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Effect of chocolate preference and cocoa content on experimentally induced muscle pain

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Abstract

Objectives Chocolate consumption has been associated with pain reduction, but it remains unclear whether this effect depends on cocoa concentration or individual preference. This study aimed to investigate whether chocolate preference and/or cocoa content modulate experimentally induced muscle pain in healthy men and women.

Methods Thirty healthy, pain-free participants (15 men, 15 women; mean age 24.6 years) underwent three randomized, double-blind, crossover sessions separated by one-week washout periods. Pain was induced twice per session by intramuscular hypertonic saline injections into the masseter muscle, before and after ingestion of white (30% cocoa), milk (34%), or dark (70%) chocolate. Peak-pain intensity and pain-duration were assessed using a visual analogue scale. Data were analyzed using multilevel Poisson regression models accounting for repeated measures.

Results Both chocolate type and individual preference significantly influenced pain outcomes. Milk chocolate produced the strongest analgesic effect, showing lower peak-pain (IRR = 0.75, 95% CI: 0.64–0.88) and shorter pain-duration (IRR = 0.64, 95% CI: 0.57–0.72; $p < 0.01$) compared with dark chocolate. White chocolate showed weaker effects for both outcomes (IRR = 1.09 and 1.11; $p < 0.01$). Participants' most preferred chocolate (milk) produced greater pain-reduction than less preferred types (IRR range 0.61–0.69; $p < 0.01$). Chocolate composition, including sugar and cocoa content, showed no clear association with pain measures under the experimental conditions.

Conclusion The analgesic effect of chocolate in this experimental setting appears to be more closely related to subjective preference and hedonic value than to cocoa concentration. Given the low dose and short exposure time, the findings primarily reflect immediate sensory-affective mechanisms rather than biochemical effects. These results suggest that reward-related processes may contribute to chocolate-induced pain modulation in acute experimental muscle pain.

Keywords Myalgia, Temporomandibular disorders, Masseter muscle, Preference, Cocoa

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Introduction

The complexity of pain and its multifaceted etiology is well known [1–4]. The latter includes biological, behavioral, psychological, social, and environmental factors [5, 6]. Pain is a global health problem that affects society, health care systems, and, not least, individuals in terms of costs and decreased quality of life [7, 8]. The unpleasant sensation of pain is connected to actual or potential tissue damage, or can be described in terms of such damage [9]. Pain can be acute or chronic, and it can be expressed as nociceptive, neuropathic, nociplastic, idiopathic, or mixed pain [10, 11]. While acute pain is a defence mechanism that stimulates reflexes to prevent injury, chronic pain has no known function and persists beyond the usual recovery period, lasting for more than 3 months [12–14]. Thus, musculoskeletal pain is a *sensory* and *emotional* experience that is mainly evoked by nociceptive pathways by the activation of noxious peripheral stimuli on free nerve endings [12–14].

Pain conditions in the orofacial region include, but are not limited to, temporomandibular disorders (TMD) [15]. These pains involve the masticatory muscles, temporomandibular joints as well as their associated structures [16]. Painful TMD is the second most common musculoskeletal disorder after chronic back pain [14, 17], has a prevalence of 5% to 12%, and affects almost twice as many women than men [16, 18–21]. Myalgia in the orofacial region, that is pain in the jaw muscles, is often described as a dull, pressing muscle pain of moderate intensity, which can be intensified when provoked [22]. It affects the mouth opening capacity, causes pain and soreness when chewing and moving the jaw, and is also often attributed to headaches [16].

Experimental pain models are used to investigate pathophysiological aspects and therapeutic approaches to better understand pain. The induced pains should be short-lasting, performed in standardized and controlled settings, with healthy and homogenous participants, without confounding factors of internal and external character, but still mimicking the subjective experience in a clinical setting [20, 23–26]. To chemically induce pain of muscular origin in the orofacial region, hypertonic saline is commonly used, which is considered both safe and valid for use [20, 24–26]. It mimics the sensation of deep and diffuse acute pain and pain referral, experienced by individuals with orofacial pain [20, 25–30].

Chocolate is a widely consumed cocoa-derived product with both nutritional and sensory properties. Cocoa contains bioactive compounds and has historically been attributed medicinal properties due to its chemical composition [31–33]. Experimental studies have suggested that cocoa-enriched diets may influence inflammatory processes and pain-related outcomes [34–36]. However,

the sensory and hedonic properties of chocolate, including sweetness, palatability, and individual preference, may also play a significant role in pain modulation. Experimental evidence indicates that palatable stimuli can activate reward-related neural circuits, including dopaminergic and opioidergic pathways, which are known to influence pain perception [37–42]. In a previous study from our research group using the same experimental model, chocolate intake reduced hypertonic saline-induced muscle pain irrespective of cocoa concentration [43]. However, that study did not specifically evaluate whether the observed analgesic effect was better explained by cocoa content or by subjective preference for a given chocolate type. Importantly, the small amount of chocolate administered and the short interval between intake and pain induction in this experimental paradigm are unlikely to allow sufficient time for systemic biochemical or anti-inflammatory effects to occur. Instead, immediate effects are more likely to reflect sensory-affective and hedonic mechanisms.

