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Relationships between craniofacial morphology and masticatory muscle activity during isometric contraction at different interocclusal distances

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Running title (not more than 40 letters and spaces):

Craniofacial morphology and jaw muscles. (40/40)

Abstract (no longer than 250 words)

Objective: The aim was to investigate relationships amongst interocclusal distances, masticatory muscle electromyographic (EMG) activity during isometric contraction of masticatory muscles, and craniofacial morphology.

Design: Twenty-eight women and 12 men (25.3 ± 3.8 years old) participated. After measuring maximal voluntary occlusal bite force (MVOBF) between the right-first premolars, the participants were asked to bite at submaximal levels of 0 (= holding the bite force transducer), 15, 22.5, and 30% MVOBF with the use of visual feedback. The thickness of a bite force transducer was set at 10, 12, 13, 14, 16, 17, 18, 19, 20, 22, and 24 mm (= interocclusal distance: IOD). Nine soft tissue craniofacial factors were assessed through digital photograph: face height, middle face height, lower face height, face width, inter-pupil distance and mandibular plane angle, lower face height / face height ratio, inter-pupil distance / facial width ratio and face width / face height ratio.

Results: In the masseter muscle, EMG activity decreased with increased IODs. The participants with higher mandibular plane angle had more negative slope coefficients of IOD-EMG graphs at 0% MVOBF especially in male temporalis and female masseter and temporalis muscles, suggesting that a greater mandibular plane angle is associated with lower EMG activity at longer IOD.

Conclusions: Overall the findings support the notion that craniofacial morphology is associated with differences in neuromuscular activity of the masticatory muscles, and suggest that the neuromuscular effects of oral appliances may be dependent on patients' craniofacial morphology and the thickness of the device.

Key words (maximum of 6 keywords):

electromyography, masticatory muscles, bite force, trigeminal nerve, craniofacial morphology.

Abbreviations (EMG may be used without definition.)

CT, computerized tomography; Go, Gonion; IOD, interocclusal distance; MAL, left masseter; MANOVA, multivariate analysis of variance; MAR, right masseter;

Me, Menton; MRI, magnetic resonance imaging; MVOBF, maximal voluntary occlusal bite force; N, Nasion; P, Pupil; R, Pearson's correlation coefficients; RMS, root-mean-square; Sn, Subnasale; T, Tragion; TAL, left-anterior temporalis; TAR, right-anterior temporalis; TMD, temporomandibular disorders; Zy, Zygion.

1. Introduction

The relationship between masticatory muscles function and craniofacial morphology has been widely assessed in individuals by bite force (Sondang et al., 2003; Sonnesen & Bakke, 2005), electromyographic (EMG) examinations (Farella et al., 2005; Serrao, Sforza, Dellavia, Antinori, & Ferrario, 2003; Tecco, Caputi, & Festa, 2007), computerized tomography (CT) (Gionhaku & Lowe, 1989; Weijs & Hillen, 1984), ultrasonography (Farella, Bakke, Michelotti, Rapuano, & Martina, 2003; Raadsheer, van Eijden, van Ginkel, & Prahl-Andersen, 1999; Satiroglu, Arun, & Isik, 2005), magnetic resonance imaging (MRI) (Hannam & Wood, 1989; Van Spronsen, 2010) and immunohistochemistry evaluations of muscular fibres (Rowlerson et al., 2005; Tuxen, Bakke, & Pinholt, 1999).

A general consensus in previous studies is that long-faced individuals have lower masticatory EMG activity (Serrao et al., 2003; Tecco et al., 2007; Ueda, Miyamoto, Saifuddin, Ishizuka, & Tanne, 2000), weaker bite force (Kiliaridis, 1995; Raadsheer et al., 1999) and wider transversal craniofacial dimensions (Weijs & Hillen, 1984) compared with short-faced individuals. Larger bite force and/or EMG activity is especially correlated with smaller mandibular plane angles and/or gonial angles (Farella et al., 2003; Pepicelli, Woods, & Briggs, 2005; Serrao et al., 2003), and larger posterior

facial height (Gionhaku & Lowe, 1989; Sondang et al., 2003).

Moreover, masseter muscle thickness (Satiroglu et al., 2005) and volume (Benington, Gardener, & Hunt, 1999) are negatively correlated with mandibular plane angle, and positively correlated with mandibular ramus height, i.e., short-faced individuals have a larger volume and greater cross-sectional area of masticatory muscles than long-faced individuals (Raadsheer et al., 1999; Van Spronsen, 2010).

However, the interaction between muscles function and morphology is complex, and the results of previous studies so far are ambiguous; other studies reported no significant relationships between craniofacial morphology and masticatory muscle activity (Farella et al., 2005; Hannam & Wood, 1989). Some studies also reported that the activity of the masseter and digastric muscles was significantly related with the vertical facial type, although temporal muscle activity presented no significant relationship with craniofacial morphology (Ueda, Ishizuka, Miyamoto, Morimoto, & Tanne, 1998; Ueda et al., 2000).

Moreover, a distinct peak force from jaw-closing muscle may exist within the length–tension relationship, and the occlusal bite force increases up to a certain range of jaw opening and then decreases at wider jaw opening (Mackenna & Turker, 1983; Paphangkorakit & Osborn, 1997). Some participants might produce the maximum

occlusal bite force at the masseter optimum length (Mackenna & Turker, 1978; Weijs, Korfage, & Langenbach, 1989), others at the temporalis optimum length (Paphangkorakit & Osborn, 1997). Most muscle parts may function in agreement with their respective active length–tension curve (Goto, Langenbach, & Hannam, 2001). However, despite there must be individual differences in masticatory muscle length, no study has considered this issue.

