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Blood flow and peripheral catecholamine release during localized hand cooling

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A unilateral cold hand immersion challenge was used to identify circulating catecholamine differences between the immersed and non-immersed arms. There were no significant differences in either plasma norepinephrine or epinephrine concentration between arms. Plasma epinephrine was unaffected by the cold hand immersion challenge, whereas plasma norepinephrine values increased appreciably. Upon rewarming, plasma norepinephrine peaked within two minutes in the non-immersed hand, while it peaked later (approximately five minutes) in the immersed hand. In a second experiment, fingertip capillary perfusion decreased upon immersion in cold water for both hands. However, the non-immersed fingertip capillary perfusion immediately began to return towards basal levels. The immersed hand demonstrated continued reductions for as long as 20 minutes after removal from the cold water stimulus. And last, no gender related differences in circulating plasma catecholamines at rest or after the cold hand immersion challenge were noted.

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INTRODUCTION

The cold hand pressor test (CHPT) is used for investigating the pain reflex, which seems to be dependent upon water temperature. The CHPT is a useful tool for isolating differences between central and peripheral catecholamine release. Epinephrine (EPI) is released from the adrenal medulla, and norepinephrine (NE) is released from peripheral nerve endings. The purpose of this investigation was to determine and assess the following:

SECTION I - the individual differences for neurotransmitter (e.g. norepinephrine) release in venous blood between arms during a unilateral cold stimulus

SECTION II - individual finger capillary perfusion response during and following a unilateral cold hand pressor test

SECTION III - the temperature range that elicits maximal sympathetic response

METHODS AND RESULTS

SECTION I - INDIVIDUAL DIFFERENCES IN NEUROTRANSMITTER RELEASE

Subjects - Military and civilian male personnel (n=8) were advised of the risks of the study, and they provided informed consent. Subject characteristics were age 27.3±6.0, height 180.8±4.1 cm, and weight 84.8±7.1 kg.

Protocol - Subjects arrived at the laboratory after a 12 hour fast. While they were in a seated position wearing shorts, socks, and a t-shirt, 18-G catheters were inserted into the antecubital vein in both arms. The catheters were kept patent using heparinized saline. Heart rate was obtained via a standard 3-lead configuration. A YSI 700 thermistor was taped to the index finger of both hands. After 30 minutes of baseline data collection, the subject immersed one hand (e.g. randomized by dominance) i cold (4° C) water for 2.0 minutes. Blood samples were obtained from both arms before immersion and at minutes 1 and 2 of immersion. Thereafter, blood samples were obtained at recovery 2.5, 5, and 10 minutes. Fingertip temperature and heart rate were recorded each minute during baseline, immersion, and recovery periods.

Plasma for catecholamine analysis was separated by centrifugation at 3,000 rpm for 10 minutes at 4° C; 1-ml aliquots were pipetted into tubes containing 6.5 mM glutathione and 8 mM ethylene glycol-bis-(B-aminoethyl ether)-N,N,N',N'-tetracetic acid (EGTA) and frozen at -70° C before analysis. Samples were extracted by alumina adsorption with 3,4-dihydroxybenzylamine (DHBA) included as an internal standard. Extracted samples were assayed by high-pressure liquid chromatography by using electro-chemical detection (Waters Division, Millipore, Milford, MA).

Results - Reductions in finger temperature were paralleled by increases (11%) in heart rate. The non-immersed arm had a baseline epinephrine concentration of 48.5 pg/ml with a peak (151%) value at 1 - 2 minutes of recovery, while the immersed arm was 44.5 pg/ml basally with a peak (144%) at 1 - 2 minutes of recovery. Plasma norepinephrine increases for either arm (NIA- 372;IA-378 pg/ml) were negligible during immersion for either arm. However, NE values rose during recovery with the non-immersed arm reaching its peak (122%) NE value at recovery minute 2.5 while the immersed arm (138%) peaked at five minutes of recovery. The difference between arms (immersed arm minus non-immersed arm) for finger temperature (T_{fing}), EPI, and NE are reported in table 1. Values are mean (S.E.). * = $p < 0.05$.

