Are serum α- and β-carotene concentrations associated with the development of advanced beta-cell autoimmunity in children with increased genetic susceptibility to type 1 diabetes?

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Received 18 August 2010; received in revised form 29 September 2010; accepted 1st October 2010
Available online 7 December 2010

Abstract

Aim. – Reactive oxygen intermediates have been implicated in mediating the destruction of insulin-producing beta cells and antioxidant nutrients thought to protect against such a process. This study aimed to assess the associations between serum α- and β-carotene concentrations, and the risk of advanced beta-cell autoimmunity, in children with HLA-conferred susceptibility to type 1 diabetes.

Methods. – This case-control study, comprising 108 case children with advanced beta-cell autoimmunity and 216 matched control children, was nested within the nutrition study of the Type 1 Diabetes Prediction and Prevention (DIPP) birth cohort. Serum α- and β-carotene samples were collected each year from the age of 1 to 6 years. For each case-control group, serum samples were analyzed up to the time of seroconversion in the case children. Associations were studied using a conditional logistic-regression model.

Results. – Neither serum α- nor β-carotene concentration was significantly associated with the risk of advanced beta-cell autoimmunity. There was marginal evidence (P = 0.049) of an inverse association between serum β-carotene concentration and the risk of developing advanced beta-cell autoimmunity at a time closest to seroconversion after adjusting for parental education, maternal age, duration of gestation, diabetes in first-degree relatives, number of earlier deliveries and maternal smoking during pregnancy.

Conclusion. – The present study data provided no clear evidence to support an association between serum α- or β-carotene concentration and advanced beta-cell autoimmunity.

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Keywords: Aetiology; Beta-cell autoimmunity; Type 1 diabetes; Children; Carotenoids

Résumé

Les concentrations sériques d’α- et de β-carotène sont-elles associées au développement d’une réaction auto-immune évoluée vis-à-vis des cellules bêta chez les enfants génétiquement prédisposés au diabète du type 1 ?

Objectif. – Les radicaux libres de l’oxygène ont été impliqués dans la médiation de la destruction des cellules bêta qui produisent l’insuline et des substances anti-oxydantes sont supposées protéger contre un tel processus. Cette étude avait pour objectif d’évaluer l’association entre les concentrations sériques d’α- et de β-carotène, et le risque d’une réaction auto-immune évoluée...
vis-à-vis des cellules bêta chez des enfants ayant une prédisposition conférée par le système HLA au diabète du type 1 (DT1).

**Méthodes.** – Cette étude cas-témoins, comprenant 108 enfants cas ayant une auto-immunité évoluée vis-à-vis des cellules bêta et 216 enfants témoins apparisés, faisait partie de la Nutrition Study of the Type 1 Diabetes Prediction and Prevention (DIPP). Des échantillons destinés à doser l’α- et le β-carotène sériques ont été collectés annuellement de l’âge d’un an à six ans. Pour chaque groupe cas-témoin, des échantillons de sérum ont été analysés jusqu’à l’âge de séroconversion de l’enfant atteint de DT1. Les associations ont ensuite été étudiées à l’aide d’un modèle de régression logistique conditionnel.

**Résultats.** – Ni les concentrations d’α- ni celles de β-carotène n’étaient significativement associées au risque d’auto-immunité évolutée des cellules bêta. Il y avait une tendance (P = 0.049) en faveur d’une relation inverse entre les concentrations de β-carotène sériques et le risque de développer une réaction auto-immune évolutée vis-à-vis des cellules bêta en période proche de la séroconversion, après ajustement sur l’éducation parentale, l’âge de la maternité, la durée de gestation, les cas de diabète chez les parents au premier degré, le nombre d’accouchements antérieurs et le tabagisme de la mère pendant la grossesse.

**Conclusions.** – Ces données ne sont pas en faveur de l’association entre les concentrations sériques d’α- et de β-carotène et le développement d’une réaction auto-immune vis-à-vis des cellules bêta.