Therefore, the aim of the present study was to investigate whether chocolate preference and cocoa content modulate experimentally induced muscle pain. It was hypothesized that chocolate preference would reduce pain, while the role of cocoa content was explored given previous mixed findings regarding compositional and hedonic influences [31, 44, 45].

Materials and methods

Study context and setting

The present study is a secondary analysis of data from a previously published randomized, double-blind, crossover study investigating the effects of chocolate intake on experimentally induced muscle pain [43]. The current analysis specifically examines whether the observed analgesic response is associated with chocolate preference and/or cocoa content. Ethical approval was obtained, a priori, from the Swedish Ethical Review Authority (Dnr: 2019/05785). The study followed the principles for medical research according to the Declaration of Helsinki, as well as the guidelines for Good Clinical Practice (GCP). Further, the study was registered on ClinicalTrials.gov (Identifier: NCT05378984). The enrollment and all experimental sessions took place between March 1st and December 20th, 2020, at the clinical research lab, Department of Dental Medicine, Karolinska Institutet, Huddinge, Sweden. Before inclusion, all participants received both written and verbal information and gave their verbal as well as written consent.

Participants

Thirty healthy, pain-free participants were included according to the predefined eligibility criteria. Detailed

descriptive characteristics of the sample are presented in the Results section, while additional cohort information has been reported previously [43].

Inclusion criteria were: 1) age-matched (± 2 years) men and women; 2) aged between 18–40 years; 3) pain-free; and 4) good general health, i.e. no known conditions. Exclusion criteria were: a) any kind of pain-related diagnosis of TMD; b) headaches; c) systemic muscular or joint diseases (such as fibromyalgia or rheumatoid arthritis); d) whiplash-associated disorders; e) neuropathic pain or neurological disorders; f) psychiatric disorders; g) pain of dental origin; h) intake of use of analgesics of non-steroidal anti-inflammatory drugs during 48 h before the three experimental sessions; and/or i) allergies to any of the substances used. Recruitment of participants was done mainly through information and advertisement at the Department of Dental Medicine at Karolinska Institutet in Huddinge, Sweden.

The minimum sample size was estimated based on a normally distributed standard deviation of 30% [20, 25, 26], a significance level (α) of 0.05 and a power (β) of 99%. The power to detect a significant effect within the 15 pairs was also guaranteed at > 0.999 (99%) using an effect (f) of 3, an error probability (α) of 0.05, and a correlation among the repeated 6 measures of 0.5. This resulted in 13 pairs of healthy and pain-free participants. Considering the risk of dropouts, 15 men and 15 women were included [43]. All participants who were included in the study completed all three sessions without any dropouts, as shown in the CONSORT Flow Diagram, Fig. 1.

Habitual physical activity levels, including type, frequency, and intensity of exercise, were not systematically recorded and were therefore not included as covariates in the present analysis.

Experimental protocol

The experimental protocol had a randomized, double-blind, controlled, and crossover design, where each participant was in his/her own control. The study comprised three separate experimental sessions lasting for approximately one hour each. In each session, experimental pain was induced, with at least one week of washout between each session, as shown in Figs. 1 and 2. Chocolate given to the participants contained a different concentration of cocoa: 30% (white), 34% (milk), and 70% (dark), and was distributed in a random and double-blind order with one sort for each experimental session. To randomize the order in which the chocolates were given to each participant, a randomization list was generated using a web-based randomization tool (www.randomization.com; Seed 19,525). The randomization was done in five blocks of six participants each, by a researcher who did not participate in the data collection (NC).

The participants were seated in a quiet environment and in the same dental chair during all three sessions. At the first session, the participants were informed about the study and interviewed for study suitability. After that, the participants completed questionnaires regarding their psychosocial status, including the screening instruments for stress (Perceived Stress Scale; PSS-10) [46], depression (Patient Health Questionnaire; PHQ-9) [47], pain catastrophizing (Pain Catastrophizing Scale-13; PCS-13) [48], anxiety (Generalized Anxiety Disorder Scale-7; GAD-7) [49], and somatization (Patient Health Questionnaire; PHQ-15) [50], all part of Axis II of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) [16]. The women were also asked about the use of contraceptives or if normally menstruating, the date of the first day of the last menses. Finally, before inclusion, a blinded clinical examination (AH for all men; and VK for all women) according to the DC/TMD Axis I protocol was followed to confirm that the participants fulfilled the inclusion criteria and none of the exclusion criteria.