TABLE 1.

	TIME					
Variable	Rest	I-1	I-2	R-2.5	R-5	R-10
T_{fing}	3.9 (1.1)	-16.0* (1.6)	-17.0* (2.1)	4.0 (0.9)	3.3 (1.1)	3.8 (1.1)
EPI	-3.5 (5.3)	2.3 (20.0)	-9.3 (18.6)	7.7 (14.0)	-3.3 (9.9)	1.5 (4.6)
NE	5.3 (22.2)	5.8 (33.1)	-25.2 (23.3)	-2.7 (49.2)	87.8 (51.2)	1.3 (54.6)

I - immersed time.
R - recovery time.

SECTION II - CAPILLARY PERFUSION AND NOREPINEPHRINE RELEASE

Subjects - Six male subjects were advised of the risks and provided informed consent. Characteristics were age 26.5±8.5, height 179.9±6.1, and weight 86.3±10.4.

Protocol - Subjects arrived at the laboratory after an overnight fast. An 18-G catheter was inserted into the antecubital vein of each arm and was kept patent via heparinized saline. The subject was instrumented with YSI 700 series thermistors on the middle finger of both hands. Laser doppler flow meters (MedPacific Incs, Seattle, WA) were placed on the index finger of both hands. Baseline data collection was obtained while subjects were in a seated position. This period lasted for 30 minutes; after which, the subject immersed one hand into cold (4° C) water for 2.0 minutes. Upon removal from the cold water, the hand was lightly towel dried and placed in front of the subject. Finger temperature and blood flow were collected each minute during the experiment. Blood for both arms was obtained prior to immersing the hand in cold water, at minutes 1 and 2 of immersion, and at minutes 2.5, 5, and 10 of recovery. Plasma catecholamines were analyzed as described in Section I.

Results - Catecholamines were not different between arms during immersion or upon rewarming (Figs. 1 and 2). Finger temperature and capillary perfusion (Fig. 3) were reduced significantly in both hands upon immersion. A modest reduction in finger capillary perfusion was still evident in the immersed finger during recovery. Individual temperature and capillary perfusion for both fingers are shown in figure 4.

SECTION III - WATER TEMPERATURE AND GENDER DIFFERENCES IN RESPONSE TO THE COLD HAND PRESSOR TEST

Subjects - Four men and four women volunteered and provided informed consent. Characteristics listed in table 2 are reported as mean ± standard deviation.

TABLE 2.

	Male	Female
Age	31±3.3	40.7±8.7
Height (cm)	181.6±4.8	172.1±9.1
Weight (kg)	84.5±6.9	59.1±4.2

Protocol - Subjects fasted overnight, and upon arrival at the laboratory, a 19-G butterfly needle was inserted into the antecubital vein of one arm. A YSI 700 series thermistor was taped to the index finger of both hands. The subjects, while in a seated position, immersed one hand in 26° C water following a 15 minute baseline period. After another baseline period, the subject immersed one hand into one of three cold (e.g. 4, 8, or 12° C) water temperatures for 2 minutes. Upon removal from the cold water, the hand was again immersed back into the warm (26° C) water for 10 minutes. After the rewarming period, the subject placed the hand in one of the remaining two cold water temperatures and repeated this procedure again for the last temperature. The presentation of the water temperatures was randomized. Finger temperature and heart rate were collected each minute throughout the protocol. Blood was collected just prior to cold water immersion, at minutes 1 and 2 during immersion, and at recovery minutes 2.5, 5, and 10. After the first immersion, recovery minute 10 represented the baseline prior to the next cold water immersion. Plasma catecholamines were analyzed as described in Section I.

