Reproducibility of estimation of blood flow in the human masseter muscle from measurements of ^{133}Xe clearance

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The reproducibility of estimations of the masseter intramuscular blood flow (IMBF) was assessed bilaterally within and between clinical sessions. The ^{133}Xe clearance in nine normal individuals was measured before, during, immediately after, and after endurance of isometric contraction at an attempted level of 50% of maximum voluntary clenching contraction. An overall low reproducibility of the estimations was found. This result was probably caused by uncertainties about the exact site of intramuscular ^{133}Xe deposition, errors in assessment of the plots of clearance, and variabilities in the relative contraction levels sustained and, especially, in the overall muscle effort. In agreement with previous reports concerning other skeletal muscles, the ^{133}Xe clearance method provided inconsistent estimates of absolute values of IMBF also in this clinical setting. Although there was a high intra-individual variation in the relative level of isometric contraction sustained, the endurance test induced distinct changes in IMBF, among which the estimate of post-endurance hyperemia was the most consistent for each individual. Therefore, measurements of ^{133}Xe clearance seem to be useful to detect intra-individual changes in masseter IMBF resulting from isometric work. □ *Clinical study; isometric contraction; physiology; radioisotope technique*

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Insufficient intra-muscular blood flow (IMBF) has been proposed as a major mechanism to explain the pain induced by sustained muscle contraction (1). The radioactive inert gas xenon-133, which diffuses freely across cell membranes and is cleared from the tissue at a rate that is largely determined by the blood flow, has been used extensively to estimate IMBF (2–7). In particular, this method has also been used in the masticatory muscles, indicating an insufficient IMBF during static and dynamic activities at a high rate (8–11). Measurements of ^{133}Xe clearance have been found to be poorly reproducible in other muscles when performed in the clinic (5, 7, 12). Nevertheless, we wished to examine whether consistent measurements of ^{133}Xe clearance from the masseter muscle of individuals without facial pain could be obtained in our particular clinical setting.

Materials and methods

Nine volunteers from among the staff and graduate students at the Dental School in Malmö agreed to participate in the study after receiving detailed information about the experiment. They were in good physical health, under no medication, free from dental or facial pain, and had symptoms and signs of craniomandibular disorders within the range presented by the normal population (13). The study was approved by the Ethical Committee at the University of Lund.

Electromyographic (EMG) activity was recorded bilaterally, with the subjects seated upright in a dental chair, using unipolar platinum wire electrodes 0.18 mm in diameter, cut at 45° at the tip. At approximately 5 mm from the tip the wire was bent around 135°, forming the insertional part of the hook,

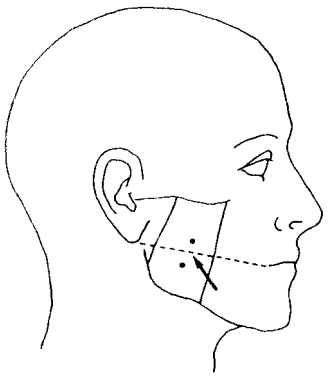


Fig. 1. The standard landmarks over the masseter muscle used to locate the two intradermal hook electrodes (dots), and site of intramuscular deposition of ^{133}Xe (arrow).

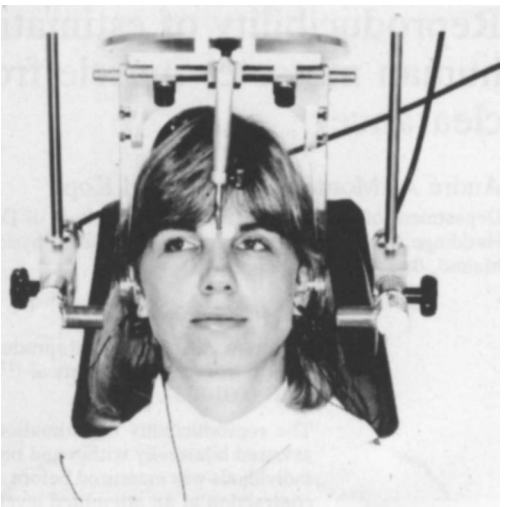


Fig. 2. A subject during a recording session, with the head fixed in an upright position in a cephalostat. The right and left collimators are assembled in the scintillation detectors and maintained in a fixed spatial relationship with the head.

