Matjaž Pirc

STOP AND SMELL THE FAT!

Exploring the role of olfaction in dietary fat perception

PROPOSITIONS

1. Fat itself does not elicit pleasant odour sensations
   (this thesis).

2. Effective solutions for fat overconsumption go beyond fat odour-related food manipulations (this thesis).

3. Studies investigating food choice need to account for factors of time, effort, and finances to be ecologically relevant.

4. Neurodivergence is an advantage in scientific careers.

5. An effective climate change mitigation plan needs to include mandatory meat rationing.

6. The greatest challenge in addressing climate change lies in overcoming our predisposition for delay discounting.

Propositions belonging to the thesis, entitled:
Stop and Smell the Fat! Exploring the Role of Olfaction in Dietary Fat Perception

Matjaž Pirc
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TABLE OF CONTENTS

1

Chapter 1

Introduction

BACKGROUND

Obesity, one of the most detrimental chronic health conditions of our time, has nearly tripled since 1975 and is currently afflicting more than 650 million adults globally (WHO, 2021). In fact, based on the European regional obesity report (WHO, 2022), not a single European Union member state is on track to reach the target of halting the rise of obesity by 2025. This potentially debilitating disease is associated with a higher risk of numerous health problems, including heart disease, diabetes, musculoskeletal disorders, and various cancers. It is therefore a major societal challenge, impacting individuals as well as the broader economy (Kumar & Kutty, 2021) and requiring multidisciplinary solutions (Samaras et al., 2019).

The primary driver of obesity is excessive caloric intake stemming from overconsumption of energy-dense foods, compounded by decreased physical activity. Among macronutrients, dietary fat is the most calorie-dense, providing nine kilocalories per gram – more than twice the energy compared to carbohydrates and protein. This translates into foods with a higher fat content inherently having a higher energy density (Drewnowski et al., 2004; Rolls et al., 2014). In addition to being energy dense, high fat foods tend to be highly palatable (Blundell & MacDiarmid, 1997; Drewnowski & Almiron-Roig, 2009), which makes them seemingly universally preferred (Drewnowski & Almiron-Roig, 2009). Given that palatability stimulates consumption (McCrickerd & Forde, 2016), this exacerbates the likelihood of overindulgence, leading to increased total energy intake and, in the long term, overweight and obesity (Blundell & MacDiarmid, 1997; Donahoo et al., 2008; Viskaal-van Dongen et al., 2009; WHO, 2023). One of the key proposed approaches towards effective weight management is therefore to reduce the intake of energy dense foods, such as high-fat ones (Rolls et al., 2014; WHO, 2022). Yet, despite the longstanding efforts aimed at curbing dietary fat overconsumption, intakes remain high, exceeding recommendations in numerous countries (Eilander et al., 2015; Shen et al., 2017; Vadiveloo et al., 2014; Van Rossum et al., 2020; WHO, 2021).

Nutritional guidelines targeting obesity frequently focus exclusively on limiting certain nutrients to decrease energy density, neglecting the sensory characteristics linked to those nutrients. To illustrate, while the recently revised World Health Organisation dietary guidelines on recommended dietary fat intakes (WHO, 2023) acknowledge the role of palatability in fat overconsumption, their recommendations primarily emphasize fat reduction without addressing how fat-related sensory characteristics could be considered. Since sensory cues play a pivotal role in the way we choose, consume and experience foods, understanding the mechanisms underlying sensory perception of fat might be crucial for the success of nutritional strategies (Forde & de Graaf, 2022).

From a sensory perspective, dietary fat is an important functional ingredient, acting as a carrier of flavour and a textural component in many food products (Drewnowski & Almiron-Roig, 2009; Sanders, 2016). Its sensory attributes depend on factors such as viscosity, fat particle size, volatile components, and the presence of other nutrients (Guichard, 2002; Guichard et al., 2018; Tepper & Kuang, 1996). While historically, the perception of fat was largely attributed to its mouthfeel and post-ingestive effects (Drewnowski & Almiron-Roig, 2009), fat perception is now understood as a complex interaction between multiple sensory modalities. This includes various senses involved in texture perception (touch, vision and hearing), the trigeminal system, taste and olfaction (Drewnowski & Almiron-Roig, 2009; Mattes, 2005; Running & Mattes, 2016). Oral fat texture or mouthfeel, characterised by sensations such as creaminess, thickness, oiliness and smoothness, mainly arises from the dynamic interaction between the food's physical properties and the somatosensory system (Engelen & Van Der Bilt, 2008). Fat taste (so-called "oleogustus"), on the other hand, appears to arise when free fatty acids bind to fatty acid taste receptors located on the tongue (Costanzo et al., 2019; Keast & Costanzo, 2015; Running & Mattes, 2016). While the consideration of fat taste as the sixth basic taste modality remains a topic of debate, there's convincing evidence that free fatty acids elicit distinct gustatory sensations. Free fatty acids can also activate the trigeminal system, producing irritation-like sensations (Running & Mattes, 2016). Whereas mouthfeel, trigeminal and taste-related sensations are primarily relevant only once food enters the oral cavity, the olfactory sense plays a role even before food reaches the mouth.

Olfaction is a dual (chemo)sensory system, capable of detecting odorants both from the external environment and from within the mouth (Rozin, 1982). When ambient odours enter the nasal cavity through the nostrils, this is described as orthonasal olfaction. Conversely, when volatile compounds (i.e. aromas) are released from food during chewing and enter the nasal cavity via the nasopharynx, this is described as retronasal olfaction. Regardless of the route, all odorants bind to the same receptors located in the olfactory epithelium, which runs along the top of the nasal cavity

(Goldberg et al., 2018) (see Figure 1 for an illustration of the two olfactory routes). Despite sharing receptors, the two olfactory routes are considered to serve distinct, yet complementary functions. In the context of food perception, orthonasal olfaction is considered a detection mechanism for food sources in our environment, which helps induce appetite in the anticipatory phase of eating. In contrast, retronasal olfaction is a principal component of food flavour perception in the consummatory phase of eating and may influence intake and satiation to a degree (Boesveldt & de Graaf, 2017; Goldberg et al., 2018).

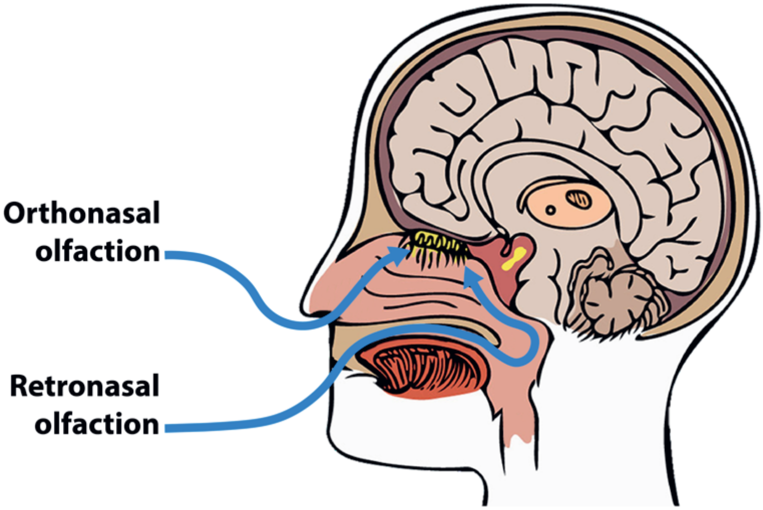

Figure 1. *Illustration of the two olfactory pathways*

Compared to oral fat perception (encompassing oral texture and taste), olfactory fat perception has received relatively little attention. While research shows that humans can detect vapour-phase fatty acids ortho- and retronasally (Bolton & Halpern, 2010; Chale-Rush et al., 2007), there is limited evidence on how olfaction is involved in fat perception within complex food matrices (i.e. real foods). Although there are studies investigating fat flavour perception in various foods, none specifically focus on the fundamental role of olfaction. Moreover, the overlap of olfaction with other sensory modalities in these studies makes it challenging to assess its distinct contribution. To truly understand the role of olfaction in fat perception, it is imperative to also assess it in isolation. The study by Boesveldt and Lundstrom (2014) stands out, as it demonstrated that humans could discriminate dairy milks differing in fat content using only orthonasal cues. However, comprehensive research on the topic, especially on retronasal olfaction, remains sparse. Notably, the scarcity of studies on retronasal olfaction can be attributed to challenges in stimulus delivery and control (due to anatomical limitations). Moreover, isolating retronasal olfaction from other modalities involved in flavour perception is inherently complex (Goldberg et al., 2018). Since olfaction plays a pivotal role in flavour perception, particularly retronasal, overcoming these limitations is crucial to expand our understanding of fat perception. In fact, given the subtlety of perceptual fatty acid taste effects (Mattes, 2005), the failure of mouthfeel-related sensations to account for certain aspects of fat perception (Jervis et al., 2014; Mattes, 2005) and the common misinterpretation of retronasal sensations as being related to taste (Goldberg et al., 2018; Spence, 2016), olfaction may be a pivotal component in the alluring flavour of fat. This is underscored by Jervis et al. (2014) who demonstrated that blocking retronasal olfaction (using nose clips) diminishes the perception of creaminess in sour cream. Considering that fat-related attributes such as creaminess are key drivers of sensory appeal (Frøst & Janhøj, 2007) facilitating intake (Forde & de Graaf, 2022), understanding olfaction's role in their perception is essential.

To summarise, it is unclear how **fat content** relates to the perception of food odours and how fat-related odours contribute to the **perception of fatty foods**. The ability of fatty odours to influence **eating behaviour** is unexplored as well, as are the **neural mechanisms underpinning fat perception**. Investigating the contribution of olfaction to fat perception, particularly in real foods, could provide vital insights into the broader sensory experience of fat. Not only would this fill relevant knowledge gaps but it could potentially lead to more effective strategies aimed at curbing the overconsumption of fat-laden foods. By understanding the influence of olfactory cues on fat perception, new food product reformulation possibilities might arise. For instance, utilising fat-related food odours as fat substitutes could be a promising strategy for reducing fat content, while preserving the pleasurable qualities associated with fatty foods.

THESIS AIM AND OVERVIEW

There is evidence supporting olfaction's role in the perception of dietary fat. However, compared to other sensory modalities involved in fat perception, olfaction has been studied less extensively, especially within complex food matrices. This thesis therefore aims at investigating the role of olfaction in the perception of dietary fat in food; assessing the potential of fat-related odours to influence food-related hedonics and steer eating behaviour; and exploring factors underpinning fat-related odour perception. See Table 1 for an overview of primary research objectives per chapter.

To establish a solid theoretical foundation for the subsequent studies described in this thesis, we initially summarised relevant evidence on the topic and identified key knowledge gaps. Accordingly, **Chapter 2** presents findings of a systematic scoping review on the contribution of olfaction to dietary fat perception in humans and rodents. The chapter serves as a general introduction to the main theme of the current thesis.

Chapter 3 describes two experiments conducted to replicate previous findings on the human ability to discriminate fat content in foods using solely orthonasal cues (Boesveldt & Lundstrom, 2014) and evaluate whether they extend to retronasal olfaction. In both experiments we isolated both olfactory routes from potentially confounding sensory modalities and investigated the human ability of discriminating food fat content in dairy milk covering a wide range of fat content. We also explored effects of habitual intake on fat content discrimination ability in both.

To establish the neural underpinnings of observations from our previous study, we delved into the realm of neuroimaging, as described in **Chapter 4**. By conducting a combination of laboratory and functional magnetic resonance imaging (fMRI) experiments, we investigated olfactory fat content discrimination and perception, and their potential relationship with brain activation resulting from exposure to fat-related odours – dairy milk varying in fat content.

Chapter 5 describes an innovative approach to studying the impact of retronasal fat-related odours on perception and eating behaviour. We carried out a combination of sensory and behavioural experiments to explore the utility of retronasal fat-related odours as fat substitutes and examined the impact of retronasal exposure to fat on subsequent ad-libitum food intake.

Lastly, **Chapter 6** reflects on our main findings and their practical implications, discusses the methodological aspects of the described studies, and offers recommendations for future research on the topic.

Table 1. *An overview of primary research objectives per chapter*

Chapter	Primary Research Objectives
2	To systematically identify and summarise relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents and highlight relevant knowledge gaps.
3	To investigate whether humans can discriminate fat content in dairy milk (ranging from 0% to 14% fat) using solely orthonasal and/or retronasal cues and assess whether this ability depends on habitual dairy intake.
4	To map brain activation in response to olfactory (orthonasal) exposure to varying levels of dietary fat embedded within dairy milk (ranging from 0% to 14% fat), and exploring potential associations between brain activation, olfactory fat content discrimination and perceptual characteristics.
5	To investigate whether sensory characteristics of a low-fat food product (0% dairy milk) can be enhanced by the addition of a fat-related aroma (cream) and assess the influence of exposure to retronasal fat-related odours on subsequent ad-libitum consumption and appetite.

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2

Chapter 2

Smells like fat: A systematic scoping review on the contribution of olfaction to fat perception in humans and rodents

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ABSTRACT

Understanding how dietary fat is perceived by the senses is crucial in developing public health strategies aimed at curbing excessive fat intakes. Olfaction is one of several sensory modalities contributing to fat perception in foods, yet the nature and extent of its involvement is relatively unclear.

A systematic scoping literature review was conducted to identify and summarise relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents and highlight relevant knowledge gaps. The review was carried out in accordance with the PRISMA methodology, using combinations of olfaction-, fat- and perception-related search terms. Following searches in Scopus, Web of Science and PubMed databases, 42 articles were ultimately included.

Overall, findings are consistent with the notion that olfaction plays a role in the perception of dietary fat in rodents and humans. Rodents can perceive dietary fat via olfactory cues, and this ability may affect their preference for fat-containing feed. Humans can detect, discriminate, and identify fat and its constituents solely by olfaction, even when embedded within a complex food matrix. Food fat content can modulate the perception of various fat- and non-fat olfactory qualities, depending on the food matrix and odorant physio-chemical properties. On the other hand, the presence of fat-related odours can modify the perception of olfactory and non-olfactory sensory qualities (e.g., mouthfeel). Several knowledge gaps were identified, namely, the role of fat-related odours in eating behaviour, the nature of chemical signals underlying olfactory fat perception and factors governing sensitivity to fat-related odours.

Keywords: fat perception, olfaction, orthonasal, retronasal, systematic scoping review

1 INTRODUCTION

Consumption of dietary fat is exceeding recommended daily intake requirements in many Western countries, including the Netherlands (van Rossum et al., 2020), in some accounting for up to 46% of the total daily energy intake (Eilander et al., 2015). Due to its high energy density and low effect on satiation, especially in obese individuals, (Blundell et al., 1993) fat is considered a major contributor to energy overconsumption and consequential development of obesity and related comorbidities (Blundell & Macdiarmid, 1997; Bray et al., 2004; Golay & Bobbioni, 1997). Fat overconsumption is further exacerbated by its flavour, texture, and aroma-enhancing properties, all of which considerably contribute towards the pleasurable experience of eating (Drewnowski, 1997a, 1997b; Drewnowski & Almiron-Roig, 2009). The interaction of these factors has recently been illustrated by Teo et al. (2022) who found that foods associated with fat-related flavours contributed most to higher energy intakes, independent of weight status.

Multiple sensory systems contribute to dietary fat perception (Drewnowski & Almiron-Roig, 2009; Guichard et al., 2018). Fat is known to impart a range of mouthfeel sensations, such as thickness, creaminess, mouthcoating and smoothness (Drewnowski, 1992; Mela, 1988; Schiffman et al., 1998), while the presence of free fatty acids can be detected in the oral cavity via taste receptors located on the human tongue (Chale-Rush et al., 2007; Keast & Costanzo, 2015; Mattes, 2009; Pepino et al., 2012; Running et al., 2015; Stewart et al., 2010). In addition to mouthfeel and taste cues, the involvement of olfactory cues in fat perception has also been established. Flavour release studies identified various volatile compounds, belonging to different chemical classes as being associated with fat-related sensations (Guichard, 2002; Guichard et al., 2018). When released from foods or beverages, these volatiles bind to receptors located throughout the olfactory epithelium in the nasal cavity, which ultimately results in odour perception (Delime et al., 2016). Orthonasal odours originate from the external environment and enter the nasal cavity via the nostrils. They are thought to be related to food source detection and the induction of appetite during the anticipatory phase of eating. Retronasal odours, on the other hand, enter the nasal cavity from the mouth during food consumption. They mainly contribute to flavour perception and may influence intake and satiation (Boesveldt & de Graaf, 2017; Bojanowski & Hummel, 2012; Delime et al., 2016; Goldberg et al., 2018). The two olfaction routes can yield distinct perceptions, even when odour intensities are matched (Sun & Halpern, 2005). In comparison to mouthfeel and taste, however, the involvement of olfaction in dietary fat perception seems to be relatively underexplored and much remains unclear about the nature and extent of its contribution.

Given the societal relevance of understanding sensory fat perception, and the lack of systematic literature reviews on this topic in academic literature, the current scoping review aimed at (1) systematically identifying and summarizing relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents, and (2) highlighting relevant knowledge gaps. The rationale behind focusing on broader literature, also involving rodents, was to gain insight from mechanistic studies, which might not be feasible or ethical to conduct in human subjects.

2 METHODS

Due to the broad nature of its aims, the current work is considered a systematic scoping review. It was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) methodology (Moher et al., 2009).

2.1 Search strategy

Three academic electronic databases (Scopus, PubMed and Web of Science) were searched for original articles published in English, without any publication date restrictions. Search strings included olfaction- (e.g. volatiles, orthonasal, aroma, odour) and fat-related words (e.g. fat, lipid, fatty acid, butter), combined with perception-related words or strings (e.g. flavour, discrimination, identification, chemosensory). Search strings for all three databases contained exclusion commands (excluding words such as cat, dog, insect, larvae from the search), to avoid articles beyond the scope of this review (e.g. insect studies). Detailed search strategies used in each database can be found in Supplementary Material A. Due to search algorithm differences, a specific search string was applied to each of the databases. It must be noted that the word “preference” in combination with fat-related words was excluded from the search string applied in the PubMed database. This was done to increase specificity, as inclusion of this combination mainly yielded articles deemed beyond the scope of this review. Early search results were evaluated to determine the relevance of obtained articles, and search term modifications were made prior to the formal search procedure. Reference lists of included articles were not searched for articles not captured by the searches. Manual searching was also not undertaken.