Baseline recordings of pain characteristics were done. After the baseline recordings, pain was induced, whereafter the assessments of pain characteristics were repeated until pain had subsided, normally within 5 min. After 30 min, one random piece of chocolate (3.6 g) was provided to the participants, and five minutes later, pain was induced (for the second time), and assessments of pain characteristics were repeated. The amount of chocolate administered and the short interval between ingestion and pain induction were chosen to standardize sensory exposure rather than to elicit systemic pharmacological or anti-inflammatory effects. This procedure was repeated during the following two sessions with different concentrations of cocoa. Thus, each participant served as their own control (pre- or post-chocolate intake). This procedure has also been explained in detail in a study by Christidis et al. (2008) [25].

At each subsequent session, the participants were asked about the presence of orofacial pain and/or headache, and the masseter was palpated to ensure that the participants were pain-free, as shown in Fig. 2.

Induction of experimental pain

The most prominent point of the right side's masseter muscle during contraction was used for all injections (i.e., the midline and approximately 2 cm superior to the lower mandibular border). To ensure that the injections were given at the same site in the masseter muscle at all sessions, the point of injection was marked on translucent paper using the participant's nose, outline of the eye, and jawline.



CONSORT 2010 Flow Diagram

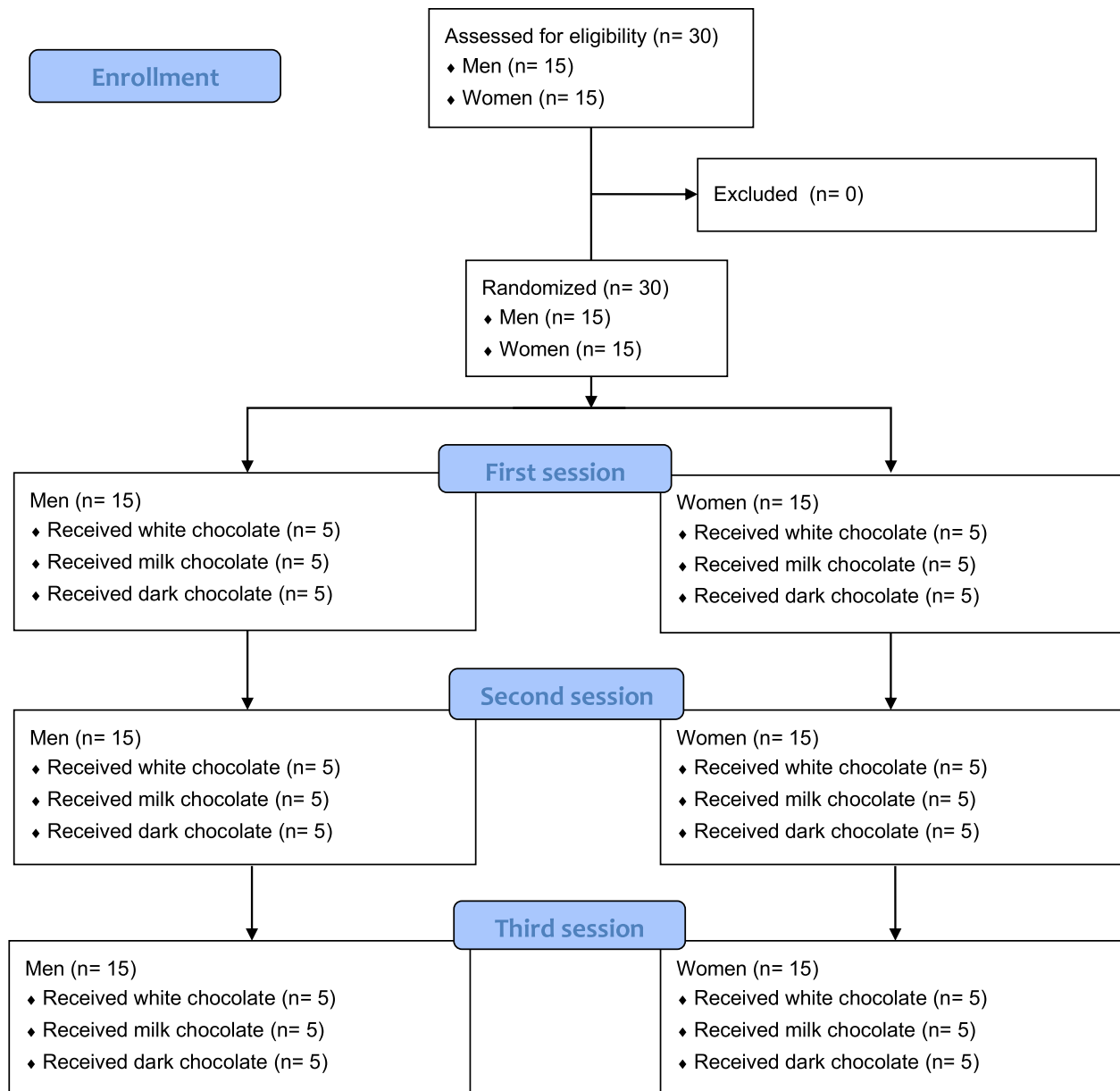


Fig. 1 The CONSORT flow diagram. The CONSORT flow diagram illustrates participant enrollment, randomization, and crossover allocation across the three experimental sessions. Thirty healthy, pain-free participants (15 men and 15 women) were randomized to receive white, milk, and dark chocolate in a double-blind, crossover design with one-week washout periods between sessions. All participants completed all three sessions, and no exclusions or dropouts occurred during the study