which was placed intradermally, providing the desired close approximation of the tip to the muscle fascia (14). Two electrodes were inserted in a bipolar arrangement along the muscle length, over the center of the superficial masseter, each approximately 8 mm away from either side of a line drawn from the base of the ear lobe to the corner of the mouth (Fig. 1). The center of the muscle was determined by palpation during voluntary clenching. Reference electrodes were attached to the right and left ear lobes. No attempt was made to determine whether the EMG activity was originating from the deep or superficial parts of the masseter muscle. The myopotentials were amplified, rectified and averaged in an EMT 42 integrator, and plotted by an ink-jet writer (Mingograph 800, Elema-Schönander).

IMBF was estimated from measurements of ^{133}Xe (Mallinckrodt Diagnostica, Holland) clearance made with an external sodium-iodide crystal detector (Meditronic 12M5), with its 5-cm-long lateral collimator with 15-mm diameter placed 5 cm from the skin over the masseter. As shown in Fig. 2, the upright head position of the subjects was fixed in a cephalostat, providing a fixed relationship between the detector tubes and the head. Each detector was connected to a single-channel analyzer (Selektronik A/S 45-22) set with a time constant of 3 sec. As shown in Fig. 3, the outputs from the right and left detectors were plotted simultaneously on a logarithmic scale (log 10, ordinate) and as a function of time on a linear scale (abscissa) (Omnigraphic 2000 Recorder, Houston Instruments). The $T^{0.5}$, the time that it takes for the counts of activity to reach half of its value (3, 4, 14), was estimated in minutes from straight lines drawn through different phases of the log clearance slope. IMBF absolute values (k) were calculated, assuming a blood-tissue partition coefficient (λ) of 0.7 (2, 3), from the equation:

$$k = (\ln 2 \times T^{-0.5}) \times \lambda \times 1000 \times \min^{-1} \times l^{-1},$$

where l is liter of muscle tissue.

Procedure

The maximum EMG activity was determined as the highest signal obtained from three consecutive maximum voluntary clenching contractions (MVC) with the teeth in the intercuspal position, each lasting approximately 2 sec and with an interval of about 3 sec. Five minutes after the three

