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# Deep and Superficial Masseter Muscle Blood Flow in Women

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## Abstract

**Purpose:** Although changes in blood perfusion have been described as being associated with temporomandibular disorder (TMD) myofascial pain, very little is known about blood flow levels in the deep and superficial masseter muscle. This study investigated blood flow in deep and superficial sites of six healthy female particpants at baseline and during intermittent and continuous biting exercises and recovery.
**Materials and Methods:** Blood flow was monitored unilaterally using a single-fiber probe laser Doppler flowmeter. The blood flow was continuously monitored at baseline and during two biting exercises: (a) intermittent at 25%, 50%, and 100% maximum voluntary bite force for 30 seconds each followed by 90 seconds rest between each biting level and (b) continuous biting at similar maximum voluntary bite force levels followed by 90 seconds rest.
**Results:** There was significantly higher blood flow in the deep sites compared to the superficial sites ($p < 0.001$) and a significant increase in blood flow during biting compared to baseline ($p < 0.001$). There were no significant changes in blood flow among the three levels of biting, between the intermittent and continuous exercises, or from baseline blood flow compared to recovery.
**Conclusions:** This study showed regional differences in masseter muscle blood flow, perhaps related to differences in muscle fiber type and pattern of muscle fiber recruitment.

The masseter muscle is a common site for myofascial pain, more often localized in the deep part of the muscle.[1,2] Although the etiology of myofascial pain remains controversial, changes in blood flow during contractions have been suggested as a reason for muscle pain and tenderness to palpation.[3] In this study, we examine possible regional muscle differences in superficial and deep masseter muscle blood flow at the capillary level.

The masseter muscle has unique physiological and histochemical characteristics, differing from both skeletal and other orofacial muscles.[4] Jaw muscles in general and the masseter muscle in particular present diverse types of muscle fibers.[5,6] Histochemical and physiological studies have shown that the deep portion of the muscle contains more slow-twitch, high oxidative fibers, and the superficial portion a higher percentage of fast-twitch fibers.[5,6] The high oxidative fibers of limb muscles have been reported to have greater capillarization to match functional demands.[7,8] However, there is no published information regarding regional differences in human masseter muscle blood flow.

Blood flow in the human masseter has been previously investigated in both healthy and myofascial pain subjects during rest, exercise, and after exercise.[9-12] These studies reported conflicting findings indicating blood flow increases,[9] decreases,[10] or both[11] during muscle contraction. These variations are related in part to different methods of recording blood flow clearance including using xenon[133],[9,12] near-infrared spectroscopy,[10] laser Doppler flowmetry (LDF),[13] and hydrogen clearance.[11] Both the near-infrared spectroscopy and xenon[133] clearance recorded blood flow from a broad area of the muscle, while LDF using an intramuscular probe recorded blood flow from a very small area, mostly in the capillary region. Each of these techniques has advantages and disadvantages. Infrared spectroscopy, using a surface measuring device, is a noninvasive technique, which eliminates the influence of trauma associated with needle insertion.[14,15] The hydrogen clearance technique is a newer method that provides an absolute value of blood flow (BF) volume, but cannot continuously record blood flow.[11]

LDF has the advantage of continuous blood flow recording from a very localized tissue area. LDF operates on the Doppler effect principle to detect the flux of red blood cells.[16] The development of a single-fiber probe has increased the capacity to study deep tissue perfusion at the capillary level under various physiological conditions.[16] The area of measurement is very discrete: approximately 2 mm in diameter from the probe tip and 1 mm penetration of light,[17] which allows recording potential differences in regional muscle perfusion.

Although differences in blood flow have been shown to vary between orofacial muscles,[18] sex,[19] age,[5] and by various interventions such as heat pack,[20] regional muscle differences within the masseter have not been reported. The jaw closing muscles are mostly involved in intermittent contractions such as chewing and swallowing; however, since most previous experiments also used sustained contractions, we investigated BF during both intermittent and continuous contractions. Our goal was to determine changes in BF level from deep and superficial sites of the masseter muscle during intermittent and continuous biting at different bite levels and compare them to the baseline blood flow level.