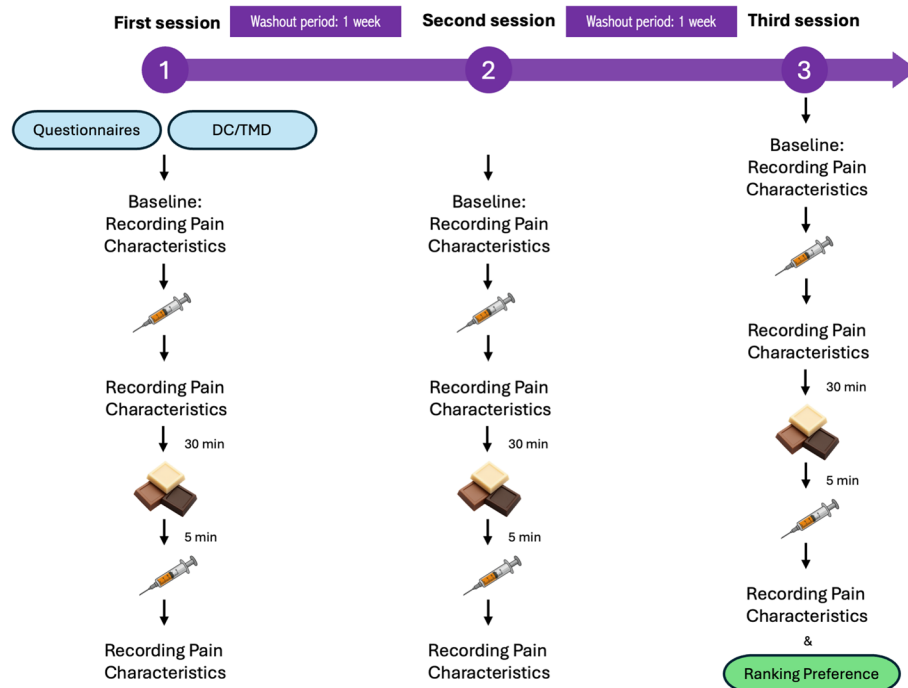


Fig. 2 Flowchart of the experimental protocol. Flowchart of the experimental protocol. Participants completed three separate sessions (first, second, and third), each including a baseline pain recording, induction of experimental pain with hypertonic saline, and reassessment of pain characteristics before and after chocolate intake (30 min post-baseline). Sessions were separated by a one-week washout period. At the final session, participants ranked their chocolate preference. *DC/TMD = Diagnostic Criteria for Temporomandibular Disorders

Acute pain was induced twice at each session, by intramuscular injections (0.2 ml) of sterile hypertonic saline (58.5 mg/ml) by a non-blinded examiner (LA for all men; and LL for all women). Before injection, the skin was cleaned with a swab containing isopropyl alcohol (70%). To ensure intramuscular injection, a cannula of 19*0.4 mm was used and inserted perpendicular to the skin surface covering the masseter muscle to a depth of 15 mm, as previously described by our research group [25].

Intake of chocolate

Three different types of chocolate were given to the participants, one random piece (3.6 g) at a time, in a randomized and double-blind order. The non-blinded examiner (LA for all men; and LL for all women) placed a random piece of chocolate in the participant's mouth, while the participants kept their eyes closed, while the blinded examiner (AH for all men; and VK for all women) did all the assessments. To ascertain that there was no possible carry-over effect, the three different types of chocolate were given at three different sessions to all participants, with at least 1 week of washout between visits. After the three sessions, the participants were asked

to rank the three chocolates based on preference, with 1 being the most preferred and 3 being the least preferred.

The chocolates used in this study were packaged at Gem Chocolates®, in Vancouver, Canada. Wafers from Belcolade manufacture in Belgium, were used. To blind the chocolates, from both the participants and examiners, they were all made uniform in shape and placed in homogeneous white bags. Each bag contained a single type of chocolate (3.6 g) of a given concentration of cocoa. Each type of chocolate is made with a variation in the concentration of cocoa mass (cocoa nibs + cocoa butter), sugar, emulsifier, natural vanilla flavoring, and other products. Please note that cocoa content comes from both cocoa butter and cocoa nibs that together make the cocoa mass (that is, two different brands of 70% dark chocolate can have different percentages of cocoa nibs and cocoa butter and still be 70%). All chocolate samples were identical in shape, texture, firmness, and aroma. Participants were therefore unable to distinguish the chocolates based on sensory properties other than taste. Consequently, the preference ratings obtained after the experimental sessions primarily reflected gustatory liking rather than differences in texture, odor, or appearance. However, because the chocolates differed in taste, complete blinding cannot be fully guaranteed, and potential

recognition based on gustatory perception should be considered when interpreting the findings.