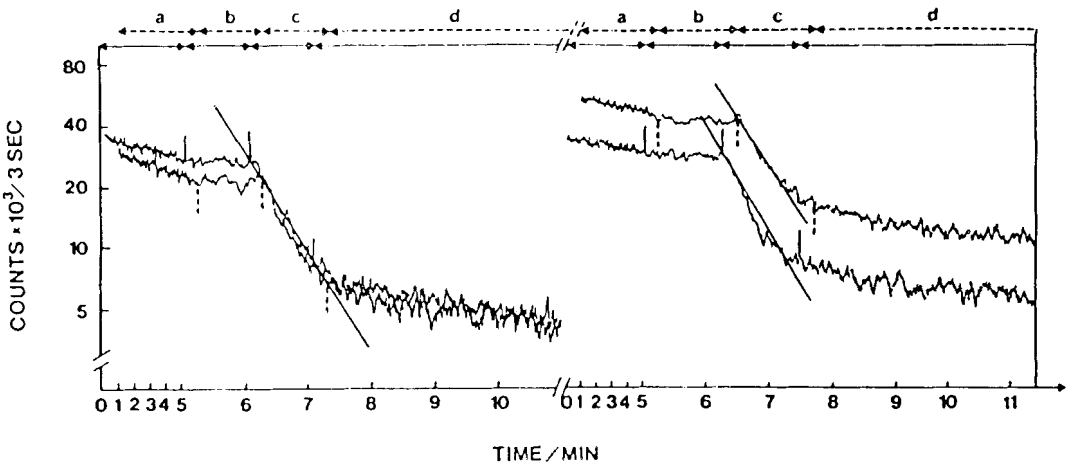


Fig. 3. Plots of two successive recordings of ^{133}Xe clearance from the same individual, taken simultaneously from the right and left masseter muscles within session 1. The paper speed during the phase of initial rest (a) was 1 cm/min, whereas the phases of endurance of isometric contraction (b), post-endurance (c), and final rest (d) are plotted with a paper speed of 4 cm/min. At the top of the figure, each of these phases and the synchronization between the plots of the right (horizontal broken line) and left sides (unbroken line) are indicated. The vertical broken and unbroken lines drawn on the plots of clearance delimit the timing of each phase for the right and left sides, respectively. The lines drawn for the $T^{0.5}$ estimation of post-endurance phase are shown.

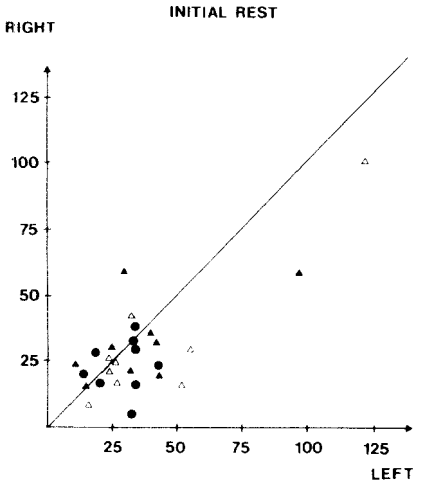
MVC a volume of 0.1 ml of saline solution containing 5 MBq of ^{133}Xe, prepared in a syringe graduated in 0.01 ml, was injected intraorally at the height of the occlusal plane into the central part of both the right and the left superficial masseter muscles, with a 19-mm-long needle with 0.4-mm diameter. The needle was kept in situ for approximately 15 sec, to prevent reflux through the injection channel. The ^{133}Xe was deposited in a location between the two EMG electrodes, as shown by the arrow in Fig. 1. Approximately 5 min after injection on the second side ^{133}Xe clearance was measured during 5 min of rest. If a straight plot was not obtained within these 5 min, a longer period of time was allowed before the endurance test. The 50% level of the maximum voluntary clenching contraction was then displayed on the oscilloscope screen. The subjects were asked to clench at this level and encouraged to sustain the contraction as long as possible. After clenching, the subjects relaxed, and the clearance was measured for another 5 min.

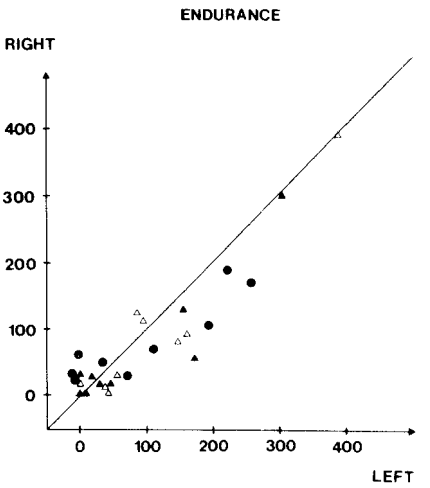
IMBF was estimated at the following phases of the logarithmic plots of clearance (Fig. 3): a) initial rest (rest before endurance); b) endurance (endurance of isometric contraction); c) post-endurance (the hyperemia that immediately follows the endurance); and d) final rest (the recovery to resting clearance rate). The %EMG activity (that is, relative EMG activity) was calculated as the EMG activity during endurance in percentage of the EMG activity during maximum voluntary clenching. The masseter effort (that is, the tension time) was calculated as the product of the %EMG activity and the contraction time.

