RESEARCH ARTICLE

A novel method for the direct measurement of urinary conjugated metabolites of α-tocopherol and its use in diabetes

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1 Introduction

Over the past 50 years, a number of metabolites of vitamin E (α-tocopherol) have been described. These can be divided into two groups. The first include α-tocopherylquinone, α-tocopheronic acid and α-tocopheronolactone (α-TL), all of which result from the oxidation and opening of the chromanol ring of α-tocopherol [1, 2]. The second group result from the successive shortening of the phytyl side chain of α-tocopherol, initially by ω-hydroxylation by a cytochrome P450-dependent mechanism followed by β-oxidation [3, 4] and include α-carboxymethyl-butyl-hydroxychroman and α-carboxy-ethyl-hydroxychroman (α-CEHC) [5–7]. The principal urinary metabolites of α-tocopherol are α-CEHC and αTL, both of which are excreted as their polar sulfate and glucuronide conjugates [5, 8]. There is, however, doubt in the literature whether the αTL observed in urine is real or an artifact produced from the oxidation of α-CEHC during the methodological work up [5]. Confirmation of the authenticity of αTL is important, as it could potentially be used as an *ex vivo* biomarker of oxidative stress.

Published methods for the measurement of urinary vitamin E metabolites typically involve deconjugation, extraction and derivatization steps, which are time consuming and risk the artifactual formation of αTL from α-CEHC [9]. Our aim was to develop a new and rapid method for the direct assay of the conjugated vitamin E metabolites and to investigate whether there was evidence for increased urinary concentrations of αTL in conditions where oxidative stress may be implicated such as diabetes.

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Abbreviations: α-CEHC, α-carboxy-ethyl-hydroxychroman; **αTL,** α-tocopheronolactone

2 Materials and methods

A method that required no sample preparation was established and validated for the direct measurement of the sulfate and glucuronide conjugates of α-CEHC, αTL, appropriate internal standards and creatinine using LC/MS/MS. The total run time *per* sample was 20 min. The m/z transitions used for the sulfate (S) and glucuronide (G) metabolites were $356.97 > 80.37$ and $453.05 > 112.79$, respectively. Two peaks, presumed to be isomers, were obtained for αTL-glucuronide and were designated αTL-G1 and αTL-G2. The intra-assay CVs for the metabolites ranged from 0.88 to 3.73% ($n = 20$) and the inter-assay CVs from 1.18 to 4.32% ($n = 20$ over 60 days). Recoveries were $\geq 90\%$.

3 Results

This method has been used to study 32 children with type 1 diabetes mellitus and age-matched controls. The results in nmol/mmol creatinine (means ± 1SEM) for the children with diabetes and controls are summarized in Table 1.

The mean concentrations of the αTL conjugates are all highly significantly increased in the diabetic patients. The concentrations of α-CEHC conjugates were also increased but not to the same degree of significance.

Table 1. Concentrations of urinary conjugated vitamin E metabolites in diabetic children and controls

	αTL-G1	αTL-G2	αTL-S	α-CEHC-G	α-CEHC-S
Diabetics	1098±279	562±166	98±24	126±16	138±33
Controls	76±13	34±9	10±2	73±19	57±12
p	0.0006	0.0018	0.001	0.04	0.03

4 Discussion

In summary, we have developed a method which provides a direct, rapid and reproducible assay for the conjugates of the metabolites of α-tocopherol without the risk of artifact formation. In previous methods, the urinary metabolites have had to be deconjugated, extracted and derivatized prior to analysis. This required relatively harsh conditions and the risk of the artifactual production of αTL from α-CEHC. The fact that in this new method the metabolites are measured directly and rapidly means that artifactual oxidation cannot occur. In addition, the run time of this method was 20 min *per* sample compared with a run time in the previous methods of 9 h [9].

The highly significant increase in urinary αTL concentrations in the patients with diabetes compared with the α-CEHC concentrations, which are only just significantly increased, suggest that the measurement of urinary αTL conjugates may provide a useful marker of oxidative stress. Further work will, however, be required to prove the usefulness of this potential biomarker.

The authors have declared no conflict of interest.

5 References

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