To the best of our knowledge, no previous study has investigated the correlation between IOD-EMG relationship and craniofacial morphology through digital photographs. Therefore, the aims of this study were to adapt a reliable non-invasive method to evaluate craniofacial morphology and to investigate the relationship amongst interocclusal distances, masticatory muscle electromyographic (EMG) activity during isometric contractions and craniofacial morphology.

2. Materials and methods

2. 1. Participants

Twelve healthy men (mean ± SD, 26.6 ± 2.7 years old) and 28 healthy women (24.7 ± 4.1 years old) with full natural dentitions were enrolled. Sample size was estimated a priori using G*Power (version 3.1.9.2) (Faul, Erdfelder, Lang, & Buchner, 2007). For a desired power of 0.8, an expected small effect size of 0.1 and an alpha of

0.05, we estimated the required sample size for 2 × 11 multiple analysis of variance (MANOVA). The minimum repeated measures correlation that we observed in this task across any pair of conditions was 0.6 producing a minimum required sample of 40 participants. All participants had no previous orthodontic treatment, deep over-bites (≥ 5 mm), obvious dental asymmetries (≥ 2 mm), missing teeth except for third molar, and history of temporomandibular disorders (TMD), which was ascertained according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (Dworkin & LeResche, 1992). None of the participants took medication that could influence EMG or psychological responses. Informed consent was obtained from each participant and the experimental protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki.

2. 2. Study design

The participants sat on a comfortable chair and first performed a maximal voluntary contraction (MVOBF: maximal voluntary occlusal bite force, Newton) on a bite force transducer between the right upper and lower first premolars. After that, the submaximal forces (15%, 22.5% and 30% MVOBF) were calculated. The interocclusal distances (IOD = thickness of bite force transducer) were set at 10, 12, 13, 14, 16, 17,

18, 19, 20, 22 and 24 mm and then, the participants performed these submaximal isometric contractions with the use of visual feedback. The order of the levels of submaximal bite forces and the IODs were randomized in each participant. Force and the corresponding electromyographic (EMG) activity (μV) of the left masseter (MAL), right masseter (MAR), left-anterior temporalis (TAL) and right-anterior temporalis (TAR) muscles were simultaneously recorded during the isometric contraction tasks, stored on a PC and later used for off-line analyses (see Electromyographic (EMG) estimation). In addition, frontal and lateral digital photographs of facial soft tissue were taken and measured for anthropometric craniofacial estimation.

2. 3. Maximal voluntary occlusal bite force (MVOBF)

MVOBF was recorded with a bite force transducer (41.0 × 12.0 × 10.0 mm, length × width × height, Aalborg University, Aalborg, Denmark) connected to an amplifier with peak-hold facility (Floystrand, Kleven, & Oilo, 1982). The analogue output of the amplifier was connected to an analogue-to-digital (A/D)-converter and stored on a PC together with the EMG data. The participants were instructed to clench their teeth as hard as they could for about 1–2 s with 1-min rest intervals. MVOBF was measured three times and averaged (Bakke et al., 1996).

2. 4. Interocclusal distances (IOD)

IODs of the bite force transducer (original thickness of transducer: 10 mm) were adjusted by bite blocks made from cold curing acrylic composite (3M ESPE, Saint Paul, Minnesota, USA). A set of 11 blocks were constructed to ensure the repositioning of the bite blocks for all the tests, and to give IODs 10, 12, 13, 14, 16, 17, 18, 19, 20, 22 and 24 mm. The order of the 11 bite blocks was randomized. Each block had two 0.7 mm stainless steel sprung wires to attach firmly to the bite force transducer.

2. 5. Submaximal isometric contractions

Pilot studies had indicated that maximum value, which most of the individuals could maintain for a 5-s isometric contraction, was 30% MVOBF (Dawson, List, Ernberg, & Svensson, 2012). Therefore, we used levels of 30% MVOBF or less. After determination of the average MVOBF at 10 mm IOD, the participants were instructed to perform four levels (0% (= only holding the bite force transducer), 15%, 22.5% and 30% MVOBF) of submaximal bite forces (target levels) in random order. The participants increased the occlusal bite forces up to the specified target level and held the contraction for about 5 s with the use of visual feedback by looking at a display of

the force transducer with 2-min rest intervals.

2. 6. Electromyographic (EMG) estimation

EMG activities during the submaximal isometric contractions were simultaneously recorded from bilateral masseter and anterior temporalis muscles. Bipolar disposable surface electrodes (Ambu, Ballerup, Denmark) were placed on the most prominent part of the muscles, perpendicular to the main direction of the muscle fibres in the superficial portion of masseter and the anterior portion of temporalis muscles. Electrode placement was based on palpation by asking the participants to clench their teeth. The interelectrode distance was about 10 mm. The EMG signals were amplified (5,000–20,000 times; Dantec Measurement Technology A/S, Skovlunde, Denmark), 20–200 Hz signal filtered by a processor box (National Instruments, Austin, Texas, USA), A/D converted with sample frequency of 512 Hz and were stored in a PC. A custom-made software program, which can determine the peak EMG amplitude during the submaximal contractions, calculated the corresponding root-mean-square (RMS) value of the EMG signal in a 5-s window (Arima et al., 2013). Each EMG-RMS value was then, expressed as the ratio to the EMG activity during the submaximal voluntary contraction at 30% MVOBF with 10-mm IOD.