Results - Although the women were significantly older than the men, there were no significant differences between groups or in temperature at any time during the two cold water immersions. Plasma catecholamines were not significantly different between water temperatures.

DISCUSSION

This series of experiments provides data about the mechanism of plasma norepinephrine release after a cold hand pressor test. As described in section I, plasma epinephrine does not appear to be released after a cold hand pressor stimulus. Furthermore, although there was a large drop in finger temperature of the immersed hand, the contralateral finger temperature remained relatively stable. While these temperature differences are not too surprising, the observation that plasma norepinephrine in the non-immersed arm was similar to that in the immersed arm indicates a salient point. Namely, a unilateral stimulus elicits a general sympathetic stimulus with norepinephrine spilling over into the circulating blood bilaterally. Moreover, the two to three minute delay in plasma NE peaks between arms may reflect a slower vasoconstrictor release in the immersed (i.e. cooler) hand.

The second section identified the degree of capillary perfusion after such a cold hand stimulus. Plasma levels of epinephrine or norepinephrine between arms were not different. As observed in figure 3, the capillary perfusion in both fingers dropped precipitously upon immersion, but the non-immersed hand immediately began to return to basal levels. This demonstrates quite nicely the effect of the general sympathetic response and the local effects of NE. Moreover, when the stimulus was released, norepinephrine was taken up and cleared much faster in the non-immersed hand as noted by the quick return to basal capillary perfusion levels. In figure 4, individual data are presented. The majority of subjects experienced a rebound in the non-immersed finger but continued to have negligible perfusion in the immersed finger lasting as long as 15 minutes post-immersion. Whether this delayed release was caused by NE remaining in the synaptic cleft (possible retardation in NE re-uptake) or by effects of the cold on the capillary musculature is not known?

The last section isolated the effects of gender and water temperature on sympathetic response. Our data supports previous research, showing no difference in the sympathetic response to a cold hand test between men and women. It is also apparent that the responses to the cold water were not distinguishably different from one another although there was a general trend for the response to follow the pattern 4° C > 8° C > 12° C. Moreover, subjects uniformly agreed that the lower water temperature elicited a greater pain response.

In conclusion, the cold hand pressor test is a useful measure for investigating the general sympathetic stimulus. Although finger temperature does not change in the non-immersed hand, there is an appreciable reduction in capillary perfusion which quickly returns once the stimulus is released. Capillary perfusion remains depressed in the immersed hand for as long as 15 minutes. Whether this is caused by localized norepinephrine remaining or because of cold effects on smooth muscle responsiveness is not clear. Furthermore, unless the cold hand pressor is used to evaluate pain perception, the use of 4° C water is not warranted for examining the effects on physiological and sympathetic parameters.