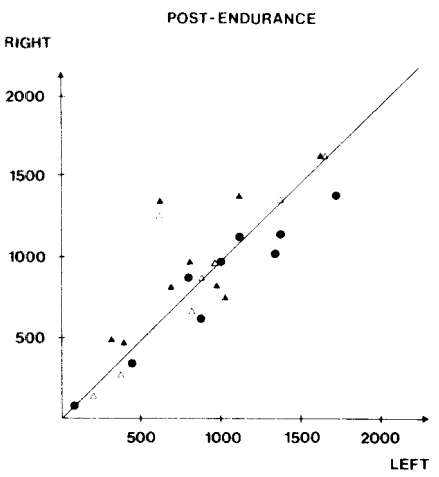
The complete sequence of procedures is termed a series (s). Two series (s1, s2) were successively registered at session (S) 1 (s1S1, s2S1), with an interval of approximately 30 min to enable washout of ^{133}Xe injected at the first series. The series was then performed once at a separate session (S2), at least 1 week later. Fig. 3 illustrates the different phases of bilateral plots of a repeated series within session 1 (s1S1, s2S1).

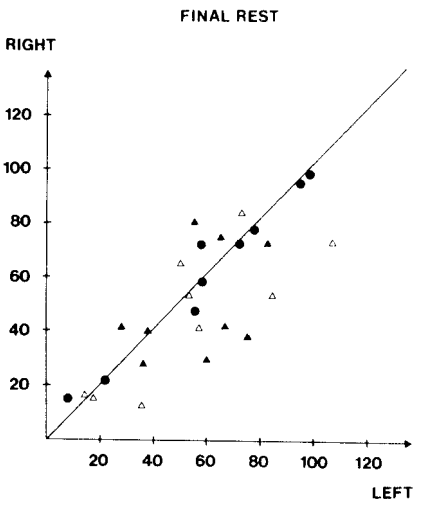
Analysis and statistics

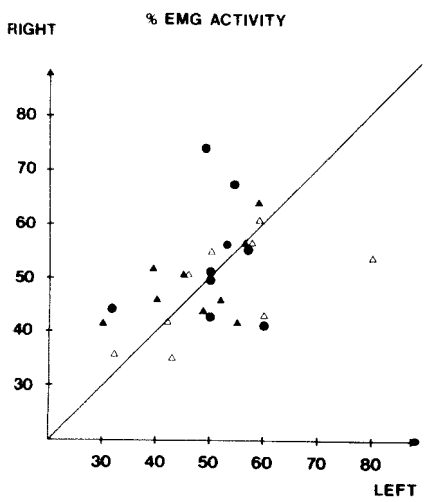
The accuracy of the ^{133}Xe measuring equipment was tested by comparing measurements of 10 aliquots of ^{133}Xe activity


INITIAL REST
RIGHT
125
100
75
50
25
25
50
75
100
125
LEFT


ENDURANCE
RIGHT
400
300
200
100
0
0
100
200
300
400
LEFT


POST-ENDURANCE
RIGHT
2000
1500
1000
500
500
1000
1500
2000
LEFT


FINAL REST
RIGHT
120
100
80
60
40
20
20
40
60
80
100
120
LEFT


% EMG ACTIVITY
RIGHT
80
70
60
50
40
30
30
40
50
60
70
80
LEFT

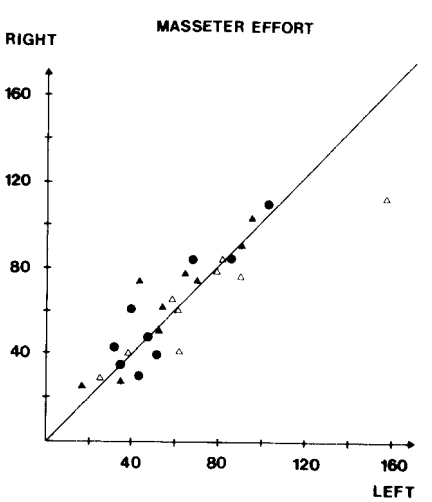

MASSETER EFFORT
RIGHT
160
120
80
40
40
80
120
160
LEFT

Fig. 4. Variation between the right and left measurements of 3 series ($n = 9$). The upper and central plots display the absolute values of IMBF, estimated in $ml \times min^{-1} \times l^{-1}$, for the different phases of clearance: The lower plots display the variables of muscle contraction during endurance: %EMG ACTIVITY is the relative EMG activity during endurance given in percentage of the EMG activity during maximum voluntary contraction. MASSETER EFFORT is the product of %EMG activity and contraction time. Closed triangles = first series session 1 (s1S1); open triangles = second series session 1 (s2S1); closed circles = the series at session 2 (sS2).