2.2 Article inclusion

Articles met eligibility criteria if they reported an investigation of olfactory exposure (ortho- or retronasal) to fat and its constituents, in isolation or via foods (real or model), beverages or emulsions in human or rodent subjects, utilising sensory evaluation. Sensory evaluation was defined as a scientific approach utilising a measure of perception, discrimination, identification, preference, acceptance and/or detection thresholds. Articles concerning the addition of fat-related aromas/flavourings to foods were included as well if their addition impacted relevant sensory attributes. Exclusion criteria involved fat perception not being the topic of research; lack of olfactory exposure to suitable fat sources (i.e. either no exposure to fat; or exposure to fat in combination with potentially confounding odour/flavour sources); lack of reporting relevant outcomes resulting from olfactory exposure; articles focusing on volatile compounds without relevant sensory evaluation measures; reviews, meta-analyses, books, or book chapters; articles lacking an abstract; full-text unavailability; non-English publications; and non-peer reviewed publications.

2.3 Article selection

Literature searches were performed up to April 2021 by three authors: PM, MS and FG. All identified items were exported to the reference software EndNote™ X9 (Clarivate Analytics) where they were organized, deduplicated and screened following the PRISMA guidelines (Moher et al., 2009). Title and corresponding abstract screening were carried out by FG. Screening reliability was determined by calculating the Cohen’s Kappa coefficient, after PM and FG screened a random sample of 116 titles and corresponding abstracts from the retrieved items (sample size was determined in accordance with the Cohen’s Kappa methodology). The interrater reliability score amounted to 0.90, which indicated a strong agreement (McHugh, 2012; Sim & Wright, 2005). Remaining potentially eligible items then underwent full-text screening, carried out by PM and MS. Any discordances regarding the ultimate inclusion of articles in the review were discussed by the reviewers until reaching a consensus. A list of citations excluded during the full-text screening process can be found in Table S1, Supplementary Material B.

2.4 Review outcomes and data synthesis

Data from articles meeting all inclusion criteria were extracted. Extracted data included outcomes of interest relevant to our research question, study population characteristics (along with relevant population specifics, if applicable), stimuli (types used along with the applied manipulation, if applicable), route of olfactory exposure (orthonasal or retronasal), and relevant findings. Data were then evaluated and interpreted by all authors, tabulated per study, and listed by author name in an ascending alphabetical order. Rodent studies were distinguished from human ones and reported in a separate table. A narrative synthesis was ultimately conducted, meta-analysis was not performed due to the indirect nature of most of the identified work and lack of relevant and comparable data.

2.5 Risk of bias assessment

To assess the quality of included studies, two authors (MP and MS) independently reviewed and evaluated each article in accordance with the Cochrane Association Risk of Bias methodology (Higgins et al., 2011). Any discrepancies in risk of bias scores were discussed to reach agreements. Due to the nature of this review's topic, specific risk assessment domains were generated per study subject type. Risk evaluation domains for rodent studies included random group generation, researcher blinding, incomplete outcome reporting and selective reporting. Human studies were evaluated on stimulus randomisation; isolation of olfaction from potentially confounding effects of taste, mouthfeel, and trigeminal sensations; participant blinding to sample identities; incomplete outcome reporting; and selective reporting. For each domain, the risk of bias was rated as "low risk", "some concern", "high risk" or "risk unclear", based on information reported in the included articles.

3 FINDINGS

An overview of the search process and its results can be seen in the PRISMA flowchart in Figure 1. Database searches resulted in the identification of 2596 items from all sources, with 1703 of them remaining after deduplication. After title and abstract screening, 93 items remained and were assessed against our eligibility criteria. In total, 51 items were excluded: 4 were not about fat perception, 11 lacked olfactory exposure to suitable fat sources, 11 did not report relevant outcomes resulting from olfactory exposure, 4 focused on volatile chemical compounds without relevant sensory evaluation measures, 17 were either meta-analyses, reviews, books, or book chapters, and 4 were inaccessible. Full-text assessment ultimately resulted in 42 articles being included in the current review.

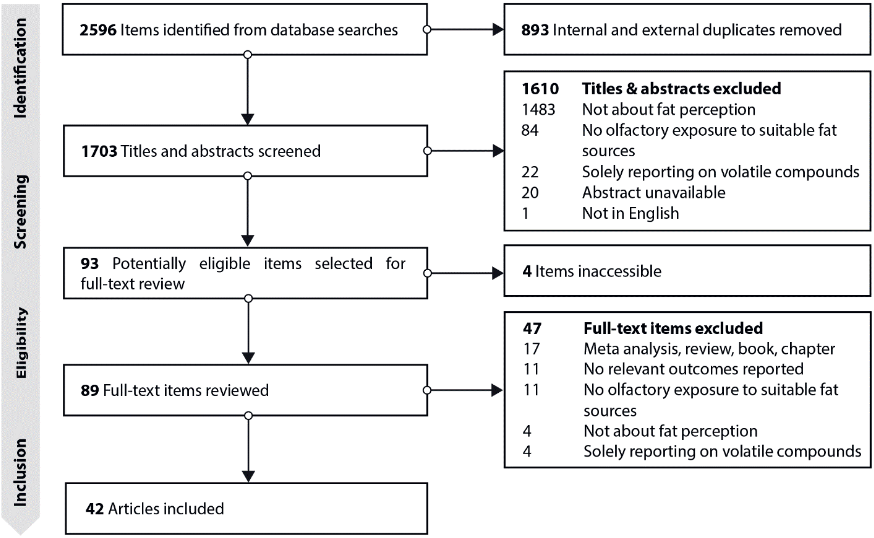

Figure 1. *Preferred Reporting Items for Systematic Reviews and Meta-Analyses flow diagram of the literature search to identify olfactory fat perception studies.*

3.1 Rodent studies

A summary of studies investigating olfactory fat perception in rodents is presented in Table 1.

Six studies employed rodent subjects, namely mice (Boone et al., 2021; Kinney & Antill, 1996; Lee et al., 2015; Takeda et al., 2001; Xavier et al., 2016) or rats (Ramirez, 1993). In all cases wild-type controls were compared to either anosmiated (Boone et al., 2021; Kinney & Antill, 1996; Lee et al., 2015; Ramirez, 1993; Takeda et al., 2001) or CD36 receptor-deficient specimens (Xavier et al., 2016). All rodent studies utilised preference paradigms in which animals were exposed to olfactory stimuli either via food varying in fat content (Boone et al., 2021; Kinney & Antill, 1996; Ramirez, 1993), scented paper (Xavier et al., 2016), sucrose-based solutions (Lee et al., 2015), or corn oil and linoleic acid (Takeda et al., 2001).

To summarize, rodents' preferences for fat-related odorants diminished when rodents were anosmiated (Kinney & Antill, 1996; Ramirez, 1993; Takeda et al., 2001) or lacked olfactory CD36 receptors (Xavier et al., 2016). Once their sense of smell was restored, preference for fat returned (Kinney & Antill, 1996). Moreover, following anosmiation, rodents lost their preference for aversion-inducing lipids (Lee et al., 2015). Anosmiation, however, did not lead to a complete preference diminishment for fat in all cases. Despite anosmiation, Boone et al. (2021) observed no preference alterations towards a high-fat diet, Ramirez (1993) observed only a decrease in preference towards fat-containing mixtures, while Takeda et al. (2001) observed a preference decrease only for corn oil containing higher fat levels.

Table 1. *Summary of studies investigating olfactory fat perception in **rodents**.*

Study	Outcome(s) of interest	Subjects	Stimuli	Relevant Findings	Interpretation
Boone et al. (2021)	Changes in feeding patterns in response to varying access to different diets (standard or standard combined with high fat).	96-120 (exact numbers per experiment n.s.) mixed-sex adult mice, either anosmiated via complete bilateral bulbectomy or sham-operated.	Standard diet (14% energy from fat) and high fat diet (60% energy from fat).	All mice, regardless of treatment (anosmiated or sham-operated) exhibited a preference for the high-fat diet.	Olfactory information is not relevant for the formation of high-fat food preferences.
Kinney and Antill (1996)	Intake of food mixtures during a 2-h preference test.	36 male albino mice: 12 underwent bilateral olfactory nerve section, 12 underwent sham surgery (control), 12 untreated mice (control).	Corn oil-based high fat (3.42 kcal/g) and mineral oil-based low-fat (2.61 kcal/g) food mixes.	Pre-treatment, all mice preferred the high-fat food mixture; post-treatment, anosmic mice showed no preference for the high-fat mixture, preference for the high-fat mixture increased in the control groups. Preference for the high-fat mixture returned to anosmic mice after olfactory nerve recovery.	Olfactory information is relevant for the formation of high-fat food preferences.
Lee et al. (2015)	Intake following two-bottle choice tests	8 – 12 week old mice (number and sex n.s.): Sham-operated (control) or anosmiated via olfactory nerve transection.	0.15 M sucrose solutions with 7.5 µM KOdiA-PC lipids (test) and without (control)	In contrast to normosmic controls, anosmiated mice exhibited preference for the solution containing the aversive KOdiA-PC lipid.	Olfaction is involved in the perception of lipids in mice.
Ramirez (1993)	Preference scores following two-bottle preference tests.	20 female rats: 12 anosmiated via bulbectomy, 8 underwent sham surgery (control).	Carbohydrate- and fat-containing mixtures. Fat-containing mixtures included 0.5% corn oil, 1% corn oil, 0.5% triolein and 1% triolein.	Preference scores for fat-containing mixtures were lower in bulbectomized rats than in sham operated ones. Bulbectomized rats still exhibited preferences for fat-containing mixtures.	Preference for fat is mediated by olfactory and non-olfactory cues.

Study	Outcome(s) of interest	Subjects	Stimuli	Relevant Findings	Interpretation
Takeda et al. (2001)	Voluntary intake of corn oil or linoleic acid; Place preference.	28 male mice: either sham-treated or anosmiated (via ZnSO4-induced olfactory blockade).	Corn oil (1, 3, 5 and 10%), linoleic acid and water.	Pre- treatment, mice preferred corn oil over the vehicle at all concentrations; post-treatment, sham-treated mice preferred corn oil over the vehicle at all concentrations, anosmiated mice preferred corn oil only at higher ones (5% and 10%), Place preference induced by corn oil was observed in both treatment conditions.	Multiple sensory modalities are involved in the perception of oil, olfactory stimuli might act as a signal for oil at low concentrations.
Xavier et al. (2016)	Innate preference for scented filter paper inferred from Investigation time.	10 mice: 6 with CD36 receptor deficiency, 4 wild type (control).	Deodorized filter paper scented with PBS (control), amyl acetate (1 mM), or a lipid concentrate.	Contrary to wild type mice (control), CD36-deficient ones showed no preference for the lipid concentrate-scented filter paper.	Receptor CD36 is involved in the perception of fat-related odorants.

3.2 Human studies

A summary of studies investigating olfactory fat perception in humans is presented in Table 2. Of the 36 studies employing human subjects, 8 presented olfactory stimuli orthonasally (Boesveldt & Lundstrom, 2014; Chen & Eaton, 2012; Dadalı & Elmacı, 2019; Fernandez et al., 2000; Glumac & Chen, 2020; Kindleysides et al., 2017; Running et al., 2017; Rychlik et al., 2006), 15 retronasally (Arancibia et al., 2015; Brauss et al., 1999; Chukir et al., 2013; Ebba et al., 2012; Frank et al., 2015; González-Tomás et al., 2007; Jervis et al., 2014; Kallas & Halpern, 2011; Kindleysides et al., 2017; Le Calvé et al., 2015; Mela, 1988; Miettinen et al., 2004; Roberts, Pollien, Antille, et al., 2003; Schoumacker et al., 2017; Yackinous & Guinard, 2000; Zhou et al., 2016) and 13 through a combination of both olfaction routes (Bolton & Halpern, 2010; Bult et al., 2007; Chale-Rush et al., 2007; de Wijk et al., 2003; Frøst et al., 2001; Han et al., 2019; Hyvönen et al., 2003; Lorenzo et al., 2015; Miettinen et al., 2003; Parat-Wilhelms et al., 2005; Running et al., 2017; Syarifuddin et al., 2016; Ventanas et al., 2010; Weenen et al., 2005). Utilised sensory methodology included perceptual ratings (Boesveldt & Lundstrom, 2014; Bult et al., 2007; Chen & Eaton, 2012; Dadalı & Elmacı, 2019; de Wijk et al., 2003; Ebba et al., 2012; Fernandez et al., 2000; Frank et al., 2015; Frøst et al., 2001; Han et al., 2019; Hyvönen et al., 2003; Jervis et al., 2014; Lorenzo et al., 2015; Mela, 1988; Miettinen et al., 2004; Miettinen et al., 2003; Parat-Wilhelms et al., 2005; Roberts, Pollien, Antille, et al., 2003; Rychlik et al., 2006; Syarifuddin et al., 2016; Ventanas et al., 2010; Weenen et al., 2005; Yackinous & Guinard, 2000; Zhou et al., 2016); discrimination testing (Boesveldt & Lundstrom, 2014; Bolton & Halpern, 2010; González-Tomás et al., 2007; Kallas & Halpern, 2011; Le Calvé et al., 2015); detection (Chale-Rush et al., 2007; Schoumacker et al., 2017), difference (Le Calvé et al., 2015; Schoumacker et al., 2017) and rejection (Running et al., 2017) threshold testing; pairwise ranking (Arancibia et al., 2015); time-intensity methods (Brauss et al., 1999; Hyvönen et al., 2003; Miettinen et al., 2004; Miettinen et al., 2003; Ventanas et al., 2010); and identification testing (Chukir et al., 2013; Glumac & Chen, 2020). In addition to sensory methods, aroma volatile release or volatile compound composition analyses (Arancibia et al., 2015; Brauss et al., 1999; Dadalı & Elmacı, 2019; Frank et al., 2015; González-Tomás et al., 2007; Miettinen et al., 2004; Miettinen et al., 2003; Roberts, Pollien, Antille, et al., 2003; Ventanas et al., 2010) and dietary intake assessments (Boesveldt & Lundstrom, 2014; Kindleysides et al., 2017) were carried out. Fatty acids were exclusively used as olfactory stimuli in six studies (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Ebba et al., 2012; Kallas

& Halpern, 2011; Kindleysides et al., 2017), with subjects being exposed to either stearic, linoleic and oleic acid (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011); taste strips containing varying levels of linoleic acid (Ebba et al., 2012); or oleic acid (Kindleysides et al., 2017). Food matrices served as olfactory stimuli in 31 human studies (Arancibia et al., 2015; Boesveldt & Lundstrom, 2014; Brauss et al., 1999; Bult et al., 2007; Chen & Eaton, 2012; Dadalı & Elmacı, 2019; de Wijk et al., 2003; Fernandez et al., 2000; Frank et al., 2015; Frøst et al., 2001; Glumac & Chen, 2020; González-Tomás et al., 2007; Han et al., 2019; Hyvönen et al., 2003; Jervis et al., 2014; Le Calvé et al., 2015; Lorenzo et al., 2015; Mela, 1988; Miettinen et al., 2004; Miettinen et al., 2003; Parat-Wilhelms et al., 2005; Roberts, Pollien, Antille, et al., 2003; Running et al., 2017; Rychlik et al., 2006; Schoumacker et al., 2017; Syarifuddin et al., 2016; Ventanas et al., 2010; Weenen et al., 2005; Yackinous & Guinard, 2000; Zhou et al., 2016). The vast majority of food matrices were dairy product-based (Arancibia et al., 2015; Boesveldt & Lundstrom, 2014; Brauss et al., 1999; Bult et al., 2007; Chen & Eaton, 2012; de Wijk et al., 2003; Frøst et al., 2001; González-Tomás et al., 2007; Han et al., 2019; Hyvönen et al., 2003; Jervis et al., 2014; Le Calvé et al., 2015; Mela, 1988; Miettinen et al., 2004; Miettinen et al., 2003; Parat-Wilhelms et al., 2005; Roberts, Pollien, Antille, et al., 2003; Rychlik et al., 2006; Schoumacker et al., 2017; Syarifuddin et al., 2016; Weenen et al., 2005; Yackinous & Guinard, 2000; Zhou et al., 2016), others included meat products (Fernandez et al., 2000; Lorenzo et al., 2015; Ventanas et al., 2010) margarine (Dadalı & Elmacı, 2019), oil and lard (Glumac & Chen, 2020), chocolate (Running et al., 2017) and agar gels (Frank et al., 2015). Most studies utilising foods added flavour/aroma volatiles to the matrices (Arancibia et al., 2015; Brauss et al., 1999; Bult et al., 2007; Frank et al., 2015; Frøst et al., 2001; González-Tomás et al., 2007; Han et al., 2019; Hyvönen et al., 2003; Le Calvé et al., 2015; Miettinen et al., 2004; Miettinen et al., 2003; Roberts, Pollien, Antille, et al., 2003; Syarifuddin et al., 2016; Ventanas et al., 2010; Yackinous & Guinard, 2000), while some added free fatty acids (Chen & Eaton, 2012; Running et al., 2017; Rychlik et al., 2006)

Studies on the human ability to smell fatty acids found that 18-carbon fatty acids, namely linoleic, oleic and stearic, can be detected orthonasally (Chale-Rush et al., 2007; Kindleysides et al., 2017) and retronasally (Chale-Rush et al., 2007), with retronasal detection thresholds being higher than orthonasal ones (Chale-Rush et al., 2007). Linoleic, oleic and stearic acids can also be discriminated from blanks ortho- and retronasally, with discrimination ability for oleic acid being lower for retronasal olfaction (Bolton & Halpern, 2010); discriminated from each other retronasally (Kallas & Halpern, 2011); and retronasally identified from blanks and each other, with their chemical structure (i.e., the number of double bonds) influencing identification (Chukir et al., 2013). Upon removing retronasal cues, the detection of linoleic acid on taste strips diminishes (Ebba et al., 2012). The addition of oleic and stearic acids to a corn starch solution had no effect on perception of creaminess odour (Chen & Eaton, 2012), whereas adding short chain fatty acids, namely acetic, butanoic and hexanoic acid, to yogurt decreased yogurt-like odour intensity while simultaneously increasing intensities of off-flavours (Rychlik et al., 2006). Chocolate containing linoleic fatty acids was rejected at lower concentrations than chocolate containing oleic acid, whereas stearic acid had no effect on rejection thresholds (Running et al., 2017).

