Four- and six-hour urinary albumin excretion is a valuable alternative to 24-h urinary albumin excretion in male db/db mice

SA Nørgaard, FW Sand, DB Sørensen and H Søndergaard

Abstract

In mouse (Mus musculus) models of diabetic nephropathy (DN), one of the most important read-outs is the 24-h urinary albumin excretion (UAE). The 24-h urine collection is usually performed by single housing mice in metabolic cages on wire mesh without enrichment. This is known to be stressful for the mice. Therefore, it was investigated if shorter urine collections would be sufficient to get reliable assessments of albuminuria. Twenty-one diabetic (C57BLKS-Leprdb/db) and ten non-diabetic mice (C57BLKS-Leprdb/+ ) were placed in metabolic cages at 15 and 20 weeks of age (WoA) for 24 h. Urine samples were taken at 4, 6, 18 and 24 h and albumin and creatinine concentration were measured. Four- and 6-h UAE was found to correlate significantly with 24-h UAE. Furthermore, a significant correlation was found between 24-h UAE and albumin:creatinine ratio (ACR) in the 4-h sample. However, the strength of the correlation between ACR and 24-h UAE was weaker than between the 4- and 24-h UAE. This suggests that normalising to creatinine may not provide additional value to the 4-h urine collection. In conclusion, the strong correlation between 4- and 6-h UAE and 24-h UAE indicates that the collection period can be considerably reduced. This refinement could reduce stress and increase welfare of the db/db model and potentially be applied to other DN models.

Keywords: albuminuria, animal welfare, db/db mice, diabetic nephropathy, metabolic cages, refinement

Introduction

Albuminuria is one of the key features of diabetic nephropathy (DN) and is used in diabetic patients to stage disease and predict progression of DN. This early manifestation of DN is caused by changes in the structure and function of the filtration barrier of the glomeruli as well as the tubular reabsorption which result in increased excretion of albumin in the urine (Gross et al 2005; Birn & Christensen 2006; Moreasco et al 2013). While animal models mimicking the more advanced changes in DN are still being developed, assessing the early changes in the kidney by measuring the urinary excretion of albumin is still a widely used read-out in these models (Breyer et al 2005; Brosius et al 2009; Azushima et al 2018). In mice (Mus musculus), albuminuria is usually measured by a total 24-h urinary albumin excretion (UAE) or albumin:creatinine ratio (ACR) in a spot urine sample. It has been shown previously that in some mouse models there is a poor correlation between ACR and UAE (Qi et al 2005). Since it is still not certain which of the two is the better read-out, many publications choose to report both which has also been recommended by the Animal Models of Diabetic Complications Consortium (AMDCC) (Brosius et al 2009). One of the major disadvantages of using the 24-h UAE in DN models is the need for a full day of urine collection. This is usually done by housing the mice in metabolic cages, where they are single housed on wire mesh without enrichment or bedding; factors which are all known to induce stress in rodents (Maner et al 1995; Bartolomucci et al 2003; Hoppe et al 2009; Banggaard Bendtsen et al 2012; Kallikoski et al 2013). Furthermore, we have observed that the db/db mouse strain, which was used here, seems unable to maintain a high food intake in the metabolic cages, which is possibly due to mobility challenges or stress. This leads to weight loss and the mice often have difficulties in recovering from being metabolic cage-housed which may, in the worst case, lead to mortality or cause early euthanasia. Moreover, consideration has been given to the possibility that mice may drink insufficiently while metabolic cage-housed, thereby becoming susceptible to dehydration. This may provide at least part of the explanation for the observed weight loss and slow recovery and perhaps also giving rise to variation in the urine read-outs.