made with our analyzer with those made with a radioisotope calibrator (Carpintec, CRC-10). The operator's accuracy in preparing the dilutions of ^{133}Xe activity in the syringes was assessed by comparing the expected and obtained measurements of activity from six different dosages. The operator's reproducibility in estimating the slopes of clearance plots was assessed from repeated estimations of 10 plots after a period of 3 months. The variability between the right and left measurements (R × L) was assessed for each of the three series (s1S1, s2S1, sS2). The reproducibility of the measurements was assessed within session 1 (s1S1 × s2S1) and between the two sessions (s1S1 × sS2).

The mean value of the intra-individual differences ($X_{/di/}$) was calculated. The absolute variability was expressed as the standard deviation for a single measurement of intra-individual differences ($S_{/di/} = \sqrt{\Sigma_{/di^2/}/2n}$). The relative variability was expressed as percentage by the coefficient of variation of a single measurement ($CV = S_{/di/} \times 100/$ mean).

Results

The equipment presented an error of 9.5% in relation to the radioisotope calibrator. The operator presented a variability of 7.3% in the preparation of the aliquot. His variability in the estimation of IMBF from the slopes of the 10 plots of clearance was 8.2% for initial rest, 98.6% for the endurance, 23.8% for the post-endurance, and 9.1% for the final rest flow.

The variation among the values estimated at the three series (s1S1, s2S1, S2) for the right and left measurements is displayed in Fig. 4. The variability between sides (R × L) and the reproducibility within session 1 (s1S1 × s2S1) and between sessions (s1S1 × S2) are shown in Table 1.

Discussion

Several factors may cause inconsistency in the initial rest clearance, in its recording, and in the estimation of IMBF. In the chronology of events within this clinical setting, the first factor should be the room temperature (16), which was not controlled. The second should be the time given to the subjects for their psychophysiologic adjustment from an 'open environment' to a 'closed environment' (17). In this regard, they were given at least 20 min to adjust to the room and understand the procedures to be done. Because muscle contraction produces changes in IMBF, an interval of approximately 5 min was given between the three MVC and the first injection. However, if the total interval of 10 to 15 min between the three MVC and the recording of initial rest clearance was not enough to enable full recovery of normal flow, the three MVC might still have been a source of error. To diminish the effect of the injection trauma, which causes an initial exponential slope that lasts for a few minutes, thin needles were used, small volumes were deposited (0.1 ml), and a delay of at least 10 sec was given between the end of injection and removal of the needle, to avoid reflux (12). To prevent the initial exponential slope, an interval of 5–10 min was given between the injection on the second side and the recording of initial rest clearance (6, 12, 19), which provided a fairly straight plot of the initial rest phase (Fig. 3). Clausen & Lassen (7) obtained a steady clearance 5–7 min after injection. They found a mean error of 17% (range, 9.6% to 22.4%) when comparing different sites of injection in the vastus lateralis muscle and a mean error of 19% (range, 10.2% to 36.8%) when comparing superficial and deep depositions of ^{133}Xe (0.5 cm and 3.0 cm below the fascia, respectively) in the anterior

Table 1. Reproducibility of clinical estimations of IMBF (upper part) and muscle contraction (lower part)