2. 7. Anthropometric craniofacial estimation

A single experienced operator (MM) located seven soft-tissue landmarks by inspection and/or palpation and marked the landmarks with coloured pencils on the face, and then the bilateral pupils were defined from the photographs. Craniofacial morphology was measured by anthropometric measurements obtained from the frontal and lateral photographs and in total nine (seven soft-tissue and two on the photographs) landmarks. The distance between each participant and a digital camera (Nikon, Tokyo, Japan) was 2 meters. The following anatomical structures were traced as follows (Fig. 1): distances (unit, mm); face height (Nasion-Menton; N-Me), middle face height (Nasion-Subnasale; N-Sn), lower face height (Subnasale-Menton; Sn-Me), face width (Zygion-Zygion; Zy-Zy); inter-pupil distance (Pupil-Pupil; P-P) and angles (unit, degrees); mandibular plane angle (NT/MeGo). Several values were then, calculated for the analysis: ratios (no unit); lower face height / face height ratio (Sn-Me/N-Me), inter-pupil distance / facial width ratio (P-P/Zy-Zy) and face width / face height ratio (Zy-Zy/N-Me) (Tartaglia, Grandi, Mian, Sforza, & Ferrario, 2009).

2. 8. Statistics

The Shapiro-Wilk test and Levene's test demonstrated that the data were normally distributed and had equality of variances, respectively. The EMG-RMS and anthropometric craniofacial measurements repeatability were quantified with intraclass correlation coefficient (ICC(1,1)), ranged between 0.747 and 0.967. All data was expressed with mean ± SD. EMG-RMS was analysed in MANOVA with repeated measures. The variable was IOD (mm) as the repeated factor and compared by gender and side (working side and balancing side). The levels of significance were adjusted for multiple pairwise comparisons with the Tukey's honest significant difference (HSD) test. Gender differences in anthropometric craniofacial measurements were analysed with simple t-tests between men and women. The relation between slope coefficients derived from the IOD-EMG graphs and anthropometric measurements was analysed using Pearson's correlation coefficients (R). The STATISTICA software (StatSoft, Tulsa, Oklahoma, USA) was used for all analyses. Significance was accepted at $P < 0.050$.

3. Results

3. 1. EMG findings during submaximal isometric contractions

The IOD-EMG relationship is shown in Fig. 2 and Fig. 3. EMG-RMS was not dependent on IOD during 0% MVOBF activity. MANOVA showed that EMG-RMS decreased with increased IOD in MAL of males and females at 15%, 22.5% and 30% MVOBF (IOD: $P < 0.001$) and MAR of females at 30% MVOBF (IOD: $P = 0.003$) (Fig. 2). Mean slope coefficients (a) derived from linear regression analysis also showed the same tendency on the masseter and the female temporalis, except for the female TAL with increasing EMG-RMS at 30% MVOBF. EMG-RMS of males was higher than those of females in MAR and TAL (MAR; interaction gender x IOD: $P = 0.035$, TAL; gender: $P = 0.012$, MANOVA, Fig. 2). In comparison with side differences (Fig. 3), there was a tendency that EMG-RMS on the working side were higher than those on the balancing side in the female masseter (22.5% MVOBF; interaction side x IOD: $P < 0.001$, 30% MVOBF; side: $P = 0.001$, MANOVA).

3. 2. Anthropometric craniofacial measurements

We compared the anthropometric craniofacial measurements with nine factors (Table 1). There were significant gender differences in the factors associated with

vertical height of face, i.e., face height (P = 0.008), middle face height (P = 0.044), lower face height (P = 0.005) and face width / face height ratio (P = 0.041). The participants with greater face height had greater face width. Longer-faced males had smaller face width / face height ratio and males with lower mandibular angle had larger inter-pupil distance / face width ratio.

3. 3. Relationships between EMG activity and anthropometric craniofacial factors

There were negative correlations between each participant's slope coefficients (a') derived from the IOD-EMG graphs and mandibular plane angles during 0% MVOBF activity in both masseter and temporalis muscle of females, while only in the right temporalis of males (Table 2). Further analysis, calculating their regression line equations ($y_{NT/MeGo} = \alpha x_{a'} + \beta$) between each participant's slope coefficients (a') of IOD-EMG graphs and mandibular plane angles at 0% MVOBF, showed that the intercepts (β) were almost equal to the mean angle (Table 1 and 3), i.e., higher-angled participants had more negative slope coefficients of IOD-EMG graphs at 0% MVOBF, while lower-angled participants had more positive slope coefficients (Fig. 4).

4. Discussion

This study indicated that within a range of 10–24 mm between the right first premolars, the shorter IOD leads to the higher EMG-RMS in masseter muscle; males have significantly longer face height than females; there is a tendency that the participants with higher mandibular plane angle had more negative slope coefficients of IOD-EMG graphs.

In this study, higher EMG-RMS activity was produced at the shortest IOD, in agreement with previous studies (Arima et al., 2013; Lindauer, Gay, & Rendell, 1993; Morimoto, Abekura, Tokuyama, & Hamada, 1996). There was no distinct peak in the individual IOD-EMG graphs, therefore, these results indicate that there is no discrete IOD at which the surface EMG required to exert a unit force is a minimum, i.e. an optimal length.