Studies investigating olfactory fat perception ability in food matrices show that humans can orthonasally distinguish rapeseed oil, lard and oleic acid from non-fat controls (Glumac & Chen, 2020) and discriminate fat content of dairy milks (Boesveldt & Lundstrom, 2014). Moreover, the presence of retronasal cues can impact the ability to discriminate fat content in white sauces, milk, and yogurt, with the impact depending on the reference fat content, direction of comparison, and other factors such as added ingredients and the presence of sensory cues from other modalities (Le Calvé et al., 2015). The presence of retronasal cues enhances the perception of fattiness in dairy-based mixtures, while their elimination increases fat content detection and difference thresholds in cottage cheese (Schoumacker et al., 2017), decreases the perception of creamy and fatty mouthfeel in vanilla custard and affects the perception of creaminess in sour cream (Jervis et al., 2014). In contrast, one study reported that elimination of retronasal cues does not affect fat content and creaminess perception in commercially available dairy products (Mela, 1988).

Fat content was reported to have differential effects on the release of flavour volatiles (Arancibia et al., 2015; Brauss et al., 1999; Dadalı & Elmacı, 2019; Frank et al., 2015; González-Tomás et al., 2007; Hyvönen et al., 2003; Lorenzo et al., 2015; Miettinen et al., 2004; Miettinen et al., 2003; Roberts, Pollien, Antille, et al., 2003; Ventanas et al., 2010) and influenced the perception of various odours in diverse food matrices. Increases in fat content were found to decrease lemon flavour intensity, while increasing that of milk flavour in dairy desserts (Arancibia et al., 2015); increase overall odour intensity in dairy milk (Boesveldt & Lundstrom, 2014); decrease flavour intensities of 2-hexenyl acetate; anethole and terpinolene in yogurt (Brauss et al., 1999); increase creamy odour intensity in fresh cream and evaporated milk, with the increase being larger in evaporated milk, despite having a lower fat content than fresh cream (Chen & Eaton, 2012); increase butter and cheese odour in margarine, while decreasing that of cream (Dadalı & Elmacı, 2019); increase blue cheese flavour in flavoured agar gel (Frank et al., 2015); decrease boiled odour in milk, while increasing creamy odour, flavour intensities and fattiness – a descriptor which was highly positively correlated with creamy aroma and flavour, and increased more in low-fat samples than in high-fat ones (Frøst et al., 2001); decrease strawberry flavour intensity in strawberry custard (González-Tomás et al., 2007); increase creaminess and butter note intensities in Gouda cheese (Han et al., 2019); decrease overall odour and flavour intensity and sharpness in strawberry ice cream (Hyvönen et al., 2003); decrease black pepper odour intensity in dry-ripened sausages (Lorenzo et al., 2015); decrease the odour intensity of linalool in dairy milk (Miettinen et al., 2003); increase linalool odour intensity in strawberry-flavoured milk while decreasing strawberry flavour intensity (Miettinen et al., 2004); decrease intensities of various coffee-related (e.g. roasty, coffee, burnt), but not milk-related (e.g. milky, butter, creamy) flavour qualities (Parat-Wilhelms et al., 2005); decrease flavour intensities of beta-damascenone, hexanal and ethyl butyrate in flavoured dairy milk (Roberts, Pollien, Antille, et al., 2003); decrease mushroom odour intensity, while increasing that of cocoa odour in mushroom and cocoa-flavoured bologna sausages (Ventanas et al., 2010); increase intensities of vanilla, caramel, milk odour and flavour, as well as cream and fat flavour in vanilla custards, while decreasing synthetic odour and chemical and sickly flavour (de Wijk et al., 2003). Fat content was not found to affect cured ham odour intensity in cooked ham (Fernandez et al., 2000) and overall odour intensity in cheese (Syarifuddin et al., 2016).

Five studies investigated the perceptual consequences of adding fat-related odours to foods. In dairy milk, the addition of a cream aroma led to an increase in perceived fattiness (Frøst et al., 2001), creaminess and thickness (Bult et al., 2007); butter aroma added to cheese enhanced perceived creaminess and texture pleasantness (Han et al., 2019) and fat content texture (Syarifuddin et al., 2016), while it enhanced fattiness when added to mashed potatoes (Yackinous & Guinard, 2000); fattiness was also enhanced after adding cream and onion aroma to potato chips (Yackinous & Guinard, 2000); the addition of a butter odour enhanced texture pleasantness in cheese (Han et al., 2019)

Table 2. *Summary of studies investigating olfactory fat perception in* ***human subjects****.*

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
STUDIES ON OLFACTORY PERCEPTION OF FAT IN ISOLATION						
Bolton & Halpern (2010)	Discrimination ability between fatty acids and blanks.	Untrained: - EXP 1 (ortho- and retronasal session): n = 30 (13F); 26.6 ± 9.3 y - EXP 2 (retronasal and oral-cavity-only session): n = 30 (16F); 26.0 ± 4.0 y	Orthonasal Retronasal (I)	Linoleic and oleic acids compared to mineral oil (control); Undiluted stearic acid compared to NaCl (control).	All fatty acids were discriminated from control, ortho- and retronasally: Orthonasally, 87% of subjects discriminated linoleic acid from blanks and 83% discriminated oleic and stearic acids; Retronasally, 93% discriminated linoleic acid from control, 57% discriminated oleic acid and 83% discriminated stearic acid. Discrimination ability did not differ between the routes for linoleic and stearic acids, it was lower for oleic acid in the retronasal condition.	Humans can ortho- and retronasally distinguish fatty acids from non-fatty acid-containing controls.
Chale-Rush et al. (2007)	Orthonasal, retronasal and multimodal detection thresholds of different fatty acids	Untrained; 6-n-Propylthiouracil tasters; n = 22 (7F); 21.2 ± 0.6 y; BMI 23.6 ± 0.4; body fat 18.3 ± 1.3 %	Orthonasal Retronasal (I, C)	Linoleic, oxidised linoleic, oleic, and stearic acids varying in concentration.	Retronasal detection thresholds were higher than those of other exposure routes for all fatty acids. Detection thresholds for linoleic acid were lowest for orthonasal olfaction compared to other exposure routes. For oxidised linoleic and oleic acids, orthonasal thresholds did not differ from those of multimodal exposure but were lower than those of taste. Stearic acid detection thresholds did not differ between orthonasal, taste and multimodal exposure. No correlations between the different thresholds were observed.	Humans can smell 18-carbon fatty acids. Olfaction contributes independently to the perception of fatty acids. Retronasal olfaction is less sensitive to the presence of fatty acids than other chemosensory systems.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Chukir et al. (2013)	Check-All-That-Apply-based linguistic identification following retronasal inhalation.	Untrained; n = 36 (24F); 18 – 71 y (median 21 y)	Retronasal (I)	Fatty acids: linoleic, oleic, and stearic; non-fatty acid stimuli (controls): geraniol and phenylethyl alcohol.	Fatty acid-containing samples received identifications that consistently differed from those ascribed to controls. Stearic acid was identified differently from linoleic and oleic acids by approximately one-third of assessors. Linoleic and oleic acids mostly received the same, partly food-related identifications: - Linoleic acid identifications included: new plastic, rubbery, sunflower, peanut oil, olive oil and oily; - Oleic acid identifications included: new plastic, rubbery, sunflower, peanut oil, margarine, olive oil and oily; - Stearic acid identifications included: new plastic, rubbery, sunflower, and oily - the proportion of rubbery for stearic acid was about twice that for linoleic and oleic acids. Identifications of the three fatty acids were consistently different from those of non-fatty acid stimuli.	18-carbon fatty acids can be identified retronasally. Linoleic and oleic fatty acids may contribute to flavour perception.
Ebba et al. (2012)	Perceptual ratings of fat-related taste quality intensities.	Untrained; n = 88 (51F); 18 – 74 y (mean 25.1 y)	Retronasal (C)	Taste strips containing mineral oil (control), linoleic acid in amounts of 1.1, 1.3, 1.5, and 1.7 µmol.	The perceived taste intensity of linoleic acid decreased by 40% when retronasal olfaction was eliminated via nose clips.	Olfaction is involved in the perception of fatty acids and can enhance fat-related taste qualities.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Kallas & Halpern (2011)	Discrimination ability between fatty acids.	Untrained; n = 40 (30F); 18 – 36 y	Retronasal (I)	Linoleic (40.5%), oleic (40.0%) and stearic acids, all at suprathreshold levels; Linoleic acid (0.005% – subthreshold level concentration) compared to mineral oil (control).	Stearic acids were discriminated from linoleic or oleic acids: 70% of subjects discriminated between stearic and linoleic acids; 65% discriminated between stearic and oleic acids. Oleic and linoleic fatty acids were discriminated by 38% of subjects. No discrimination occurred in "negative control" trials.	Humans can discriminate 18-carbon fatty acids using solely retronasal olfaction.
Kindleysides et al. (2017)	Fatty acid olfactory detection thresholds. Dietary intake of key food groups.	Untrained; n = 50F; 18 – 45 y (median 26 y); median BMI 24 (31 normal-weight, 11 overweight, 8 obese)	Orthonasal	Oleic acid (combined with mineral oil), varying in concentration (6, 12, 24, 48, 95, 190, and 380 mM).	Olfactory detection curves increased with higher concentration of oleic acid. Oleic acid taste and olfactory detection abilities were positively correlated. Oleic acid olfactory sensitivity was not related to body composition. Dietary intakes of nuts, nut spreads, and seeds were positively correlated with high olfactory sensitivity to oleic acid.	Oleic fatty acid can be detected orthonasally. While olfactory sensitivity to oleic fatty acid is independent of body composition, it is related to the habitual consumption of fat-containing foods and gustatory sensitivity to oleic acid.
STUDIES ON OLFACTORY PERCEPTION OF FAT EMBEDDED WITHIN FOOD MATRICES						
Arancibia et al. (2015)	Relative intensities of lemon and milk flavours assessed via pairwise ranking. Aroma release parameters following nose-space sampling.	- Sensory session: trained; n = 28 (16F); 23 – 55 y - Aroma release session: n = 8	Retronasal (C)	Lemon-flavoured (added linalool and cis-3-hexen-1-ol) dairy desserts with added thickeners and varying in fat content: 0.14% and 3.5% fat.	Lemon flavour intensity was higher in dairy desserts with a lower fat content, while milk flavour intensity was higher in desserts with a higher fat content. Linalool release was lower in desserts with a higher fat content.	Fat content influences *in vivo* release of certain flavour compounds, which affects their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Boesveldt & Lundstrom (2014)	Orthonasal discrimination ability between fat levels in dairy milk. Perceptual ratings of intensity, pleasantness. Habitual fat intake.	- EXP 1: untrained; n = 30 (16F), 27.3 ± 4.2 y, BMI 23.1 ± 3.1 - EXP 2: untrained; n = 18 (12F), 22.1 ± 1.2 y, BMI 22.7 ± 3.1 - EXP 3: Normal-weight – untrained; n = 30 (15F), 25.0 ± 3.7 y, BMI 22.5 ± 1.8; Overweight – untrained; n = 30 (18F), 30.6 ± 7.2 y, BMI 35.6 ± 8.4	Orthonasal	Manipulated milk samples varying in fat content (skimmed, semi-skimmed, whole):	Skimmed milk samples were discriminated from whole milk ones in all experiments. In EXP 1 and EXP 2, skimmed milk was not discriminated from whole milk; in EXP 3 skimmed milk was not discriminated from semi-skimmed milk. There was no difference between normal-weight and overweight subjects discrimination performance. In EXP 1 and EXP 2, perceived intensity increased with increasing fat content, while pleasantness decreased. In EXP 2, perceived pleasantness did not differ between the samples. In EXP 3, perceived intensity, but not pleasantness was lower in the overweight group. Discrimination ability was not correlated to BMI or habitual dairy fat consumption parameters in any of the experiments.	Humans can smell differences between dairy milks differing in fat level, using solely orthonasal olfaction. This ability seems independent from habitual dairy fat consumption and BMI.
Brauss et al. (1999)	Time-intensity parameters related to the perception of flavourings (2-hexenyl acetate, anethole and terpinolene) and roma release parameters after nosespace sampling.	Trained; n = 10	Retronasal (C)	Flavoured yogurts varying in fat content (0.2, 3.5 and 10%).	Flavour compound volatility and perceived flavour intensities decreased with increasing fat content.	Fat content diminishes the volatility of certain flavour compounds, which affects their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Bult et al. (2007)	Perceptual ratings of flavour intensity, thickness, creaminess (whle ingesting of milk-like foods in the presence of cream odours).	Untrained; n = 11 (3F); 41 ± 11 y	Orthonasal Retronasal (I, C)	Fresh skim milk (0.075% fat content) with an added cream aroma.	The odour stimulus increased intensities of thickness and creaminess, but only when the odour was presented retronasally. This was most pronounced when odours coincided with swallowing.	Fat-related retronasal odours can enhance fat-related mouthfeel sensations via cross-modal interactions.
Dadalı & Elmacı (2019)	Perceptual ratings of butter, creamy, cheesy, animal-like, margarine and oxidised aroma. Relative amounts of volatiles in the headspace following fat and emulsifier content manipulation.	Trained; n = 10 (8F); 23 – 54 y	Orthonasal	Model margarines varying in fat content (60, 70 and 80%).	The release of 2,3-butanedione and butanoic acid was higher in model margarines with 70% and 80% fat content. The release of 2-heptanone, 2-nonanone, 2-undeca-none, hexanoic acid, and delta-decalactone was higher in margarines with a lower fat ratio. Fattier margarines were rated higher in terms of butter and cheese aroma. Cream aroma was rated as being more intense in lower-fat margarines.	Fat content influences the volatility of certain flavour compounds, which affects their perception.
Fernandez et al. (2000)	Perceptual ratings of smell intensity.	Trained; n = 12	Orthonasal	Cooked ham varying in fat content (≤2%; 2-3%; 3-4%; >4%).	Smell intensity of cured ham (pork) was not affected by fat content.	Fat content alterations do not necessarily modify smell intensity.
Frank et al. (2015)	Perceptual ratings of blue cheese flavour and overall flavour intensities. Aroma release parameters following headspace sampling.	Trained; n = 10	Retronasal (C)	Agar gels varying in fat content (0%, 10%) and aromatised with blue cheese-related volatiles.	Fat-containing agar gels were rated as more intense in terms of blue cheese flavour. Fat content had differential effects on the release of several volatiles, depending on their solubility and lipophilicity.	Fat content influences the volatility of certain flavour compounds, which affected their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Frøst et al. (2001)	Perceptual ratings of creamy aroma, cream flavour, and total fattiness (meta descriptor).	Trained; n = 7	Orthonasal Retronasal (C)	Commercially available dairy milk varying in fat content (0.1, 1.3 and 3.5%) with added cream aroma (0 or 0.75 g/L), thickener (0 or 1 g/L) and whitener (0 or 1 g/L).	With increasing fat content, creamy odour and flavour intensities increased, while that of boiled milk odour decreased. The magnitude of perceived difference in fattiness was much larger between 0.1 and 1.3% fat samples than between 1.3 and 3.5% ones. Samples with added cream aroma scored higher in terms of total fattiness, which was highly positively correlated with creamy odour and flavour.	The addition of fat-related odours to milk enhanced the perception of milk fat content.
Glumac & Chen (2020)	Proportion of correct answers to the question: "Is this perceived as oil/fat?", posed following exposure via various sensory modalities.	Untrained; n = 30 (15F); 27.3 ± 2.0 y; BMI 18.5 – 25.9	Orthonasal	Commercial rapeseed oil, commercial lard, plant-sourced oleic acid, food grade silicone oil, food-grade glycerol, and food-grade xanthan gum solution.	Using orthonasal cues, subjects identified rapeseed oil, lard and oleic acid as oil/fat-containing, while silicon, glycerol and xanthan gum solution were correctly identified as non-oil/fat. Aroma was more informative for oil/fat recognition than tactile and taste.	Humans can identify fat-containing food samples using solely orthonasal cues. For fat recognition, orthonasal cues are more informative than tactile and taste-related ones.
González-Tomás et al. (2007)	Relative intensity of strawberry flavour assessed via pairwise comparison. Aroma release parameters following nose-space sampling.	Aroma release: n = 10 Sensory evaluation: Trained; n = 39	Retronasal (C)	Model, strawberry-flavoured custards varying in fat content (0.14% and 3.5%), starch and emulsifier concentrations.	Fat content influenced strawberry flavour intensity and release: - Strawberry flavour of 0.14% fat samples was more intense than that of 3.5% ones. - Volatile release was higher in 0.14% milk fat samples than in 3.5% ones.	Increases in fat content may diminish the volatility of certain flavour compounds, in turn modulating their perceived intensity.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Han et al. (2019)	Perceptual ratings of cheese creaminess, butter note, overall flavour and cheese texture pleasantness following consumption of cheese cubes in the presence of either ortho- or retronasal butter odour delivered at various points of the oral processing cycle.	Untrained; n = 20 (8F); 25 – 29 y	Orthonasal Retronasal (C)	Butter odour at two concentrations: low (just above the detection threshold) and high (well above the detection threshold) Gouda cheese varying in fat content (20, 30 and 40%).	Creaminess, butter note intensity and texture pleasantness were enhanced by the addition of a butter odour – effects were more pronounced when a low odour concentration was presented and varied with the timing of odour presentation and cheese fat content: - Creaminess increased when butter odour was presented retronasally at the start of chewing. - Butter note intensity peaked when the odour was delivered retronasally during chewing in regardless of the butter odour concentration. - Texture pleasantness was enhanced when butter odour was delivered orthonasally before chewing. - Creaminess and butter note intensities increased with increasing fat content.	Fat content affects the olfactory perception of creaminess. Fat-related odours can enhance the perception of cheese-related attributes via cross-modal interactions. These enhancements are more pronounced at lower odour concentrations.
Hyvönen et al. (2003)	Time-intensity parameters related to the perception of strawberry flavour release and melting. 6 perceptual ratings, including fattiness and creaminess.	Untrained: - Time-intensity panel: n = 15 (9F); 28 y (SD n.s.) - Descriptive panel: n = 35 (23F); 31 y (SD n.s.)	Orthonasal Retronasal (C)	Strawberry-flavoured ice cream varying in fat content (0, 5, 9, 14 and 18%), prepared using dairy and vegetable fat.	Flavour release from vegetable fat ice cream samples was slightly faster than from dairy fat-based ones. Intensity and sharpness of ice cream aroma and flavour were higher in fat-free than fat-containing samples. No differences in the intensities of aroma and flavour attributes were observed in samples containing 5% of fat or more.	Fat type may influence the volatility of certain odour/flavour compounds, without affecting their perception. Fat content influences the volatility of certain odour/flavour compounds, which may affect their perception.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Jervis et al. (2014)	Perceptual creaminess ratings of sour cream in various conditions: Normal consumption (control); visual exposure only; visual exposure while stirring; stirring while blindfolded; tasting while blindfolded; tasting while blindfolded and wearing a nose clip; tasting while only wearing a nose clip.	Untrained: - Control session: n = 274 - Test sessions: n = 100 – 111	Retronasal (C)	12 samples representing the sensory space of commercial sour creams, with fat content ranging between 0 and 33%.	When the retronasal pathway was inhibited using a nose clip, creaminess perception was different from control (where all sensory modalities were used) – perceived creaminess decreased in most cases. Inhibition of retronasal olfaction had the greatest impact on creaminess perception compared to other modalities.	Retronasal olfaction is involved in the perception of creaminess.
Le Calvé et al. (2015)	Discrimination ability and fat difference thresholds between various food matrices varying in fat.	Trained; n = 35 - 50	Retronasal (C)	Different food matrices varying in fat, sugar and flavouring content: White sauces containing 7.5 - 32.5% fat; dairy milks containing 0 - 3.8% fat; yogurts containing 0 – 3.5% fat.	Sauces: Adding olfactory cues during tasting modulated discrimination ability, depending on the reference fat content and comparison direction. Milk: Fat content discrimination was possible only when olfactory and/or vision cues were involved. In sucrose-enriched samples, olfaction's involvement reduced discrimination ability. The addition of flavours had no effect on discrimination. Yoghurt: Fat discrimination was impossible in the absence of olfactory and/or visual cues. Aame results were observed in sucrose-enriched samples. In samples with added flavour and/or fruit preparation fat discrimination was possible, but the ability was reduced.	Retronasal fat discrimination ability depends on product type and reference fat content. Retronasal olfaction, along with other sensory systems, is involved in food fat content discrimination.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Lorenzo et al. (2015)	Perceptual ratings of flavour intensity and black pepper odour.	Trained; n = 10	Orthonasal Retronasal (C)	Sausages varying in fat content: 10, 20 and 30%.	Whereas fat content had no effect on flavour intensity, perceived black pepper odour intensity decreased with increasing fat content.	Increases in fat content may diminish the volatility of certain odour/flavour compounds, in turn modulating their perceived intensity.
Mela (1988)	Perceptual ratings of fat content and creaminess.	Untrained: - EXP 1: n = 20 (12F); 27 y (SD n.s.) - EXP 2: n = 20 (12F); 23 y (SD n.s.)	Retronasal (C)	Commercially available skim milk (0.5%), whole milk (3.3%), light cream (11.6%), a mixture of light and heavy cream (24%) and heavy cream (36%).	Elimination of olfactory cues had no effect on fat content perception in both experiments.	Fat perception is not driven by olfactory cues. Inhibiting olfactory cues might influence hedonic perception.
Miettinen et al. (2003)	Perceptual intensity ratings of diacetyl and linalool aromas. Time-intensity parameters related to the perception of diacetyl and linalool aromas. Aroma release parameters following headspace sampling.	Trained; n = 12; 28 y (SD n.s.)	Orthonasal Retronasal (C)	Commercial non-fat milk with added rapeseed oil at levels of 0%, 1%, 5%, and 10% (v/v) and flavoured with either diacetyl or linalool.	With increasing fat content, linalool was retained in the matrix, while the release of diacetyl was not affected. Adding 1% of fat to the matrix reduced the headspace linalool concentration and orthonasal, but not retronasal intensity. Linalool aroma perception in the sample containing most fat lasted a shorter time than in samples containing less fat.	Increases in fat content may diminish the volatility of certain odour/flavour compounds, in turn modulating their perception.
Parat-Wilhelms et al. (2005)	Perceptual ratings of coffee-related odour and taste/retronasal odour attributes: buttery, milky, creamy, sour, caramel, aromatic, roasted, coffee, butter, burnt.	Trained; n = 15	Orthonasal Retronasal (C)	Coffee beverages with or without added milk, varying in fat content (0, 3.5 and 7.0%).	An increase in milk sample fat content (from 3.5% to 7.0%) led to a decrease in the perceived intensity of coffee-realted descriptors. No differences in the milk-related descriptors were found between the two milk samples.	Increases in fat content may diminish the perception of certain flavours.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Roberts et al. (2003a)	Flavour compound intensities Aroma release parameters following headspace and nosespace sampling.	Trained; n = 5	Retronasal (C)	Food matrices varying in fat content with added aroma compounds: Water, skim (0.033% fat), semi-skim (2.7% fat) and whole milk (3.8% fat) containing either beta-damascenone, hexanal, ethyl butyrate, benzaldehyde and 2,3-butanedione.	Fat content influenced perceived intensities of the different compounds in all conditions. Volatility and Intensities of the most lipophilic compounds (beta-damascenone, hexanal and ethyl butyrate) decreased with increasing fat content.	Increases in fat content may diminish the volatility of certain flavour compounds, in turn reducing their perceived intensity.
Running et al. (2017)	Orthonasal (linoleic, oleic) and flavour (stearic) rejection thresholds.	Untrained: - Linoleic acid test: n = 75 (49F); 31.1 y (SD n.s.) - Oleic acid test: n = 69 (48F); 34.3 y (SD n.s.) Stearic acid test: n = 80 (21F); 32.1 y (SD n.s.)	Orthonasal	Dark chocolate containing different concentrations (0.04 – 2.5%; w/w) of free fatty acids: linoleic, oleic, stearic.	Chocolate containing the polyunsaturated fatty acid (linoleic) was rejected at lower concentrations than the one containing monounsaturated fatty acid (oleic) in orthonasal and taste conditions. Stearic acid-containing chocolate was not rejected at any concentration.	Fatty acid addition to foods may negatively alter their odour qualities. The degree of fatty acid unsaturation influences rejection upon orthonasal exposure (the more unsaturated, at lower concentrations it gets rejected). Saturated fatty acids do not seem to contribute to flavour preference.
Syarifuddin et al. (2016)	Perceptual ratings of several odour, taste and texture attribute intensities, including perceived fat content.	Untrained; n = 31 (21F); 10 – 61 y	Orthonasal Retronasal (C)	Model cheeses varying in fat content (20%, 40%), added aroma (none, sardine, butter), salt (0.5%, 1.5%) and pH at renneting (5.0, 6.2).	Perceptual ratings of fat content texture increased after the addition of a butter aroma. Fat content had no effect on overall odour intensity, regardless of the added aroma.	The addition of fat-related odours can enhance the perception of fat-related texture sensations.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Ventanas et al. (2010)	Perceptual ratings of mushroom and cocoa odour and flavour. Time-intensity parameters related to the perception of mushroom flavour. Aroma release parameters following headspace sampling.	Trained; n = 8 (5F); 25 – 59 y	Orthonasal Retronasal (C)	Mushroom and cocoa-flavoured cooked bologna sausages varying in NaCl and fat content (from 4.4 to 22.5% fat).	With increasing fat content, mushroom odour intensity decreased, while that of cocoa odour increased. With increasing fat content, mushroom and cocoa flavour intensities decreased. Duration of mushroom flavour perception decreased with increasing fat content. Fat content influenced the volatility of mushroom but not cocoa flavour-related volatiles.	Increases in fat content may diminish the volatility of certain odour/flavour compounds, in turn modulating their perception.
Weenen et al. (2005)	Perceptual ratings of 60 odour, taste/flavour, mouthfeel, and aftertaste-related attributes.	Trained; n = n.s.	Orthonasal Retronasal (C)	10 commercially available vanilla custards (fat content between <0.5 and 3.5%); Mayonnaises (fat content between 0 and 80%); Warm sauces (mainly starch-based).	The use of nose clips decreased the perception of creamy and fatty mouthfeel in vanilla custards.	Retronasal, cues contribute to the perception of fat-related mouthfeel sensations.
de Wijk et al. (2003)	Perceptual ratings of 66 descriptive attributes, including 6 odour and 11 flavour-related sensations.	Trained; n = 9 (7F) (22 – 49 y)	Orthonasal Retronasal (C)	16 vanilla-flavoured model custards varying in fat content (0 and 4.5%), carrageenan and starch.	Compared to 0% fat custard samples, 4.5% fat ones were rated as more intense in terms of vanilla, caramel and milk odour, and less intense in terms of synthetic odour. 4.5% custard samples were also rated as more intense in terms of vanilla, caramel, milk, cream and fat flavour and less intense in terms of chemical and sickly flavour.	Increases in fat content may alter the perception of certain odour and flavour-related sensory qualities.