FIGURE LEGEND

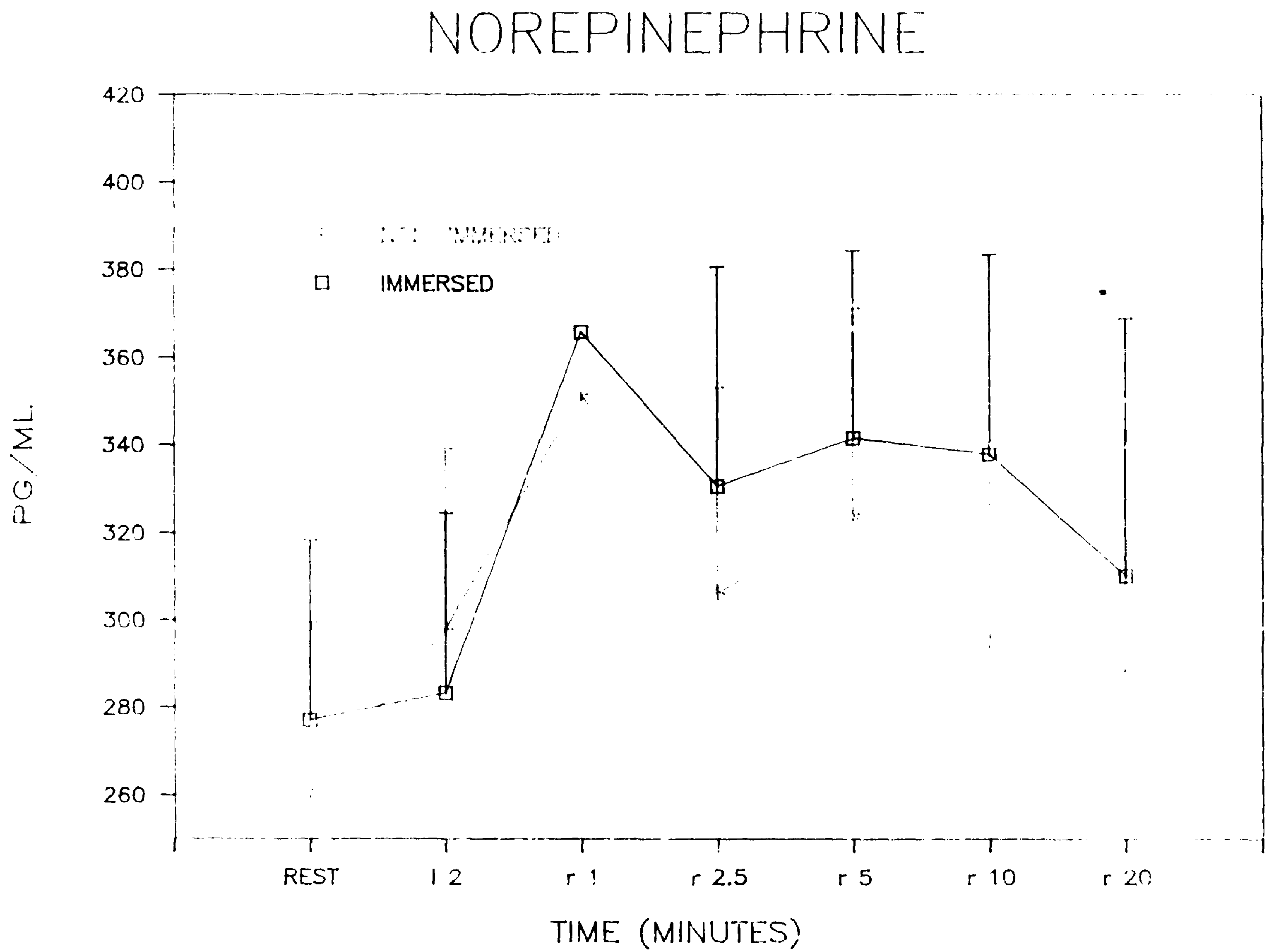
Figure 1. Plasma norepinephrine in seven men obtained while performing a unilateral cold hand immersion challenge. Blood samples obtained at rest, after two minutes of immersion, and 20 minutes of recovery.

Figure 2. Plasma epinephrine in seven men during a unilateral cold hand immersion challenge. Time as described in figure 1.

Figure 3. Fingertip capillary perfusion in seven men performing a unilateral cold had immersion challenge. Time as described in figure 1.

Figure 4. Individual fingertip temperature and fingertip capillary perfusion in six men while performing a unilateral cold hand immersion challenge. Dotted line represents non-immersed fingertip temperature, and dashed line represents immersed fingertip temperature. Solid line illustrates perfusion for immersed hand, and small dash represents non-immersed fingertip perfusion.