In this study, the composition of the three types of chocolate is presented in Table 1, while the nutritional information of each chocolate is presented in Table 2. The manufacturer also advises that their product may contain traces of soy and eggs.

Assessment of pain variables

The perceived pain intensity was continuously graded on a 0 to 100 mm Visual Analogue Scale (VAS), with 0 representing “no pain” and 100 representing “worst imaginable pain”. Pain intensity was marked every fifteen seconds on command for 5 min. At the end of the last grading, 5 min after injection, the participants were asked to mark the maximal subjective induced pain spread using a chart drawing with two lateral views of the head, one extraoral

Table 1 Characteristics of the participants, ranking of chocolate preference, and composition of chocolates according to the three types of included chocolate (white – 30%; milk – 34%; dark – 70%)

Variables	White chocolate	Milk chocolate	Dark chocolate
Sex [n (%)]			
Men	15 (50)	15 (50)	15 (50)
Women	15 (50)	15 (50)	15 (50)
Age [mean (SD)^a]	24.6 (3.0)	24.6 (3.0)	24.6 (3.0)
Chocolate preference [n = 28 (%)]			
Best	0 (0)	28 (100)	0 (0)
Middle	16 (57)	0 (0)	12 (43)
Least preferred	12 (43)	0 (0)	16 (57)
Peak pain [mean (SD)^a] before intake	74.3 (27.1)	74.8 (20.1)	70.1 (24.5)
Peak pain [mean (SD)^a] after intake	72.5 (22.9)	67.9 (26.4)	66.6 (29.2)
Pain duration [mean (SD)^a] before intake	210.6 (121.7)	193.5 (93.2)	193.8 (84.3)
Pain duration [mean (SD)^a] after intake	182.8 (91.2)	168.8 (78.8)	170.7 (72.2)
Composition (%)			
Cocoa beans	0	9.8	67.5
Cocoa butter	30	25.9	3.2
Sugar	43.2	42.9	28.3
Whole milk powder	21.2	21	0.5
Sunflower lecithin	0.6	0.4	0.4
Natural vanilla extract	0.04	0.04	0.005
Dried skimmed milk	5	0	0

^a SD, standard deviation

Table 2 Nutritional information of the three types of chocolate (white – 30%; milk – 34%; dark – 70%) given to 15 healthy and pain-free men and 15 healthy and pain-free age-matched women before induction of experimental muscle pain

Values per 100 g	White chocolate	Milk chocolate	Dark chocolate
Energy (Kcal)	576	557	566
Energy (Kj)	2 402	2 312	2 351
Fat	37.1	35.0	42.0
Fat, of which saturated sat	22.5	21.4	26.2
Carbohydrate	54.3	53.1	32.6
Carbohydrate, of which sugars	54.3	52.2	28.3
Fibre	0	1.9	11.0
Protein	6.2	6.9	8.2
Salt	0.2	0.2	0.01

and one intraoral. The complete course of the study is shown in Fig. 2.

For each injection, peak pain intensity was defined as the highest VAS value recorded during the 5-min assessment period following the hypertonic saline injection. Pain duration was defined as the time in seconds from the injection until the participant reported no pain (VAS=0), or until the end of the 5-min observation period. These outcomes were obtained for both the pre-intake (baseline) and post-intake injections performed within each experimental session.

Statistical analysis

Statistical analysis was performed on data regarding peak pain intensity as well as pain duration using STATA 14.0 (Stata Corporation, College Station, TX, USA). A descriptive analysis was carried out according to the different types of chocolate. A Pearson correlation between the composition of the chocolates and the outcomes "peak of pain" and "pain duration (in seconds)" was conducted. To test the normality of the data, the Shapiro–Wilk's test was used.

A multilevel Poisson regression model for repeated measures was used to evaluate the impact of chocolate preference and/or type of chocolate on pain reducing effect. The model used a fixed effects model with a random intercept. The repeated measures of outcomes over time (level 1) were nested in the participants (level 2). Variables that presented $p \leq 0.20$ in the unadjusted analysis were included in the adjusted model. Results were presented as Incidence Rate Ratio (IRR) and 95% confidence interval (95% CI). The significance level was set to $p < 0.05$ for all tests.