Study	Outcome(s) of interest	Study Population Characteristics	Exposure	Stimuli	Relevant Findings	Interpretation
Yackinous & Guinard (2000)	Perceptual ratings of fattiness, intensity and liking.	Untrained; n = 106 (66F); 19.3 ± 1.6 y; BMI 21.9 ± 2.7	Retronasal (C)	4 foods varying in fat and flavour concentration: - Butter-flavoured mashed potatoes: (0.5% fat + 0.08% flavour; 15% fat + 3.75% flavour); - Dairy-flavoured vanilla pudding (4% fat + 0.05% flavour; 28% fat + 1.75% flavour); - Sour cream and onion-flavoured potato chips (1% fat + 0.05% flavour; 5% fat + 1.00% flavour); - White chocolate-flavoured chocolate drink (5.29% fat + 4.50% flavour; 15.87% fat + 7.00% flavour).	The use of nose clips reduced the perception of fattiness across all investigated foods. A product-specific effect of flavour concentration on fattiness ratings was observed: The addition of high levels of fatty-type flavours enhanced the perception of fattiness in mashed potatoes and potato chips.	Olfaction contributes to the perception of fat in food. Adding fat-related flavours to foods can enhance the perception of their fattiness.
Zhou et al. (2016)	Perceptual ratings of fattiness intensity following exposure to mixtures differing in fat content via various combinations of sensory modalities (taste, taste + odour, taste + mouthfeel, all modalities).	- EXP 1: n = 46 (21F); 19 – 53 y; BMI 16.5 – 43.5 EXP 2: n = 51 (35F); 18 – 55 y; BMI 17.0 – 39.3	Retronasal (C)	EXP 1: Five mixtures differing in fat content (0, 7.5, 10, 15 and 20%) produced from non-fat skimmed milk, single cream (19.1% fat) and double cream (50.5% fat). EXP 2: Two more mixtures differing in fat content were added (2.5 and 5.5%).	Perceived fattiness intensity rated from taste + odour (without nose clips) was higher than that from just taste (with nose clips) or all modalities. Perceived fattiness intensity rated from all modalities was higher than just from taste + mouthfeel.	Retronasal olfaction contributes to the perception of fat.

EXP, experiment; **n.s.**, not specified; **n**, sample size (**F**, female); **y**, years of age (mean ± SD / range); **BMI**, body mass index, expressed in kg/m2 as mean ± SD or range); **I**, isolated from taste and mouthfeel (e.g. inhalation); **C**, combined with taste and mouthfeel (e.g. during ingestion);

3.3 Risk of bias assessment

Risk of bias evaluations of included rodent studies are presented in Figures S1 and S2 in Supplementary Material C. No information reported in rodent studies indicated a high bias risk or concerns in any of the evaluated domains. Overall, there was a considerable amount of unclear risk of bias due to lack of explicit reporting, particularly not stating whether the researchers were blinded to treatments.

Risk of bias evaluations of included human studies are presented in Figures S3 and S4 in Supplementary Material C. In human studies, there was a moderate amount of unclear risk of bias due to lack of explicit reporting on stimulus presentation orders and participant blinding. Moreover, incomplete outcome reporting (i.e. attrition bias) could not be assessed in several studies due to lack of clarity regarding the inclusion of all participants in the final outcome reports. Not isolating olfaction from effects of potentially confounding sensory modalities, namely taste, mouthfeel and trigeminal sensations was identified as a common source of high bias risk or concerns. Most of the "some concerns" judgements in this domain were given when mouthfeel and taste effects were clearly eliminated, but potential involvement of the trigeminal system could not be ruled out completely, or when orthonasal exposure was combined with non-isolated retronasal exposure.

4 DISCUSSION

This systematic scoping review aimed at (1) identifying and summarizing relevant evidence on the contribution of olfaction to dietary fat perception and (2) highlighting relevant knowledge gaps. It yields consistent evidence supporting the notion that olfaction is involved in the perception of dietary fat in rodents and humans. Olfaction alone is sufficient for detecting fat and its components (i.e. fatty acids), whether they are present on their own or as part of a complex food matrix. Food fat content plays a considerable role in modulating the perception of various fat- and non-fat-related olfactory qualities, depending on the food matrix and odorant properties. Furthermore, the perception of fat in food can be influenced by the addition of fat-related odours, which may enhance olfactory, as well as non-olfactory fat-related attributes, such as mouthfeel.

Albeit limited, evidence from rodent studies supports the involvement of olfaction in fat perception. With the exception of Boone et al. (2021), all studies demonstrated that olfactory cues contribute to the formation of preferences towards fat-related odorants (Kinney & Antill, 1996; Lee et al., 2015; Ramirez, 1993; Takeda et al., 2001; Xavier et al., 2016). Anosmiation having no effect on preference in the case of Boone et al. (2021), and preference partially diminishing following anosmiation in the case of Ramirez (1993) and Takeda et al. (2001), suggests that preference for fat in rodents is mediated by olfactory, as well as non-olfactory cues. Moreover, anosmiation eliminating preference only for low-fat stimuli, as shown by Takeda et al. (2001), points towards olfaction in rodents acting as a signalling mechanism for fat at lower concentrations. Lastly, as suggested by (Xavier et al., 2016), receptor CD36 seems to play a role in detecting fat-related stimuli in rodents.

Findings of human studies utilising free fatty acids as olfactory stimuli are aligned in suggesting that humans possess the ability of perceiving fatty acids via the olfactory system (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Ebba et al., 2012; Kallas & Halpern, 2011; Kindleysides et al., 2017; Running et al., 2017; Rychlik et al., 2006). The interpretation of some findings, however, requires caution. It must be acknowledged that although most studies (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011; Kindleysides et al., 2017), attempted to isolate olfactory inputs from potentially confounding effects of non-olfactory systems (e.g., vision, gustation, somatosensation), only Bolton and Halpern (2010) verified the absence of trigeminal system involvement. They did so by demonstrating that the presentation of fatty acids to the oral cavity resulted in no discrimination from blanks. As the oral cavity is innervated by trigeminal but not olfactory nerve branches (Halpern, 2014), this shows that the discrimination observed by Bolton and Halpern (2010) was indeed olfaction-based and provides the most convincing evidence of 18-carbon fatty acids being effective olfactory stimuli. The involvement of olfaction in fatty acid perception is further corroborated by the fact that elimination of retronasal cues considerably decreases the perceived taste intensity of linoleic acid presented to the oral cavity (Ebba et al., 2012).

Clearly, sensations elicited via olfactory exposure to fat in its isolated form (i.e., fatty acids) are sufficient to evoke perception. However, since fat-related odorants are usually perceived in conjunction with a multitude of other stimuli present in a particular food matrix, the more relevant question is whether fat can be smelled when embedded within a food matrix, and if so, how does that influence perception. Various studies on the matter demonstrated that, even when dietary fat is embedded within a food matrix, olfactory cues enable or facilitate its perception. Using solely olfaction, humans are able to distinguish natural oils and oleic acid from non-fat controls (Glumac & Chen, 2020) and discriminate between fat content differences in dairy milk (Boesveldt & Lundstrom, 2014). The latter has been replicated by our own experiments as well (not included in this review as they were unpublished at the time of search), where we observed that ortho- or retronasal cues in isolation are sufficient to allow for dairy fat content discrimination (Pirc et al., 2022), and identified headspace composition differences underlying the ability (Mu et al., 2022). The involvement of olfaction in detecting food fat content differences seems to be particularly relevant in certain food products, as demonstrated by Le Calvé et al. (2015), who observed that fat content discrimination in milk and yoghurt was possible only after retronasal cues were added to those of other sensory modalities. They also showed that, despite olfaction not being crucial for discriminating fat content in white sauces, retronasal cues can modulate fat content discrimination, depending on the fat content levels being compared and added sweeteners or flavours. Similarly, elimination of retronasal cues via the use of nose clips has been reported to hinder food fat content discrimination (Schoumacker et al., 2017) and affect the perception of fat-related qualities (Jervis et al., 2014; Zhou et al., 2016). The role of olfaction in perceiving fat embedded within food is further underscored by findings that the addition of fatty acids to a food matrix unfavourably alters odour-related qualities by producing off-odours (Rychlik et al., 2006), which may lead to rejection, depending on fatty acid type (Running et al., 2017). All in all, although relatively limited, evidence suggests that olfactory cues are integral for the perception of fat in food (Jervis et al., 2014; Le Calvé et al., 2015; Schoumacker et al., 2017; Zhou et al., 2016). They not only signal its presence (Glumac & Chen, 2020; Rychlik et al., 2006), but may also provide information about its quantity (Boesveldt & Lundstrom, 2014; Mu et al., 2022; Pirc et al., 2022) or type (Running et al., 2017). These findings, in combination with those from studies on fatty acids, indicate that humans possess a functional olfaction-based system for detecting dietary fat in isolation or when part of a food matrix.