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**Mots clés :** Étiologie ; Auto-immunité des cellules bêta ; Diabète de type 1 ; Enfants ; Caroténoïdes

1. **Abbreviations**

   - BHT butylated hydroxytoluene
   - DIPP Type 1 Diabetes Prediction and Prevention study
   - GADA antibodies to the 65-kDa isoform of glutamic acid decarboxylase
   - IAA insulin autoantibodies
   - IA-2A antibodies to the tyrosine phosphatase-related islet antigen 2
   - ICA islet-cell antibodies

2. **Introduction**

   Type 1 diabetes is an autoimmune disease resulting from the destruction of insulin-secreting beta cells in the pancreatic islets of genetically susceptible individuals. Although the molecular mechanisms behind beta-cell destruction have yet to be determined, there is evidence implicating the involvement of reactive oxygen intermediates [1]. Animal models of type 1 diabetes have indicated that dietary antioxidants may protect beta cells from oxidative damage [2,3]. Hence, there is growing interest in the possibility that dietary antioxidants may help to delay, or even prevent, the clinical manifestation of type 1 diabetes in humans.

   Carotenoids are an important group of dietary phytochemicals with antioxidant properties. It has long been suggested that carotenoids may account for the health effects of fruits and vegetables, mainly due to their antioxidant properties *in vitro* and, thus, their ability to reduce excess oxidative stress [4]. The provitamin A carotenoids α- and β-carotene are among the most abundant carotenoids in the human diet, and are essential for many important cell functions [4]. An area as yet unexplored, but nevertheless pertinent, is the role of carotenoids in the aetiology of type 1 diabetes.

   To investigate the association between serum α- and β-carotene concentrations and the risk of advanced beta-cell autoimmunity, we conducted a nested case-control study within the DIPP nutrition study cohort of children with HLA-conferred susceptibility to type 1 diabetes.

3. **Subjects and methods**

   The DIPP project is an ongoing prospective cohort study that started in 1994 by enrolling children with HLA-DQB1-conferring susceptibility to type 1 diabetes from the areas of three university hospitals in Finland [5]. Participants in the DIPP study are monitored for diabetes-associated autoantibodies [6], growth, viral infection and nutrition at 3- to 12-month intervals. The case and control children for the present study came from the cohort of at-risk children born between October 1996 and July 2004 at Oulu and Tampere University Hospitals (n = 5787; 76% of invited children). All parents of the participating children gave their written informed consent. The study was also approved by the local ethics committees.

   Details of the selection process and follow-up for the present case-control series have been described elsewhere [7]. In brief, ICA were used as a primary screening tool for beta-cell autoimmunity. When a child had an ICA-positive sample for the first time, all of that child’s previous and subsequent samples were analyzed for IAA, for GADA and for the protein tyrosine phosphatase-related IA-2 molecules (IA-2A). Of the 5787 children in the study, 119 children seroconverted to advanced beta-cell autoimmunity, defined here as having repeated positivity for ICA plus at least one other autoantibody. In addition, there were six children who had presented with one or more autoantibodies in a single sample either before or at the time of diabetes diagnosis, and three persistently seronegative children who had had their last blood sample drawn 1.9, 3.3 and 5.2 years, respectively, before diabetes diagnosis [7]. For this reason, clinical type 1 diabetes was included in the autoantibody endpoint. The term ‘advanced beta-cell autoimmunity’ here refers to the composite endpoint, and the term ‘séroconversion’ refers to the time at which the composite endpoint was reached. For each case child, two control children who fulfilled matching criteria (birth date within 3 months, same gender, same hospital of birth and same genotype) were randomly selected. However, 20 case children who had no serum carotenoid samples drawn until the age of seroconversion were, therefore, omitted from the analyses, together with their matched controls, resulting in a final total of 108 case children.

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3.1. Determination of α- and β-carotene concentrations

Non-fasting blood samples were taken by venipuncture for the serum α- and β-carotene analyses [7]. Serum α- and β-carotene concentrations were measured using reversed-phase high-performance liquid chromatography (HPLC) [8] at the Biomarker Laboratory, National Institute for Health and Welfare. Each case-control group was analyzed as one batch and, within each group, the samples were in random order in relation to case-control status. The laboratory technicians were unaware of the case-control status of the samples.

