Eur J Appl Physiol (2000) 82: 487–492

ORIGINAL ARTICLE

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Re-evaluation of the cold face test in humans

Accepted: 13 March 2000

Abstract The cold face test has been found to be a simple clinical test to elicit the diving reflex, which assesses function of the sympathetic and parasympathetic nerve systems at the same time. However, there is no consensus about how the test should be performed without confounding the results by eliciting other reflexes, such as the oculocardiac reflex. The object of this study was to compare and standardize methods for performing the cold face test. Reproducibility of results was assessed. Groups of 6 to 11 subjects participated in each protocol. To act as a cold stimulus a bag filled with iced-water and having a wet surface was used. The effects of allowing breathing to continue, of different masses of the bag, and of avoiding ocular pressure by wearing diving goggles were investigated. Blood pressure and heart rate were measured beat to beat using an automatic blood pressure measuring device. The cold stimulus used in this study was too small to elicit the oculocardiac reflex: wearing diving goggles and different masses of the bag had no influence on the response. The prevention of breathing, however, tended to enhance the fall in heart rate during the cold stress. Reproducibility was highest when the subjects were habituated to the intensity of the stimulus. We recommend practising the test method in advance and performing it in a setting where the subject is unable to breathe.

Key words Autonomic function · Diving reflex

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Introduction

A large range of cardiovascular reflex tests is used clinically to assess autonomic function. Most of these tests determine predominantly sympathetic (isometric handgrip test, cold pressor test) or parasympathetic (deep breathing, Valsalva manoeuvre) nerve function. The diving test has been associated with a considerable fall in heart rate (Andersen 1966; Craig 1963) due to parasympathetic activation and, simultaneously, a marked peripheral vasoconstriction (Andersen 1966; Heistad et al. 1968; Khurana et al. 1980), due to peripheral sympathetic activation. The fall in heart rate and the peripheral vasoconstriction persist for the duration of the test. Thus, this test is unique in its ability to separate the effects of simultaneously operating activation of the parasympathetic and sympathetic nerve systems.

Previous studies (Khurana et al. 1980; Whayne and Killip 1967) have shown that application of cold to the face can replace facial immersion as a stimulus for eliciting the diving reflex in the assessment of the function of the autonomic nerve system. Therefore, it is a simple clinical test to perform. This test can be useful in patients in whom autonomic function is difficult to assess, for example in patients with rheumatoid arthritis.

However, there is no consensus on how to perform the cold face test. Heath and Downey (1990) have proposed a method which has allowed breathing to continue during the test, other authors have used methods which do not (Trouerbach et al. 1994). Deep breathing normally causes heart rate variations, which have been found to be parasympathetically mediated (Wheeler and Watkins 1973). On the other hand, taking a deep breath before the diving test may elicit an effect comparable to that of the Valsalva manoeuvre, i.e. an early fall in pulse pressure. Immediately related to whether breathing should be allowed to continue or not, is the question of whether the duration of the test affects the responses of heart rate and blood pressure. There has also been a lack of uniformity in the degree of coverage of the eyes and eyelids during the test. The oculocardiac reflex (Van Brocklin et al. 1982), which has been shown to be caused by increases in ocular pressure, causes sinus bradycardia. The reflex is believed to have its afferent link through the ophthalmic portion of the trigeminal nerve and the efferent link through the vagal nerve to the heart. It has been suggested that increases in ocular pressure should be excluded to avoid the oculocardiac reflex occurring during the cold face test (Khurana et al. 1980). Also, the effect of the mass of whatever is used to provide the cold stimulus has not been investigated. Therefore, the first aim of our study was to establish a clinical method of performing a test to elicit the diving reflex, taking into account the presence or absence of breathing, the extent to which the eyes and eyelids are covered and the mass of the stimulus. The second aim of this study was to assess the reproducibility of the results obtained.