Studies investigating the effects of fat content on odour perception found that fat content impacts (i.e. accentuates or diminishes) intensities of various fat and non-fat olfaction-related qualities, in a range of diverse food matrices (Arancibia et al., 2015; Boesveldt & Lundstrom, 2014; Brauss et al., 1999; Chen & Eaton, 2012; Dadalı &

Elmacı, 2019; de Wijk et al., 2003; Frank et al., 2015; Frøst et al., 2001; González-Tomás et al., 2007; Han et al., 2019; Hyvönen et al., 2003; Lorenzo et al., 2015; Miettinen et al., 2004; Miettinen et al., 2003; Parat-Wilhelms et al., 2005; Ventanas et al., 2010). Some qualities, such as creaminess, seem to be positively related to fat content (Chen & Eaton, 2012; Dadalı & Elmacı, 2019; Frøst et al., 2001; Han et al., 2019), yet the relationship is not always linear (Chen & Eaton, 2012; Frøst et al., 2001). It has to be acknowledged that fat content alterations do not always modulate olfaction-related qualities, as was the case in Fernandez et al. (2000) and Syarifuddin et al. (2016). Olfaction-related quality or intensity shifts following fat content alteration, likely arise from changes in the volatility of odorous compounds contained in the food matrix. Various factors, such as lipophilicity and solubility (Guichard, 2002; Guichard et al., 2018), modulate their release, which influences subsequent perception, as demonstrated by several studies included in the current review (Arancibia et al., 2015; Brauss et al., 1999; Dadalı & Elmacı, 2019; Frank et al., 2015; González-Tomás et al., 2007; Hyvönen et al., 2003; Lorenzo et al., 2015; Miettinen et al., 2004; Miettinen et al., 2003; Roberts, Pollien, Antille, et al., 2003; Ventanas et al., 2010). In most instances, increases in fat content seem to accentuate the perception of fat-related flavour volatiles, while diminishing that of non-fat-related ones. There are, however, exceptions. For example, as demonstrated by Dadali & Elmaci, the release of Hexanoic acid, a fat-related odorant responsible for eliciting fatty, waxy or cheesy qualities, decreased despite an increase in fat content. Further discussion about the intricacies behind factors that influence fat-related volatile release are beyond the scope of the current review - for further information on the matter, see the review on flavour compound and food ingredient interactions and their influence on flavour perception by Guichard (2002). In summary, fat content clearly has an influence on the perception of food-related odours and/or flavours. Olfaction-related perceptual consequences of fat content alteration depend on the food matrix and physio-chemical properties of the odorants in question (Guichard et al., 2018).

Conversely, the perception of fat content-related attributes can be modified by the presence of odours associated with fat. All studies exploring perceptual effects of adding fat-related odours to foods observed an enhancement of fat-related qualities (Bult et al., 2007; Frøst et al., 2001; Han et al., 2019; Syarifuddin et al., 2016; Yackinous & Guinard, 2000). The enhancement, however, is not limited solely to olfaction-related attributes, but may also affect non-olfactory ones, such as thickness (Bult et al., 2007), fat-related mouthfeel (Syarifuddin et al., 2016), and texture pleasantness

(Han et al., 2019). The enhancing effects of odours on other sensory modalities have also been demonstrated by Ebba et al. (2012), observing that the removal of retronasal cues diminished taste intensity of linoleic acid, and Weenen et al. (2005), where their absence diminished creamy and fatty mouthfeel. These findings underscore the multi- and cross-modal nature of fat perception (Guichard et al., 2018), wherein the presence of fat-related odours can enhance fat-related mouthfeel and even taste sensations. For additional information on the taste-enhancing potential of odours, see the reviews by Ai and Han (2022) and Spence (2022). For insights on fat-related odour-mouthfeel interactions, see the review by Guichard et al. (2018).

All human studies included in this review, with the exception of Mela (1988), demonstrated that olfaction is involved in the perception of fat or fat-related odours to some degree. Several even found that dietary fat can be perceived using solely olfactory cues (Boesveldt & Lundstrom, 2014; Bolton & Halpern, 2010; Chukir et al., 2013; Glumac & Chen, 2020; Kallas & Halpern, 2011; Le Calvé et al., 2015). We speculate that the low sample serving temperature (4°C) in the study of Mela et al (11) might have reduced the volatility of fat-related odorants, thus hindering the perception of sensory differences between the fat content of their samples. Since fat perception is multi-modal, the exact contribution of olfaction to the overall flavour percept is difficult to approximate. Not only because of the inherent difficulty in disentangling olfactory inputs from non-olfactory ones, but also due to complex cross-modal interactions occurring between olfaction and other modalities, as discussed above. Nevertheless, findings of the current review clearly show that olfaction has a relevant, even independent, role to play in the perception of dietary fat in humans.

Another relevant point that requires discussion is on the differential role the two olfactory routes might play in fat perception, given that they seem to serve distinct purposes in the context of eating (Boesveldt & de Graaf, 2017; Goldberg et al., 2018). Few studies included in the current review aimed specifically at comparing the two routes. Nevertheless, some observations can be highlighted. Although free fatty acids can be perceived by either route, retronasal olfaction seems to be less sensitive to their presence (Chale-Rush et al., 2007). The two routes, however, are relatively comparable in discriminating between specific fatty acid types (Bolton & Halpern, 2010). As demonstrated by our recent work on the topic (Pirc et al., 2022) the routes are also comparable in discriminating fat content of dairy milk. When it comes to perception of fat-related odours in the context of food, Han et al. (2019) compared the two routes and observed differential effects on perception of butter aroma delivered during consumption of cheese, depending on the route of delivery. Specifically, when delivered retronasally, butter aroma enhanced creaminess and butter note intensity, while orthonasally it enhanced texture pleasantness. In contrast, Bult et al. (2007) reported enhancements to creaminess and thickness in dairy milk following retronasal, but not orthonasal exposure to cream aroma. In summary, there seem to be differences in fat perception between the olfactory routes. However, to reach reliable conclusions, more research focusing specifically on the distinctions between the two is needed. For an overview of distinctions between ortho- and retronasal olfaction in the context of flavour perception in general, see the review by Goldberg et al. (2018)

The current work has identified several other relevant knowledge gaps that require attention in order to further our comprehension of the topic. One of the more relevant blind spots is the potential impact of olfactory fat perception on subsequent eating behaviour. Apart from six studies, whose findings on fat odour-related hedonics (Boesveldt & Lundstrom, 2014; Han et al., 2019; Jervis et al., 2014; Running et al., 2017; Syarifuddin et al., 2016; Yackinous & Guinard, 2000) merely hint at possible behavioural implications without experimentally determining them, no other study included in this review aimed at investigating the potential behavioural consequences of fat-related odours. It must be acknowledged that much is still unclear about how, and under what circumstances, food odours impact eating behaviour. Although it has been established that orthonasal food odours can induce appetite specific for the cued product during the anticipatory phase of eating, findings on their effects on food choice and intake are limited and conflicting (Boesveldt & de Graaf, 2017). The effect of retronasal exposure to food odours on eating behaviour has received even less attention. While there is some evidence of their influence on appetite (Ruijschop et al., 2008), which does not seem to translate into actual food intake (Boesveldt & de Graaf, 2017), reports on their potential role in food choice are practically non-existent, even more so when it comes to behavioural consequences of fat-related odours. Future studies should therefore aim to fill this important knowledge gap by investigating potential effects of exposure to various ambient and retronasal fat-related odours on appetite, food choice and intake. One of the key prerequisites to this approach is the elucidation of the exact nature of fat-related olfactory chemical signals. Although fatty acids seem to be effective olfactory stimuli on their own (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011), most fat-related odours largely originate from volatile compounds bound to dietary fats – which are known to act as volatile compound reservoirs (Carrapiso, 2007; Doyen et al., 2001; Haahr, 2000; Roberts, Pollien, & Watzke, 2003). Future research should thus aim to identify effective fat-related olfactory stimuli; extend the knowledge on headspace compositions of different fat-based food matrices, varying in fat content and type; and establish which volatiles underly specific fat-related olfactory qualities (e.g., using gas chromatography-olfactometry or proton transfer reaction-mass spectrometry). Efforts should also be focussed towards identifying fat-related olfactory receptors and elucidating their role. Examining the exact role of receptor CD36, which was suggested to be involved in the perception of fat-related odorants in rodents (Xavier et al., 2016), appears a reasonable initial step. Lastly, and similar to previous work for fat taste (Tucker et al., 2017), additional work is required to illuminate factors governing olfactory sensitivity to fat-related odorants. Sensitivity to fat-related odours seems independent of body composition (Boesveldt & Lundstrom, 2014; Kindleysides et al., 2017; Pirc et al., 2022), and has been found to be related with gustatory sensitivity to oleic acid (Kindleysides et al., 2017). Moreover, our own findings show that olfactory fat content discrimination ability is independent of habitual consumption (Mu et al., 2022; Pirc et al., 2022). However, the evidence base is limited, which warrants further investigation. Future studies should thus aim to replicate initial findings on the topic and seek other potential influences (e.g., genetics). Lastly, expanding the knowledge on mouthfeel and taste-enhancing qualities of specific fat-related odours might also prove worthwhile, especially for commercial applications. Specifically, the addition of fat-related odours to foods as fat substitutes seems a potentially viable approach for reducing food fat content in various food products, without compromising on their appealing fat-related sensory characteristics and negatively impacting food choice and intake. Considering that fat flavour-related foods seem to contribute most to energy intakes (Teo et al., 2022), the development of such sensory optimised foods might help maintain existing dietary flavour patterns, while moderating dietary energy density, as suggested by (Teo et al., 2022) and (Forde & de Graaf, 2022). Findings on the interactions between olfaction and other sensory modalities involved in fat perception could thus prove instrumental in developing strategies aimed at curbing excess dietary fat intakes.

The current review is the first to summarize findings specific to olfactory fat perception. It yields consistent evidence supportive of olfaction's contribution to the perception of fat, yet conclusions are inherently influenced by the studies selected for inclusion. Our choices of search strings, literature eligibility criteria and their appraisal, and the decision to forgo manual literature searching and sifting through reference lists of included articles are likely to have resulted in the omission of other relevant studies. Publication bias remains a possibility as well. Furthermore, potential bias sources should be considered when interpreting reported findings, particularly those that arise from interactions between olfaction and potentially confounding sensory modalities (see Figures 3 and 5), namely taste, mouthfeel and trigeminal sensations. The risks of cross-modal interactions are, however, generally difficult to avoid, mainly due to the inherent complexity in separating retronasal olfaction from other sensations, particularly when it comes to flavour release studies. Even when olfaction is completely isolated from mouthfeel and taste, prying it apart from trigeminal sensations is virtually impossible. Since most odorants can activate the trigeminal system (Goldberg et al., 2018), we decided to take a conservative approach when scoring this domain, to raise caution when interpreting results. This resulted in multiple studies receiving "some concerns" bias risk scores. Nevertheless, we deem the methodological quality and validity of findings reported in this review as high. Especially considering that findings from the vast majority of included studies are aligned. Furthermore, the main conclusions of this review were drawn from studies where the bias risk due to potentially confounding effects of other sensory modalities was minimised. Future work on olfactory fat perception should consider employing control conditions, where possible, wherein the potential involvement of the trigeminal system can be established (as demonstrated by Bolton and Halpern (2010)).

5 CONCLUSION

Our findings support the notion that olfaction contributes to the perception of dietary fat in rodents and humans. The identified evidence base, although relatively heterogenous and limited in some areas, is consistent in showing that olfaction is involved in detecting, discriminating, and identifying fat and its constituents, when either isolated or embedded within a complex food matrix. When embedded within complex food matrices, fat content and type can modulate the perception of various fat- and non-fat related olfactory qualities, likely by influencing the volatility of odorous compounds. Furthermore, the addition of fat-related odorants to a food matrix may modulate not only its olfactory, but also non-olfactory sensory characteristics, such as mouthfeel. This demonstrates that, although olfaction can act as an independent fat-sensing modality, it also interacts with other sensory systems. Several knowledge gaps have been identified by the current review, including the role of fat-related odours in the choice and intake of various foods; the nature of chemical signals underlying olfactory fat perception; and factors governing olfactory sensitivity to fat-related odours. Replication of included studies and examination of suggested knowledge gaps are warranted given the public health and commercial relevance of this topic. Potentially, the cross-modal nature of olfactory cues in fat perception could be exploited in product reformulation. Specifically, fat-related odorants could be used as dietary fat substitutes, to enhance palatability in various low-fat or reduced-fat food products. The current systematic scoping review is the first of its kind focusing specifically on the olfactory component of fat perception. It provides an extensive overview of the topic, which has the potential of facilitating future research and providing useful information to the food industry.

6 REFERENCES

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SUPPLEMENTARY MATERIAL A

The following search strings (per online database) were applied to perform the literature search.

Scopus: (((TITLE-ABS-KEY (volatile OR volatiles OR orthonasal OR orthonasally OR retronasal OR retronasally OR aroma OR aromas OR olfaction OR olfactory OR smell OR smells OR smelling OR odorous OR odorant OR odorants OR odourant OR odourants OR odor OR odors OR odour OR odours)) AND (TITLE-ABS-KEY (fat OR fats OR lipid OR lipids OR "fatty acid" OR "fatty acids" OR fatty OR fattiness OR creamy OR creaminess OR greasiness OR greasy OR oiliness OR oily OR butter OR buttery OR butteriness OR rancid OR rancidness OR rancidity))) AND ((TITLE-ABS-KEY ((flavor) AND (creaminess OR creamy))) OR (TITLE-ABS-KEY ("fat flavor")) OR (TITLE (perception OR discriminat* OR preference)) OR (TITLE-ABS-KEY ((discrimination OR absolute OR difference OR flavor OR olfactory OR odor OR smell OR identification OR sensory OR detection) PRE/3 (threshold))) OR (TITLE-ABS-KEY ((preference) W/10 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY ((perception) W/3 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY ((identification) W/3 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY (chemosensation OR chemosensory OR chemosensitivity)) OR (TITLE-ABS-KEY ((sense) W/15 (fat OR lipid OR "fatty acid"))) OR (TITLE-ABS-KEY ((sensation) W/15 (fat OR lipid OR "fatty acid"))))) AND NOT (cat OR dog OR "honey bee" OR bioelectronic OR electronic OR "bank voles" OR larvae OR larval OR insect OR beetle OR mosquito) AND (LIMIT-TO (LANGUAGE , "English"))

Web of Science: (((TS=(volatile OR volatiles OR orthonasal OR orthonasally OR retronasal OR retronasally OR aroma OR aromas OR olfaction OR olfactory OR smell OR smells OR smelling OR odorous OR odorant OR odorants OR odourant OR odourants OR odor OR odors OR odour OR odours)) AND (TS=(fat OR fats OR lipid OR lipids OR "fatty acid" OR "fatty acids" OR fatty OR fattiness OR creamy OR creaminess OR greasiness OR greasy OR oiliness OR oily OR butter OR buttery OR butteriness OR rancid OR rancidness OR rancidity))) AND ((TS=((flavor OR flavors OR flavour OR flavours) and (creaminess OR creamy))) OR (TS=("fat flavor" OR "fat flavour")) OR (TI=(perception)) OR (TI=(discriminat*)) OR (TI=(preference)) OR (TS=((discrimination OR absolute OR difference OR flavor OR flavors OR flavour OR flavours OR olfactory OR odor OR odors OR odour OR odours OR smell OR identification OR sensory OR detection) NEAR/3 (threshold$))) OR (TS=((preference$) NEAR/10 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=((perception$) NEAR/3 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=((identification$) NEAR/3 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=(chemosensation OR chemosensory OR chemosensitivity)) OR (TS=((sense) NEAR/15 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids"))) OR (TS=((sensation$) NEAR/15 (fat or fats OR lipid OR lipids OR "fatty acid" OR "fatty acids")))) NOT(ALL=(cat OR dog OR "honey bee" OR bioelectronic OR electronic OR "bank voles" OR larvae OR larval OR insect OR beetle OR mosquito))) AND LANGUAGE: (English)

Indexes=SCI-EXPANDED, SSCI, A&HCI, ESCI Timespan=All years

Pubmed: ((volatile[Title/Abstract] OR volatiles[Title/Abstract] OR orthonasal[Title/Abstract] OR orthonasally[Title/Abstract] OR retronasal[Title/Abstract] OR retronasally[Title/Abstract] OR aroma[Title/Abstract] OR aromas[Title/Abstract] OR olfaction[Title/Abstract] OR olfactory[Title/Abstract] OR smell[Title/Abstract] OR smells[Title/Abstract] OR smelling[Title/Abstract] OR odorous[Title/Abstract] OR odorant[Title/Abstract] OR odorants[Title/Abstract] OR odourant[Title/Abstract] OR odourants[Title/Abstract] OR odor[Title/Abstract] OR odors[Title/Abstract] OR odour[Title/Abstract] OR odours[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract] OR fatty[Title/Abstract] OR fattiness[Title/Abstract] OR creamy[Title/Abstract] OR creaminess[Title/Abstract] OR greasiness[Title/Abstract] OR greasy[Title/Abstract] OR oiliness[Title/Abstract] OR oily[Title/Abstract] OR butter[Title/Abstract] OR buttery[Title/Abstract] OR butteriness[Title/Abstract] OR rancid[Title/Abstract] OR rancidness[Title/Abstract] OR rancidity[Title/Abstract])) AND (((flavor[Title/Abstract] OR flavors[Title/Abstract] OR flavour[Title/Abstract] OR flavours[Title/Abstract]) AND (creaminess[Title/Abstract] OR creamy[Title/Abstract])) OR (“fat flavor”[Title/Abstract] OR “fat flavour”[Title/Abstract]) OR (perception[Title]) OR (discriminat*[Title]) OR (preference[Title]) OR ((discrimination[Title/Abstract] OR absolute[Title/Abstract] OR difference[Title/Abstract] OR flavor[Title/Abstract] OR flavors[Title/Abstract] OR flavour[Title/Abstract] OR flavours[Title/Abstract] OR olfactory[Title/Abstract] OR odor[Title/Abstract] OR odors[Title/Abstract] OR odour[Title/Abstract] OR odours[Title/Abstract] OR smell[Title/Abstract] OR identification[Title/Abstract] OR sensory[Title/Abstract] OR detection[Title/Abstract]) AND (threshold[Title/Abstract] OR thresholds[Title/Abstract])) OR ((perception[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract])) OR ((identification[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract])) OR ((chemosensation[Title/Abstract] OR chemosensory[Title/Abstract] OR chemosensitivity[Title/Abstract])) OR ((sense[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract])) OR ((sensation[Title/Abstract]) AND (fat[Title/Abstract] OR fats[Title/Abstract] OR lipid[Title/Abstract] OR lipids[Title/Abstract] OR “fatty acid”[Title/Abstract] OR “fatty acids”[Title/Abstract]))) NOT (cat[All] OR dog[All] OR “honey bee”[All] OR bioelectronic[All] OR electronic[All] OR “bank voles”[All] OR larvae[All] OR larval[All] OR insect[All] OR beetle[All] OR mosquito[All])