		Between sides			Within Session 1				Between sessions		
Phases	Measurement	$X_{/di/}$	$S_{/di/}$	CV	Sides	$X_{/di/}$	$S_{/di/}$	CV	$X_{/di/}$	$S_{/di/}$	CV
Estimations of IBMF											
Initial rest	s1S1	4.6	13.5	38.4							
	s2S1	10.2	11.8	32.4	R	1.6	14.1	44.0	9.3	9,3	32.9
	S2	5.7	9.5	35.7	L	3.9	26.4	66.8	8.2	18.0	53.7
	X	6.8	11.6	35.5	X	2.7	20.2	55.4	8.7	13.6	43.3
Endurance	s1S1	13.3	28.9	39.3							
	s2S1	13.7	26.7	25.2	R	32.3	35.1	42.3	16.6	37.3	49.6
	S2	14.9	36.9	40.6	L	32.7	49.3	51.1	18.1	68.9	77.2
	X	13.9	30.8	35.1	X	32.5	42.2	46.7	17.3	53.1	63.4
Post-endurance	s1S1	125.0	209.5	23.3							
	s2S1	128.8	226.1	23.9	R	47.3	383.7	38.9	88.1	333.2	36.3
	S2	98.4	127.3	13.8	L	43.5	287.4	33.5	135.3	417.0	46.1
	X	117.4	187.6	20.3	X	45.5	335.5	36.2	111.7	375.1	41.2
Final rest	s1S1	6.7	14.9	37.3							
	s2S1	9.4	13.7	27.0	R	4.1	13.6	28.5	12.4	23.4	39.5
	S2	1.2	4.1	6.6	L	1.4	14.2	25.3	4.5	23.0	38.9
	X	5.7	10.9	23.6	X	2.7	13.9	26.9	8.4	23.2	39.2
Contractions											
%EMG activity	s1S1	2.8	5.9	12.0							
	s2S1	3.8	7.8	15.6	R	1.1	3.2	6.7	4.7	9.1	17.6
	S2	3.4	8.7	16.6	L	4.7	7.9	15.9	3.2	5.1	10.4
	X	3.3	7.5	14.7	X	2.9	5.5	11.3	3.9	7.1	14.0
Masseter effort	s1S1	2.1	5.9	9.9							
	s2S1	7.3	12.5	17.9	R	5.5	19.9	31.5	0.7	22.0	36.4
	S2	4.5	8.0	13.9	L	15.6	27.6	42.0	2.3	18.4	32.4
	X	4.6	8.8	13.9	X	10.5	23.7	36.7	1.5	20.5	34.4

The top row marks the columns displaying the variability between sides and the reproducibility within session 1 and between sessions. The upper part of the vertical axis displays the different phases of clearance: initial rest (rest before endurance), endurance (endurance of isometric contraction), post-endurance (the hyperemia that immediately follows the endurance), and final rest (the recovery to initial rest clearance rate). The lower part of the vertical axis displays the variables of muscle contraction during endurance: %EMG activity (that is, the relative EMG activity, which is the EMG activity during endurance given in percentage of the EMG activity during maximum voluntary contraction) and masseter effort (the product of the %EMG activity and contraction time). The variability between sides (R × L) is shown for each of the three series separately (s1S1, s2S1, sS2; $n = 9$) and also for the mean of series means. The reproducibility of the series within session 1 (s1S1 × s2S1) and between sessions (s1S1 × sS2) is given for the right (R; $n = 9$) and left (L; $n = 9$) sides separately and also for the mean of the right and left means. The expressions of variability are $X_{/di/}$ = the mean value of intra-individual differences; $S_{/di/}$ = the absolute variability, expressed as the standard deviation of the intra-individual differences; CV = the relative variation, expressed in percentage by the coefficient of variation of intra-individual differences.

tibial muscle, which indicates that the clearance rate varies with the site of deposition, owing to inhomogeneous perfusion in different parts of the muscle tissue (7). The high standard deviations of the resting flow also found by Tönnesen (5) in the gastrocnemius muscle clearly indicates that ^{133}Xe clearance presents a high intra-individual variability. The variation in resting IMBF is dependent not only on the total blood flow through the muscle but also on the state of local capillary exchange at the site of ^{133}Xe deposition (19). In turn, the capillary exchange varies also at the same location in the muscle over time, contributing to the time-induced variation in muscle perfusion at a specific site. The right masseter was injected before the left, which caused a systematic lower estimation of right IMBF as compared with the left and a higher repro-