For the analysis, 0.4 mL of a 50% ethanol solution, containing ascorbic acid and BHT, and 50 μL of the internal standard echinenone were added to 50 μL of plasma. After mixing, analytes were extracted with 1-mL hexane. A 0.8-mL hexane aliquot was evaporated under nitrogen, and the residue dissolved in 100 μL of mobile phase. The serum α- and β-carotenoids were separated with a Nova-Pak C18 column (3.9 × 150 mm, 4 μm; Waters Corporation, Milford, MA, USA). The carotenoids were detected at 450 nm. Peak height/internal standard ratios were compared with the ratios of reference plasma, the values of which were traceable to the National Institute of Standards and Technology (NIST) certified serum standard reference material (SRM) 968b (National Institute of Standards and Technology, Gaithersburg, MD, USA). The interassay coefficient of variation was 9.4%.

3.2. Statistical analysis

Conditional logistic regression was used to estimate any potential associations between development of the endpoint and serum α- and β-carotene concentrations in the nested case-control design. To assess the sensitivity of the time interval between the endpoint and carotene measurement, analyses were performed in three settings in the case children: at the age of 1 year; at least 6 months before seroconversion; and at the time of seroconversion. In addition, the explanatory variables were aggregated into low (less than the 25th percentile), intermediate (within the interquartile range) and high (above the 75th percentile) values within each age group, based on the carotenoid concentrations of both the case and control children. Putative confounders (Table 1) were adjusted for in the multiple logistic-regression models. All analyses were carried out using SAS version 9.1.3 software (SAS Institute, Cary, NC, USA), and the selected statistical significance was set at 5%.

4. Results

Both case and control children were largely comparable, as described elsewhere [7]. Within each age group, the median serum α- and β-carotene concentrations were similar in both case and control children (Fig. 1). Also, both median α- and β-carotene serum concentrations were highest at the age of 1 year, and tended to decline gradually thereafter.

In the first analysis of the 1-year-olds, no significant associations were observed between serum α- and β-carotene concentrations and the risk of advanced beta-cell autoimmunity (Table 1). In the second analysis, for which serum samples were drawn at least 6 months before seroconversion in the case children, no associations were observed between serum carotenoid concentrations and the risk of advanced beta-cell autoimmunity (data not shown). In the third analysis, serum samples were drawn at the time of seroconversion in the case children (Table 1). Serum α-carotene concentration was not significantly associated with the risk of advanced beta-cell autoimmunity. However, after adjusting for background factors, there was a marginally
<table>
<thead>
<tr>
<th></th>
<th>Crude (Cases (n)</th>
<th>Controls (n)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
<th>Adjusted (Cases (n)</th>
<th>Controls (n)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
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<tr>
<td>Values of α-carotene&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97</td>
<td>199</td>
<td>0.78 (0.41–1.46)</td>
<td>0.404</td>
<td>96</td>
<td>172</td>
<td>0.62 (0.27–1.38)</td>
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<td>21</td>
<td>53</td>
<td>0.41–1.46</td>
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<td>17</td>
<td>45</td>
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<td>48</td>
<td>100</td>
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<td>44</td>
<td>88</td>
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<tr>
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<td>28</td>
<td>46</td>
<td>1.29 (0.69–2.39)</td>
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<td>39</td>
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<td>Values of β-carotene&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>199</td>
<td>0.77 (0.40–1.48)</td>
<td>0.532</td>
<td>86</td>
<td>172</td>
<td>0.52 (0.22–1.27)</td>
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<td>Values of α-carotene&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>216</td>
<td>0.72 (0.41–1.26)</td>
<td>0.472</td>
<td>96</td>
<td>187</td>
<td>0.57 (0.27–1.20)</td>
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<tr>
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<td>99</td>
<td>1.00</td>
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<tr>
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<td>22</td>
<td>50</td>
<td>0.26–1.14</td>
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</table>

<sup>a</sup> Taken at age 1 year in case and control children; median (range) time lag between carotene measurement and advanced beta-cell autoimmunity was 1.0 (0.0–5.4) years.

<sup>b</sup> Low (0, q25), intermediate (q25, q75) and high (q75, q100) values of α- and β-carotene.

<sup>c</sup> Reference group.