Males had significantly longer faces than females (Table 1) in agreement with the previous studies (Farkas, 1994, Zhuang, Landsittel, Benson, Roberge, & Shaffer, 2010). Males with lower mandibular angle had larger inter-pupil distance / face width ratio. These results indicated that, in males, the individual difference of growth was greater in the vertical dimension than in the horizontal dimension and the horizontal growth may be affected by mandibular plane angle indicating masticatory muscles fibre direction.

Anthropometric craniofacial measurements showed that there were negative correlations between each participant's slope coefficients (a') derived from the IOD-EMG graphs and mandibular plane angles during 0% MVOBF activity in masticatory muscles (Table 2). Many papers also have showed mandibular plane angle variation with bite force and/or EMG activity (Pepicelli et al., 2005; Serrao et al., 2003; Tuxen et al., 1999). Recent investigations have demonstrated that low-angled individuals have a significantly larger masseter muscle thickness (Satiroglu et al., 2005) and volume (Benington et al., 1999) than high-angled individuals. These results indicate that craniofacial morphology and jaw growth are associated with EMG activity differences in the masticatory muscles.

Further analysis in this study demonstrated that the higher-angled participants had more negative slope coefficients of IOD-EMG graphs at 0% MVOBF, while the lower-angled participants had more positive slope coefficients (Table 3 and Fig. 4). In other words, higher-angled individuals decreased their muscle activity as increased IOD, but lower-angled individuals increased. The present findings support the notion that craniofacial morphology is associated with differences in neuromuscular activity of the masticatory muscles (Hannam & Wood, 1989; Sondang et al., 2003), and suggest that higher-angled individuals more easily may reduce their muscle activity while wearing

an oral appliance.

A possible limitation of the present study is that the IOD was set within a thicker range of 10–24 mm than the thickness of most oral appliances. Therefore, further research will be needed to assess masticatory muscles activity within the IOD of 8 mm or less, in order to further the insights into the relationships between masticatory muscles function and craniofacial morphology. Another limitation of the present study is that it might be difficult to evaluate gender differences because of the numbers of males and females were different. There seems to be the tendency in relationships between slope coefficients derived from the IOD-EMG curves and mandibular plane angle in male temporalis and female masseter and temporalis muscles. This study was not specifically designed to test potential gender differences, however, the discordances between the genders were seen in Table 2. This might, indeed, indicate a possible gender difference. Also, the regression line equations only indicated more spurious relationships in males. These results may be due to the lack number of subjects especially for males. Further studies will be needed to address this issue. A minor limitation may also be that the success in terms of actually reaching and maintaining the different force target levels was not assessed in the present study. Therefore, individual differences in the variability of the different target forces, e.g. due to fatigue, are not

considered to have significantly influenced the present observations. Finally, this study was designed for applying the current knowledge to the clinic, thus, used 2D (dimensional) -ordinary digital camera. However, as 3D cameras are becoming more accessible future studies should incorporate this feature in the methodology.

5. Conclusions

The present study demonstrated that higher EMG activity could be generated between the first premolars at shorter interocclusal distances. The main new finding in this study was that there might be an effect of mandibular plane angle on masticatory muscle activity at 0% MVOBF. It is suggestive that different craniofacial morphologies are associated with differences in neuromuscular activity of the masticatory muscles. However, a full understanding of the mechanisms underlying these relationships between muscle activity and interocclusal distance require further studies but could be important for the understanding of physiological effects of occlusal splints.

Conflicts of interest statement

The authors report no conflicts of interest.

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Contributors

Tamiyo Takeuchi-Sato

Substantial contributions to the analysis and interpretation of data, and drafting the article.

Taro Arima

Substantial contributions to revising the article critically for important intellectual content.

Michael Mew

Substantial contributions to the conception and design of the study, and the acquisition of data.

Peter Svensson

Substantial contributions to the conception and design of the study, and revising the article critically for important intellectual content.

All authors have read and approved the final article.

Landmarks	Units	Males	Females	P value
N-Me	mm	54.9 ± 6.0	48.0 ± 7.6	0.008*
N-Sn	mm	23.1 ± 3.0	20.5 ± 3.9	0.044*
Sn-Me	mm	31.7 ± 3.5	27.5 ± 4.4	0.005*
Zy-Zy	mm	64.4 ± 5.3	59.5 ± 9.5	0.098
P-P	mm	29.6 ± 2.4	27.7 ± 4.1	0.147
NT/MeGo	degree	35.7 ± 7.1	31.9 ± 6.9	0.130
Sn-Me/N-Me		0.579 ± 0.023	0.573 ± 0.036	0.619
P-P/Zy-Zy		0.460 ± 0.018	0.469 ± 0.052	0.567
Zy-Zy/N-Me		1.18 ± 0.08	1.24 ± 0.09	0.041*

	Pearson's correlation coefficients (R)								
Landmarks	**N-Me**	**N-Sn**	**Sn-Me**	**Zy-Zy**	**P-P**	**NT/ MeGo**	**Sn-Me/ N-Me**	**P-P/ Zy-Zy**	**Zy-Zy/ N-Me**
N-Me	1	0.921**	0.939**	0.766*	0.868**	0.313	-0.236	0.200	-0.646*
N-Sn	0.907**	1	0.732*	0.729*	0.862**	0.209	-0.594*	0.252	-0.575
Sn-Me	0.927**	0.682**	1	0.698*	0.758*	0.361	0.111	0.125	-0.627*
Zy-Zy	0.892**	0.798**	0.835**	1	0.871**	-0.060	-0.256	-0.242	-0.006
P-P	0.934**	0.869*	0.847**	0.848**	1	0.383	-0.370	0.264	-0.321
NT/MeGo	0.078	0.012	0.126	0.054	-0.026	1	0.138	0.891**	-0.558
Sn-Me/N-Me	-0.101	-0.508*	0.277	-0.085	-0.158	0.136	1	-0.203	0.081
P-P/Zy-Zy	0.024	0.096	-0.041	-0.341	0.206	-0.166	-0.158	1	-0.605*
Zy-Zy/N-Me	-0.218	-0.197	-0.204	0.244	-0.183	-0.058	-0.023	-0.801	1