Methods

Subjects

Tests were undertaken by 11 healthy persons (3 women, 8 men) with a mean age of 30 (range 21–45) years in protocol I, 10 in protocol II (4 women, 6 men), mean age 32 (range 21–54) years and 6 in protocol III (3 women, 3 men) with a mean age of 24 (20–35) years. During the tests the subjects lay in a supine position in a quiet room having a constant temperature of 22 °C. The blood pressure and the heart rate were recorded beat to beat at heart level using a Finapres instrument (type Ohmeda 2300, Englewood, Colo., USA). Before starting the cold face test baseline values of the blood pressure and heart rate were measured. Between tests an interval of at least 5 min was taken to allow the heart rate and blood pressure to return to their baseline values. All tests were sustained to maximal endurance.

As a cold stimulus a plastic bag filled with melting ice-water at 0 °C was used. The same bag with the same amount of iced-water was used in all tests carried out on any individual subject. In between tests the bag was kept in a bucket containing iced-water, so that the exterior of the bag was wet also. The plastic bag was flexible and not completely filled to allow direct contact with the part of the face to be covered in each of the settings described below.

The study was performed in accordance with the Declaration of Helsinki and all subjects gave informed consent. The Medical Ethics Committee of our hospital approved the protocol.

Protocol I

The purpose of protocol I was to investigate whether the continuation of breathing or increases in ocular pressure influence the diving reflex. To this end every subject undertook three variations of the cold face test:

A. The subject was not able to continue breathing as a result of the bag filled with ice-water covering the whole face. Eyes and eyelids were also covered by the plastic bag.
B. The test was performed as described above, but the person wore diving goggles to prevent cooling of, and pressure on, the eyes and eyelids.
C. The test was repeated allowing the subject to continue breathing, by the plastic bag covering the forehead and cheeks, but not the nose and mouth. The subject was wearing diving goggles to prevent a possible confounding of the results by the oculocardiac reflex on the effect of the continuation of breathing.

Each person performed each variation twice and the tests were carried out in a randomized sequence. Each cold face test was announced 1 min in advance and just before the test, so the subject could take a breath. They were instructed to take a normal breath just before the start of the test and not to perform a Valsalva manoeuvre during the test. Also, they were instructed to breathe normally and not to take deep breaths when variation C of the cold face test was performed. The same ice-bag with the same mass of contents was used for each subject. We made certain that the ice-bag covered the face completely.

The tests were performed in the early afternoon, after the subjects had had lunch.

Protocol II

The aim of protocol II was to assess whether the mass of the bag containing ice-water had any effect on the cold face test by activating the oculocardiac reflex. The plastic bag was filled half-and-half with ice and water weighing 800, 1,100 or 1,400 g. With each of the three different masses the cold face test was performed twice in a random sequence among the subjects. All tests were performed according to variation A as described in protocol I. The tests were performed in the early afternoon, after the subjects had had lunch.

Protocol III

The purpose of protocol III was to assess the reproducibility of the cold face test. On 3 days (days 1, 2 and 15) subjects carried out the cold face test three times immediately following one another. Each day, tests were performed in the morning, at the same time, after an overnight fast. The diving reflex was assessed as described in protocol I, variation A. The weight of the bag was 1,100 g. Subjects were instructed to take a normal breath just before the start of each test and not to perform a Valsalva manoeuvre during the test.

Analysis, including statistics

The baseline heart rate was defined as the median heart rate over a period of 30 s. This measurement was performed 1 min prior to the next cold face test, before the subject was informed of the imminent occurrence of the test starting an average of 30 s later. The baseline heart rate was compared to the minimal heart rate during the test. The changes in heart rate were compared in absolute terms and, because the baseline heart rate can be a determinant of the decrease in heart rate, they were expressed also in terms of a percentage.

As blood pressure can vary widely from beat-to-beat, the mean of three successive systolic as well as diastolic blood pressures during the tests was calculated. The highest of these means was compared with the average blood pressure during the 30 s before starting the tests.

Differences between tests were analysed using Wilcoxon matched pairs signed rank test. Differences between different masses were analysed using Friedman's two-way analysis of variance with the Bonferroni-Holm method for multiple comparisons. A result was considered significant if $P < 0.05$. To determine correlation the Pearson correlation was used.

