were 1) other types of diabetes; 2) severe hyperlipidaemia and family history data to support diagnosis of familial hypercholesterolaemia; 3) established hypertension unrelated to DKD; 4) prior exposure to the AddIT investigational products [statins and/or angiotensin converting enzyme inhibitors (ACEi)]; 5) other comorbidities considered unsuitable by the investigator (excluding treated hypothyroidism and coeliac disease); and, 6) proliferative retinopathy. Participants were initially assessed at baseline during adolescence (2009-2013) and subsequently followed up as young adults (2019-2021). Medical management according to prevailing best practice was provided by the participants' local health care teams during the study period. Written informed consent was obtained from all participants and for those under the age of 18 years, from their legal guardian in addition to participant assent. The study conformed to the provisions of the Declaration of Helsinki and was approved by the Human Research Ethics Committee at each of the participating centres.

Clinical assessments

All participants were required to be systemically well without intercurrent illness on the day of assessment. Data on age, duration of diabetes and anthropometry (weight and height) were collected at each visit. Brachial blood pressure was measured twice in the seated position (Omron M6 Blood Pressure Monitor; Kyoto), using an appropriate cuff size. The average of the two blood pressure measurements was used in the analysis. Body mass index (BMI) was calculated using the formula: $\text{BMI} = \text{weight (kilograms)} / \text{height (metres)}^2$. A BMI z-score was calculated for individuals <18 years old at time of visit using the Centers for Disease Control and Prevention, National Center for Health Statistics 2000 growth charts, U.S.¹⁵.

Heart rate variability was assessed using the LabChart-Pro device (ADInstruments, Sydney Australia). Indices of heart rate variability were derived from the electrocardiographic R-R intervals and included time-domain measures [heart rate, SD of mean NN intervals (SDNN), and root mean squared difference of successive NN intervals (RMSSD)], frequency-domain measures [low-frequency (LF), and high-frequency (HF) components, and the LF-to-HF ratio] and triangular index. Cardiac autonomic dysfunction was defined by the presence of at least 2 abnormal indices.

Biochemical assessments

Three consecutive early morning urine samples collected at two time points, approximately six months apart during screening, were averaged and represented the baseline UACR. These urine samples were analysed centrally at The WellChild Laboratory at The Evelina Children's Hospital, London. Urine albumin was measured using nephelometric immunoassay (Siemens BN Prospec) and for concentrations <2.1 mg/L by ELISA using rabbit antibodies to human albumin

(RRID:AB_578465, Agilent Technologies, Santa Clara, USA; Original Manufacturer Dako, High Wycombe, Buckinghamshire, U.K)^{16,17}. Urine creatinine was measured using a chromatographic stable isotope dilution electrospray mass spectrometry-mass spectrometry method (AB SCIEX API5000). At follow-up, 3 consecutive early morning urine samples were collected with urinary albumin and creatinine measured locally using either a VITROS 5600 analyser (Ortho Clinical Diagnostics, New Jersey, USA) or Immulite 1000 analyser (Siemens Healthineers, Erlangen, Germany).

All other biochemical assessments for this study were measured locally at each centre. HbA1c was analysed using Diabetes Control and Complications Trial (DCCT) aligned methods. Plasma creatinine and lipids were measured using a VITROS 5,1 or VITROS 5600 analyser (Ortho Clinical Diagnostics, New Jersey, USA). Estimated GFR (eGFR) (millilitres per minute per 1.73 metres squared) was calculated using a modified Schwartz formula: $42 \times \text{height (centimetres)} / \text{plasma creatinine (micromoles per litre)}$ ¹⁴.

Urinary mtDNA:nDNA

Aliquots of unspun urine collected at baseline and follow-up were used to measure urinary mtDNA:nDNA. DNA was extracted from concentrated urine using QIAamp MiniElute Virus Kits (Qiagen, The Netherlands) with concentrations determined using a Nanodrop Lite Spectrophotometer (NanoDrop Technologies, Wilmington, DE). A multiplexed, quantitative polymerase chain reaction (qPCR) technique (QuantiStudio7Flex qPCR Machine with TaqMan TaqPath ProAmp Master Mix, Thermo Fisher, MA, USA) was then employed with probes and primers for the mitochondrial encoded gene (mtDNA) *NADH-ubiquinone oxidoreductase chain 1*

(*ND-1*) and nuclear encoded gene (nDNA) *Cystic fibrosis transmembrane conductance regulator* (*CFTR*) (Supplementary Figure 1) ¹⁸. Relative gene expression (mtDNA:nDNA) was calculated using the $\Delta\Delta C_t$ method ¹⁹ whereby the mean ΔC_t value of the non-hyperfiltration group at baseline was used as the calibrator at baseline and follow-up (Supplementary Figure 1) ¹⁸.