## Materials and methods

### Study population

Six female particpants with an average age of 28 $\pm$ 3.2 years participated in this study. Inclusion criteria were fully dentate (with or without third molars), good health, and not taking medications. Exclusion criteria included a current or recent history of smoking; temporomandibular symptoms; or difficulty clenching due to muscle, joint, or tooth pain. The University's Human Subjects Committee (UCSF, San Francisco, CA) approved the study, and all participants signed an informed consent statement.

### Equipment

Bite force was measured by means of an intraoral bite force transducer (West Coast Research Co., Santa Monica, CA) positioned midline to record at the first molar area during bilateral biting. The participant sat upright in a dental chair. For participant comfort and to ensure a reproducible placement, an acrylic resin stent was fabricated to adapt the transducer to the first molar area, producing a vertical separation of 3 mm to 4 mm. The force signal from the transducer was amplified and displayed on a monitor screen in front of the participant. The maximum voluntary bite force (MVBF) was determined prior to blood flow recordings by asking the participant to bite briefly as hard a possible five times. There was a 30-second rest interval between each biting effort, and the highest value was determined as the MVBF.

Blood flow was continuously recorded for each participant at baseline, during exercises, and the intervening recoveries using a 0.45 mm single-fiber probe (Moor MBF3D, Moor Instruments, Devon, UK). The instrument's upper limit of filter bandwidth was set at 14.9 kHz and a constant of 0.1 seconds. The LDF analog signal (0 V to 2.5 V) was processed at 40 Hz using a Moor software program for data processing.

The probe was placed percutaneously inside the muscle via a 22 gauge plastic cannula (BD Angiocath, Franklin Lakes, NJ) based on information regarding masseter muscle volume.[21] The horizontal landmark for probe insertion was the midpoint anterior-posterior of the palpated masseter muscle, and the vertical landmark was the midpoint between the origin and insertion of the superficial masseter. Probe placement was aimed at two sites (the superficial and deep masseter muscle) with approximately 8 mm separation. A random order for probe placement was used.

### Protocol

Each participant completed two exercises: intermittent and continuous. First, an intermittent exercise: biting for 30 seconds at each of three biting levels: 25%, 50%, and 100% MVBF, with a 90-second rest between each level. After a 10-minute recovery period, the continuous exercise began, with participants biting for 20 seconds at each of three biting levels without rest followed by a 90-second recovery.

Blood flow was recorded continuously for approximately 12 minutes at each muscle site: baseline recording (1.5 minutes), followed by intermittent exercise and recovery periods (8 minutes), and then continuous exercise and recovery (2.5 minutes). Blood flow or flux was expressed in arbitrary units (AU) and ranged from 0 AU to 1000 AU per second. The flux value was used as a measure of blood flow, consistent with other investigations.[16,18]

### Sample size validation

Sample size calculations were estimated using one-way repeated measures ANOVA with alpha = 0.05 to yield 80% power; a sample size of 6 was estimated to detect an effect size of 1.435 for a two-level factor, an effect size of 1.270 for a three-level factor, and an effect size of 1.224 for a four-level factor.

### Data analysis

Blood flow was recorded continuously. For data analysis we used the mean from 30-second blocks for each participant. Blood flow values were expressed as percent change from baseline and were then compared to recordings from 25%, 50%, and 100% MVBF during exercises and recovery periods.

To compare blood flow level changes from baseline for the various experimental factors, a repeated ANOVA was fitted using four within-person factors: probe site (superficial, deep), recovery time (exercise and three consecutive 30-second recordings after biting), biting exercise (intermittent, continuous), and biting level (25%, 50%, 100%, MVBF). Model residuals were examined to assess model assumptions (e.g., normally distributed model residuals). The Box-Cox power transformation identified the natural logarithm transformation as needed to normalize the data; however, log transformed data still yielded nonnormally distributed model residuals. Thus, logged data were examined for possible outliers and were Winsorized at 85% of extremes to trim back the outliers to provide a more robust model estimation.[22] The reference group (intercept) for the repeated measures ANOVA model was deep muscle undergoing 100% MVBF continuous exercise (see the footnote of Table 1 for further detail). Statistical significance was set at $p \leq 0.05$ throughout.