<sup>d</sup> Taken from case and control children at the time closest to seroconversion in case children; median (range) time lag between carotene measurement and advanced beta-cell autoimmunity was 0 (0–3.3) years.
significant inverse association between serum β-carotene concentrations and the risk of advanced beta-cell autoimmunity.

5. Discussion

In this nested case-control study, there was no clear evidence of any association between serum α- or β-carotene concentration and the risk of advanced beta-cell autoimmunity. Although a marginal inverse association was observed between serum β-carotene concentrations and advanced beta-cell autoimmunity at the time of seroconversion in the case children, this finding needs to be interpreted with caution because of the number of tests made, the lack of internal consistency of results and the sensitivity of results to the choice of time interval.

A major strength of the present study was the use of a large, population-based cohort with a well-defined study population. The prospective design allowed the concentrations of α- and β-carotene to be measured longitudinally. Another study strength was the definition of our endpoint, which used repeated positivity for ICA plus at least one other autoantibody, thereby reflecting a progressive process that rarely reverts [9]. There was also a large number of progressors in the early-age groups. On the other hand, one limitation of the present study was the use of single measurements of serum carotenoids, which have been argued to reflect only short-term dietary intakes [10].

Our results, which showed no clear association between serum α- and β-carotene concentrations and seroconversion, are in line with those of an earlier study showing no indication of reduced plasma antioxidant activity among Finnish children and adolescents with an increased genetic risk of type 1 diabetes [11]. However, before interpreting the present results, it should be borne in mind that our present study was limited to only two serum carotenoids. Other important carotenoids with antioxidant properties are also present in the human diet, and have been suggested to play a protective role in several diseases [4]. Furthermore, the antioxidant capacity of carotenoids is influenced by a range of factors, such as the mix of carotenoids and other dietary antioxidants in the diet [4,12]. Another point that needs to be highlighted is the possibility that antioxidant nutrients may merely delay the autoimmune process or alter the sensitivity of beta cells to autoimmune disease, an idea that could not be analyzed in our present study.

Recent epidemiological findings have also given cause for further consideration of the role of carotenoids in the aetiology of type 1 diabetes, as the early introduction of fruits, berries and roots has been associated with an increased risk of advanced beta-cell autoimmunity [13], and the early consumption of vegetables with an increased risk of type 1 diabetes [14]. Indeed, while the antioxidant nutrients found in fruits and vegetables have been associated with a possible protective role against type 1 diabetes, they also contain potentially diabetogenic compounds, such as the natural toxins found in Streptomyces-infected root vegetables [15], which could counteract such protection. This is especially pertinent in Finland, where root vegetables are the most common weaning foods. The exceptionally high consumption of industrial vegetable and fruit ‘baby purées’ in Finland, as well as homemade ones, is reflected by the raised serum α- and β-carotene concentrations seen in the 1-year-olds in our present study (Fig. 1).

6. Conclusion

To continue the investigation into the role of carotenoids in human health, it is important to expand our study to include the children’s carotenoid intakes from diet. This would help to further explore the role of carotenoids in the aetiopathology of type 1 diabetes and, in addition, would allow either confirmation or repudiation of the present finding of a marginal inverse association between serum β-carotene concentrations and advanced beta-cell autoimmunity at the time of seroconversion to advanced beta-cell autoimmunity.

Conflict of interest statement

The authors declare no conflicts of interest associated with this manuscript.

Acknowledgements

The skilled assistance of I. Salminen and H. Haponen is gratefully acknowledged. We also thank the DIPP research staff for their excellent collaboration, and are indebted to all the families who participated in the study. This research was supported by the Academy of Finland (grants 63672, 79685, 79686, 80846, 201988, 210632), European Foundation for the Study of Diabetes, Finnish Paediatric Research Foundation, Juho Vainio Foundation, Yrjö Jahnsson Foundation, Competitive Research Funding of the Pirkanmaa Hospital District, Tampere University Hospital, Medical Research Funds, Turku and Tampere University Hospitals, Juvenile Diabetes Research Foundation (grants 197032, 4-1998-274, 4-1999-731, 4-2001-435), Novo Nordisk Foundation and EU Biomed 2 (BMH4-CT98-3314).

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