As a measure of reproducibility the coefficient of repeatability was calculated as proposed by Bland and Altman (1986). First, the difference against the mean for two successive measurements was plotted for each subject. Secondly, if repeatability could be assessed using these data, the coefficient of repeatability was calculated as follows: all the differences were squared, added, divided by the number of subjects and the square root to get the standard deviation of the differences. The coefficient of repeatability was twice this standard deviation. The pooled within-subject standard deviation was calculated to assess repeatability for measurements on successive days (Chinn 1990).

Results

Protocol I

The cold face test (Table l) was performed all six times by all but one person. For this single person the results of four tests have been included. The median baseline heart rate was 84 (range 63–98) beats · min^{-1}.

No differences appeared between the first and second time a test was performed, except for the duration of the cold face test and the time till the minimal heart rate in test variation A ($P = 0.01$). Protocol I revealed that the fall in heart rate was larger if the subject was not able to continue breathing. Wearing diving goggles did not influence the heart rate changes (Fig. 1).

In all subjects, on all but two occasions, the blood pressure rose during the cold face test from a baseline median systolic blood pressure of 141 (range 102–155) mmHg and a median diastolic blood pressure of 75 (range 67–90) mmHg (Fig. 2). Wearing diving goggles and being able to breathe did not influence the increase of systolic or diastolic blood pressure during the cold face test. Being able to breathe prolonged the duration of the test, as did wearing diving goggles.

Protocol II

The median baseline heart rate was 85 (range 55–113) beats · min^{-1}. No significant differences were found in decreases of heart rate among the different weights. The median baseline systolic and diastolic blood pressures were 115 (range 94–133) mmHg and 63 (range 54–68) mmHg, respectively. All blood pressures rose during the tests and no differences in systolic or diastolic, were seen among the various weights (Table 2).

Protocol III

The mean difference of the heart rate and blood pressure tended to be different from 0 for the first two tests on the first and second day. On the third day the mean difference of the last two measurements showed a trend of difference from 0. Therefore, the coefficient of repeatability for two successive measurements on 1 day was calculated from the two last measurements on the 2nd day the cold face tests were performed. Repeatability was also assessed for measurements on successive days.

Repeatability was lowest when performing the cold face tests on the first 2 successive days and it was greatest for the last two measurements on the 2nd day the tests were performed; differences found were mean falls in heart rate of 32 compared to 33 beats · min^{-1}, coefficients of repeatability of 25 compared to 18 beats · min^{-1}; mean decreases of systolic blood pressure of 15 compared to 15 mmHg, coefficients of repeatability of 22 compared to 12 mmHg; mean decreases in diastolic

Table 1 The results of the three variations of the test method of the cold face test are shown. *A* The subject was not able to breathe because the bag filled with ice-water covered the whole face, eyes and eyelids also being covered, *B* the same test as described above, but the person was also wearing diving goggles to prevent cooling of, and pressure on, the eyes and eyelids, *C* the subject continued to breathe, because the plastic bag covered the forehead and cheeks, but not the nose and mouth. The subject was wearing diving goggles. The results from the first test (*A1*, *B1*, *C1*) and from a repeat (*A2*, *B2*, *C2*) are shown. *P* Probability, *BP* blood pressure

Test	No breathing, no goggles				No breathing, with goggles				Breathing, with goggles				Significance, Tests A compared to B	Significance, Tests B compared to C
	Test A1		Test A2		Test B1		Test B2		Test C1		Test C2			
Parameter	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range		
Fall in heart rate (beats · min^{-1})	23	11–54	25	2–50	20	10–51	20	9–40	12	4–54	17	12–42	NS	$P = 0.05$
Fall in heart rate (%)	30.5		32.8	3–54	28.5	11–59	27.2	13–46	15.7	6–55	22.1	15–48	NS	$P = 0.08$
Increase in systolic BP (mmHg)	20	8–40	23	−9 to 50	20	−8 to 37	23	8–49	20	8–50	26	11–57	NS	NS
Increase in diastolic BP (mmHg)	18	6–29	16.5	3–36	12	6–26	17	5–31	1	4–36	18	10–37	NS	NS
Time to minimal heart rate (s)	22*	3–44	33.5*	6–82	34	7–60	41	13–64	25	11–78	44	5–115	$P = 0.05$	NS
Duration of test (s)	28*	4–50	41.5*	7–89	37	14–62	48	15–140	58	19–180	120.5	26–242	$P = 0.003$	$P < 0.0001$