## Results

### Individual data

A typical LDF recording (participant 3) is shown in (Fig 1). The trace represents approximately 12 minutes of blood flow recordings from the deep muscle site. The baseline recording

**Table 1** Within-subject blood flow log percent changes from baseline (ANOVA)

| Effect | Estimate | Lower 95% CL | Upper 95% CL | *p*-value |
|---|---|---|---|---|
| Intercept† | 5.03 | 4.79 | 5.26 | <0.001 |
| Superficial | −0.13 | −0.20 | −0.06 | <0.001 |
| Intermittent | 0.07 | −0.02 | 0.15 | 0.132 |
| 25% MVBF‡ | 0.02 | −0.09 | 0.12 | 0.713 |
| 50% MVBF‡ | −0.01 | −0.11 | 0.10 | 0.907 |
| 30s Recovery* | −0.50 | −0.61 | −0.39 | <0.001 |
| 60s Recovery* | −0.60 | −0.71 | −0.49 | <0.001 |
| 90s Recovery* | −0.60 | −0.71 | −0.49 | <0.001 |

†Intercept refers to deep muscle undergoing 100% MVBF continuous exercise, so "Superficial" is the difference between deep and superficial probe placement, "Intermittent" is the difference between continuous and intermittent exercise, "25% MVBF" is the difference between 100% MVBF and 25% MVBF effort, "30s Recovery" is the difference between exercise and 30-second recovery, and so on.

‡ F test for bite force: 2 d.f. F = 0.13, $p = 0.883$.

* F test for recovery time: 3 d.f. F = 62.09, $p < 0.001$.

(1.5 minutes) shows a blood flow level of around 40 AU, followed by an increase to approximately 100 AU during the 30-second biting at 25% MVBF. At 50% MVBF the blood flow increased to about 60 AU, and at 100% MVBF to 75 AU. After each biting level, the blood flow level returned to around 30 AU to 40 AU within 30 seconds, as represented by the stable trace. During the continuous biting exercise at 25%, 50%, and 100% MVBF for 20 seconds at each level, the blood flow increased to 80 AU, 70 AU, and 60 AU, respectively, followed by a rapid recovery to baseline levels.

Figure 2 represents a more detailed display of the LDF recordings from participant 3 before the continuous exercise (Fig 2A) and during recovery following 50% MVBF (Fig 2B) and 100% MVBF (Fig 2C). As is seen, the baseline level of blood flow recorded before the beginning of exercises was around 40 AU, and the level of blood flow during recoveries (Figs 2B, 2C) was maintained between 25 and 45 AU. In addition to pulsation, a wave pattern of five or six cycles per minute was noted and considered evidence of vasomotility, as it was present in all three traces.

**Table 2** Log percent changes from baseline for recovery times*

| Recovery time (seconds) | Estimate | Lower 95% CL | Upper 95% CL |
|---|---|---|---|
| 30 | 4.53 | 4.28 | 4.78 |
| 60 | 4.43 | 4.18 | 4.68 |
| 90 | 4.43 | 4.18 | 4.68 |

*The Box-Cox transformation was natural log (percent change from baseline in blood flow + 80), so comparing recovery to baseline corresponds to ln (0 + 80) = 4.38. The 95% confidence limits are fairly narrow and include the zero change from baseline value of 4.38.

## Group data

Intersubject variability of LDF recordings was found both during exercises and recovery at both the superficial and deep recording sites. Participants 1 and 2 showed a greater increase in blood flow from baseline during both intermittent and continuous exercises compared to the other four; however, the increase was not linear with the increase in bite force (i.e., a lower level of BF was generally observed at 100 MVBF compared to 50 and 25 MVBF). Similar to the changes demonstrated by the superficial recording, participants 1 and 2 showed a substantial increase in blood flow from baseline during exercises. In contrast, particpants 3 through 6 demonstrated a much lower increase in blood flow at 25 and 50 MVBF and almost an occlusion at 100% MVBF. Interestingly, the same particpants who showed blood occlusion during intermittent exercise in the superficial site also showed occlusion in the deep site.

Due to the variability of the data and small number of partipants, Winsorized logged data were analyzed by a repeated measures ANOVA using the four within-person factors. Table 1 presents the within-person factor results and their statistical significance (e.g., 100% MVBF to 25% MVBF and to 50% MVBF; exercise to 30-second recovery, 60-second recovery, and 90-second recovery). There was no statistically significant difference in BF measurements during intermittent and continuous biting, nor between the three biting levels; however, there was significantly higher blood flow in the deep sites compared to the superficial sites ($p < 0.001$) and a significant increase during biting compared to baseline ($p < 0.001$).

