The Effect of Folate and Vitamin B6 on Endothelial Function in Children with Type 1 Diabetes

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A Thesis Submitted for the Degree of Doctor of Philosophy
The Effect of Folate and Vitamin B6 on Endothelial Function in Children with Type 1 Diabetes

Dr Karen E MacKenzie
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Abstract

Introduction

Endothelial dysfunction is a precursor of vascular disease. Children at high risk of vascular disease including children with type 1 diabetes (T1DM) have marked endothelial dysfunction. Endothelial dysfunction is reversible occurring early in the time-line of atherosclerosis. The detection of endothelial dysfunction in childhood allows the study of interventions at an early and potentially reversible stage of vascular damage.

We have previously shown that endothelial dysfunction is common in children with T1DM and relates to folate status (Wiltshire, Gent et al. 2002) despite higher serum and red cell folate levels and lower total plasma homocyst(e)ine (tHcy) than healthy controls (Wiltshire, Thomas et al. 2001; Wiltshire and Couper 2004). Even with these higher folate levels, in a pilot, cross-over study we have shown that folate supplementation improves endothelial function in children with T1DM (Pena, Wiltshire et al. 2004).

Beneficial effects of folate on endothelial function are being demonstrated in increasing numbers of studies (Verhaar, Wever et al. 1998; Woo, Chook et al. 1999; Doshi, McDowell et al. 2001; Thambyrajah, Landray et al. 2001; van Etten, de Koning et al. 2002; Woo, Chook et al. 2002). Improvement in endothelial function, has also been observed within hours of additional oral folate (Doshi, McDowell et al. 2002) and within minutes of intravenous 5-methyltetrahydrofolate (MTHF), the active form of folate (Verhaar, Wever et al. 1998; van Etten, de Koning et al. 2002).

Treatment with combination folate and vitamin B6 lowers markers of endothelial activation (Constans, Blann et al. 1999; Vermeulen, Stehouwer et al. 2000). However, there is limited literature examining the effect of B6 alone on the endothelium. Vitamin B6 improves endothelial function in cardiac transplant recipients (Miner, Cole et al. 2001). There is no data examining the effect of supplemental vitamin B6 in T1DM or children at risk of vascular disease.

Atherosclerosis is an inflammatory process and high-sensitivity C-reactive protein (Hs-CRP), a marker of inflammation, predicts cardiovascular events in adults. Elevated Hs-
CRP in otherwise healthy children is associated with impaired endothelial function. Similar studies in children with T1DM have not been performed.

We therefore aimed to determine the effects, acutely, of folate and vitamin B6 on endothelial function, and over eight weeks, of folate and vitamin B6, alone and in combination, on endothelial function. In addition, we sought to determine whether Hs-CRP, is associated with vascular endothelial and smooth muscle dysfunction, in children with T1DM and healthy control subjects.

**Methods**

A randomised, double-blind, placebo-controlled study of folate 5mg daily and vitamin B6 100mg daily in 124 children with T1DM determined the immediate and eight week effects of these vitamins, alone and in combination, on endothelial function. Endothelial function, assessed by flow mediated dilatation (FMD) and glyceryl-trinitrate (GTN)-induced dilatation using high resolution ultrasound of the brachial artery, was measured at baseline, at two and four hours after the first dose (n=35), and at four and eight weeks of treatment (n=122). Serum and red cell folate, serum vitamin B6, Hs-CRP, tHcy, HbA1c and blood glucose were measured at each assessment of endothelial function.

Hs-CRP and endothelial function, were measured at baseline, in 121 subjects with T1DM. 31 subjects with T1DM that were randomised to receive placebo treatment were studied at four and eight weeks and were included in the longitudinal analysis of Hs-CRP and endothelial function. Hs-CRP and endothelial function were also studied in 33 age-matched, healthy control subjects.

**Results**

FMD normalised in all treatment groups. At baseline and eight weeks FMD [mean(SD)] on folate improved from 2.6(4.3)% to 9.7(6.0)% (p<0.001), on vitamin B6 from 3.5(4.0)% to 8.3(4.2)% (p<0.001), and on folate/vitamin B6 from 2.8(3.5)% to 10.5(4.4)% (p<0.001) respectively. This improvement in FMD occurred within two hours and was maintained
over eight weeks for each treatment. FMD in the placebo group, and GTN-induced dilatation in all groups, did not change. Increase in serum folate, red cell folate, and vitamin B6 related to increase in FMD. Improvement in FMD was independent of change in tHcy, glucose, HbA1c and Hs-CRP. Baseline red cell folate and baseline diastolic blood pressure inversely related to improvement in FMD. Serum triglycerides and LDL-cholesterol inversely related to baseline FMD.