Filter: English language

SUPPLEMENTARY MATERIAL B

Table S1: *Studies excluded during full-text screening*

Reference	Title	Exclusion Reason
Bisulco and Slotnick, 2003	Olfactory discrimination of short chain fatty acids in rats with large bilateral lesions of the olfactory bulbs	Lack of olfactory exposure to suitable fat sources.
Borg and Seubert, 2017	Lipids in Eating and Appetite Regulation – A Neuro-Cognitive Perspective	Review, meta-analysis, book, chapter
Burseg et al., 2009	Flavor perception in biscuits; Correlating sensory properties with composition, aroma release, and texture	No relevant outcomes resulting from olfactory exposure reported.
Calkins and Hodgen, 2007	A fresh look at meat flavor	Review, meta-analysis, book, chapter
de Roos, 1997	How lipids influence food flavor	Unavailable
De Roos, 2005	How lipids influence flavor perception	Review, meta-analysis, book, chapter
Delahunty, 1996	Comparison of dynamic flavour release from hard cheeses and analysis of headspace volatiles from the mouth with flavour perception during consumption	Lack of olfactory exposure to suitable fat sources.
Drake et al., 2010	Impact of fat reduction on flavor and flavor chemistry of Cheddar cheeses	Lack of olfactory exposure to suitable fat sources.
Drake et al., 2010	Influence of fat on flavour and flavour development in cheddar cheese	Review, meta-analysis, book, chapter
Drewnowski, 1997	Why do we like fat?	Review, meta-analysis, book, chapter
Feyzi et al., 2020	A study on aroma release and perception of saffron ice cream using in-vitro and in-vivo approaches	Lack of olfactory exposure to suitable fat sources.
Folkenberg and Martens, 2003	Sensory properties of low fat yoghurts. Part A: Effect of fat content, fermentation culture and addition of non-fat dry milk on the sensory properties of plain yoghurts	Unavailable
Francis and Eldeghaidy, 2015	Imaging methodologies and applications for nutrition research: what can functional MRI offer?	Review, meta-analysis, book, chapter

Reference	Title	Exclusion Reason
Frank et al., 2011	Proton transfer reaction mass spectrometry and time intensity perceptual measurement of flavor release from lipid emulsions using trained human subjects	Focusing on volatile compounds without relevant sensory evaluation measures
Fuentes et al., 2013	Effect of intramuscular fat content and serving temperature on temporal sensory perception of sliced and vacuum packaged dry-cured ham	Lack of olfactory exposure to suitable fat sources.
Garvey et al., 2019	Factors influencing the sensory perception of reformulated baked confectionary products	Review, meta-analysis, book, chapter
Guichard, 2001	Interactions between flavor compounds and food ingredients and their influence on flavor perception	Review, meta-analysis, book, chapter
Guichard and Relkin, 2008	Flavor release from food emulsions varying in their composition in fat and proteins and its effect on flavor perception	Unavailable
Guichard et al., 2013	Flavour release and sensory perception in cheeses	Review, meta-analysis, book, chapter
Guichard et al., 2018	Physiological mechanisms explaining human differences in fat perception and liking in food spreads-a review	Review, meta-analysis, book, chapter
Hatakeyama et al., 2014	Optimising aroma quality in curry sauce products using in vivo aroma release measurements	Focusing on volatile compounds without relevant sensory evaluation measures
Hatchwell, 1996	Implications of Fat on Flavor	Review, meta-analysis, book, chapter
Henneberry et al., 2015	Sensory quality of unheated and heated Mozzarella-style cheeses with different fat, salt and calcium levels	Lack of olfactory exposure to suitable fat sources.
Kanta et al., 2019	Eliciting the Sensory Modalities of Fat Reformulated Yoghurt Ice Cream Using Oligosaccharides	Lack of olfactory exposure to suitable fat sources.
Larue, 1978	Oral cues involved in the rat's selective intake of fats	Unavailable
Le Calvé et al., 2019	Capturing key sensory moments during biscuit consumption: Using TDS to evaluate several concurrent sensory modalities	No relevant outcomes resulting from olfactory exposure reported.

Reference	Title	Exclusion Reason
Lim et al., 2010	Effect of flaxseed oil towards physicochemical and sensory characteristic of reduced fat ice creams and its stability in ice creams upon storage	No relevant outcomes resulting from olfactory exposure reported.
McDaniel et al., 1969	Influence of Free Fatty Acids on Sweet Cream Butter Flavor	No relevant outcomes resulting from olfactory exposure reported.
Morquecho-Campos et al., 2020	Smelling our appetite? The influence of food odors on congruent appetite, food preferences and intake	Review, meta-analysis, book, chapter
Neugebauer et al., 2020	Characterization of the Key Odorants in High-Quality Extra Virgin Olive Oils and Certified Off-Flavor Oils to Elucidate Aroma Compounds Causing a Rancid Off-Flavor	Focusing on volatile compounds without relevant sensory evaluation measures
Nishimura and Saiga, 2019	Umami compounds and fats involved in koku attribute of pork sausages	Review, meta-analysis, book, chapter
Overington et al., 2010	Flavour release and perception in cheese bases	No relevant outcomes resulting from olfactory exposure reported.
Pepino and Mennella, 2014	Cigarette smoking and obesity are associated with decreased fat perception in women	No relevant outcomes resulting from olfactory exposure reported.
Postma et al., 2020	Food preferences and intake in a population of Dutch individuals with self-reported smell loss: An online survey	Not about fat peception
Schlutt et al., 2007	Sensory-directed identification of creaminess-enhancing volatiles and semivolatiles in full-fat cream	Focusing on volatile compounds without relevant sensory evaluation measures
Shepard et al., 2013	Relating sensory and chemical properties of sour cream to consumer acceptance	Not about fat perception
Shiota et al., 2011	Model studies on volatile release from different semisolid fat blends correlated with changes in sensory perception	Not about fat peception
Shojaei et al., 2006	Measurement and manipulation of aroma delivery allows control of perceived fruit flavour in low- And regular-fat milks	Lack of olfactory exposure to suitable fat sources.
Stevenson et al., 2016	Chemosensory Abilities in Consumers of a Western-Style Diet	Lack of olfactory exposure to suitable fat sources.
Strugnell, 1995	Consumer acceptance of fat substitutes	Review, meta-analysis, book, chapter

Reference	Title	Exclusion Reason
Summo et al., 2020	Effectiveness of Oat-Hull-Based Ingredient as Fat Replacer to Produce Low Fat Burger with High Beta-Glucans Content	Lack of olfactory exposure to suitable fat sources.
Tamsma et al., 1969	Contribution of Milk Fat to the Flavor of Milk	No relevant outcomes resulting from olfactory exposure reported.
Tepper and Kuang, 1996	Perception of fat in a milk model system using multidimensional scaling	No relevant outcomes resulting from olfactory exposure reported.
Tomaschunas et al., 2013	Changes in sensory properties and consumer acceptance of reduced fat pork Lyon-style and liver sausages containing inulin and citrus fiber as fat replacers	Lack of olfactory exposure to suitable fat sources.
Tsuruta et al., 1999	The orosensory recognition of long-chain fatty acids in rats	No relevant outcomes resulting from olfactory exposure reported.
Tucker et al., 2012	Olfactory ability and object memory in three mouse models of varying body weight, metabolic hormones, and adiposity	Not about fat perception
Ulla et al., 2016	Genetic basis of flavor sensitivity and food preferences	Review, meta-analysis, book, chapter
Upadhyay et al., 2020	Perception of creaminess in foods	Review, meta-analysis, book, chapter
Van den Oever, 2006	Fat reduction in foods: Microstructure control of oral texture, taste, and aroma in reduced oil systems	Review, meta-analysis, book, chapter
Yackinous and Guinard, 2001	Relation between PROP taster status and fat perception, touch, and olfaction	No relevant outcomes resulting from olfactory exposure reported.
Yackinous et al., 1999	Internal preference mapping of hedonic ratings for Ranch salad dressings varying in fat and garlic flavor	No relevant outcomes resulting from olfactory exposure reported.

SUPPLEMENTARY MATERIAL C

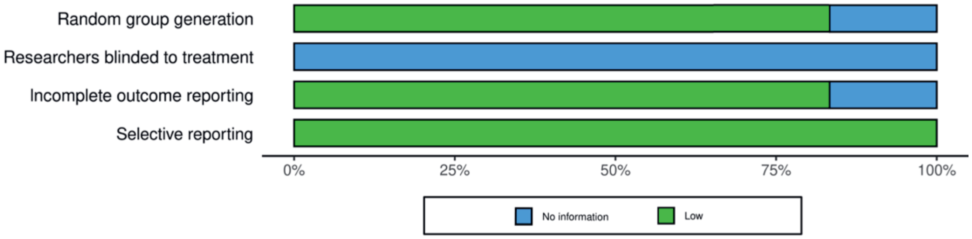
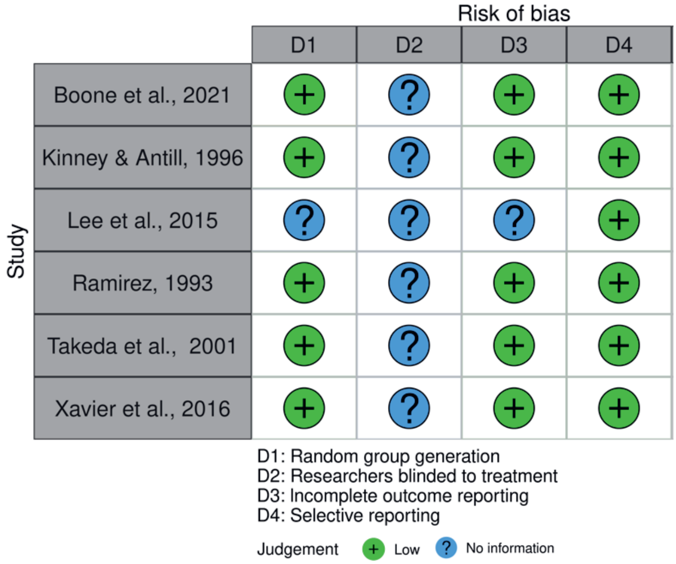


Figure S1. *Risk of bias assessment graph for rodent studies.*



Risk of bias

Study	D1	D2	D3	D4
Boone et al., 2021	+	?	+	+
Kinney & Antill, 1996	+	?	+	+
Lee et al., 2015	?	?	?	+
Ramirez, 1993	+	?	+	+
Takeda et al., 2001	+	?	+	+
Xavier et al., 2016	+	?	+	+

D1: Random group generation
D2: Researchers blinded to treatment
D3: Incomplete outcome reporting
D4: Selective reporting

Judgement + Low ? No information

Figure S2. *Risk of bias assessment summary table for rodent studies.*

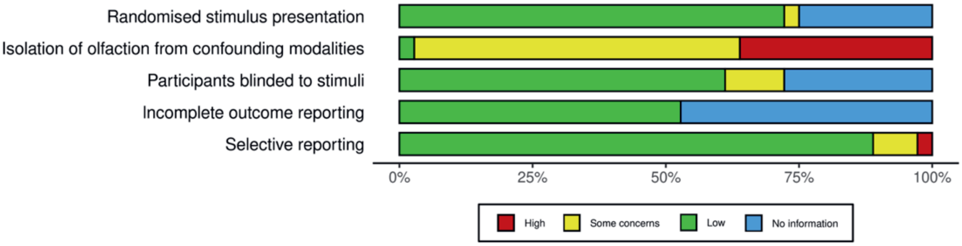
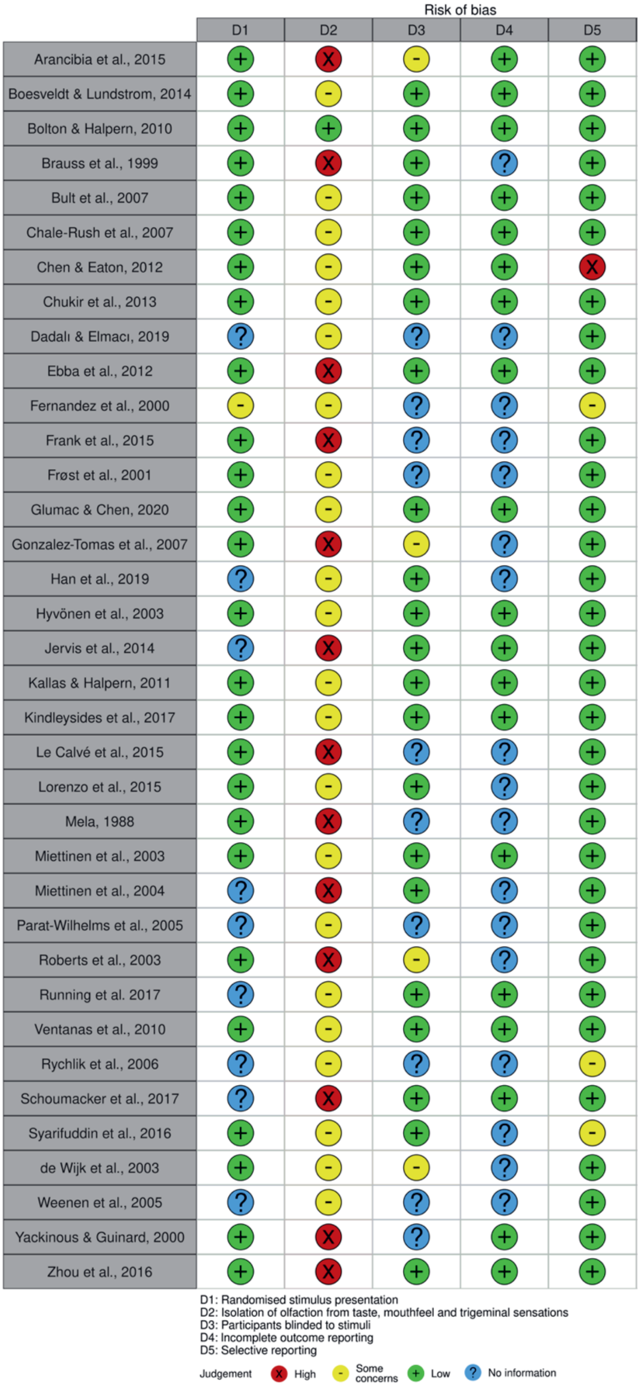


Figure S3. *Risk of bias assessment graph for human studies.*



	Risk of bias				
	D1	D2	D3	D4	D5
Arancibia et al., 2015	+	X	-	+	+
Boesveldt & Lundstrom, 2014	+	-	+	+	+
Bolton & Halpern, 2010	+	+	+	+	+
Brauss et al., 1999	+	X	+	?	+
Bult et al., 2007	+	-	+	+	+
Chale-Rush et al., 2007	+	-	+	+	+
Chen & Eaton, 2012	+	-	+	+	X
Chukir et al., 2013	+	-	+	+	+
Dadalı & Elmacı, 2019	?	-	?	?	+
Ebba et al., 2012	+	X	+	+	+
Fernandez et al., 2000	-	-	?	?	-
Frank et al., 2015	+	X	?	?	+
Frøst et al., 2001	+	-	?	?	+
Glumac & Chen, 2020	+	-	+	+	+
Gonzalez-Tomas et al., 2007	+	X	-	?	+
Han et al., 2019	?	-	+	?	+
Hyvönen et al., 2003	+	-	+	+	+
Jervis et al., 2014	?	X	+	+	+
Kallas & Halpern, 2011	+	-	+	+	+
Kindleysides et al., 2017	+	-	+	+	+
Le Calvé et al., 2015	+	X	?	?	+
Lorenzo et al., 2015	+	-	+	?	+
Mela, 1988	+	X	?	?	+
Miettinen et al., 2003	+	-	+	+	+
Miettinen et al., 2004	?	X	+	?	+
Parat-Wilhelms et al., 2005	?	-	?	?	+
Roberts et al., 2003	+	X	-	?	+
Running et al. 2017	?	-	+	+	+
Ventanas et al., 2010	+	-	+	+	+
Rychlik et al., 2006	?	-	?	?	-
Schoumacker et al., 2017	?	X	+	+	+
Syarifuddin et al., 2016	+	-	+	?	-
de Wijk et al., 2003	+	-	-	?	+
Weenen et al., 2005	?	-	?	?	+
Yackinous & Guinard, 2000	+	X	?	+	+
Zhou et al., 2016	+	X	+	+	+

D1: Randomised stimulus presentation
D2: Isolation of olfaction from taste, mouthfeel and trigeminal sensations
D3: Participants blinded to stimuli
D4: Incomplete outcome reporting
D5: Selective reporting

Judgement X High - Some concerns + Low ? No information

Figure S4. *Risk of bias assessment summary table for human studies*

3

Chapter 3

Humans possess the ability to discriminate food fat content solely based on retronasal olfaction

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ABSTRACT

Dietary fat overconsumption contributes to the development of obesity and related comorbidities; however, its sensory perception is poorly understood. Although humans can discriminate between vapor-phase fatty acids, both ortho- and retronasally, evidence of orthonasal fat discrimination in real foods is limited, and non-existent for retronasal olfaction.

In two experiments, we investigated the human ability of olfactory food fat content discrimination in dairy milk and assessed whether this ability is affected by habitual dairy intake. Participants undertook a series of DR A-not A discrimination tests (analysed with R-index analyses) coupled with perceptual ratings and a questionnaire on dairy consumption habits.

In the first experiment (n = 66), ortho- and retronasal discrimination was evaluated using dairy milk samples manipulated to contain 0%, 1.5% and 3.5% fat. Subjects could discriminate between all three fat levels orthonasally ($p < .001$), whereas retronasally they were able to do so between 0–1.5% ($p < .001$) and 0–3.5% ($p < .001$). The second experiment (n = 44) focused only on retronasal discrimination, using (manipulated) dairy milk samples of 3.5%, 7%, 10.5% and 14% fat. Here, discrimination was possible between 3.5–14% ($p < .001$) and 7–14% ($p < .05$) samples. No effects of total dairy fat intake, total dairy product intake or dairy exposure frequency were observed on discrimination ability in both experiments.

To our knowledge, this is the first study demonstrating that humans are capable of discriminating food fat content solely based on retronasal olfaction. Results also suggest that this ability is unaffected by habitual intake.