Table 2 presents log percent changes from baseline and 95% confidence intervals for all recovery times. Although all three

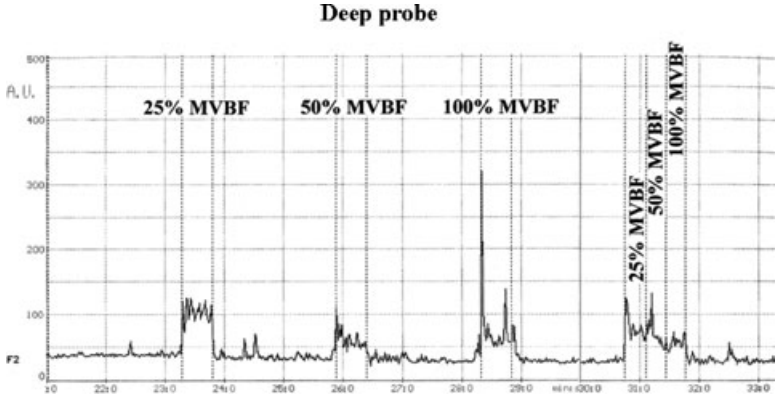


**Figure 1** Compressed blood flow recording from a nonpain subject. The horizontal axis represents approximately 12 minutes of recording. The bite force levels are indicated on the upper part of the chart. The lower trace represents the LDF recording of blood flow from the deep penetration. The blood flow is expressed in arbitrary units (AU) on the vertical axis. Recordings from two types of biting exercises: intermittent and continuous and their respective recoveries can be seen.

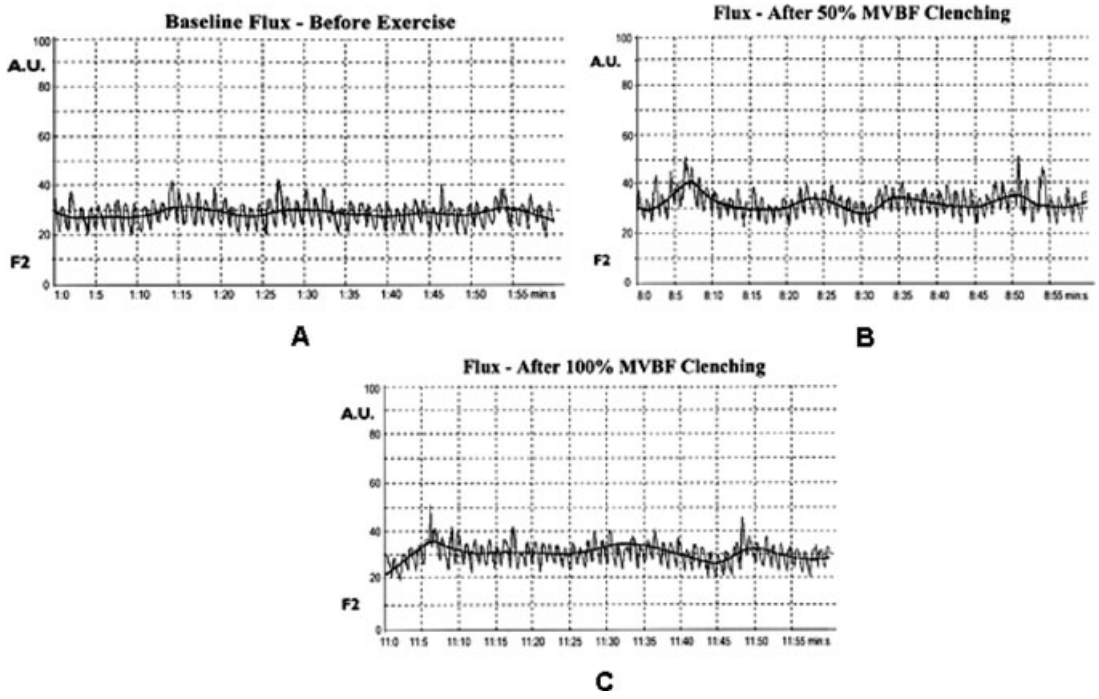


**Figure 2** (A) Baseline blood flow recording prior to any exercise. The horizontal axis represents approximately 1.55 minutes. Blood flow is expressed in arbitrary units (AU) on the vertical axis. (B) Blood flow recording after 50% MVBF. (C) Blood flow recording during recovery after 100% MVBF.

recovery times differed significantly from exercise, there were no statistically significant differences among the three recovery times (the CI's overlap with each other), showing that recovery occurred within 30 seconds after biting.