Table 1. Descriptive statistics of anthropometric craniofacial measurements and Pearson's correlation coefficients (R) between nine anthropometric craniofacial factors. Right-upper triangle comprises correlations amongst males and left-lower triangle comprises correlations amongst females. *Significant difference in gender by t-test (*P < 0.050). Correlation is significant (*P < 0.050, **P < 0.001).

		Males				Females			
	%MVOBF	MAL	MAR	TAL	TAR	MAL	MAR	TAL	TAR
N-Me									
	0	0.002	-0.095	-0.361	-0.119	-0.091	-0.216	-0.136	-0.023
	15	-0.401	0.209	-0.341	0.539	-0.049	-0.159	0.090	-0.158
	22.5	-0.270	0.399	-0.456	0.670*	-0.115	-0.246	-0.129	-0.072
	30	-0.185	0.391	-0.291	0.667*	0.174	0.171	-0.337	-0.140
N-Sn									
	0	-0.035	-0.224	-0.258	-0.070	0.030	-0.091	-0.069	0.054
	15	-0.295	0.238	-0.522	0.434	0.125	0.004	0.232	-0.012
	22.5	-0.347	0.431	-0.576	0.566	-0.020	-0.128	0.090	-0.001
	30	-0.144	0.483	-0.374	0.527	0.243	0.162	-0.082	-0.044
Sn-Me									
	0	0.041	0.031	-0.397	-0.147	-0.183	-0.293	-0.172	-0.087
	15	-0.435	0.154	-0.292	0.554	-0.196	-0.281	-0.050	-0.263
	22.5	-0.164	0.318	-0.289	0.669*	-0.184	-0.314	-0.303	-0.124
	30	-0.194	0.254	-0.178	0.699*	0.082	0.150	-0.511	-0.204
Zy-Zy									
	0	-0.137	0.150	-0.436	-0.088	-0.125	-0.228	-0.146	-0.098
	15	-0.273	0.014	0.012	0.389	-0.040	-0.147	0.022	-0.208
	22.5	-0.245	0.243	0.050	0.539	-0.141	-0.219	-0.169	-0.085
	30	0.040	0.386	0.195	0.577*	0.276	0.207	-0.310	-0.098
P-P									
	0	-0.136	-0.147	-0.462	-0.315	-0.018	-0.131	-0.080	0.040
	15	-0.267	0.141	-0.288	0.444	0.006	-0.151	0.111	-0.092
	22.5	-0.443	0.319	-0.328	0.439	-0.081	-0.312	-0.105	-0.058
	30	-0.088	0.473	-0.085	0.464	0.286	0.139	-0.325	-0.804
NT-MeGo									
	0	-0.028	-0.449	-0.337	-0.584*	-0.589**	-0.497*	-0.598**	-0.583*
	15	-0.091	0.251	-0.374	0.289	-0.225	-0.257	-0.124	0.064
	22.5	-0.413	0.172	-0.502	-0.063	-0.152	-0.132	-0.147	0.069
	30	-0.283	0.192	-0.326	-0.063	-0.432*	-0.313	-0.160	0.055
Sn-Me/N-Me									
	0	0.112	0.315	-0.100	-0.071	-0.250	-0.206	-0.092	-0.164
	15	-0.062	-0.113	0.426	0.038	-0.435*	-0.351	-0.310	-0.330
	22.5	0.279	-0.212	0.492	-0.038	-0.217	-0.212	-0.446*	-0.186
	30	-0.049	-0.382	0.333	0.034	-0.234	-0.021	-0.490*	-0.243
P-P/Zy-Zy									
	0	-0.004	-0.566	-0.072	-0.462	0.198	0.186	0.127	0.107
	15	0.005	0.236	-0.579*	0.113	0.098	0.031	0.168	0.223
	22.5	-0.386	0.138	-0.725*	-0.201	0.132	-0.109	0.135	0.063
	30	-0.244	0.163	-0.532	-0.218	-0.046	-0.106	0.017	-0.155
Zy-Zy/N-Me									
	0	-0.194	0.313	0.039	0.108	-0.044	-0.004	-0.008	0.027
	15	0.273	-0.275	0.678*	-0.335	0.071	0.041	-0.149	-0.088
	22.5	0.104	-0.305	0.768*	-0.367	-0.027	0.066	-0.078	-0.022
	30	0.303	-0.123	0.680*	-0.335	0.230	0.046	0.082	0.116

Table 2. Pearson's correlation coefficients (R) between each participant's slope

coefficients (a') of IOD-EMG graphs and anthropometric measurements. Correlation is significant (*P < 0.050, **P < 0.001).

Regression line equation	Males				Females			
$y_{NT/MeGO} = \alpha x_{a'} + \beta$	MAL	MAR	TAL	TAR	MAL	MAR	TAL	TAR
α	-	-	-	-39.0	-23.3	-19.2	-29.3	-21.2
β	-	-	-	35.5	30.9	31.3	31.8	31.3

Table 3. Regression line equation ($y_{NT/MeGo} = \alpha x_{a'} + \beta$) between each participant's slope coefficients (a') of IOD-EMG graphs and mandibular plane angles (NT-MeGo) at 0% MVOBF (α: slope coefficient of regression line equation, β: intercept).