ducibility of IMBF on the right side. The operator's error in estimating the slopes of the different phases of the plots of clearance may be an important factor contributing to the overall reproducibility of the method. Estimations made from slightly or steeply inclined plots are prone to present high variability, whereas more reproducible estimations are obtained from plots that approximate a 45° line (12). In an attempt to obtain a good compromise between an approximate inclination and length of the initial rest clearance, it was recorded for 5 min and plotted with a paper speed of 1 cm/min. The finding that the reproducibility of the method of drawing a line through the slope of each phase of clearance was the same as that of the least squares method (20) cannot be generalized, since this ability varies between observers. Further, the ability should also vary within any observer for the estimations of the different phases of clearance, owing to their different characteristics. In this study, the operator's error was smallest for the estimations of initial rest clearance, and, although of relevance, this may be considered a minor factor affecting the variability of the initial rest IMBF. To summarize, the factors that may eventually affect the reproducibility of the estimations of initial rest IMBF are the room temperature, the resting period between performance of the three MVC and the injection, the injection trauma, reflux, the time allowed between the injection and measurement of clearance, the site of ^{133}Xe deposition, the time-induced variation in the muscle perfusion at a specific site, and error in the estimation of the clearance plots.

The reproducibility of IMBF estimations in the subsequent phases is further related to additional factors. The changes in the slopes of the measurements of ^{133}Xe clearance due to isometric contraction is related to the relative level and time sustained. Therefore, the reproducibility of our measurements should be affected if the subjects were not able to repeat the same masseter effort owing to arbitrary changes such as attention, fatigue, and boredom or systematic changes such as the level of understanding, performing, and adapting to different phases of the task (17). In an attempt to control the arbitrary changes, subjects were encouraged to maintain the 50% level of EMG activity during endurance as long as possible. The operator's low reproducibility in the estimation of the endurance IMBF from the plots, as compared with the other phases, may be partly related to the slight inclination of the plots of endurance clearance. For instance, 2 of the 10 plots used to re-estimate IMBF after an interval of 3 months had its phase of endurance clearance estimated at 20.8 and 15.1 ml $\times$ min^{-1} during the second estimation compared with the previous 1 ml $\times$ min^{-1} $\times$ l^{-1}. The sustained bite effort may be interplayed by different jaw closing muscles (21), in contrast to endurance of the limb, where the effort is performed mostly by contraction of one muscle. Since the visual feedback of the EMG activity assists the subjects to control the level of contraction and the feedback provided in this study was derived from the right masseter only, the consistency of the left masseter contraction is expected to be lower than the right masseter. This may be a reason for the higher reproducibility of the right %EMG activity as compared with the left, within session 1, and also for its higher average reproducibility within session 1 than between sides. Its higher reproducibility within session 1 than between sessions was probably also caused by the mark displayed on the oscilloscope screen representing 50% of the maximum of EMG activity that was not altered within session 1, thus providing a better opportunity for the subjects to endure at the same relative level. The masseter effort was more reproducible between sides than within and between sessions, because the time factor was then constant. The consequence of the higher inconsistency of the %EMG activity between sessions than within session 1 is demonstrated by the lower reproducibility of the estimations of endurance, post-endurance, and final rest phases between sessions.

Compared with the other phases, the slope of clearance immediately after endurance presented a much higher steepness, which indicates a distinct post-endurance hyperemia. Although there was a high variation

in the relative isometric contraction (that is, %EMG activity) and time sustained, the post-endurance hyperemia was the most reproducible phase. As indicated by the lower reproducibility of the initial rest IMBF within than between sessions, the influence of time variables seems less relevant than the trauma from double injection within one session. In contrast, Tönnesen (5) found approximately the same high standard deviation of the resting flow in both groups when comparing the effect of one and three depots injected into the gastrocnemius muscle.

The overall low reproducibility of the estimation of IMBF from measurements of ^{133}Xe clearance in this clinical setting is similar to those of previous reports from other skeletal muscles and indicates that the method is not reliable to estimate absolute values of blood flow in the masseter muscle. However, the ^{133}Xe clearance method, as utilized in this clinical setting, is valuable to detect intra-individual changes in IMBF induced by isometric contraction.

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