Figure 1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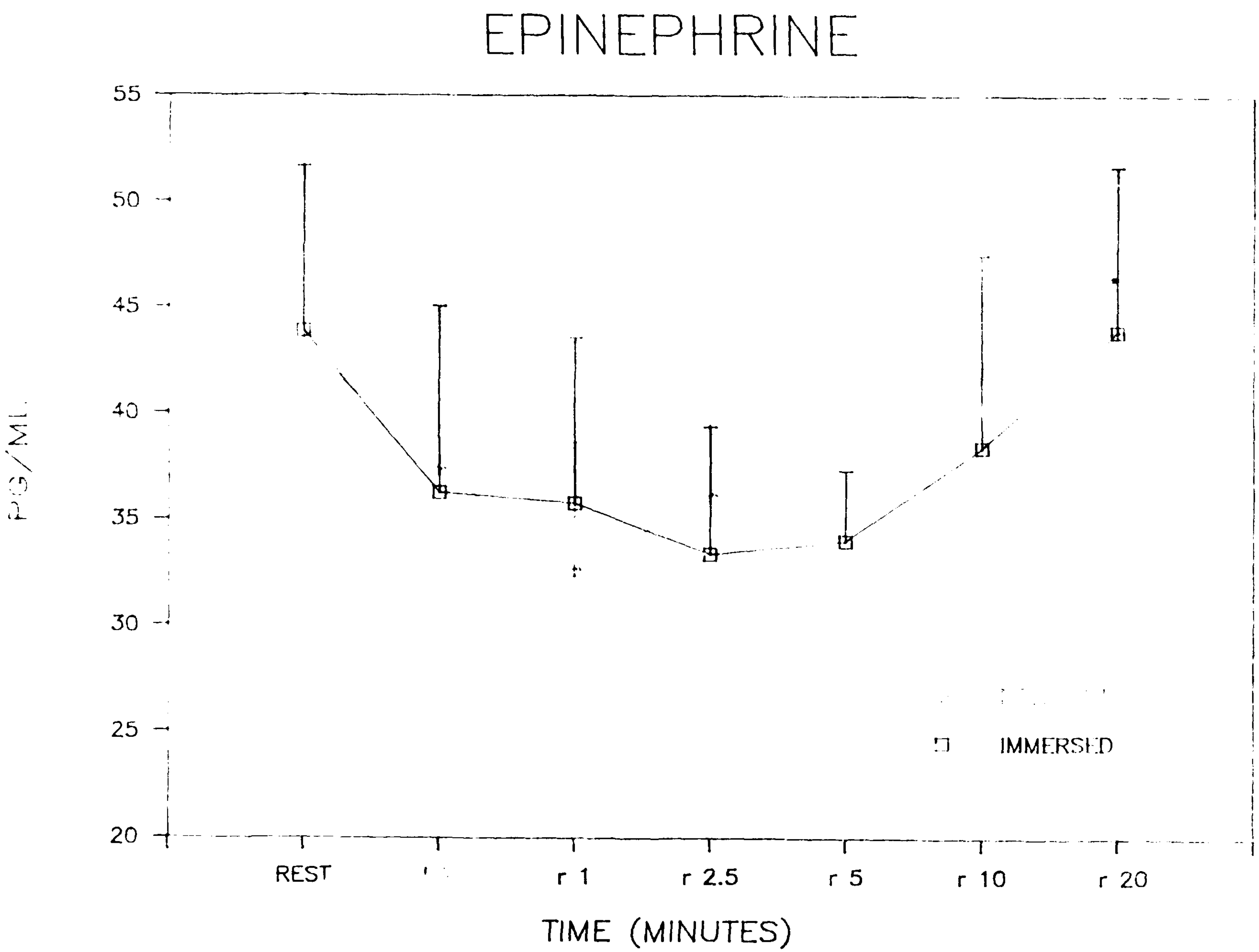
TIME (MINUTES)