Study outcomes

Participants were assigned to one of two groups according to the presence or absence of hyperfiltration at baseline with hyperfiltration defined by an $eGFR > 135 \text{ mL/min/1.73m}^2$ ²⁰. While there is no formal consensus on the definition of hyperfiltration, this threshold is consistent with earlier reports of hyperfiltration in people with diabetes, including those that have examined youth with type 1 and type 2 diabetes ^{21,22}. The primary outcome measure was a difference in the annual rate of change in $eGFR$ ($\text{mL/min/1.73m}^2/\text{year}$) between the two groups using a linear $eGFR$ slope.

Secondary outcome measures included ‘rapid $eGFR$ decline’ ($>3 \text{ mL/min/1.73 m}^2/\text{year}$) ¹¹, temporal changes in UACR as well as trends in HbA1c and cardiovascular risk markers such as blood pressure, lipid levels and heart rate variability. Albuminuria was defined as a $UACR > 3 \text{ mg/mmol}$ on at least two out of three early morning urine samples ¹⁴. Exploratory analyses were performed to examine potential associations between baseline urinary mtDNA:nDNA and hyperfiltration status. We also explored baseline urinary mtDNA:nDNA as a predictor of change in $eGFR$ and UACR, as well as risk for rapid $eGFR$ decline.

Statistical analysis

Data are summarised as mean (SD) or median (interquartile range) unless otherwise specified. Non-normally distributed variables were log transformed before analysis. Between-group baseline comparisons were performed by two sample t-test or Wilcoxon rank-sum test for continuous variables and by Pearson's chi-squared test for categorical variables.

For both the primary and secondary outcomes, generalised linear models were used to assess the effect of the baseline eGFR group (hyperfiltration vs non-hyperfiltration). Firstly, univariable analyses were performed to identify statistically significant associations. Multivariable models were then derived by including clinically and/or statistically significant covariates. Generalised linear models were similarly used to examine baseline urinary mtDNA:nDNA as a predictor of change in kidney markers. Statistical analyses were performed using R statistical package with graphical data produced using Prism. Associations with P values <0.05 were considered significant.

RESULTS

Study population and baseline characteristics

The study cohort consisted of participants who completed both a baseline [age 14.2 (1.5) years; mean diabetes duration 6.7 (3.0) years] and follow-up [age 23.4 (2.3) years; mean diabetes duration 15.9 (3.0) years] assessment. Twenty-five (31%) participants had hyperfiltration whilst fifty-five (69%) participants had absence of hyperfiltration ('non-hyperfiltration') at baseline. The mean duration of follow-up for the study was 9.2 (1.3) years.

The general characteristics of study participants at baseline and follow-up are shown in Table 1. Adolescents in the hyperfiltration group were slightly younger and, by definition, had higher eGFR at baseline. There were more females than males with hyperfiltration. While diabetes duration did not differ significantly between groups at baseline, this difference was found to be statistically significant at follow-up. Baseline anthropometric measures, HbA1c, blood pressure, lipid levels, UACR and urinary mtDNA:nDNA were also similar between the two groups. Two participants had moderate albuminuria at baseline, both in the non-hyperfiltration group. At follow-up, participants in the hyperfiltration group demonstrated higher eGFR and modestly lower triglyceride level.

Temporal trend in eGFR

Univariable linear regression showed a trend towards greater total change [hyperfiltration -12.42 (27.89) mL/min/1.73m² vs non-hyperfiltration -3.58 (22.14) mL/min/1.73 m², P=0.13] and annual rate of change in eGFR [hyperfiltration -1.46 (3.07) mL/min/1.73 m²/year vs non-hyperfiltration -0.51 (2.48) mL/min/1.73 m²/year, P=0.15] in the hyperfiltration group (Figure 1). However, this association was found to be significant following multivariable adjustment for sex, diabetes duration and HbA1c (Table 2). Annual rate of change in eGFR was also negatively associated with male sex. In total, there were 16 (20%) participants who demonstrated rapid eGFR decline [hyperfiltration 8/25 (32%) vs non-hyperfiltration 8/55 (14.5%)]. Multivariable logistic regression analysis with adjustment for sex, diabetes duration and HbA1c showed that hyperfiltration was associated with greater risk of rapid eGFR decline over 9 years compared to participants without baseline hyperfiltration [OR 14.11, 95% CI (2.30-86.60), P=0.004] (Table 3). Male sex was also associated with increased risk of rapid eGFR decline [OR 31.33, 95% CI 4.19-234.25, P<0.001].

Diabetes duration, baseline HbA1c and UACR did not predict the annual rate of change in eGFR in univariable analysis (Supplementary Table 1)¹⁸. There were no participants with an impaired eGFR of <60 mL/min/1.73 m² in the study.