Because the outcome variables (peak pain intensity and pain duration) were non-negative and exhibited right-skewed distributions, multilevel Poisson regression models with a log link function were applied to estimate relative differences between conditions while accounting for repeated measures within participants. The main analyses focused on post-intake outcomes following each chocolate condition. The pre-intake injection served as a standardized within-session baseline but was not modeled as a separate repeated factor in the primary multilevel analysis. Although Poisson models are traditionally used for count data, they have also been applied to non-negative skewed outcomes in experimental settings. However, alternative approaches such as linear mixed-effects models may be more appropriate for continuous outcomes such as VAS scores and should be considered in future studies. Model assumptions, including distributional characteristics and overall model fit, were

evaluated to ensure appropriateness of the chosen analytical approach.

Results

The study included 30 healthy, pain-free participants (15 men and 15 women) with a mean age of 24.6 years. Baseline psychosocial measures were generally low, and no marked differences were observed between men and women or in the randomization order of chocolate administration, consistent with previously reported cohort characteristics [43].

Twenty-eight out of 30 participants provided complete preference rankings. Milk chocolate was considered the favorite kind of chocolate for 28 out of 30 (15 men and 13 women) participants on a scale from 1 to 3. White chocolate was considered the second-favorite kind of chocolate for 16 (7 men and 9 women), while dark chocolate was the least-favored kind of chocolate and considered second favorite kind of chocolate for 12 participants (8 men and 4 women).

Type of chocolate

The type of chocolate significantly influenced both pain outcomes. As shown in Table 3, adjusted multilevel Poisson regression demonstrated that milk chocolate produced the strongest analgesic effect, with significantly lower peak pain intensity (IRR=0.75, 95% CI 0.64–0.88, $p < 0.01$) and shorter pain duration (IRR=0.64, 95% CI 0.57–0.72, $p < 0.01$) compared with dark chocolate (reference). In contrast, white chocolate was associated with higher pain ratings and longer pain duration (IRR=1.09 and 1.11, $p < 0.01$), indicating a weaker pain-reducing effect. These findings confirm that milk chocolate was most effective, followed by dark and white chocolate, respectively.

Preference

Chocolate preference also showed a clear effect. As illustrated by Table 3, participants' most favored chocolate (milk) produced significantly lower peak pain and pain duration compared with their second (white) and least preferred (dark) types (IRR range=0.61–0.69, $p < 0.01$), further supporting that individual preference enhances the analgesic response.

Composition of chocolate

When it comes to the compositions of the chocolate, none of the ingredients seem to correlate to peak pain intensity or pain duration. As Table 4 shows, the Pearson correlation coefficient was weak and ranged between -0.07 to 0.07 for peak pain intensity and -0.05 to 0.08 for pain duration.

Table 3 Multilevel Poisson regression showing associations between chocolate type, preference, and pain outcomes (lower IRR = greater pain reduction) ($n = 30$)

Variables	Peak of pain		Pain duration (seconds)	
	IRR ^a Unadjusted (95% CI) ^b	P-value	IRR ^a Unadjusted (95% CI) ^b	P-value
Sex				
Men	1	0.94	1	< 0.05
Women	0.98 (0.76–1.28)		0.64 (0.45–0.90)	
Age (in years)	0.94 (0.91–0.98)	< 0.01	0.93 (0.88–0.99)	< 0.05
Chocolate type				
Dark	1	0.09	1	< 0.01
White	1.05 (0.99–1.11)		1.08 (1.04–1.12)	
Milk	1.05 (0.99–1.11)		0.99 (0.96–1.03)	
Chocolate preference				
Best (Milk)	1	< 0.01	1	> 0.05
Middle (White)	0.91 (0.85–0.97)		1.00 (0.96–1.04)	
Least preferred (Dark)	0.94 (0.88–1.00)		1.01 (0.97–1.05)	
<i>Composition</i>				
Cocoa beans	0.99 (0.99–1.00)	0.06	0.99 (0.99–0.99)	< 0.01
Cocoa butter	1.00 (0.99–1.00)	0.06	1.00 (1.00–1.00)	< 0.01
Sugar	1.00 (0.99–1.00)	0.06	1.00 (1.00–1.00)	< 0.01
Whole milk powder	1.00 (0.99–1.00)	0.06	1.00 (1.00–1.00)	< 0.01
Sunflower lecithin	1.13 (0.87–1.46)	0.35	1.52 (1.30–1.77)	< 0.01
Natural vanilla extract	4.32 (0.96–19.43)	0.06	3.28 (1.34–8.01)	< 0.01
Dried skimmed milk	1.00 (0.99–1.01)	0.35	1.01 (1.01–1.02)	< 0.01
Chocolate type				
Dark	1	< 0.01	1	< 0.01
White	1.09 (1.02–1.16)		1.11 (1.07–1.15)	
Milk	0.75 (0.64–0.88)		0.64 (0.57–0.72)	
Chocolate preference				
Best (Milk)	1	< 0.01	1	< 0.01
Middle (White)	0.66 (0.56–0.78)		0.61 (0.55–0.69)	
Least preferred (Dark)	0.69 (0.59–0.82)		0.64 (0.57–0.71)	