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Figure legends

Fig. 1. Diagrammatic representation of frontal and lateral anthropometric landmarks. Abbreviations indicate; N, Nasion; P, Pupil; Zy, Zygion; Sn, Subnasale; Me, Menton; T, Tragion; Go, Gonion. Two dotted lines indicate the mandibular plane angle.

Fig. 2. IOD-EMG graphs of the data with regression line within each masticatory muscle and linear regression analysis between IOD and EMG-RMS. Each EMG-RMS value represents the mean ± SD. * Significant changes from EMG-RMS at 10-mm IOD within each level of submaximal bite force (solid: male and grey: female) and ↑ significant difference in gender by post-hoc test (Tukey: $P < 0.050$). Mean slope coefficient (a) and Pearson's correlation coefficients (R) (solid: male and grey: female). Correlation is significant (*$P < 0.050$, **$P < 0.001$).

Fig. 3. IOD-EMG graphs of the data within each masticatory muscle and gender. Each value represents the mean ± SD. * Significant changes from EMG-RMS at 10-mm IOD within each level of submaximal bite force (solid: the right (working) side and grey: the left (balancing) side) and ↑ significant difference in side by post-hoc test (Tukey: $P < 0.050$).

Fig. 4. Schematic diagram for an example of regression line equation ($y_{NT/MeGo} = \alpha x_{a'} + \beta$).

Fig. 1.

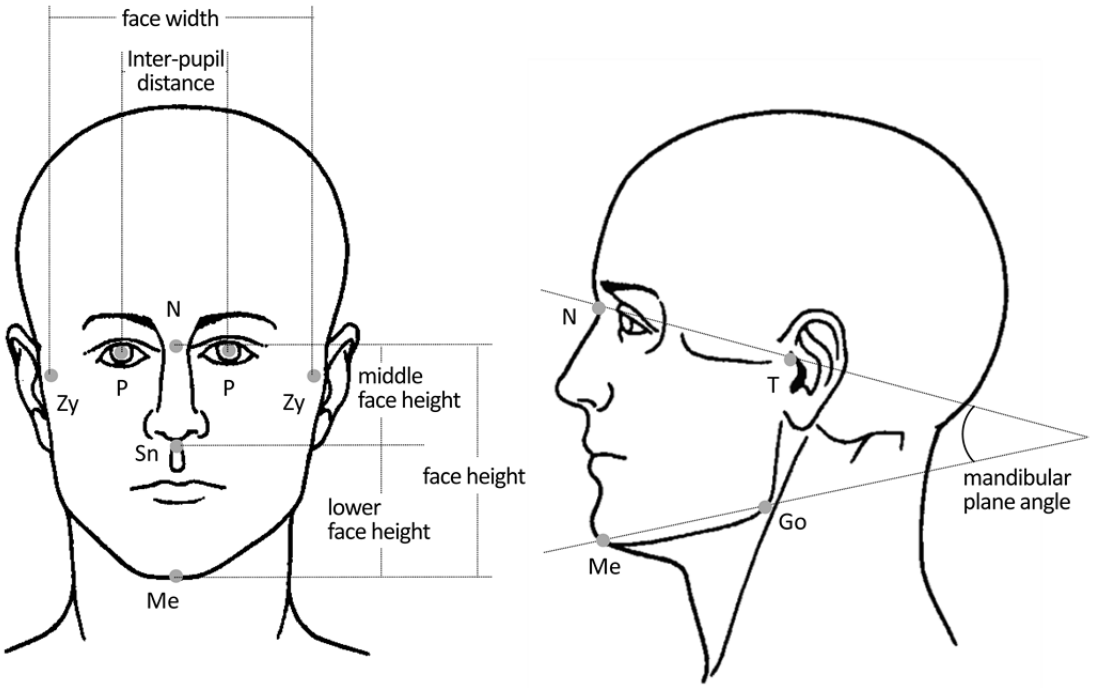

face width
Inter-pupil distance
N
Zy
P
P
Zy
middle face height
Sn
face height
lower face height
Me
N
T
Go
Me
mandibular plane angle

Fig. 2.