Hs-CRP did not differ between subjects with T1DM and healthy, age-matched controls. In both controls and subjects with T1DM, Hs-CRP did not relate to FMD or GTN-induced dilatation at baseline or at intervals over eight weeks in subjects with T1DM. Hs-CRP did not change over time. In T1DM, but not healthy controls, Hs-CRP related to BMI z-score($r=0.47, p<0.001$), weight z-score($r=0.41, p<0.001$) and female sex($p=0.008$).

**Conclusions**

High dose folate and vitamin B6 rapidly normalise endothelial dysfunction in children with T1DM. This effect is maintained over eight weeks with ongoing supplementation. Combination treatment over eight weeks does not confer additional benefit.

Hs-CRP is not associated with early vascular dysfunction in children with T1DM. However, in children and adolescents with T1DM, Hs-CRP is associated with female sex and children with higher BMI suggesting these groups may be at greater cardiovascular risk.

In addition to optimising metabolic control, intervention with folate or vitamin B6, at an early stage in childhood, could have a major impact on long-term diabetic vascular complications, and requires further investigation. Maintenance of a healthy BMI may be important in the prevention of vascular disease of T1DM.
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Abbreviations

2D Two-dimensional
ACE Angiotensin converting enzyme
AER Albumin excretion rate
AGEs Advanced glycation end products
AST Aspartate aminotransferase
BH₂ Dihydrobiopterin
BH₄ Tetrahydrobiopterin
cGMP cyclic 3’5’ guanosine monophosphate
CHD Coronary heart disease
CRP C-reactive protein
CYWHS Children, Youth and Women’s Health Service
DAG Diacylglycerol
DCCT Diabetes Control and Complications Trial
DKA Diabetic ketoacidosis
ECG Electrocardiogram
EDIC Epidemiology of Diabetes Interventions and Complications
EDRF Endothelium derived relaxing factor
EDTA Ethylene-diamine-tetra acetic acid
eNOS Endothelial nitric oxide synthase
ET-1 Endothelin-1
FDA Food and Drug Administration
FMD Flow mediated dilatation
GFR Glomerular filtration rate
GTN Glyceryl trinitrate
HbA1c Haemoglobin A1c
HDL High density lipoprotein
Hs-CRP High sensitivity C- reactive protein
IDDM Insulin dependent diabetes mellitus
IDF International Diabetes Federation
Ig Immunoglobulin
IMT Intimal medial thickness
IMVS Institute of Medical and Veterinary Science
LDL Low density lipoprotein
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>MTHF</td>
<td>Methyltetrahydrofolate</td>
</tr>
<tr>
<td>MTHFR</td>
<td>Methylene tetrahydrofolate reductase</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate (reduced)</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor kappa B</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>ORPS</td>
<td>Oxford Regional Prospective Study</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen activator inhibitor-1</td>
</tr>
<tr>
<td>PGA</td>
<td>Pteroylmonoglutamate</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
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<td>Pyridoxal 5'-phosphate</td>
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<tr>
<td>RAGE</td>
<td>Receptors for AGE</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SAH</td>
<td>S-adenosyl-L-homocysteine</td>
</tr>
<tr>
<td>T1DM</td>
<td>Type 1 Diabetes Mellitus</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Transforming growth factor-β</td>
</tr>
<tr>
<td>tHcy</td>
<td>Total plasma homocyst(e)ine</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VD</td>
<td>Vessel diameter</td>
</tr>
<tr>
<td>VLDL</td>
<td>Very low density lipoprotein</td>
</tr>
<tr>
<td>vWF</td>
<td>von Willebrand Factor</td>
</tr>
<tr>
<td>WCH</td>
<td>Women’s and Children’s Hospital</td>
</tr>
</tbody>
</table>
Units

\[
\begin{align*}
\mu g/\text{min} & : \text{micrograms per minute} \\
\mu mol/l & : \text{micromoles per litre} \\
\mu g & : \text{micrograms} \\
\mu g/l & : \text{micrograms per litre} \\
\degree C & : \text{degrees Celsius} \\
cm & : \text{centimetres} \\
kg & : \text{kilogram} \\
kg/m^2 & : \text{kilograms per square metre} \\
m/s & : \text{metres per second} \\
mg/day & : \text{milligrams per day} \\
mg/l & : \text{milligram per litre} \\
mm & : \text{millimetre} \\
mmHg & : \text{millimeters mercury} \\
mmol/l & : \text{millimoles per litre} \\
nmol/l & : \text{nanomoles per litre} \\
\text{units/kg} & : \text{units per kilogram}
\end{align*}
\]