Keywords: Dietary fat perception, retronasal olfaction, orthonasal olfaction, discrimination ability, dairy milk

1 INTRODUCTION

Overconsumption of dietary fat is considered a major contributing factor to the development of obesity and related comorbidities. Due to our innate inclination for energy-dense nutrients, a preference for fatty foods appears to be a universal human trait and the overconsumption of fat-laden foods is further exacerbated by the pleasurable sensory characteristics of fat (Drewnowski, 1997; Drewnowski & Almiron-Roig, 2009). Since fat consumption is exceeding intake recommendations in many Western diets, the understanding of its sensory perception is crucial in developing public health strategies aimed at reducing its excessive intake (Drewnowski & Almiron-Roig, 2009; WHO, 2018).

The alluring flavour of fat arises from a synergy between gustation, somatosensation, as well as olfaction (Drewnowski & Almiron-Roig, 2009; Zhou et al., 2016). Whereas orthonasal odours are related to food source detection and the induction of appetite during the anticipatory phase of eating, retronasal odours are considered fundamental contributors to flavour perception during food consumption and may influence intake and satiation (Boesveldt & de Graaf, 2017; Bojanowski & Hummel, 2012). An increasing body of evidence underscores the importance of olfaction in fat perception, with findings that humans are not only capable of detecting (Chale-Rush et al., 2007) and discriminating between vapour-phase fatty acids ortho- and retronasally (Bolton & Halpern, 2010; Kallas & Halpern, 2011), but also identifying different types retronasally (Chukir et al., 2013). Despite demonstrating ability for olfactory fat discrimination and identification, the ecological validity of studies using vapour-phase fatty acids as olfactory stimuli is limited: fatty acids in food are present in conjunction with other odorous constituents which can mask or influence olfactory perception. Therefore, olfactory fat perception needs to be studied in the context of real foods as well. The first to do so were Boesveldt and Lundstrom (2014), who demonstrated that humans can discriminate between different fat concentrations in dairy milk using solely orthonasal olfactory cues. To our knowledge, fat content discrimination in a real food context based solely on retronasal olfactory cues has not yet been reported. In fact, relatively little is known about the exact contribution of retronasal odours to fat perception. Yackinous and Guinard (2000) and Zhou et al. (2016), have demonstrated that retronasal odours enhance fat flavour intensity in various real foods, while Schoumacker et al. (2017) observed a decrease in fat detection and discrimination thresholds when cottage cheese varying in fat was evaluated without nose clips (with the involvement of the retronasal route). Similarly, Jervis et al. (2014) showed that inhibition of the retronasal pathway (using nose clips) diminishes the perception of creaminess in sour cream. This suggests that the perception of creaminess, which seems to be related to fat levels and considered a key driver of sensory appeal in fatty foods (Frøst & Janhøj, 2007), is assessed via retronasal olfactory mechanisms. A similar reduction in the perception of fat-related attributes was observed by Weenen et al. (2005), who demonstrated that the use of nose clips decreased the perception of creaminess and fattiness in custard desserts. Moreover, Martin et al. (2016) observed that the perception of naturally occurring cream aroma in cottage cheese was positively related to fat content and suggested that (retronasal) olfactory cues are one of the main contributors to fat perception in foods. Nevertheless, none of these studies evaluated the retronasal component in isolation, separating it from confounding factors such as gustatory, thermal, and mechanical sensations.

Chemosensory fat detection abilities in humans (Kindleysides et al., 2017; Stevenson et al., 2016; Stewart et al., 2010) and rats (Thiebaud et al., 2014) seem to be modulated by habitual fat intake to a degree, possibly via exposure effects. However, results of Boesveldt and Lundstrom (2014) show that olfactory fat discrimination is independent of habitual intake, suggesting that it might be an innate ability. From an evolutionary perspective this seems reasonable: An innate ability to detect fat content, and hence energy content, in foods via the olfactory system prior to and during consumption, would support energy-efficient foraging within fluctuating ancestral food environments. This line of thought is supported by findings of de Vries et al. (2020), who observed that when exposed to olfactory food cues, individuals were better at recalling locations of odours signalling high-calorie foods, compared to matched low-calorie counterparts, regardless of explicit hedonic odour evaluations or odour familiarity. Evidence therefore points towards olfaction being an effective innate mechanism for gauging the energy content of potential food sources, yet further corroboration is needed.

The contribution of olfaction, retronasal olfaction in particular, to fat perception remains to be clarified. The first step in filling this knowledge gap is to assess whether humans possess the ability to retronasally discriminate fat content in real foods. The aim of the present study was therefore to explore whether humans can discriminate fat content in different versions of dairy milk and assess whether this ability is dependent on habitual dairy intake. To confirm findings on orthonasal discrimination of fat content in food by Boesveldt and Lundstrom (2014), and extend those of Bolton and Halpern (2010) on retronasal fatty acid discrimination, two experiments were carried out. In the first experiment, we determined ortho- and retronasal discrimination ability between three milk samples manipulated to contain ecologically relevant fat levels. To gain insight on the sensory differences between the samples and allow for a more in-depth comparison between the two olfaction routes, ratings of fat odour intensity and liking were evaluated as well. In the second experiment, we focused solely on retronasal olfaction while expanding the fat sample range. In the attempt to better understand the differences in discrimination ability between the fat concentrations, perceptual ratings of creaminess were evaluated as well. Potential effects of habitual dairy consumption on discrimination ability were assessed in both experiments.

2 MATERIALS & METHODS

All participants were informed about the experimental protocol and provided written informed consent in accordance with the Declaration of Helsinki prior to participation. All study aspects were approved by the Wageningen University Medical Ethics Review Board. Data that support the findings of this study are available on the Open Science Framework Repository with the identifier DOI 10.17605/OSF.IO/NXFQZ (Pirc et al., 2021).

2.1 Experiment 1

The main aim of experiment 1 was to assess ortho- and retronasal discrimination ability in dairy milk consumers, using dairy milk samples containing 0%, 1.5% and 3.5% fat. Effects of habitual dairy consumption, along with perceptual ratings of fat odour intensity and liking were assessed as well.

2.1.1 Participants

A total of 66 participants (MAge = 24 ± 3.3 years; MBMI = 22.7 ± 2.4 kg/m2; 31 males) recruited from Wageningen (The Netherlands) and its surroundings took part in the study. All were consumers of dairy milk and met eligibility criteria of being between 18 and 55 years of age, healthy, non-smoking, normosmic (assessed with the Sniffin' Sticks 16-item odour identification test (Hummel et al., 2007)), nondieting currently or in the past two months, non-pregnant, non-lactating, not being lactose-intolerant or having any other dairy-related allergies.

2.1.2 Stimuli & stimulus presentation

Three versions of dairy milk, containing fat levels resembling those found in commercially available skimmed, semi-skimmed and whole milk, respectively, were used as odour stimuli: 0% (F0), 1.5% (F1.5) and 3.5% (F3.5). They were produced by combining fresh, pasteurised skimmed milk (0% fat - AH Magere melk, Albert Heijn B.V.) with fresh, pasteurised full-fat cream (35% fat - AH Verse Slagroom, Albert Heijn B.V.), both processed within the same dairy processing facility (Arla Foods B.V., Nijkerk, Netherlands – EC approval number: NL Z0055 EG), to minimise between-sample variation. Sample mixtures were prepared fresh at the beginning of each testing day with the use of a magnet stirrer and kept in air-tight containers until presented. To ensure sample stability, 0.5% kappa (κ) carrageenan water-based solution was added to all three milk versions. Sample ingredients and corresponding nutritional values can be found in Table A1 in the supplementary material.

Samples were presented in 60 ml amounts at 20 ± 1 °C, using containers adapted from the design used by Bolton and Halpern (2010) (see Figure 1). They consisted of an opaque, black polypropylene cup (Ø 95 mm x H 40 mm; volume 150 ml), covered with a black, reusable silicone coffee cup lid. A 2-ml micro tube with its bottom portion cut away (Ø 10 mm x H 25 mm) was inserted into the lid’s drinking hole to serve as an air inlet. The retronasal container version had a single drinking straw piece inserted into the silicone lid, whereas the orthonasal version had two (12 mm apart). Straw pieces were 65 mm long and inserted into holes made in the lid with a hole punch (Ø 5 mm), with 48 mm protruding above the lid surface. Due to elasticity of silicone all elements fit tightly, with the straws being adjustable in angle. When not in use, all openings were covered with caps.

Figure 1. *Retronasal (left) and orthonasal (right) delivery containers.*

2.1.3 Study design and procedures

Participants attended three sessions, spread across separate days and carried out in sensory booths. They were given instructions not to consume anything other than water two hours prior to testing and to avoid using any scented products on testing days.

The first session included bodyweight and height measurements, followed by an olfactory function assessment and a short training procedure. Participants were instructed not to lift the containers or blow air into the straws and to make steady, moderately intense inhalations, lasting approximately two seconds. The importance of producing consistent inhalations across all trials was emphasised. For orthonasal inhalation, they were instructed to insert straw tips into the nostrils, inhale, remove straws from the nostrils and exhale through the nose. For retronasal inhalation, they were instructed to put on a nose clip before inserting the straw tip into their mouth, inhale, remove the nose clip and exhale through the nose, while keeping the mouth closed. A demonstration on proper container handling and inhalation techniques was also given at this point.

The training procedure was followed by two blocks of intensity and liking ratings – a retronasal and an orthonasal one (order counterbalanced across participants). In both blocks, participants were presented with the three milk samples (one at a time, in a random order), instructed to smell them and rate the perceived odour intensity and liking on 100-unit Visual Analogue Scales (VAS). To prevent olfactory adaptation Pellegrino et al. (2017), samples were separated by 30-s pauses, whereas a 5-min break was implemented between the two blocks. The session concluded with a dairy food frequency questionnaire (DFFQ) (adapted from Boesveldt and Lundstrom (2014)), containing questions about participants' habitual dairy product consumption.

The remaining two sessions – one orthonasal, the other retronasal, with the order counterbalanced across participants, both comprised of discrimination testing. Participants undertook the dual reminder A-not A (DR A-not A) test (see Mun et al. (2019)) with a pairwise design (Hautus et al., 2018). In this version of the A-not A test, two reference stimulus presentations precede a single test stimulus presentation. Participants thus had to smell the reference sample twice prior to smelling the test sample once and responding whether the test sample was the reference (SA) or not (S not A). Each discrimination testing block began with a familiarisation procedure, during which participants were presented with both stimuli used in that block. They were told which sample was the reference and which was different from the reference, and instructed to smell them twice, in an alternating manner (SA, S not A, SA, S not A). This was implemented to stabilise participants' cognitive decision criteria (Lee, van Hout, & O'Mahony, 2007). They then completed three blocks of four tests, each block consisting of only two stimulus levels: either 0% and 1.5% (F0-1.5); 0% and 3.5% (F0-3.5); or 1.5% and 3.5% (F1.5-3.5). Block order was randomised. The sample with the lower fat concentration always served as the reference, whereas the test sample could be either of the stimuli in that pair. For each stimulus level combination, there were two possible presentation sequences: SA – SA – SA or SA – SA – S not A. Within a block, each presentation sequence was provided twice, in a random order. To counteract olfactory adaptation, inter-test and inter-block intervals of approximately 30 and 3 min were implemented, respectively. Responses were collected in terms of six categories: "it is the reference – I am sure", "it is the reference – I am unsure", "I am guessing it is the reference", "I am guessing it is not the reference", "it is not the reference – I am unsure", "it is not the reference – I am sure". See Figure 2 for an overview of the first experiment.

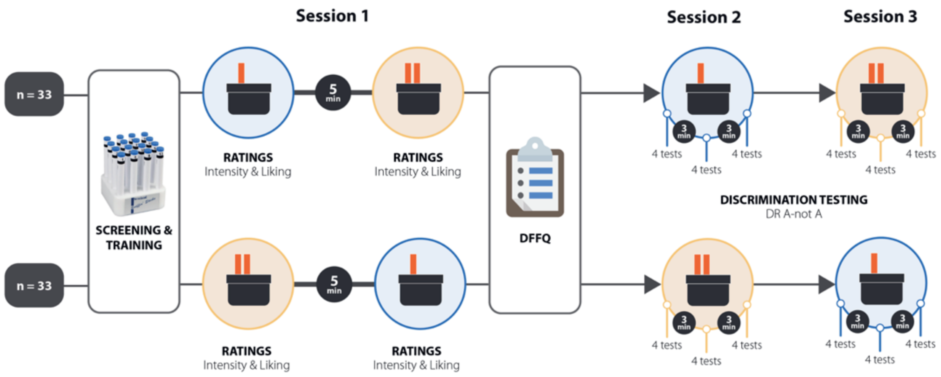

Figure 2. *Overview of experiment 1. Retronasal trials = shaded blue; orthonasal trials = shaded orange.*

2.1.4 Statistical analyses

Discrimination ability was assessed with R-index analyses carried out in accordance with the protocols described by Lee and van Hout (2009). To account for replicated testing, R-indices were computed based on weighted means of individual R-index values (derived from 4 signal / noise tests per judge) (Bi, 2015). Statistical significance was established by calculating the R-index critical value, using R statistical software (R-Core Team, 2020) and the code provided by Bi and O'Mahony (2020). The R-index critical value for 132 control and 132 test samples in a one-sided test at the .05 significance level amounts to 55.81. Apart from R-index analyses, all other statistical procedures were carried out using IBM SPSS Statistics, version 27. Differences in discrimination ability (mean individual R-index values) between olfaction routes for each of the fat concentration comparisons were analysed using Wilcoxon signed-ranks tests. Potential learning or warm-up effects during discrimination testing were assessed by evaluating frequencies of hits, misses, correct rejections and false alarms across the test repetitions, using chi-square tests of independence.

Effects of olfaction route and fat concentration on perceived odour intensity and liking were analysed with linear mixed models (LMM), using intensity or liking as dependent variables, milk fat sample concentrations and olfaction routes as fixed factors, and subjects as a random one. For significant main effects, post-hoc pairwise comparisons with Bonferroni corrections were applied to compare ratings between olfaction routes and fat concentrations.

To assess habitual dairy consumption, DFFQ responses were converted into total dairy product intakes (in g / day), total dairy fat intakes (in g / day) and dairy product consumption frequencies (number of times / day). This was done with the help of the Dutch Food Composition Database (NEVO), published by the Dutch National Institute for Public Health and Environment (RIVM). Effects of these habitual dairy consumption parameters on discrimination ability were evaluated with LMM analyses, using R-indices as dependent variables, either total dairy product intakes, total dairy fat intakes or dairy product consumption frequencies as fixed factors and subjects a random one.

2.2 Experiment 2

Results from experiment 1 confirmed previous findings of Boesveldt and Lundstrom (2014) on orthonasal fat content discrimination and revealed that fat content discrimination is also possible retronasally. The main aim of experiment 2 was to further explore retronasal discrimination ability, by evaluating whether and how it is affected by larger fat concentration magnitude differences. The fat sample range was expanded to contain dairy milk samples with 3.5%, 7%, 10.5% and 14% fat. Effects of habitual dairy consumption on discrimination ability, along with perceptual ratings of fat odour intensity, creaminess and liking were assessed as well. Creaminess was added as an attribute following multiple reports from participants taking part in the first experiment, claiming that their discrimination testing decision was based on differences in creaminess between the samples.

2.2.1 Participants

A total of 44 participants (mean age 23.8 ± 3.2 years; 21 men; mean BMI 22.2 ± 2.1 kg/m2) recruited from Wageningen (the Netherlands) and its surroundings participated in the study. All met the same inclusion criteria as described for Experiment 1 (see section 2.1.1).

2.2.2 Stimuli and stimulus presentation

Four versions of dairy milk, containing fat levels resembling those found in commercially available whole milk, quark, sour cream and reduced-fat cooking cream, respectively, were used as odour stimuli: 3.5% (F3.5), 7% (F7), 10.5% (F10.5) and 14% (F14). They were produced by combining fresh, pasteurised skimmed milk (0% fat content) (AH Magere melk, Albert Heijn B.V.) with fresh, pasteurised full fat cream (35% fat content) (AH Verse Slagroom, Albert Heijn B.V.). Sample mixtures were prepared fresh at the beginning of each testing session, using a dispersing machine (T 25 digital Ultra-Turrax, IKA®-Werke GmbH & Co. KG) set at 4000 rpm for

2 minutes. They were presented as described for Experiment 1 (see section 2.1.2). Sample ingredients and corresponding nutritional values can be found in Table A2 in the supplementary material.

2.2.3 Study design and procedures

Participants attended four sessions spread across separate days. Apart from excluding orthonasal inhalation procedures, the timeline of the first session, provided instructions and training were as described for Experiment 1 above. After training, participants were presented with the four milk sample versions, instructed to inhale them retronasally and rate the perceived odour intensity, creaminess and liking on 100-unit VAS. Samples were presented in a random order, one at a time, with 45-s pauses in between. The session concluded with the DFFQ.

The remaining three sessions involved discrimination testing, using the DR A-not A methodology as described for Experiment 1 (see section 2.1.3). Each discrimination testing session comprised of two blocks of six tests, with each block consisting of two stimulus levels: either 3.5% and 7% (F3.5-7); 3.5% and 10.5% (F3.5-10.5); 3.5% and 14% (F3.5-14); 7% and 10.5% (F7-10.5); 7% and 14% (F7-14); 10.5% and 14% (F10.5-14). Inter-test and inter-block intervals of 45 s and 5 min were implemented to counteract olfactory adaptation. All other aspects of discrimination testing procedures were identical to those described for Experiment 1. See Figure 3 for an overview of the second experiment.

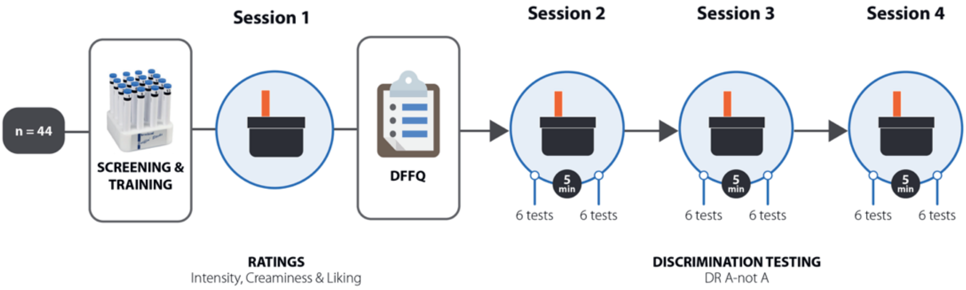

Figure 3. *An overview of experiment 2.*

2.2.4 Statistical Analyses

Discrimination ability was assessed with R-index analyses as described for Experiment 1 (see section 2.1.4). Potential learning or warm-up effects during discrimination testing were assessed as described for Experiment 1. Effects of fat concentration on perceived odour intensity, creaminess and liking were analysed with LMM, using intensity, creaminess or liking as dependent variables, fat concentrations as fixed factors and subjects as random ones. For significant main effects, post-hoc pairwise comparisons with Bonferroni corrections were applied to compare these ratings between fat concentrations. Habitual dairy consumption and its effect on discrimination ability were analysed as described for Experiment 1.