Temporal changes in UACR

There was no difference in the change in UACR over time between the two groups [hyperfiltration 0.07 (0.36) vs non-hyperfiltration 0.04 (0.44), P=0.80] (Supplementary Figure 2)¹⁸. Change in UACR was significantly associated with baseline UACR (Supplementary Table 2)¹⁸.

Among participants who had three urine samples available for assessment at follow-up (90%), two exhibited evidence of moderate albuminuria (Table 1), one of whom was on an ACEi. Another participant from the non-hyperfiltration group had severe albuminuria and was also on ACEi therapy at follow-up. Both participants who had moderate albuminuria at baseline were normoalbuminuric at follow-up without documented use of a renin-angiotensin system (RAS) inhibitor.

Other secondary outcomes

No significant differences were observed in temporal changes of other clinical risk markers including HbA1c, blood pressure, or lipid levels between the two groups (Supplementary Table 3)¹⁸. Overall, there was a low prevalence of abnormal heart rate variability at each visit with no between group differences observed (Table 1). There were low self-reported rates of smoking across both groups at each study assessment.

Urinary mtDNA:nDNA

Participants in the two groups demonstrated similar urinary mtDNA:nDNA at baseline [hyperfiltration -0.17 (1.43) vs non-hyperfiltration 1.64×10^{-10} (1.18)] and follow-up [hyperfiltration -0.17 (1.15) vs non-hyperfiltration -0.10 (1.13)] (Supplementary Figure 3)¹⁸. Baseline urinary mtDNA:nDNA was not associated with baseline hyperfiltration but was associated with male sex and diabetes duration (Supplementary Table 4)¹⁸. Furthermore, univariable linear regression showed baseline urinary mtDNA:nDNA was negatively associated with both annual rate of change in eGFR (Table 4) and rapid eGFR decline (Table 5). However, these associations did not remain significant in the fully adjusted model (Table 4 and Table 5).

There was no association between baseline urinary mtDNA:nDNA and temporal change in UACR when baseline UACR was included in multivariable modelling (Supplementary Table 5)¹⁸. Change in urinary mtDNA:nDNA was similar between those in the hyperfiltration and non-hyperfiltration groups (Supplementary Table 6)¹⁸. Male sex remained independently associated with urinary mtDNA:nDNA ratio at follow-up (Supplementary Table 7)¹⁸. Follow-up urinary mtDNA:nDNA was also strongly associated with baseline urinary mtDNA:nDNA (Supplementary Table 8)¹⁸.

DISCUSSION

This study showed that in a cohort of 80 young individuals with type 1 diabetes, aged 23.4 (2.3) years at follow-up, the presence of hyperfiltration during adolescence was associated with a greater decline in eGFR and increased odds of rapid eGFR decline when adjusted for important covariates including sex, diabetes duration and HbA1c. Interestingly, the results also highlighted a potential

1 relationship between baseline urinary mtDNA:nDNA and both greater annual decline in eGFR and
2 higher risk for rapid eGFR decline.

3
4 Of the many devastating, long-term complications of diabetes, DKD is considered one of the most
5 important given the associated morbidity and mortality. Approximately one third of people with
6 type 1 diabetes and 40% with type 2 diabetes will develop kidney disease, with diabetes now being
7 the most frequent cause of kidney failure globally ²³. Moderate albuminuria is one of the earliest
8 clinical signs of DKD and a number of observational studies have demonstrated a link to increased
9 future risk of kidney failure and cardiovascular disease ²⁴. However, more contemporary studies
10 have shown that spontaneous remission is not uncommon ²⁵. In a more recent analysis of the
11 Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and
12 Complications (DCCT/EDIC) Study, the 10-year cumulative incidence of progression to severe
13 albuminuria was 28% while regression to normoalbuminuria was 40% ²⁶. Therefore, moderate
14 albuminuria in the absence of other features of DKD may not necessarily portend irreversible and
15 progressive kidney damage. Indeed, this provides a possible explanation for the observed outcome
16 in two participants who spontaneously transitioned from moderate albuminuria to
17 normoalbuminuria between visits. While more advanced stages of DKD may take decades to
18 develop and present clinically, there is increasing evidence that kidney disease begins earlier in
19 diabetes than previously recognised ^{4,5} and the search for reliable markers of these early
20 pathological changes demands greater attention.

21
22 Hyperfiltration can be an early indicator of DKD risk, often preceding elevations in urinary
23 albumin excretion and GFR decline ⁹. It is associated with alterations to intrakidney

1 haemodynamics that can lead to structural changes including glomerular basement membrane
2 thickening and mesangial expansion ²⁷. Indeed, attenuation of hyperfiltration is a well-recognised
3 effect shared by approved DKD treatments including RAS and sodium-glucose cotransporter-2
4 (SGLT2) inhibitors ²⁸. While some studies have shown hyperfiltration to be a risk factor for the
5 development and progression of DKD in adults ^{11,29}, less is known about its long-term implications
6 when first detected in adolescence, a period often characterised by accelerated growth and
7 difficulties in achieving optimal glycaemic control.