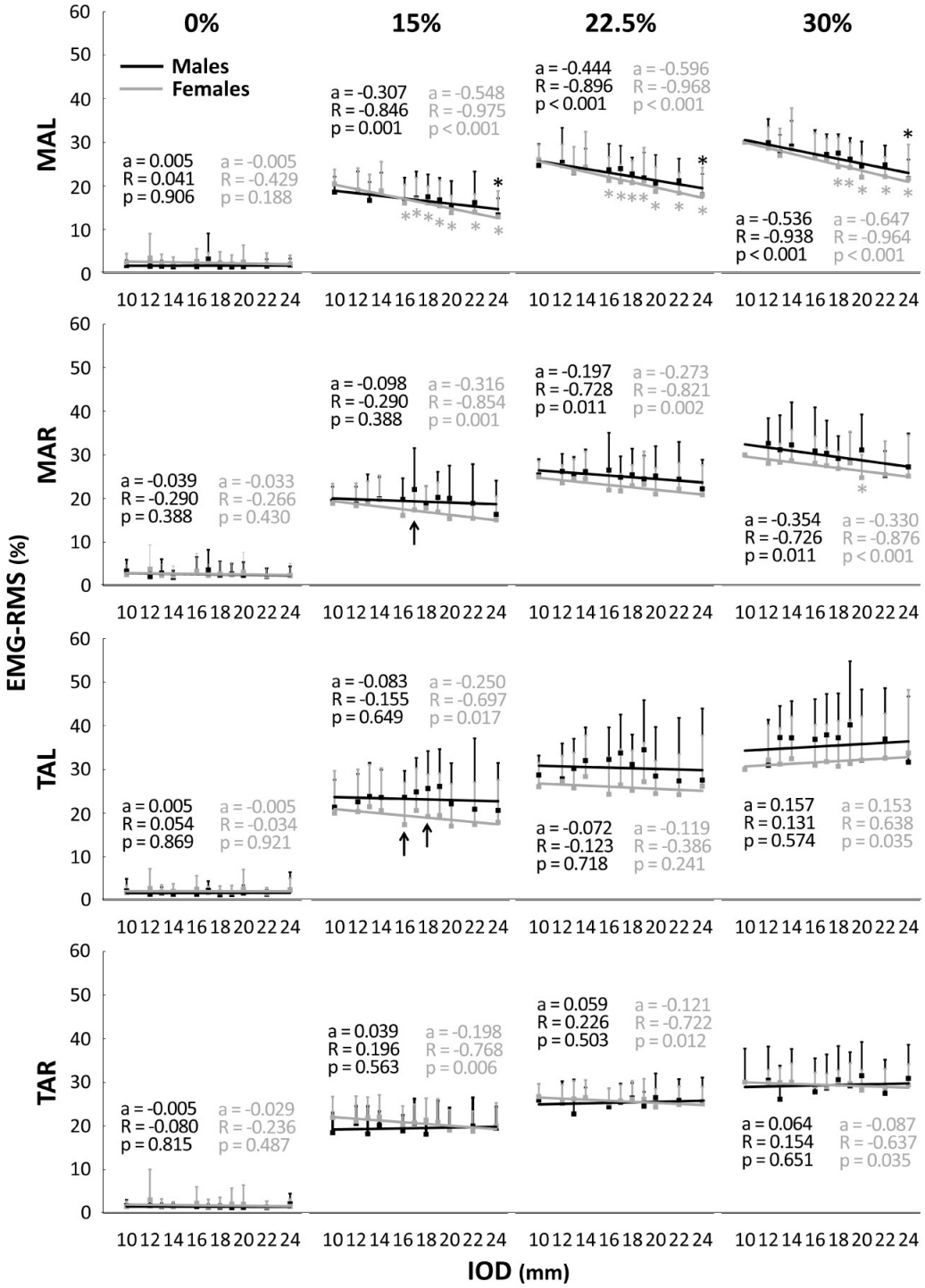

0%
15%
22.5%
30%
Males
Females
MAL
MAR
TAL
TAR
EMG-RMS (%)
IOD (mm)
a = 0.005
R = 0.041
p = 0.906
a = -0.005
R = -0.429
p = 0.188
a = -0.307
R = -0.846
p = 0.001
a = -0.548
R = -0.975
p < 0.001
a = -0.444
R = -0.896
p < 0.001
a = -0.596
R = -0.968
p < 0.001
a = -0.536
R = -0.938
p < 0.001
a = -0.647
R = -0.964
p < 0.001
a = -0.039
R = -0.290
p = 0.388
a = -0.033
R = -0.266
p = 0.430
a = -0.098
R = -0.290
p = 0.388
a = -0.316
R = -0.854
p = 0.001
a = -0.197
R = -0.728
p = 0.011
a = -0.273
R = -0.821
p = 0.002
a = -0.354
R = -0.726
p = 0.011
a = -0.330
R = -0.876
p < 0.001
a = 0.005
R = 0.054
p = 0.869
a = -0.005
R = -0.034
p = 0.921
a = -0.083
R = -0.155
p = 0.649
a = -0.250
R = -0.697
p = 0.017
a = -0.072
R = -0.123
p = 0.718
a = -0.119
R = -0.386
p = 0.241
a = 0.157
R = 0.131
p = 0.574
a = 0.153
R = 0.638
p = 0.035
a = -0.005
R = -0.080
p = 0.815
a = -0.029
R = -0.236
p = 0.487
a = 0.039
R = 0.196
p = 0.563
a = -0.198
R = -0.768
p = 0.006
a = 0.059
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p = 0.503
a = -0.121
R = -0.722
p = 0.012
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p = 0.035

Fig. 3.