* Durations of tests *A1* and *A2* and time till the minimal heart rate of tests A1 and A2 were significantly different, $P = 0.01$

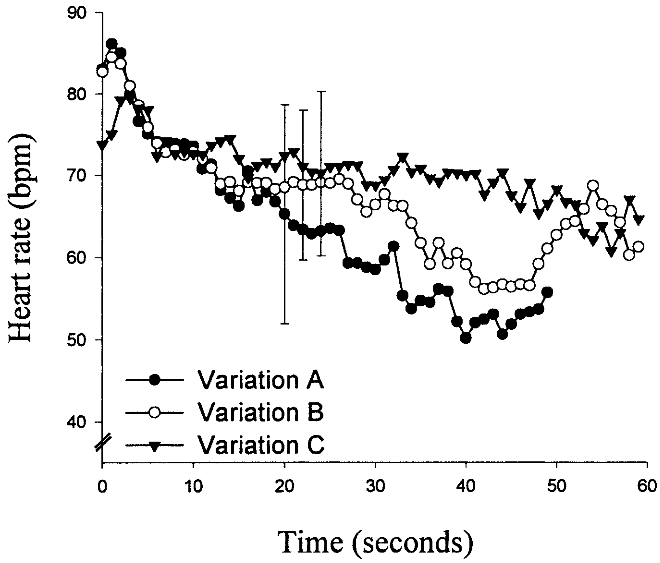

Fig. 1 Mean changes in heart rate during the three variations of protocol I (*variation A* no breathing, no goggles, *B* no breathing, with goggles, *C* breathing, with goggles) in second-by-second curves. Typical standard deviations are indicated. The cold face tests started at time 0

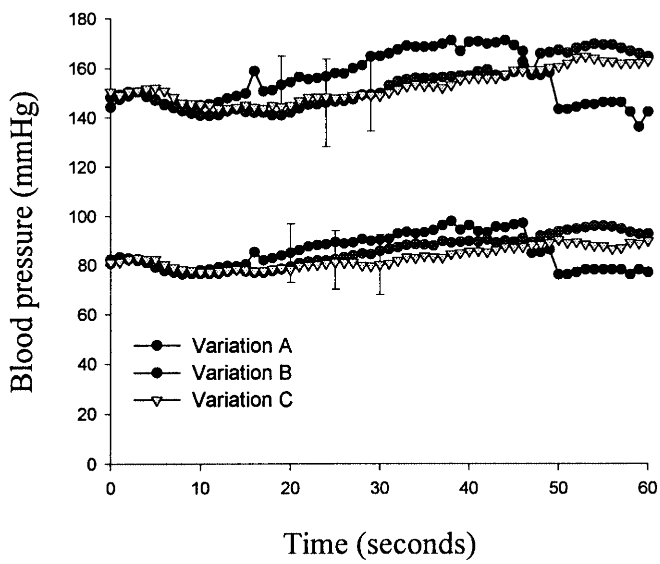

Fig. 2 The time courses of mean systolic and diastolic blood pressures for the three variations of protocol I in second-by-second curves. Typical standard deviations are indicated. The cold face tests started at time 0. There are no differences between the three tests (for definitions see Fig. 1)

blood pressure of 12 compared to 12 mmHg, coefficients of repeatability of 14 compared to 10 mmHg.

Other factors influencing the response

When comparing the results (protocols I–III) of variation A – subjects not being able to breathe, and not wearing diving goggles – of all 27 subjects, the mean was calculated for each subject and this was used in the statistical analysis. The duration of the cold face test correlated well with the heart rate decrease ($r = 0.58$, $P < 0.001$; Fig. 3). In subjects of older ages, the fall in heart rate was less pronounced ($r = -0.47$, $P = 0.014$; Fig. 4), but the duration of the test was also shorter with increasing age ($r = -0.59$, $P < 0.001$; Fig. 5).