8
9 There have been relatively few studies that have examined the effect of early hyperfiltration in
10 diabetes and the change in GFR during the transition from adolescence to young adulthood,
11 although similar trends have been demonstrated previously. Longitudinal data from the Oxford
12 Regional Prospective Study (ORPS), which followed a cohort of children from diagnosis of type
13 1 diabetes for a median duration of 10.9 years, showed that hyperfiltration was predictive of the
14 development moderate albuminuria ³⁰. Our observations relating to eGFR changes also mirror
15 findings from a study that examined the evolution of kidney hyperfiltration in a well characterised
16 group of 18 adolescents with uncomplicated type 1 diabetes ³¹. A decline in measured GFR and
17 filtration fraction was observed after 7 years in participants with baseline hyperfiltration while
18 those without hyperfiltration displayed a more stable GFR. This propensity towards a decline in
19 eGFR following hyperfiltration was similarly demonstrated in participants with type 1 diabetes
20 from the large SEARCH cohort, whereby male sex and higher baseline eGFR were significantly
21 associated with greater decline in eGFR ³². However, the longer-term impact of early
22 hyperfiltration and whether this sets a young individual on a higher risk trajectory of persistent
23 eGFR decline have not been extensively described in the literature and warrants further

1 examination. While diabetes duration differed slightly between those with and without
2 hyperfiltration at follow-up, it was not found to be associated with temporal changes in eGFR or
3 UACR. This may reflect our relatively short period of observation when compared to the typically
4 longer natural history of chronic kidney disease.

5
6 A steeper decline of the eGFR slope has been shown to be a predictor of significant kidney disease
7 progression, worse cardiovascular outcomes and higher mortality ³³. While eGFR changes in older
8 age and following longer diabetes duration are more likely to reflect underlying CKD processes,
9 data from the Joslin Kidney Cohorts indicate that even early eGFR loss within the ‘normal’ (>90
10 mL/min/1.73 m²) eGFR interval approximates the eGFR slope during more advanced stages of
11 CKD ³³. The Kidney Disease: Improving Global Outcomes KDIGO Guidelines define rapid
12 progression as annual rate of eGFR decline >5 mL/min/1.73 m²/year ³⁴. However, an eGFR
13 decline of >3 mL/min/1.73 m²/year in the setting of pre-existing hyperfiltration has been found to
14 be associated with incident kidney disease in type 1 diabetes ¹¹. Conversely, parallel meta-analyses
15 of clinical trials and cohort studies by Levey et al. (2020) have shown that a reduction of 0.5-1.0
16 mL/min/1.73 m²/year on the total eGFR slope, especially in early stages of CKD, is associated
17 with improved kidney outcomes and lower risk of disease progression (HR~0.7) ³⁵, which further
18 supports the need for early intervention.

19
20 In contrast, a recent retrospective analysis of kidney outcomes in the DCCT/EDIC cohort
21 demonstrated that while early rapid eGFR loss (slope $\leq -3\%$ per year) was associated with
22 increased risk of impaired eGFR (<60 mL/min/1.73 m²), this association was not independent of
23 baseline eGFR level ³⁶. While these findings challenge the suggestion that early rapid eGFR

1 decline may be a predictor of risk of reduced kidney function and kidney disease progression, there
2 are important demographic differences that may influence risk in this cohort including their older
3 age at diagnosis (mean of 21 years) and relatively high socioeconomic status . Furthermore,
4 adolescents and those with hyperfiltration were excluded from this recent analysis. Ultimately,
5 robust prospective data are still needed to determine whether attenuating the rate of eGFR decline
6 or reversing hyperfiltration in this early stage can prevent or delay the development of impaired
7 GFR and kidney failure in younger individuals. Although emerging data from effective novel
8 treatment classes for DKD, including mineralocorticoid receptor antagonists ³⁷ and endothelin-A
9 receptor antagonists ³⁸, continue to reinforce hyperfiltration as a potentially important target in the
10 complex pathophysiology and management of DKD.

11
12 From a metabolic standpoint, the complex diabetic milieu provokes changes to normal cellular
13 metabolism including abnormalities in substrate delivery and alterations to utilisation of fuel
14 sources, with mitochondria playing a central role. Kidney function is tightly coupled to metabolic
15 processes including glucose reabsorption. Surprisingly, some young adults with type 1 diabetes
16 but without complications exhibit hyperfiltration rather than normofiltration during clamped
17 euglycemia ³⁹ suggesting that other novel factors may be at play. Indeed, mitochondrial
18 dysfunction is associated with both diabetic and non-diabetic kidney disease ⁴⁰ and the potential
19 to exploit mitochondrial-derived products as novel biomarkers and therapeutic targets in diabetes
20 is promising, although still in the early stages of research development ^{13,41}.