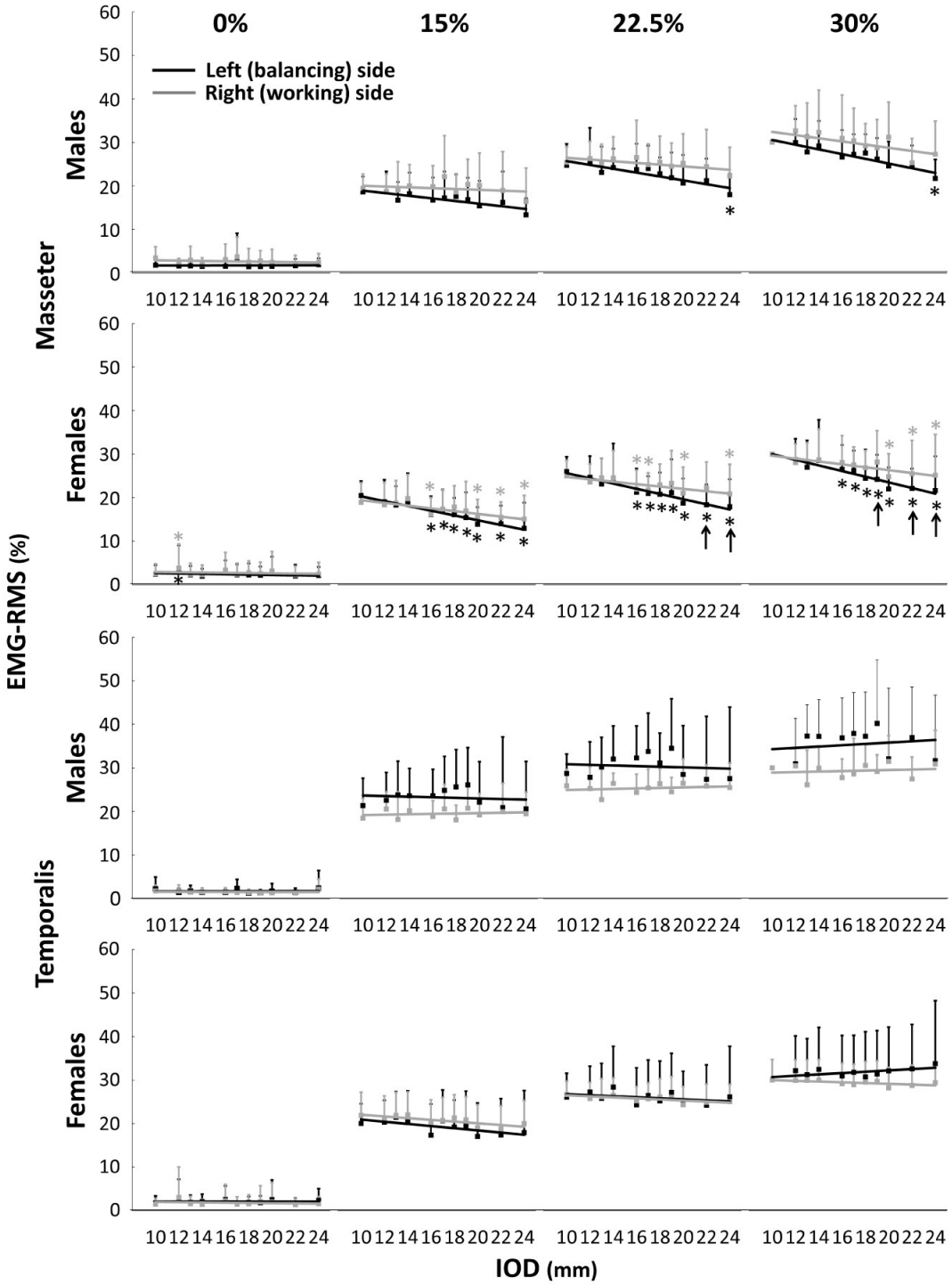
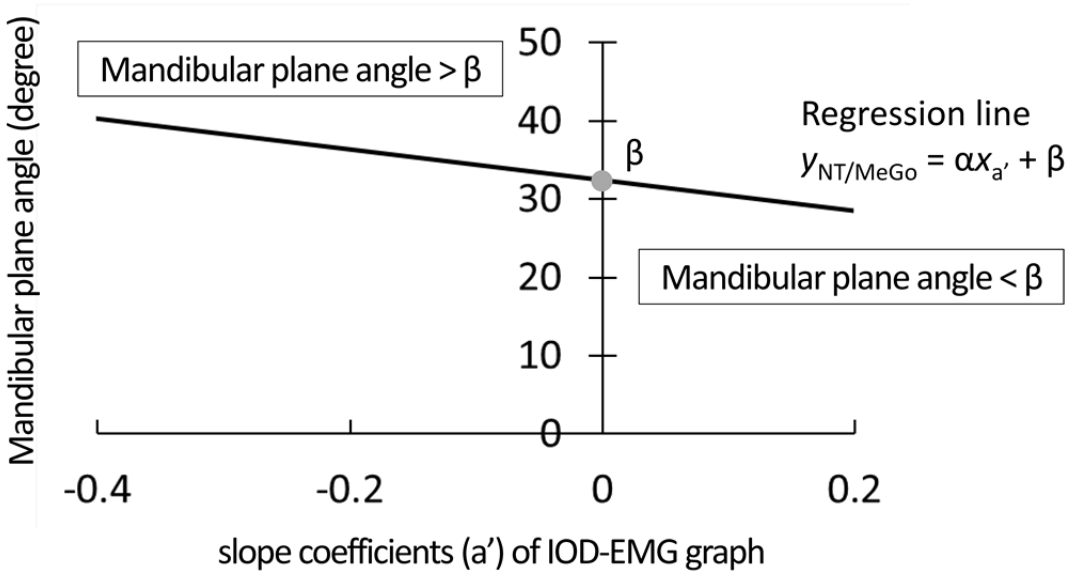

0%
15%
22.5%
30%
Left (balancing) side
Right (working) side
Masseter
Temporalis
Males
Females
EMG-RMS (%)
IOD (mm)

Fig. 4.


Mandibular plane angle (degree)
Mandibular plane angle > β
50
40
β
30
20
10
0
Regression line
$y_{NT/MeGo} = \alpha x_{a'} + \beta$
Mandibular plane angle < β
-0.4
-0.2
0
0.2
slope coefficients (a') of IOD-EMG graph