Discussion

Our results and comparisons with previous studies illustrate that standardization of the ability to breathe during the cold face test is warranted. In the study of Heath and Downey (1990) 0 °C compresses applied bilaterally to the face for 40 s have been proposed to produce the most pronounced bradycardia and peripheral vasoconstriction. In their study the subjects were able to breathe during the cold face test. As stated before, breathing has been shown to influence the heart rate (Wheeler and Watkins 1973). This may explain why the heart rate slows down less when the subject is able to breathe during the cold face test.

Trouerbach et al. (1994) found no differences between breath-holding and the continuation of breathing during a simulated diving manoeuvre. This can partly be explained by the difference in measuring the effect of the cold stimulus. In their study baseline values and blood pressure values during the manoeuvres were measured at a single point and not continuously, as in our study. In contrast to their study, in which the cold stimulus was placed only on the forehead, a larger part of the face was covered with the cold stimulus during the various tests in our study. Therefore, the differences found could also be due to a different coverage of the face. However, Khurana et al. (1980) studied the effect of coverage of the face on the cold face test. They found that the ophthalmic division of the trigeminal nerve is the most sensitive pathway in eliciting the diving reflex (Khurana et al. 1980) and no additional effect of covering the whole face was found.

Bert (1870) first described the diving reflex in ducks and chickens. These and other animals were not able to breathe during the submersion. In the facial immersion test subjects also hold their breath. So a cold face test with breath-holding seems most close to the original tests. The marked decrease in heart rate and the lack of an effect on the blood pressure response support execution of the cold face test with breath-holding.

Wearing diving goggles was not shown to influence the heart rate and blood pressure response to the cold face test. Apparently, ocular pressure during the cold face test, as we performed it, was too small to elicit the oculocardiac reflex. When comparing different masses of the bag used as a cold stimulus, no significant differences could be found either.

No differences were noted between the first and second time a test was performed, except for the duration of the cold face test and the time until the minimal heart

Table 2 The median results from both occasions the cold face test was performed in variation A (during which the subject was not able to breathe as a result of the bag filled with ice-water of various masses covering the whole face. Eyes and eyelids were also covered). No significant differences were found. *HR* Heart rate, *BP* blood pressure

Parameter	Mass of bag					
	800 g		1100 g		1400 g	
	Median	Range	Median	Range	Median	Range
Fall in HR (beats · min^{-1})	25	1–62	31	4–52	29	8–66
Fall in HR (%)	33.3	1–50	39	6–60	40.6	8–56
Systolic BP changes (mmHg)	32	15–61	37	6–70	28.5	7–58
Diastolic BP changes (mmHg)	20	9–43	20	4–38	19.5	6–34
Time till minimal heart rate (s)	26	6–77	23	9–74	25.5	1–77
Duration of the test (s)	32.5	8–81	26	12–84	31	4–81

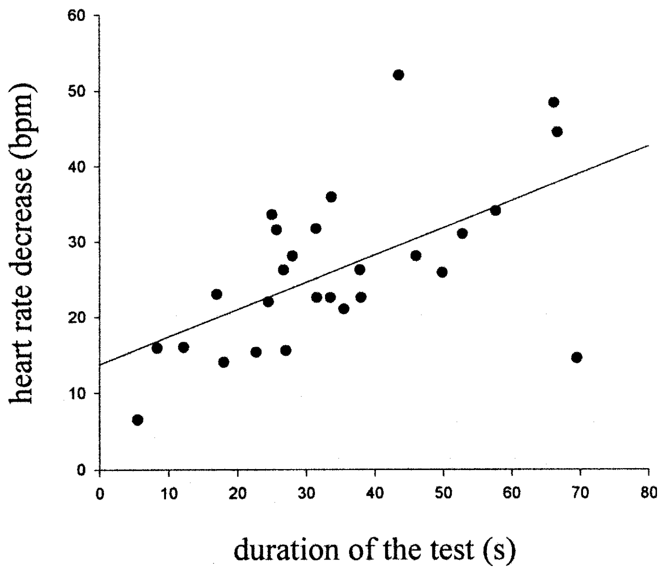

Fig. 3 The relationship between the durations of the test and the decreases in heart rate ($r = 0.58$, $P < 0.001$)