21
22 The limited number of individuals with clinically confirmed DKD in this study prevents a clear
23 relationship to be established between early changes in markers of mitochondrial function and

1 incidence of DKD. However, to our knowledge, this study is the first to demonstrate that individual
2 variations in urinary mtDNA:nDNA can be detected early in the course of type 1 diabetes and that
3 this earlier measurement aligns with a greater annual reduction in eGFR and higher risk of rapid
4 eGFR decline during the transition between adolescence to young adulthood although these
5 relationships did not remain significant when fully adjusted for clinical covariates.

6
7 Like other biomarkers, levels of urinary mtDNA:nDNA may be dynamic and reflect the underlying
8 metabolic stress and cellular injury, with hypoxia being a potentially important mediator. An
9 inherent mismatch between oxygen supply and demand is often observed in diabetes leading to
10 increased expression of hypoxia-inducible factor 1 α (HIF-1 α), metabolic switching to less efficient
11 fuel sources and downstream alterations to mitochondrial bioenergetics ^{40,42}. In support of this,
12 both pre-clinical and clinical data have shown that elevations in urinary mtDNA correlate with the
13 degree of kidney hypoxia and ischaemic injury ⁴³. Disruption to cellular and mitochondrial
14 integrity can also result in release of mtDNA into the extracellular space where it can act as
15 damage-associated molecular patterns (DAMPs) and trigger a pro-inflammatory response leading
16 to tissue injury ⁴⁴. Hyperfiltration can further increase the energy and oxygen demand from the
17 kidneys and exacerbate the underlying metabolic derangements associated with diabetes. While
18 we did not observe a difference in urinary mtDNA:nDNA between participants in the
19 hyperfiltration and non-hyperfiltration group in our cohort (possibly from being underpowered),
20 higher levels have been detected in older adults with kidney hyperfiltration in the presence of
21 obesity and hypertension ⁴⁵. Consideration should be also given to the potential impact of rapid
22 GFR decline on a background burden of chronic hypoxia. Moreover, with increasing age and
23 diabetes duration, the development of important co-morbidities such as hyperlipidaemia and

1 atherosclerosis further increases kidney vascular compromise and accelerates the risk for DKD.
2 Longitudinal studies with the incorporation of functional mitochondrial assays will further help
3 define the role of urinary mtDNA:nDNA as a non-invasive measure of mitochondrial dysfunction
4 in the development and progression of DKD.

5
6 There are important limitations to consider in the present study, including the observational design,
7 relatively small sample size and use of an eGFR. Reference methods which directly measure GFR
8 offer greater accuracy but are often challenging and costly to implement in clinical practice ⁴⁶.
9 Whilst eGFR has become an important tool in both the clinical and research setting, it is subject
10 to increased variability at higher levels of GFR. Furthermore, the performance of creatinine-based
11 equations originally derived and validated in populations with CKD is less well understood in
12 individuals with diabetes. Adding to this challenge, the ideal eGFR formula for adolescents and
13 young adults remains a subject of debate. Recent data from a large cohort of adolescents and young
14 adults showed that when compared against a measured GFR, the Schwartz equation was
15 considered more accurate and less biased than the CKD-EPI equation ⁴⁷. The current study utilised
16 a modified Schwartz equation derived from previous studies of GFR in a similar cohort of
17 participants with type 1 diabetes in which the Inutest was used as the standard ¹⁴. Further, the
18 decision to use a consistent eGFR equation reduces the potential bias and discontinuity from the
19 switching of equations in this transition period. Finally, whilst we cannot exclude that the observed
20 changes in eGFR may be partly influenced by regression to the mean, or simply represent
21 resolution of the underlying hyperfiltration, important earlier studies have highlighted that
22 individuals with early rapid eGFR decline may still represent a higher risk phenotype. These

1 individuals warrant closer monitoring, especially as they begin to enter their third and fourth
2 decade of life when physiological decline in GFR also takes effect.

4 **CONCLUSION**

5 In summary, we report significant associations between hyperfiltration and both a reduction in
6 eGFR and rapid eGFR decline following the transition from adolescence to young adulthood in
7 type 1 diabetes. We also showed that the novel biomarker, urinary mtDNA:nDNA, measured
8 during adolescence may be a useful marker of early changes to kidney function. Future evaluation
9 of this cohort will provide a more comprehensive understanding of the next phase of this GFR
10 trajectory and the impact of early hyperfiltration and mitochondrial dysfunction as risk markers of
11 DKD.