Figure 2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TIME (MINUTES)
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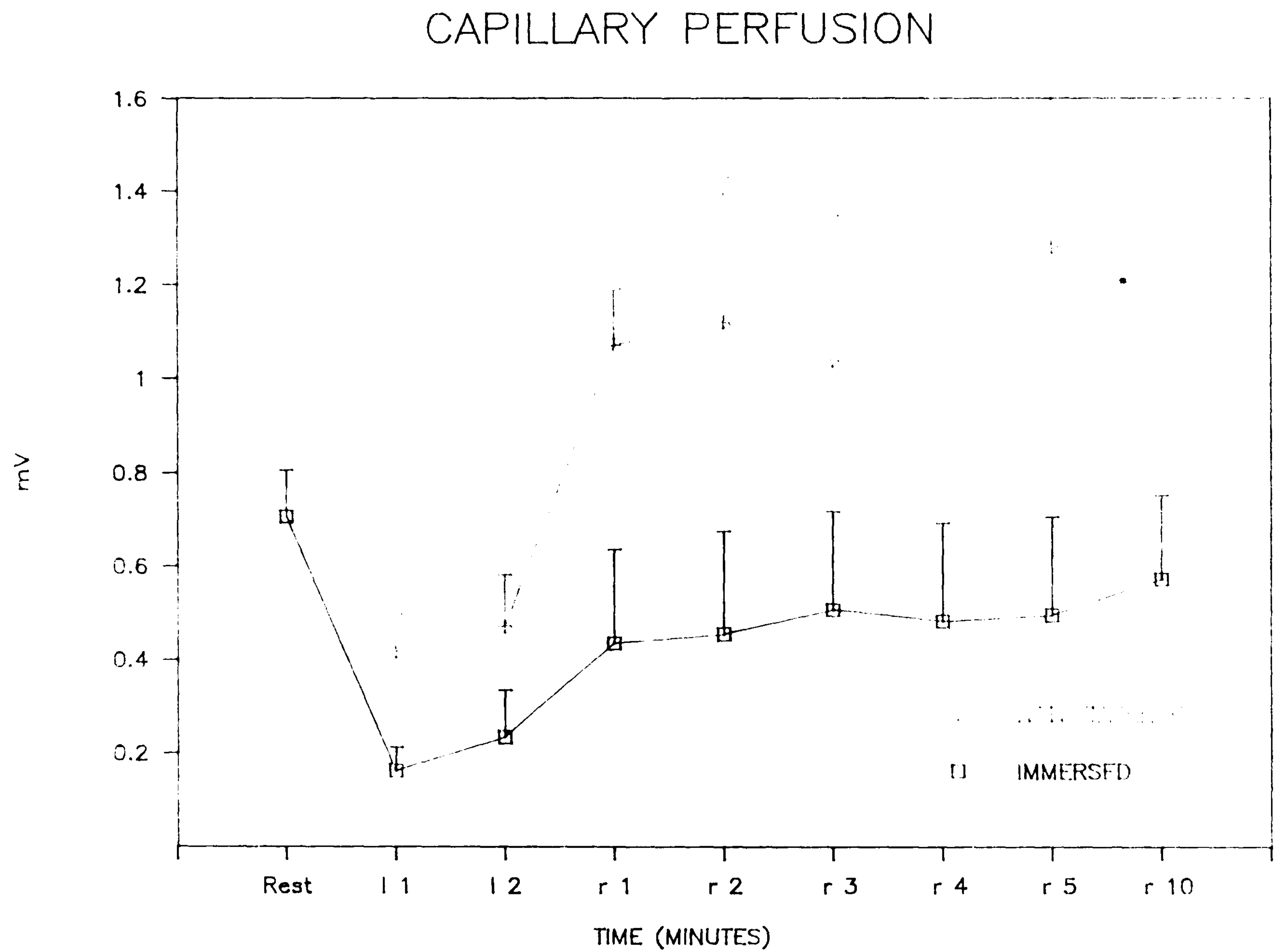
Figure 3