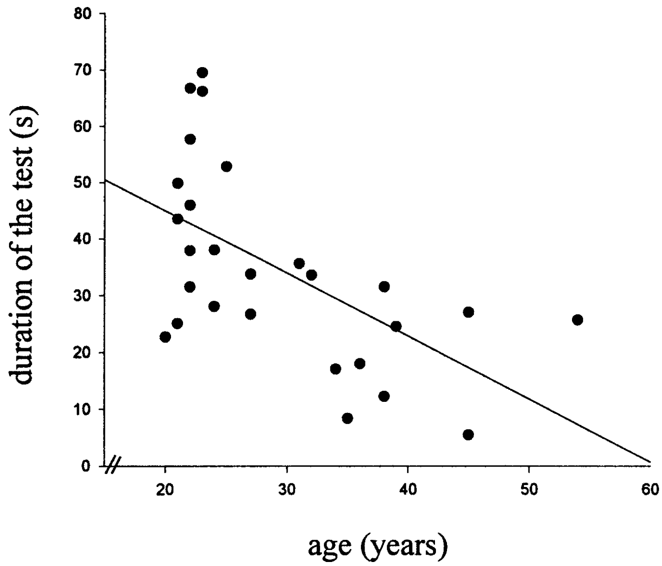

Fig. 5 The relation between age and the duration of the cold face test ($r = -0.59$, $P < 0.001$)

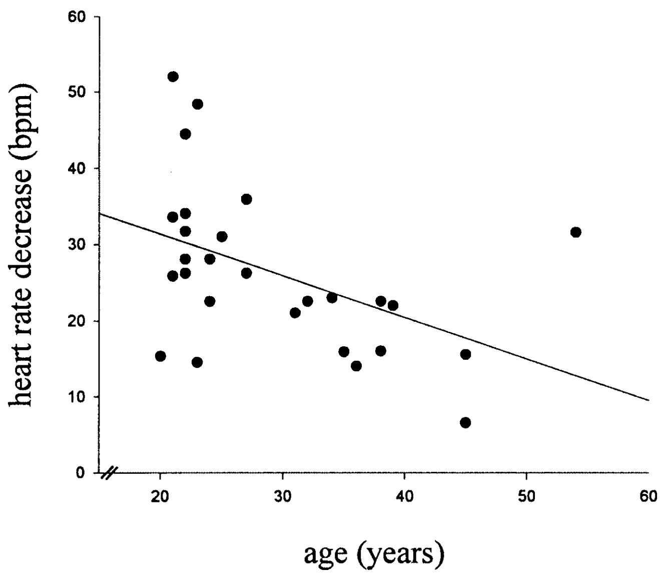

Fig. 4 The relationship between age and the decrease of the heart rate during the cold face test ($r = -0.47$, $P = 0.014$)

rate in A of protocol I. Subjects were taken by surprise by the intensity of this stimulus and, presumably, had to habituate to it. But, in spite of the intensity of the stimulus, the response was not always maximal: the extent of the response did vary.

Our study shows that the fall in heart rate is influenced by the duration of the cold face test, but is inconclusive as to whether it is influenced by the age of the subject. Like other parameters of the response of the parasympathetic nerve system (Wieling 1992), it has seemed that the heart rate changes diminish in older subjects, but the duration of the cold face test, which enhances the fall in heart rate, is also reduced with increasing age.

As the fall in heart rate is influenced by the duration of the test, it is plausible that we did not always measure the maximal possible response to the cold stimulus. Standardizing the cold face test will provide a means of comparing responses among subjects. Heath and Downey (1990) have proposed that the cold stimulus should be applied for 40 s. Of our 27 volunteers, 13 could sustain the stress for at least 40 s. These volunteers were significantly younger than those subjects who could not sustain the stimulus for that long [mean age 23 (SD 3) compared to 35 (SD 10) years, $P = 0.0023$]. However, our population was not representative for that in which, clinically, autonomic function will be determined: these patients are generally older. We expect that a duration of 40 s would be feasible for most persons when they are encouraged to sustain the stimulus and have had some practice. Further investigations are needed to reveal whether the fall in heart rate is influenced by the duration of the test, the age of a person or by a combination of the two.

