Effects of Immersion in Artificial Carbon Dioxide on Endothelial Function Assessed with Flow-Mediated Dilation in Patients with Type 2 Diabetes

Naoki MAKINO¹, Toyoki MAEDA¹, Nobuyuki ABE²

Abstract

Purpose: The aim of present study was to investigate the endothelial function of immersion of patients with diabetes in carbon dioxide (CO₂)-enriched water

Methods: Sixteen diabetic patients with minor complications were immersed in CO₂-enriched water for 4 weeks, and 8 patients were immersed in normal spa water for the same duration. To assess endothelial function, forearm flow-mediated dilation (FMD) was measured in those patients, and %FMD at pre-immersion was compared to that at post-immersion in CO₂-enriched water. The pulse wave velocity (PWV) was also measured to determine whether vascular stiffness was affected in those patients. The percent coefficient of variation of R-R intervals was examined as CVR-R (%). All patients were medicated with antidiabetic drugs, which were not changed during the study.

Results: %FMD showed no significant difference in any patients between pre- and post-CO₂-enriched water bathing. However, %FMD was significantly increased in patients under 8.0% of HbA1c after CO₂-enriched water bathing (p<0.05), but it was not significantly increased in patients over 8.0 of HbA1c. PWV and CVR-R (%) were significantly reduced in all patients after CO₂-enriched water bathing.

Conclusion: CO₂-enriched water immersion had a positive effect on endothelial function, and reduced arterial wall stiffness in patients with diabetes. These findings suggest that CO₂-enriched water bathing may improve microcirculation, as well as subjective symptoms, in patients with controlled diabetes.

Keywords: diabetes, flow-mediated dilation, CO₂ water, endothelial function, PWV

1 INTRODUCTION

Type 2 diabetes is associated with a high prevalence of atherosclerosis and with several-fold increases in cardiovascular events. This prevalence increases with male gender, aging, longer duration of diabetes, and the presence of additional cardiovascular risk factors, nephropathy, retinopathy, and peripheral or carotid occlusive arterial disease¹, ². Endothelial dysfunction is an early phenomenon during diabetic atherogenesis³ and has been associated with a poor
cardiovascular prognosis in the diabetic population\textsuperscript{4, 5}. Therefore, it is now known that peripheral endothelial dysfunction is considered an integrator of cardiovascular risk. The association between endothelial and smooth muscle dysfunction was evaluated previously by flow-mediated dilation (FMD) in patients with type 2 diabetes\textsuperscript{4, 6}. Some of those authors reported a higher prevalence of angiopathy in patients with abnormal FMD\textsuperscript{6}.

Immersion in water enriched with carbon dioxide (CO\textsubscript{2}) repeatedly had positive microcirculatory effects\textsuperscript{7, 8} and increased blood flow to a much higher extent than did immersion in plain water. Toriyama et al.\textsuperscript{9} demonstrated that CO\textsubscript{2} immersion improved the limb salvage rate in patients with critical limb ischemia without a revascularization option. CO\textsubscript{2} immersion was recently shown to induce local plasma vascular endothelial growth factor (VGEF) production, resulting in NO-dependent new capillary formation associated with mobilization of endothelial progenitor cells\textsuperscript{10}. These results suggest that CO\textsubscript{2} immersion could be effective in atherosclerosis as an adjunctive treatment in diabetic patients. The aims of the present study were to investigate type 2 diabetic patients with minor complications who underwent CO\textsubscript{2} immersion for 4 weeks, and to assess whether this therapy was associated with endothelial dysfunction as evaluated by FMD measurements.

\section*{II METHODS}

\subsection*{Study populations}

We conducted a prospective study in patients with type 2 diabetes who had not achieved their treatment goal with diet, exercise, and antidiabetic drugs (sulfonyl-urea, metformin, or DPP-4 inhibitors or insulin). We recruited 24 patients (men and women) ranging from 50 to 75 years of age (Table 1). All patients had had diabetes for more than 5 years and also had at least two cardiovascular risk factors. Diabetes mellitus was defined as fasting glucose >126 mg/dL or the use of hypoglycemic medications. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or the use of medication prescribed for hypertension. Body mass index was calculated as weight (kg)/height (m\textsuperscript{2}). Total and high-density lipoprotein (HDL) cholesterol levels were measured from blood samples obtained after a 12-hour fast. Low-density lipoprotein (LDL) cholesterol was estimated by the Friedewald equation\textsuperscript{11}. The exclusion criteria were as follows: treatment with type 1 diabetes, HbA1c ≥ 9.0%, systolic blood pressure ≥ 160 mmHg, and serum creatinine ≥ 1.5 mg/dL at baseline. Patients were also excluded from the study if they had clinically infected skin, severe heart failure, malnutrition, or a history of disease affecting the vascular system. Patients were excluded if they had ever enrolled in a clinical evaluation of another wound-care device or drug.