Very small effect sizes reflect minimal variability and should be interpreted as indicating no meaningful association

Reference category for chocolate type = dark; reference category for preference = best (milk)

^a IRR, incidence rate ratio

^b CI, confidence interval

Discussion

The primary findings of this randomized, double-blind, crossover experimental study were that both chocolate type and subjective preference were associated with pain outcomes following intramuscular hypertonic saline injections into the masseter muscle. Milk chocolate was associated with lower peak pain intensity and shorter pain duration compared with dark chocolate, and the most preferred chocolate produced the greatest analgesic response. In contrast, exploratory analyses of chocolate composition did not demonstrate meaningful associations with pain outcomes within the limited dose and short time window applied in this study. Therefore, the present findings suggest that the

immediate analgesic effects observed are more consistent with sensory-affective and hedonic mechanisms rather than immediate biochemical effects of cocoa constituents under the conditions tested.

The analysis of chocolate type revealed notable differences in its analgesic outcomes. Although dark chocolate contains a higher cocoa solid content (70%) and fiber levels (11.0 g/100 g), its analgesic benefit was not superior. Instead, milk chocolate (34% cocoa) yielded the most substantial reduction in both pain duration and intensity. The findings suggest that in acute experimental pain, the influence of chocolate type, palatability, and balanced sensory characteristics may outweigh purely biochemical components. This interpretation aligns with literature

Table 4 Pearson correlation between the composition of the chocolates and the outcomes "peak of pain" and "pain duration (in seconds)"

Chocolate	Peak of pain	Pain duration (in seconds)
Composition (%)		
Cocoa beans	−0.07	−0.05
Cocoa butter	0.07	0.05
Sugar	0.07	0.04
Whole milk powder	0.07	0.04
Sunflower lecithin	0.03	0.08
Natural vanilla extract	0.07	0.04
Dried Skimmed milk	0.03	0.08

No correlations reached statistical significance

demonstrating that palatable foods activate endogenous opioid and dopaminergic circuits, particularly the nucleus accumbens and periaqueductal gray, facilitating a descending pain inhibition [37, 38]. Neuroimaging studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) further corroborate these mechanisms, demonstrating opioid release during feeding and reduced pain-related cortical activity after sweet taste stimulation [39, 40]. Thus, the analgesic effects associated with chocolate type seem to derive primarily from general palatability features, rather than compositional metrics such as cocoa solids or fiber content.

Preference analyses extended these findings by emphasizing the subjective dimension of reward. Milk chocolate, ranked as the most favored option by the majority of participants (28/30), provided the strongest pain reduction, underscoring the role of hedonic valuation in pain modulation. These findings align with broader literature on reward-induced analgesia. Specifically, this mechanism is well-established in some studies, where reward anticipation and consumption of a desired stimulus attenuate pain through opioidergic and dopaminergic circuits [41, 42]. Previous studies have demonstrated that individual preference taps into motivational expectation and can strongly influence pain perception through placebo-like mechanisms, mediated by cortical and sub-cortical regions involved in reward and emotion [51, 52]. This raises compelling parallels with taste hedonics research, where individual differences in sweet-liking phenotypes (e.g., extreme sweet likers vs. sweet dislikers) have been shown to influence not just food preference but emotional processing and even pain sensitivity [53, 54]. Preference appears to amplify pain analgesia,

possibly via a combined dopaminergic-opioidergic pathway that integrates reward salience and nociceptive gating. The findings emphasize the subjective preference over its objective nutritional or pharmacological properties (e.g., cocoa content). However, interpretation of the preference findings should be made with caution, as chocolate type and preference were strongly aligned in this sample, with milk chocolate being preferred by the majority of participants. Therefore, the independent effects of chocolate type and preference cannot be fully disentangled.

Despite differences in sugar, fat, protein, and cocoa-related composition, no meaningful associations were observed between chocolate composition and pain outcomes. However, this finding should be interpreted with caution. The amount of chocolate administered (3.6 g) and the short interval between intake and pain induction were not designed to capture delayed pharmacological or anti-inflammatory effects. Cocoa flavanols are known to exert biological effects in a dose- and time-dependent manner [35, 55], and therefore the present results primarily reflect immediate sensory exposure rather than systemic biochemical effects. Accordingly, the lack of association between composition and pain outcomes in this study does not exclude potential effects under different dosing or temporal conditions.