Baseline systolic blood pressure was higher in protocol I than in protocol II. None of the persons participating was known to have isolated systolic hypertension. This difference was probably due to measuring the blood pressure with a Finapres machine. The Finapres machine is more accurate when measuring changes in blood pressure than in measuring the absolute blood pressure. The systolic blood pressure, particularly, has often been found to be higher when measured with a Finapres machine than with a sphygmomanometer (Imholz et al. 1998).

The repeatability was lowest when performing cold face tests on the first 2 successive days. This, and the fact that the mean difference had a tendency to be different from zero for the first two tests on days 1 and 2, suggests that subjects habituated to the test. Therefore, it is advisable that the test should be practised before actually assessing the diving reflex response. When investigating possible autonomic failure the test can be practised just before assessing the diving reflex. When performing the test to investigate the effect of intervention, it can best be practised on another day, so that adjustment to the stimulus does not interfere with the assessment of the effect of intervention.

The responses to the cold stimulus did vary between protocols I and II. The increase in systolic blood pressure in protocol II seemed higher than in protocol I. Further investigations in healthy volunteers are needed to establish age-related normal responses to the cold face test.

Because the cold face test is a simple method for measuring the effects of the sympathetic and parasympathetic nerve systems simultaneously, it may be attractive to use the test in patients who have difficulties performing other autonomic function tests (e.g. the isometric handgrip test in patients with rheumatoid arthritis or standing up in patients with systemic amyloidosis).

In conclusion we recommend performing the cold face test in a setting where the subject does not continue breathing during the test. The cold stimulus used for the test should be large enough to cover the whole face of the subject, but increasing the mass does not seem to amplify the diving reflex. A test duration of 40 s seems appropriate in subjects under the age of 40. The procedure should be practised in advance of the diagnostic test.

References

Andersen HT (1966) Physiological adaptations in diving vertebrates. Physiol Rev 46: 212–243

Bert P (1870) Trentième leçons: de l'acte du plonger chez les mammifères et chez les oiseaux. In: Bert P (ed) Leçons sur la physiologie comparée de la respiration. Baillière, Paris, pp 533–553

Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet I: 307–310

Chinn S (1990) The assessment of methods of measurement. Stat Med 9: 351–362

Craig AB Jr (1963) Heart rate responses to apneic underwater diving and to breath holding in man. J Appl Physiol 18: 854–862

Heath ME, Downey JA (1990) The cold face test (diving reflex) in clinical autonomic assessment: methodological considerations and repeatability of responses. Cli Sci 78: 139–147

Heistad DD, Abboud FM, Eckstein JW (1968) Vasoconstrictor response to simulated diving in man. J Appl Physiol 25: 542–549

Imholz BP, Wieling W, van Montfrans GA, Wesseling KH (1998) Fifteen years experience with finger arterial pressure monitoring: assessment of the technology. Cardiovasc Res 38: 605–616

Khurana RK, Watabiki S, Hebel JR, Toro R, Nelson E (1980) Cold face test in the assessment of trigeminal-brainstem-vagal function in humans. Ann Neurol 7: 144–149

Trouerbach J, Duprez D, De Buyzere M, De Sutter J, Clement D (1994) Cardiovascular responses elicited by different simulated diving manoeuvres. Eur J Appl Physiol 68: 341–344

Van Brocklin MD, Hirons RR, Yolton RL (1982) The oculocardiac reflex: a review. J Am Op Assoc 53: 407–413

Whayne TF Jr, Killip T III (1967) Simulated diving in man: comparison of facial stimuli and response in arrhythmia. J Appl Physiol 22: 800–7

Wheeler T, Watkins PJ (1973) Cardiac denervation in diabetes. BMJ IV: 584–586

Wieling W (1992) Recording of heart rate and blood pressure. In: Bannister R, Mathias CJ (eds) Autonomic failure. Oxford University Press, Oxford, pp 298–299