\subsection*{Study protocol}

Sixteen enrolled patients bathed in 40°C water in a bathtub (200 L), in which 50 g of artificial CO\textsubscript{2} bathing agents (Kao Corporation, Tokyo, Japan) were dissolved and the concentration of CO\textsubscript{2} in the bathtub was 145 ± 11 ppm at 10 minutes after mixing. They soaked for 10 min at a time at least four times a week for 4 weeks. As control subjects, 10 diabetic patients bathed
in water without the addition of artificial CO$_2$. All patients were medicated with antidiabetic, antihypertensive, and other medications, which were not changed during the study. Before and after CO$_2$ bathing, each patient’s blood pressure, %FMD, brachial intima-media thickness (bIMT), pulse wave velocity (PWV), % coefficient of variation of R-R intervals (%CVR-R), serum lipid profiles, and HbA1c were measured. The study protocol was approved by the Ethics Committee of Kyushu University Graduate School of Medicine. Written informed consent was obtained from all patients before any study procedure was undertaken.

**Flow-mediated dilation (FMD)**

Endothelium-dependent dilation was assessed as a parameter of vasodilation according to the guidelines for ultrasound assessment of FMD of the brachial artery in the fasting state$^6$. Using a 10-MHz linear-array transducer probe (Unex, Nagoya, Japan), longitudinal images of the brachial artery at baseline were recorded with a stereotactic arm, and the artery diameter was measured after supine rest for ≥5 min, as previously described by us$^{12}$. Artery diameter was measured from clear anterior (media-adventitia) and posterior (intima-media) interfaces, which were manually determined. Then, suprasystolic compression (50 mmHg higher than systolic blood pressure) was performed at the right forearm for 5 min, and the artery diameter was measured continuously from 30 sec before to ≥2 min after cuff release. Maximum vasodilation was then evaluated from the change in artery diameter after the release of occlusion (%FMD). FMD is known to be affected by a wide range of biological, environmental, and methodological factors$^{13}$. Simultaneously, intima-media thickness (IMT) was defined as the distance from the leading edge of the first acrogenic line to the leading edge of the second echogenic line on the scans$^{14}$. The highest value among three averaged IMTs was used as a representative value for each individual. To quantify inter- and intra-observer reproducibility, baseline brachial diameter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n=8)</th>
<th>CO$_2$ bath (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y.o.)</td>
<td>61±3.6</td>
<td>64.7±4.8</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/3</td>
<td>12/4</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1±2.1</td>
<td>24.7±2.1</td>
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<tr>
<td>Hypertension</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
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<tr>
<td>Duration (y)</td>
<td>6.4±0.50</td>
<td>8.6±0.81</td>
</tr>
<tr>
<td>Diabetes Complications</td>
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<tr>
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<td>4</td>
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<tr>
<td>Neuropathy</td>
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DM; diabetes mellitus, BMI; body mass index, CVD; cardiovascular disease. Data are means ± SD or number patients.
and FMD were measured by three individuals. Inter- and intra-observer coefficients were high ($r > 0.90$).

**Measurement of aortic PWV**

baPWV was measured using a volume-plethynographic apparatus (form PWV/ABI version-112; Colin, Komaki, Japan). This instrument records PWV, ankle brachial index (ABI), blood pressure, electrocardiogram, and heart sounds simultaneously. Details of the method, the validity, and the reproducibility of this approach were described previously\(^\text{15}\). The interobserver and intraobserver variation coefficients were 8.4 and 10.0%, respectively.

**Statistical analysis**

The results are expressed as mean ± SD. Differences in efficacy measures between baseline and 4 weeks after CO\(_2\) bathing were compared using the paired Student’s t-test. Values of $P < 0.05$ were considered statistically significant.

### III RESULTS

Characteristics of the enrolled patients are summarized in Table 1. No significant differences were seen in age, BMI, or duration of diabetes in all patients, although complications for diabetes in both groups were not different. Table 2 shows the data for diabetic patients before and after CO\(_2\) bathing. Blood pressure, %FMD, b-IMT, PWV, CVR-R (%), and HbA1c were not significantly different between control and pre-CO\(_2\) bathing subjects. Compared to pre-CO\(_2\) bathing, at post-CO\(_2\) bathing, both sides of PWV were significantly decreased, and CVR-R (%) was increased. We did not find any difference in %FMD between pre- and post-CO\(_2\) bathing in

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=8)</th>
<th>CO(_2) bathing (n=16)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-CO(_2)</td>
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<tr>
<td>s-BP(mmHg)</td>
<td>141±14</td>
<td>134±11</td>
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<tr>
<td>d-BP(mmHg)</td>
<td>76±5.8</td>
<td>80±5.1</td>
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<tr>
<td>%FMD</td>
<td>5.4±.51</td>
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<tr>
<td>b-IMT(mm)</td>
<td>0.31±0.03</td>
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<tr>
<td>ABI(Rt)</td>
<td>1.16±0.06</td>
<td>1.23±0.08</td>
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<tr>
<td>ABI(Lt)</td>
<td>1.12±0.04</td>
<td>1.25±0.06</td>
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<tr>
<td>rt-PWV(m/sec)</td>
<td>1792±128</td>
<td>1840±125</td>
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<tr>
<td>lt-PWV(m/sec)</td>
<td>1821±170</td>
<td>1879±132</td>
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<tr>
<td>CVR-R(%)</td>
<td>2.33±0.17</td>
<td>2.68±0.12</td>
</tr>
<tr>
<td>BS(2hr,mg/dl)</td>
<td>148±14</td>
<td>162±12</td>
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<tr>
<td>HbA1c(%)</td>
<td>7.42±.51</td>
<td>7.71±.41</td>
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</table>