In an exploratory secondary finding, women showed shorter pain duration than men. Evidence has demonstrated that sex-related biological factors, including hormonal influences and receptor-level differences in opioidergic and dopaminergic pathways, can shape immediate pain responses [56, 57]. Based on this, the shorter pain duration observed in women in the present study suggests that hedonic modulation of pain may be partly sex dependent. Importantly, such findings in acute experimental models must be distinguished from the epidemiology of chronic orofacial pain, where women present higher prevalence rates and greater symptom burden [58]. Chronic pain involves complex and long-term processes such as central sensitization, neuroimmune interactions, and psychosocial comorbidities, which may override or even reverse the modulatory advantages observed in acute contexts [59]. Because sex differences were not a primary objective and the study was not powered for this analysis, this finding should be interpreted with caution.

The clinical implications of these findings should be interpreted cautiously. Although the results suggest that hedonic and preference-related factors may influence acute pain perception, the study was conducted in healthy young adults under controlled experimental conditions. Therefore, the findings do not directly translate to clinical pain populations. Rather than supporting

specific dietary interventions, the results highlight the potential importance of individual preference and sensory-affective factors in modulating pain. Future studies should investigate whether such mechanisms can be leveraged in clinical settings, particularly in patients with TMD or chronic pain conditions.

Study strengths and limitations

This study presents several methodological strengths. The double-blind, randomized, cross-over design minimized both interindividual variability and expectancy bias, with each participant serving as their own control and ≥ 1 -week washout intervals effectively reducing potential carry-over effects. The psychological homogeneity of the cohort was ensured through validated psychometric screening instruments (PSS-10, GAD-7, PHQ-9, PCS-13, PHQ-15), thereby strengthening internal validity. Experimental pain induction was conducted using a rigorously standardized hypertonic saline protocol, a well-established method that enhances reproducibility and construct validity. Moreover, all chocolate samples were professionally standardized in shape, packaging, and dosage, which reinforced the integrity of the blinding process and minimized perceptual confounds.

Limitations include a sample consisting of healthy young adults, reducing generalizability to chronic pain or older populations. Preference rankings were retrospective, introducing minor recall bias despite blinding and randomization. Laboratory settings also cannot fully replicate the psychosocial context of pain modulation. As this manuscript constitutes a secondary analysis of an existing dataset, outcome variables, including pressure pain thresholds and pain area, were intentionally not reanalyzed to prevent duplicate reporting of previously published results. Future research should include dose-response testing, neuroimaging studies, and TMD patient populations to explore clinical translation. Another limitation relates to the choice of statistical model. Although multilevel Poisson regression was applied to account for the non-negative and skewed nature of the data, the outcome variables, particularly VAS-based peak pain intensity, are continuous measures. Alternative modeling approaches, such as linear mixed-effects models, may provide a more appropriate framework and should be considered in future studies. Furthermore, habitual physical activity was not assessed, which may have influenced pain sensitivity and endogenous pain modulation.

Conclusions

This study suggests that chocolate's pain-reducing effect in an acute orofacial muscle pain model is more closely related to hedonic and affective mechanisms associated with individual preference than to its biochemical

composition under the tested conditions. Milk chocolate, which was most strongly preferred, yielded the most pronounced reduction in both peak pain intensity and pain duration, whereas dark and white chocolate were less effective. The absence of correlation between chocolate composition and pain outcomes indicates that immediate analgesia is unlikely to arise from nutritional or pharmacological properties, but rather from activation of reward-related neural pathways.

These findings highlight the importance of subjective liking and reward in modulating pain perception and suggest that hedonic stimuli could potentially enhance endogenous pain inhibition. Future research should explore the neurobiological underpinnings of this phenomenon using neuroimaging and examine its translational potential in clinical pain management, particularly for conditions such as temporomandibular disorders.

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Authors' contributions

Maria Christidis: Conceptualization, Formal analysis, Writing – Original draft preparation; ***Dyanne Medina Flores:*** Formal analysis, Writing – Original draft preparation; ***Anastasios Grigoriadis:*** Conceptualization, Methodology, Writing—Review & Editing; ***Abhishek Kumar***: Writing—Review & Editing. ***Giuliana Estefanía Lunecke Serendero:*** Formal analysis, Data curation, Writing—Review & Editing; ***Mario Brondani***:*** Conceptualization, Methodology, Writing—Review & Editing; ***Alexandra Hajati:*** Formal analysis, Investigation, Data curation, Writing—Review & Editing; ***Giancarlo De la Torre Canales:*** Conceptualization, Methodology, Data curation, Formal analysis, Writing—Review & Editing; ***Bruna Brondani:*** Data curation, Statistical analysis, Writing—Review & Editing; ***Nikolaos Christidis:*** Conceptualization, Methodology, Data curation, Formal analysis, Resources, Writing—Review & Editing, Supervision.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Swedish Ethical Review Authority with (Dnr: 2019/05785), and the project followed the principles for medical research according to the declaration of Helsinki, as well as Good Clinical Practice (GCP) guidelines.

Consent for publication

All participants have given written consent.

Competing interests

The authors declare no competing interests.

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