Fig 3 is a boxplot of the log percent change in blood flow from baseline by exercise/recovery times at the two muscle sites. The blood flow levels were higher in the deeper sites with respect to baseline.

## Discussion

Overall, our results show that both exercises induced a significant increase in blood flow compared to baseline, and these changes returned to baseline levels within seconds after biting. Furthermore, there was no linear increase in blood flow with an increase in the bite force level or between continuous and intermittent exercises; however, our unique findings are the first to show the deeper site had higher blood flow levels during both exercises and recoveries. These results may have clinical relevance, as during prolonged contractions, muscle regions with higher levels of capillaries may be more prone to vasodilation through neurogenic inflation and thus more predisposed to pain and tenderness.[14,23]

A potential limitation of LDF recording is that the needle probe may induce local tissue damage and release of vaso-active substances that could influence microcirculation[24,25] or movement of the probe during contractions; however, this probability is low, since the blood flow returned to a very stable level that was not significantly different from initial baseline recordings (Fig 1). Additionally, the recordings at baseline and recovery demonstrated the presence of low frequency rhythmic waves of five to six cycles per minute (Fig 2). These rhythmic waves detected by the LDF probe have been described and interpreted as vasomotion of the terminal arterioles or flow motion within the capillary bed.[26] The presence of this vasomotion cyclic pattern is considered to indicate a lack of inflammation induced by the intramuscular probe.[27,28] Therefore, in this respect, our results should represent a good example of physiological changes in blood flow at the capillary or arteriolar level without significant influence from the probe.[29]

The two exercises were designed to simulate the functional activity of the masseter muscle: intermittent to parallel most functional rhythmical activity such as chewing and swallowing; and continuous to mimic parafunctional activity such as clenching. To further investigate the muscle hemodynamics, different levels of bite force were used for both exercises. Although there was a significant increase in blood flow from baseline during biting, there were no significant changes during the different levels of bite force. This is consistent with other investigators[18] and may be due to the unique characteristics of jaw closing muscles.

Compared to other skeletal muscles, jaw muscles are very different with regard to fiber types and composition and their contractile properties. The masseter muscle has a high content of hybrid and cardiac fiber types.[5,6] In limb muscles, these fibers are considered to represent a transition between type I (slow) and type II (fast) fibers. In the masseter, these fibers are thought to provide a continuum in the contractile properties of the muscle necessary for a wide range of the jaw's motor tasks, which require constant changes in load and speed.[5]

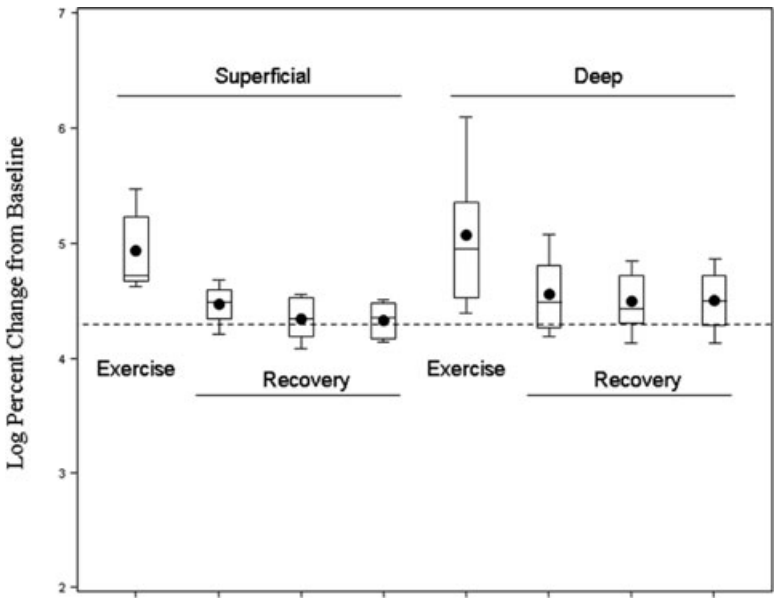


**Figure 3.** Boxplots of log percent change in blood flow from baseline by muscle site and exercise/recovery time. Dashed line represents 0% change from baseline (since the Box-Cox transformation was natural log (percent change + 80) and ln(0 + 80) = 4.38).