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Data Availability

Some or all datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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5

6

Table 1. Participant characteristics at baseline and follow-up.

	BASELINE				FOLLOW-UP			
	Study cohort	Hyperfiltration	Non-hyperfiltration	<i>P</i>	Study cohort	Hyperfiltration	Non-hyperfiltration	<i>P</i>
	80	25	55		80	25	55	
Sex				0.04				0.04
<i>Male</i>	39	8 (32.0)	31 (56.4)		39	8 (32.0)	31 (56.4)	
<i>Female</i>	41	17 (68.0)	24 (43.6)		41	17 (68.0)	24 (43.6)	
Age at visit (years)	14.2 (1.5)	13.5 (1.3)	14.5 (1.5)	0.005	23.4 (2.3)	22.4 (1.9)	23.9 (2.3)	0.01
Diabetes duration (years)	6.7 (3.0)	5.8 (2.7)	7.1 (3.1)	0.09	15.9 (3.0)	14.7 (2.8)	16.4 (3.0)	0.02
Time between baseline and follow-up (years)						9.2 (8.1-10.8)	8.9 (8.5-9.5)	0.36*
BMI (kg/m ²)	21.5 (19.0-24.1)	20.5 (18.8-24.0)	21.7 (19.5-24.2)	0.45*	26.1 (22.9-29.1)	24.1 (22.1-29.7)	26.2 (23.6-29.1)	0.45*
BMI z-score	0.58 (0.79)	0.60 (0.93)	0.57 (0.72)	0.89*				
HbA1c (%)	8.4 (1.1)	8.4 (1.4)	8.4 (1.0)	0.80	8.5 (1.7)	8.6 (1.8)	8.5 (1.7)	0.75
HbA1c (mmol/mol)		68.6 (15.4)	67.9 (11.1)			70.8 (19.2)	69.3 (19.1)	
Systolic blood pressure (mmHg)	110.6 (11.7)	110.4 (11.4)	110.7 (11.9)	0.91	120.4 (9.7)	118.4 (9.3)	121.3 (9.8)	0.22

Diastolic blood pressure (mmHg)	63.1 (8.2)	62.0 (8.3)	63.5 (8.2)	0.45	70.3 (9.3)	69.4 (8.8)	70.8 (9.6)	0.56
Total Cholesterol (mmol/L)	4.6 (0.9)	4.7 (0.9)	4.6 (0.9)	0.77	4.7 (0.9)	4.5 (0.7)	4.7 (1.0)	0.22
HDL-Chol (mmol/L)	1.5 (0.3)	1.5 (0.3)	1.5 (0.3)	0.52	1.4 (0.4)	1.5 (0.5)	1.4 (0.4)	0.17
LDL-Chol (mmol/L)	2.6 (0.8)	2.5 (0.7)	2.6 (0.8)	0.60	2.7 (0.8)	2.6 (0.8)	2.8 (0.9)	0.27
Triglycerides (mmol/L)	0.9 (0.7-1.3)	0.9 (0.6-1.3)	0.9 (0.7-1.3)	0.85*	0.9 (0.8-1.4)	0.8 (0.7-1.0)	1.0 (0.8-1.5)	0.02*
eGFR (mL/min/1.73 m ²)	124.7 (20.5)	148.6 (13.6)	113.9 (12.1)	<0.001	124.7 (13.8)	136.2 (33.7)	110.3 (21.0)	0.001
UACR (geometric mean)	0.68 (0.53-0.91)	0.74 (0.53-0.88)	0.66 (0.52-0.95)	0.91*	0.60 (0.41-1.12)	0.60 (0.41-0.94)	0.55 (0.41-1.12)	0.75*
UACR (mean of log10)	-0.16 (0.22)	-0.18 (0.24)	-0.16 (0.21)	0.63	-0.12 (0.39)	-0.11 (0.36)	-0.12 (0.41)	0.98
Albuminuria ^a n (%)	2 (2.5)	0 (0)	2 (3.6)	0.85	3 (3.75)	1 (4.5)	2 (4)	1.00
Abnormal heart rate variability ^b	6 (10.2)	3 (15.0)	3 (7.7)	0.38	21 (26.6)	7 (28.0)	14 (25.9)	0.85
mtDNA:nDNA ^c	-0.05 (1.26)	-0.17 (1.43)	1.64x10 ⁻¹⁰ (1.18)	0.59	-0.12 (1.13)	-0.17 (1.15)	-0.10 (1.13)	0.82

1 Data are *n* (%), mean (SD), or median (interquartile range). Pearson's chi-squared test for categorical data. Two
2 sample t-test for continuous data unless noted.