S-BP: systolic blood pressure, d-BP: diastolic pressure, % FMD: %flow mediated dilation, b-IMT: brachial intima-media thickness, ABI: ankle brachial index, PWV: pulse wave velocity, CVR-R (%): % coefficient of variation of R-R intervals, BS: blood sugar level. Data are means ± SD. \(^*\): $p < 0.05$ vs control subjects; \(^\dagger\): $p < 0.05$ vs values at before.
those patients (Figure 1). From these data on %FMD, we furthermore separated subjects into those with under vs over 8.0% in HbA1c. Figure 2 shows that %FMD was significantly increased in patients under 8.0 in HbA1 (p<0.05), but was not significantly increased in patients over 8.0% in HbA1 (p<0.05).

At the end of the study, we asked the subjects to complete several questionnaires regarding their subjective feelings about whether CO$_2$-enriched bathing was beneficial, neutral, or aggravating. Figure 3 summarizes the results: 14 patients felt the CO$_2$ bath kept them warm, 13 patients sweated during the bath, 12 experienced reduced fatigability, 10 patients experienced release from lower back pain or muscle pain, and 9 patients reported that they had a good night’s sleep after bathing.

**IV DISCUSSION**

The present study demonstrates that CO$_2$-enriched bathing enhances %FMD in
diabetic patients under 8.0% in HbA1c. The results indicate the improvement of endothelial function after CO₂ bathing for one month. In fact, the benefits of CO₂ bathing have been described for the past 50 years, and this therapy is now thought to be effective for the treatment of peripheral vascular disorder\(^{16}\). However, the mechanism or mechanisms underlying this traditional therapy remain poorly defined. The effect of CO₂-enriched water on cutaneous circulation depends primarily on the vasodilation elicited by the CO₂ that diffuses into the subcutaneous tissue through the skin layers\(^{17}\). Our results showed that CO₂ bathing significantly reduced arterial stiffness in the peripheral arteries of diabetic patients. This reduction may have contributed to the induction of local VEGF synthesis associated with activation of the NO-cGMP pathway\(^{10}\), resulting in increased peripheral blood flow. We have also shown that CVR-R (%), a marker of parasympathetic nerve activity, was increased in diabetic patients who were treated with CO₂ bathing. These observations therefore suggest that CO₂ bathing induces peripheral vasodilation and may increase parasympathetic nerve activity or may decrease sympathetic nerve activity as previously described by Toriyama et al.\(^{9}\).

Each group in this study was constituted a small number of patients who had no serious complications and various levels of HbA1c in diabetes, as well as control patients (Table 1). Our data indicated no difference in %FMD between pre- and post-CO₂ bathing, although CO₂ bathing did reduce arterial stiffness in patients with diabetes. However, CO₂ bathing was beneficial for patients under 8.0 in HbA1c because of the resulting increase in %FMD. On the other hand, this treatment was not beneficial for patients over 8.0 in HbA1c (Table 2). Although the
mechanisms underlying the effects of CO$_2$ water bathing remain unknown, there is evidence of a link between ROS damage$^{18}$ and the development of microvascular or macrovascular complications in diabetes$^{19,20}$. It is also possible that levels of ROS products, including serum concentrations of advanced oxidation protein products, advanced glycation end products, and lipoprotein lipase, are higher in patients over 8.0 in HbA1c than in those under 8.0%$^{21}$. Those producing factors may induce endothelial dysfunction in patients with high levels of HbA1c.

FMD studies have been reported to predict cardiovascular events$^{22}$. Diameter changes after an increase in flow depend on the endothelium, mainly through a nitric oxide-dependent mechanism but also through vascular smooth muscle cell contraction and relaxation. Endothelial dysfunction may be considered a cardiovascular risk factor or at least a cardiovascular risk marker$^{6,23}$, but impairment of vascular smooth muscle cell function has also been reported in diabetic patients$^{24}$ and may be involved in altered FMD. The mechanism underlying impairment of FMD cannot be explained by our results, since we did not test vascular smooth muscle cell function specifically. The important fact is that an impaired FMD response per se, whatever its mechanism, predicted a poor cardiovascular prognosis in several large population studies$^{25}$. The present study was short-term and consisted of small numbers of subjects in both groups. Our findings therefore need to be confirmed in a large cohort of patients with type 2 diabetes and with a long observation period.

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Conflict of Interest
No potential conflicts of interest were disclosed.

References