Furthermore, the typical classification of slow and fast fiber characteristics for the limb muscles does not fit those of the masseter muscle. The jaw muscle type II fibers have a smaller cross-sectional area than type I fibers, while the opposite is found in limb and trunk muscles.[5] The observed variability in fiber type provides evidence for increased adaptive capacity of the masseter muscle and, thus, large interindividual variation in fiber-type expression.[5,6]

The structural and functional diversity of these unique fibers are matched for their metabolic support by the blood flow at the capillary level. Diversity in capillary density was demonstrated between aerobic oxidative and glycolytic fibers; in human skeletal muscles, the number of capillaries making contact with slow and IIA fibers were reported as higher than for fast II fibers.[30] Since the deep part of the masseter muscle contains a greater number of type I oxidative fibers with a larger cross-sectional area,[5] these fibers may provide a richer capillary supply. Therefore, it is not surprising that we found regional differences with higher blood flow in the deep portion of the masseter during both biting exercises and recovery periods (Fig 2).

There was no linear increase in blood flow with an increase in bite level, nor was the blood flow totally arrested or occluded at the highest bite level. Furthermore, a rather fast recovery to baseline (within 30 seconds) was found after biting. These findings demonstrate that human masseter fibers are richly supplied by capillaries. Our previous study of masseter $P^{31}$NMR (Phosphorus 31 Nuclear Magnetic Resonance) demonstrated that under similar experimental conditions of heavy biting, PCr (phosphocreatine) depletion and drop in pH was much less than other skeletal muscles.[31] Therefore, under normal heavy exercises, the aerobic capacity of the masseter could not be exceeded, as can occur in other muscles. Taken together, this information explains, in part, the increase in blood flow during contraction followed by fast recoveries of blood flow after biting. Furthermore, the biting force is the result of four pairs of jaw closing muscles, for which it is difficult to predict the exact involvement of a particular muscle or muscle portion to the total biting force.[6] The neural integration among these muscles may involve switching in the recruitment pattern among them to avoid surpassing their aerobic capacity. The nonlinear increase in blood flow with linear increase of bite force may be due to the unique neural control involving the switching among these muscles.

The large intersubject variability in blood flow levels among the six participants could be due to differences in fiber type composition and the unique pattern of muscle recruitment seen during biting[5,6] or by the inherent problems related to the recording technique. Higher values of blood flow in some participants may be due to recordings near larger vessels, rather than capillaries. Avoiding the placement of the probe near a large vessel could not always be controlled.

The differences between our findings showing increases in blood flow during contraction and previous studies reporting a decrease[10,12] are probably due to different techniques used to measure blood flow. For example, the use of near-infrared spectroscopy provides a more global measure of changes from venous and arteriolar blood flow within the muscle and, because the device is positioned over the skin of the masseter muscle, it also includes signals from the skin vasculature.[10] The optical path of near-infrared spectroscopy is approximately 2.5 cm in human skeletal muscle,[32] while LDF measures blood flow from an area of approximately 2 mm.[17]

Blood flow recovery to baseline levels in this study was also found to be more rapid than in previous investigations.[9,12] Both Rasmussen et al[9] and Monteiro and Kopp[33] reported that recovery took approximately 2 minutes after exercises, while we observed recovery within 30 seconds. This difference again may reflect different aspects of blood flow being measured, as both previous studies[9,33] provided a global measure of venous and arteriole blood flow within the muscle, while LDF measures blood flow at the capillary level.

## Conclusion

This is the first study to show regional differences in blood flow between the superficial and deep regions of the masseter muscle. These findings support the histological and functional differences previously reported. These results will provide normative data for future studies on TMD myofacial pain patients.

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