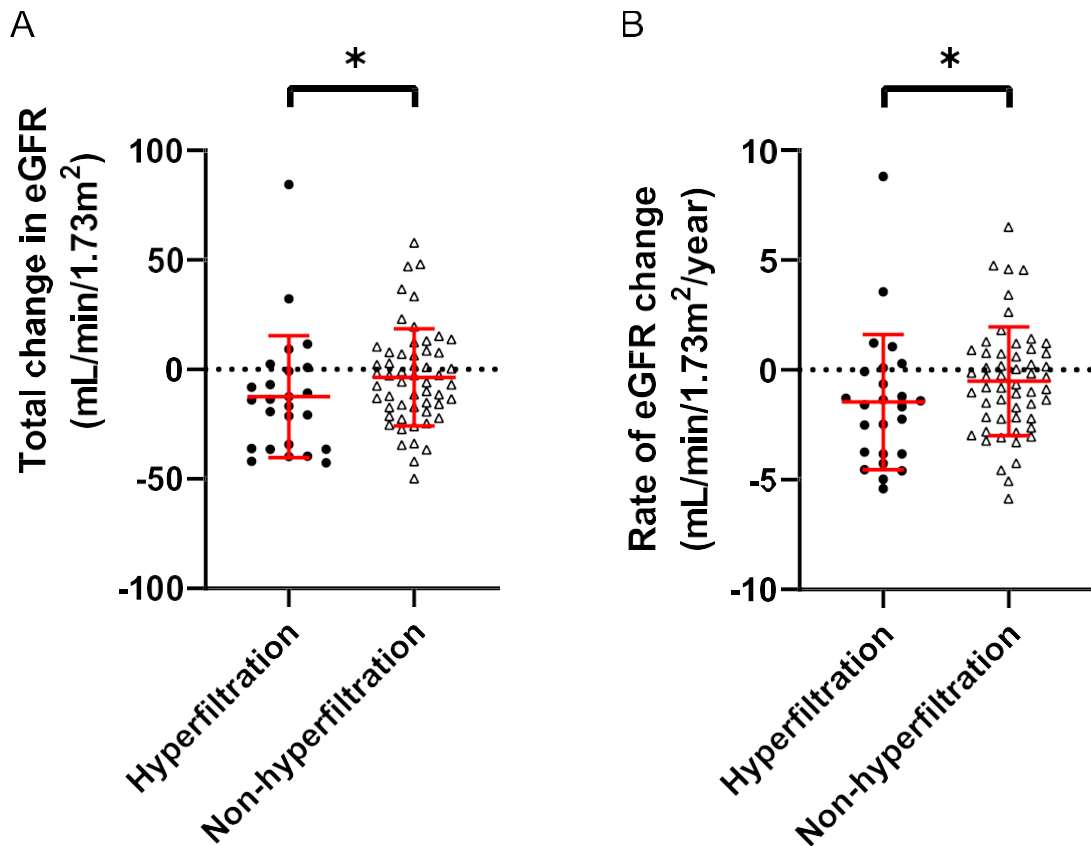
3 * Wilcoxon rank-sum test.

4 a: Based on detection of at least 2 out of 3 urine UACR > 3mg/mmol.

5 b: Based on presence of at least 2 abnormal heart rate variability indices.

6 c: Natural log transformed values, $\ln(2^{-\Delta\Delta CT})$.

Figure 1. Change in eGFR between baseline and follow-up.



A: Total change in eGFR between baseline and follow-up with mean (SD), hyperfiltration -12.42 (27.89) mL/min/1.73m² vs non-hyperfiltration -3.58 (22.14) mL/min/1.73 m².

$P=0.13$, from univariable (unadjusted) linear regression analysis.

$P=0.04$, from multivariable linear regression analysis (adjusted for sex and diabetes duration).

B: Annual rate of eGFR change between baseline and follow-up with mean (SD), hyperfiltration -1.46 (3.07) mL/min/1.73m²/year vs non-hyperfiltration -0.51 (2.48) mL/min/1.73 m²/year.

$P=0.15$, from univariable (unadjusted) linear regression analysis.

$P=0.04$, from multivariable linear regression analysis (adjusted for sex and diabetes duration).

Table 2. Linear regression analyses showing the relationship between baseline hyperfiltration and annual rate of change in eGFR with adjustments for co-variables.

Dependent: annual rate of change in eGFR		
	β -estimate (SE)	<i>P</i>
Unadjusted model ($r^2=0.015$)		
Hyperfiltration	-0.95 (0.65)	0.15
Model 1 ($r^2=0.136$) ($P_{\text{model}}=0.003$)		
Hyperfiltration	-1.50 (0.63)	0.02
Sex (male)	-2.02 (0.58)	<0.001
Diabetes duration at baseline	-0.05 (0.10)	0.64
Model 2 ($r^2=0.144$) ($P_{\text{model}}<0.001$)		
Hyperfiltration	-1.55 (0.63)	0.02
Sex (male)	-2.02 (0.58)	<0.001
Diabetes duration at baseline	-0.06 (0.10)	0.51
Baseline HbA1c	0.33 (0.25)	0.19

Results are from univariable and multivariable linear regression models. Data reported as β -estimate and standard error (SE). β -estimates are equal to the mean difference in annual rate of change in eGFR in the presence of an independent categorical variable or for every unit increase in the independent continuous variable. Adjusted r^2 shown for multivariable models.

