INTRODUCTION
Transcutaneous application of carbon dioxide (CO₂) therapy, such as by spa or CO₂-enriched water bathing has been applied for hundreds of years. The effect of this has been explained by an increase in the pressure of O₂ in tissues known as the Bohr effect. However, there have been no reports investigating oxygen dissociation of haemoglobin during transcutaneous application of CO₂ in vivo. We designed a novel system of transcutaneous application of CO₂ and investigated changes in oxygenated and deoxygenated haemoglobin during its application in the human body.

METHOD
These study were approved and permitted by the Ethical Committee of Kobe University Graduate School of Medicine and informed consent was obtained from all subjects before the start of the study. Validation of transcutaneous CO₂ absorption accelerating hydrogel using a measuring device for transcutaneous CO₂ absorption
The animal experiment plan was reviewed and approved by the Animal Research Committee of Kobe University Graduate School of Medicine. CO₂ hydrogel was applied to the skin specimen in the Gel (+) groups, and the chamber was filled with pure CO₂ gas. After filling, the pH of the solution was measured in 30-s intervals by a pH meter for 15 min.

Measurement of intramuscular pH in vivo using ³¹P-MRS during transcutaneous application of CO₂ in vivo.
Subject: Five healthy male volunteers participated in this study. Subjects were 23–38 years of age (average: 33.0 ± 6.6. ³¹P-MRS: The intramuscular pH was measured in the triceps surae muscle. All MR studies were performed with a 1.5-T superconducting imaging system and a surface coil. CO₂ hydrogel was applied to the subject’s lower leg. A plastic CO₂ adaptor was then applied on the subject’s lower leg, and then the surface coil was positioned over the adaptor. After preparing for measurements, pure CO₂ gas was flowed into the adaptor. Data were collected at before infusion of CO₂ and every 10 min after infusion.

Measurement of oxygenated and deoxygenated Hb concentration during transcutaneous application of CO₂ in vivo.
Subjects: Seven healthy male volunteers participated in this study. Subjects were 27–40 years of age (average: 32.0 ± 4.6). A near-infrared spectroscopy (NIRS) was used for Hb concentration measurements. The recording probe was attached to the inner side of the subject’s forearm. A pneumatic tourniquet system commonly used in orthopedic surgery was used for avascularization of the arm. NIRS probes were attached to the subject’s forearm. A tourniquet was wound around their upper arms, and then Hb concentration was measured. After confirming that the oxy/deoxy-Hb ratio had stabilized, the tourniquet was inflated to a pressure of 250 mmHg. Eight minutes after the inflation, CO₂ hydrogel was applied to the subject’s forearm, and the entire arm was enclosed by a CO₂ adaptor. Ten minutes after the inflation, pure CO₂ gas or air (control) was allowed to flow into the adaptor. The relative concentrations of the oxy- and deoxy-Hb were measured at 2-s intervals using NIRS. The duration of tourniquet inflation was limited to a maximum of 20 min to avoid ischemic damage to subjects’ tissues.

RESULTS
Validation of transcutaneous CO₂ absorption accelerating hydrogel using a measuring device for transcutaneous CO₂ absorption
The pH of the solution decreased time-dependently in the CO₂ (+) groups, and the pH values were significantly lower in the CO₂ (+) Gel (-) group compared to the CO₂ (+) Gel (+) group after 3.5 min (Fig.1). These results show that CO₂ hydrogel can actually enhance CO₂ gas permeation through the rat skin.

Measurement of intramuscular pH in vivo using ³¹P-MRS during transcutaneous application of CO₂ in vivo.
The intramuscular pH of the triceps surae muscle decreased significantly 10 min after transcutaneous application of CO₂ (Fig. 2). The results showed that the intramuscular pH decreased by transcutaneous application of CO₂ using this system in vivo.

DISCUSSION
The evidence provided by our study leads to the conclusion that the Bohr effect actually occurs in vivo. Our experimental results also show scientific evidence that transcutaneous CO₂ application can cause an “artificial Bohr effect.” This artificial Bohr effect may be a potential new therapy for disorders in which a high quantity of O₂ in local tissues is required for treatment.