CAPILLARY PERFUSION
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1.2
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TIME (MINUTES)
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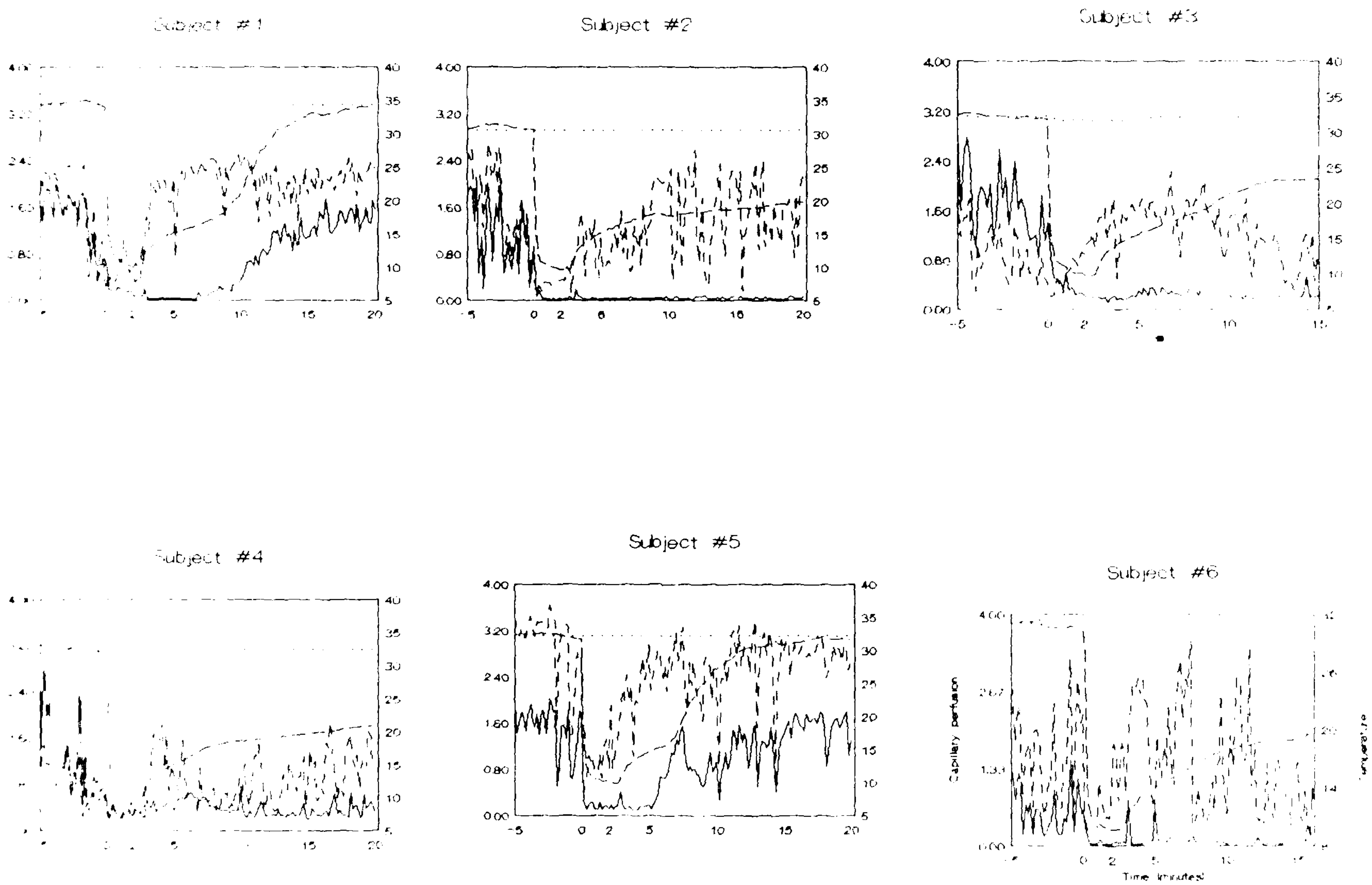
Figure 4


Subject #1
Subject #2
Subject #3
Subject #4
Subject #5
Subject #6
Capillary perfusion
Time (minutes)