Table 3. Logistic regression analyses showing the relationship between baseline hyperfiltration and rapid eGFR decline (>3 mL/min/1.73 m²/year) with adjustments for co-variates.

Dependent: rapid eGFR decline		
	OR (95% CI)	<i>P</i>
Unadjusted model		
Hyperfiltration	2.76 (0.90-8.53)	0.08
Model 1		
Hyperfiltration	13.38 (2.23-80.12)	0.004
Sex (male)	31.62 (4.20-238.21)	<0.001
Diabetes duration at baseline	1.13 (0.90-1.42)	0.28
Model 2		
Hyperfiltration	14.11 (2.30-86.60)	0.004
Sex (male)	31.33 (4.19-234.25)	<0.001
Diabetes duration at baseline	1.06 (0.91-1.45)	0.25
Baseline HbA1c	0.82 (0.44-1.55)	0.55

Results are from univariable and multivariable logistic regression models. Data are presented as odds ratio (95% CI). OR represents the increase in odds of rapid eGFR decline in the presence of an independent categorical variable or for every unit increase in the independent continuous variable.

Table 4. Linear regression analyses showing baseline urinary mtDNA:nDNA [$\ln(2^{-\Delta\Delta CT})$] as predictor of annual rate of change in eGFR with adjustment for co-variates.

Dependent: annual rate of change in eGFR		
	β -estimate (SE)	<i>P</i>
Unadjusted model ($r^2=0.006$)		
$\ln(2^{-\Delta\Delta CT})$ (baseline)	-0.82 (0.24)	0.001
Model 1 ($r^2=0.157$) ($P_{\text{model}}=0.002$)		

ln(2 ^{-ΔΔCT}) (baseline)	-0.64 (0.27)	0.02
Sex (male)	-1.33 (0.64)	0.04
Diabetes duration at baseline	0.03 (0.10)	0.80
Model 2 (r²=0.1992) (P_{model}<0.001)		
ln(2 ^{-ΔΔCT}) (baseline)	-0.54 (0.26)	0.04
Sex (male)	-1.69 (0.65)	0.01
Diabetes duration at baseline	0.00 (0.10)	1.00
Baseline eGFR	-0.03 (0.02)	0.04
Model 3 (r²=0.1915) (P_{model}=0.002)		
ln(2 ^{-ΔΔCT}) (baseline)	-0.50 (0.27)	0.07
Sex (male)	-1.74 (0.66)	0.01
Diabetes duration at baseline	-0.01 (0.10)	0.89
Baseline eGFR	-0.03 (0.02)	0.03
Baseline HbA1c	0.16 (0.27)	0.54

1 Results are from univariable and multivariable linear regression models. Data reported as β-estimate and standard
2 error (SE). β-estimates are equal to the mean difference in annual rate of change in eGFR in the presence of an
3 independent categorical variable or for every unit increase in the independent continuous variable. Adjusted r² shown
4 for multivariable models.

5

6 **Table 5.** Logistic regression analyses showing the relationship between baseline urinary
7 mtDNA:nDNA [$\ln(2^{-\Delta\Delta CT})$] and rapid eGFR decline (>3 mL/min/1.73 m²/year) with adjustments
8 for co-variables.

Dependent: rapid eGFR decline		
	OR (95% CI)	<i>P</i>
Unadjusted model		
$\ln(2^{-\Delta\Delta CT})$ (baseline)	2.34 (1.28-4.26)	0.005
Model 1		
$\ln(2^{-\Delta\Delta CT})$ (baseline)	1.72 (0.86-3.44)	0.13
Sex (male)	14.76 (1.71-127.44)	0.01
Diabetes duration at baseline	1.00 (0.78-1.29)	0.97
Model 2		
$\ln(2^{-\Delta\Delta CT})$ (baseline)	1.75 (0.85-3.62)	0.13
Sex (male)	14.62 (1.69-126.22)	0.01
Diabetes duration at baseline	1.00 (0.77-1.29)	0.99
Baseline HbA1c	1.08 (0.56-2.08)	0.83

1 Results are from univariable and multivariable logistic regression models. Data are presented as odds ratio (95% CI).
2 OR represents the increase in odds of rapid eGFR decline in the presence of an independent categorical variable or for
3 every unit increase in the independent continuous variable.