3 RESULTS

3.1 Experiment 1

3.1.1 Discrimination ability

Results of R-index analyses (Figure 4) show that orthonasally, participants were able to discriminate between all three fat sample comparisons: $F_{0\text{-}1.5}$ ($M_{R\text{-index}}$ = 68.4 ± 29.1, $p < .001$); $F_{0\text{-}3.5}$ ($M_{R\text{-index}}$ = 74.8 ± 32, $p < .001$); $F_{1.5\text{-}3.5}$ ($M_{R\text{-index}}$ = 58.5 ± 32.2, $p < .01$). Retronasally, they were able to do so between $F_{0\text{-}1.5}$ ($M_{R\text{-index}}$ = 72 ± 31.2, $p < .001$); $F_{0\text{-}3.5}$ ($M_{R\text{-index}}$ = 65.3 ± 32.4, $p < .001$); but not between $F_{1.5\text{-}3.5}$ ($M_{R\text{-index}}$ = 53.6 ± 31, $p > .05$).

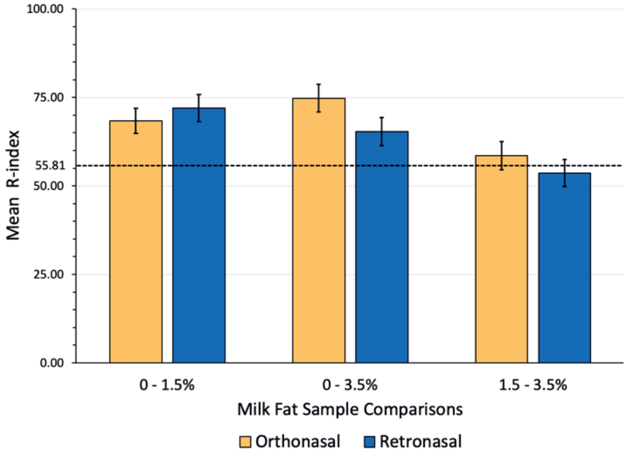

Figure 4. *R-index analyses results of Experiment 1. The dashed line indicates discrimination above statistical significance at p = 0.05 (error bars represent ± 1 SE).*

No statistically significant differences in mean individual R-index values within fat sample comparisons were observed between ortho- and retronasal conditions ($F_{0\text{-}1.5}$: Z = -.675, p = .499; $F_{0\text{-}3.5}$: Z = -1.936, p = .053; $F_{1.5\text{-}3.5}$: Z = -.827, p = .408), indicating that discrimination ability was similar between the two olfaction routes for all fat sample comparisons.

No learning or warm-up effects were observed across the four test repetitions per participant, for any of the fat sample comparisons (see Table A3 in the supplementary material).

3.1.2 Intensity and liking ratings

Mean odour intensity and liking ratings per fat sample comparison, for both olfaction routes, are shown in Figure 5.

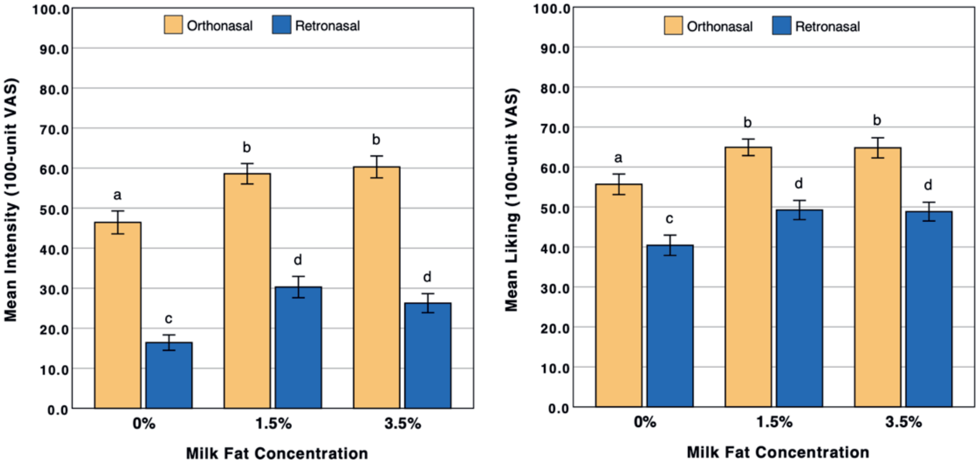

Figure 5. *Mean odour intensity and liking ratings for the three fat concentrations from Experiment 1, per olfaction route (error bars represent ± 1 SE). Mean differences between elements denoted with different letters (a, b, c, d) are statistically significant at p = 0.05.*

LMM analyses show that fat concentration and olfaction route had main effects on intensity (fat concentration: F(2, 327) = 23.45, p < .001; olfaction route: F(1, 327) = 321.02, p < .001) and liking (fat concentration: F(2, 327) = 13.36, p < .001; olfaction route: F(1, 327) = 92.61, p < .001). No interactions were observed between olfaction route and fat concentration for both, intensity (F(2, 325) = 0.97, p = .380) and liking (F(2, 325) = 0.02, p = .984). For both olfaction routes, intensity of the F_0 sample ($M_{orthonasal}$ = 46.4 ± 23.2; $M_{retronasal}$ = 16.4 ± 15.6) was rated significantly lower (p < .001) than intensities of $F_{1.5}$ ($M_{orthonasal}$ = 58.6 ± 20.7; $M_{retronasal}$ = 30.3 ± 21.6) and $F_{3.5}$

samples ($M_{orthonasal}$ = 60.3 ± 22.2; $M_{retronasal}$ = 26.3 ± 19.8). No significant differences in intensity ratings were observed between $F_{1.5}$ and $F_{3.5}$ samples for both olfaction routes ($p = 1.000$). Similarly, the F_0 sample ($M_{orthonasal}$ = 55.7 ± 20.8; $M_{retronasal}$ = 40.4 ± 20.6) was rated as being significantly less liked ($p < .001$) than $F_{1.5}$ ($M_{orthonasal}$ = 64.9 ± 16.8; $M_{retronasal}$ = 49.2 ± 19.4) and $F_{3.5}$ samples ($M_{orthonasal}$ = 64.8 ± 20.5; $M_{retronasal}$ = 48.8 ± 19.1) in both olfactory conditions. Liking ratings between F1.5 and F3.5 samples did not differ significantly between the routes ($p = .893$). Intensity of all three fat samples was rated as being lower in the retronasal condition ($p < .001$). Likewise, the three fat samples were less liked in the retronasal condition ($p < .001$).

3.1.3 Effects of habitual dairy consumption on discrimination ability

Mean reported daily dairy fat and dairy product intakes of participants were 8.4 ± 5.5 g/day and 364.1 ± 188.7 g/day, respectively. The average reported dairy consumption frequency amounted to 2.3 ± 0.9 times/day. No effects of total dairy fat intake ($F(1, 62) = 0.008$, $p = .927$), total dairy product intake ($F(1, 62) = 0.434$, $p = .512$) or dairy consumption frequency ($F(1, 62) = 0.036$, $p = .849$) were observed on discrimination ability.

3.2 Experiment 2

3.2.1 Discrimination ability

Results of R-index analyses (Figure 6) show that participants were able to retronasally discriminate between F3.5-14 ($M_{R\text{-}index}$ = 60.9 ± 26.1, $p < .001$) and F7-14 ($M_{R\text{-}index}$ = 56.6 ± 27, $p < .05$), but not between $F_{3.5\text{-}7}$ ($M_{R\text{-}index}$ = 54.2 ± 22.7, $p > .05$), $F_{3.5\text{-}10.5}$ ($M_{R\text{-}index}$ = 54.4 ± 28.1, $p > .05$), $F_{7\text{-}10.5}$ ($M_{R\text{-}index}$ = 54.7 ± 23.6, $p > .05$) and $F_{10.5\text{-}14}$ ($M_{R\text{-}index}$ = 44.9 ± 24.7; $p > .05$).

No learning or warm-up effects were observed across the four test repetitions per participant for any of the fat concentration comparisons (see Table A4 in the supplementary material).

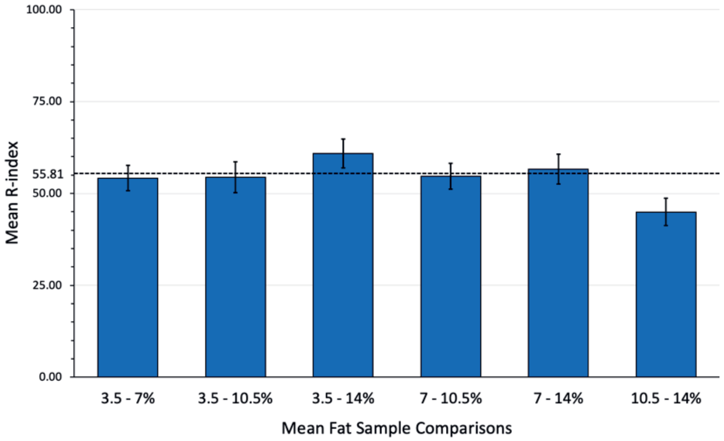

Figure 6. *R-index analyses results of Experiment 2. The dashed line indicates discrimination above statistical significance at p = 0.05 (error bars represent ± 1 SE).*

3.2.2 Intensity, creaminess and liking ratings

Mean odour intensity, creaminess and liking ratings per milk fat sample comparison are displayed in Figure 7. See Table B1 in the supplementary material for means with SD.

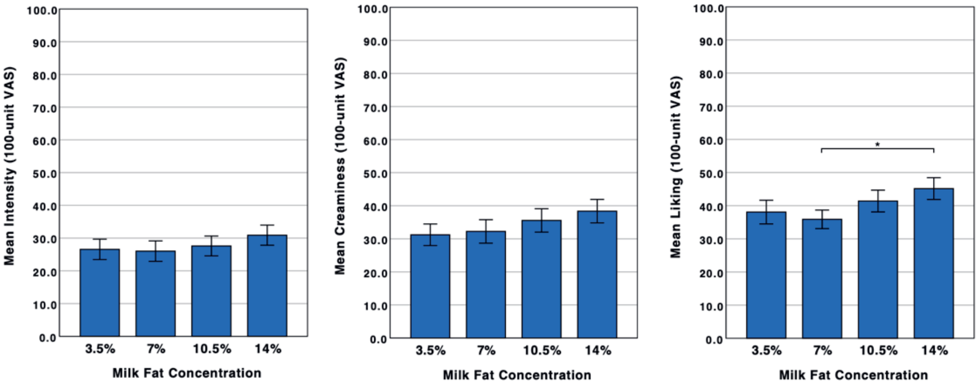

Figure 7. *Mean odour intensity, creaminess and liking ratings for the four fat concentrations used in Experiment 2 (error bars represent ± 1 SE).*

Based on LMM analyses, fat concentration had no main effect on intensity (F(3, 129) = 1.154, p = .330) or creaminess (F(3, 129) = 2.160, p = .096). It did, however, have an effect on liking (F(3, 129) = 3.855, p = .011). The F14 (M = 45.2 ± 21.9) sample was significantly (p = .011) more liked than the F7 (M = 35.9 ± 18.6) sample. No differences in liking were observed between other fat concentrations (p > .05).

3.2.3 Effects of habitual dairy consumption on discrimination ability

Mean reported daily dairy fat and dairy product intakes of participants were 8.8 ± 5.9 g/day and 288 ± 226 g/day, respectively. The average reported dairy consumption frequency amounted to 2.1 ± 1.1 times/day. No effects of total dairy fat intake (F(1, 40) = .376, p = .543), total dairy product intake (F(1, 40) = .154, p = .679) or dairy consumption frequency (F(1, 40) = 1.097, p = .301) were observed on discrimination ability.

4 DISCUSSION

The present research aimed at gaining insight on the human ability of retronasal fat content discrimination, using an ecologically relevant olfactory stimulus – dairy milk samples varying in fat concentration. This is the first study to demonstrate that humans are capable of discriminating fat content in a real food product, using solely retronasal olfactory cues. Furthermore, this ability does not appear to be related to habitual dairy intake. Although samples were perceived as being less intense and less liked in the retronasal condition, fat content discrimination between the two olfactory routes was comparable.

Previous research on ortho- and retronasal perception of vapour-phase fatty acids (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011) and orthonasal perception of fat levels in dairy milk (Boesveldt & Lundstrom, 2014) has indicated that humans possess a functional olfaction-based system for detecting food fat content. The present research replicates findings on orthonasal fat content discrimination in real foods and, more importantly, extends those on retronasal perception of vapour-phase fatty acids to a real-food context. Not only were subjects in our experiments able to retronasally discriminate between non-fat and fat-containing samples, they were able to do so between different levels of fat as well. Furthermore, the fact that we separated the olfactory component from confounding effects of taste and mouthfeel sensations, clearly demonstrates that retronasal olfaction in isolation is sufficient for discriminating fat levels in food and further emphasises its importance in fat perception.

Subjects were able to orthonasally discriminate between all three fat level comparisons used in our first experiment. This is in line with the study of Boesveldt and Lundstrom (2014), who observed the same in a comparable set of samples, albeit with some inconsistencies: in two of the three experiments participants could not discriminate semi-skimmed milk from whole milk; in one experiment they were unable to discriminate skimmed milk from semi-skimmed milk. Disparities between the latter and our study might have occurred due to differences in fat concentration steps between the experiments, or different methodological approaches to discrimination testing. Whereas Boesveldt and Lundstrom (2014) applied the triangle discrimination method, the current study implemented the DR A-not A approach. The A-not A method not only tends to be more powerful than the triangle procedure (Bi & Ennis, 2001), sensitivity variations between the two discrimination approaches are also to be expected (Lee, van Hout, & Hautus, 2007; Mun et al., 2019).

Discrimination ability between the two olfaction routes was similar overall, however, individual comparisons revealed that in contrast to the orthonasal condition, subjects were not able to retronasally discriminate between F1.5-3.5. This could be because retronasal detection thresholds are generally higher than orthonasal ones (Goldberg et al., 2018), which also seems to be the case for fatty stimuli (Chale-Rush et al., 2007). Our second experiment demonstrated that retronasal discrimination between different fat levels, not just between non-fat and fat-containing samples, is also possible, as subjects were able to discriminate between F3.5-14 and F7-14 comparisons. It has to be acknowledged that despite a comparable absolute, but smaller relative difference in fat levels, the F7-14 comparison could be discriminated, while the F3.5-10.5 could not. There is a possibility that the sample size implemented in our experiment was insufficient, resulting in the lack of statistical power for this particular comparison. Alternatively, perhaps quality differences between stimuli are more relevant than their intensities when it comes to olfactory fat discrimination. Indeed, as recently demonstrated by Ravia et al. (2020), quality differences between odorant pairs might be key to olfactory discrimination. Since unlike for JNDs in odour intensity (Cain, 1977), no framework for JND in odour quality exists, this remains to be elucidated. Future studies should therefore aim at establishing JND types and ranges relevant for fat odour discrimination and ensure sufficient sample sizes. Although a combination of the aforementioned causes is likely to have influenced our results, overall, they clearly show that humans can retronasally discriminate between various levels of fat in food and indicate that this ability seems to be comparable between the two olfaction routes. It has to be noted, however, that discrimination between non-fat-containing and fat-containing samples seems to be relatively straightforward, whereas larger fat difference magnitudes are seemingly required for discrimination between fat-containing samples. Based on the outcomes, it seems relevant for future studies to focus on individual sensitivity measurements and individual factors that might affect discrimination ability.

No perceptual rating differences were observed between the two fat-containing samples in our first experiment; however, they were both perceived as more intense and more liked compared to the non-fat sample. In comparison, Boesveldt and Lundstrom (2014), using a set of samples comparable to the one described here, observed a decrease in pleasantness with increasing fat content in one, but not their other two experiments. In congruence with the notion that orthonasal stimuli are generally perceived as more intense than retronasal ones (Goldberg et al., 2018), orthonasal intensity and liking for all three fat levels in our experiment was higher compared to the retronasal condition. Despite olfactory route-dependent perceptual rating differences and the lack of perceptual rating differences between the two fat-containing samples which could be discriminated orthonasally, discrimination ability was similar between the two conditions. This suggests that discrimination likely did not depend on intensity differences between the samples and supports the idea that quality, not intensity differences between stimuli might be crucial for olfactory fat discrimination, as mentioned in the previous paragraph.

This reasoning was also put forward by Boesveldt and Lundstrom (2014), who suggested that the addition of other relevant perceptual descriptors, namely creaminess, could help elucidate perceptual differences responsible for olfactory fat content discrimination. Therefore, creaminess was added as a perceptual rating in our second experiment. In contrast to the first experiment, we observed no perceptual differences between the samples, apart from a difference in liking between F7 and F14. Considering this was the only perceptual difference among our set of samples and three perceptual variables, we speculate it is likely a coincidental finding. All in all, it is plausible that intensity differences contributed towards discrimination results between non-fat and fat-containing samples in our experiment, however, perceptual differences responsible for discrimination between fat-containing ones remain unclear. Perhaps a larger sample size or the addition of other fat-related descriptors might reveal perceptual differences accounting for the current discrimination results.

The ability to discriminate between fat levels was not affected by habitual dairy consumption in either of our experiments. This is consistent with findings of Boesveldt and Lundstrom (2014), who observed no associations between BMI or dairy consumption habits and orthonasal fat discrimination. Similarly, Stevenson et al. (2016) reported no associations of a Western-style diet, rich in fat and sugar, on general odour discrimination or olfactory thresholds. They did, however, find that consumers of a Western-style diet performed worse during odour identification trials and were poorer at discriminating fat levels during multisensory testing. Relatedly, Kindleysides et al. (2017) observed that a higher intake of fatty foods, namely seeds, nuts and nut spreads, was associated with a higher olfactory sensitivity to oleic acid. An additional observation, supporting our findings of olfactory fat content discrimination being independent of past exposure, at least in the short term, is that no learning or warm-up effects were observed during discrimination testing trials in the current study. However, since subjects in our experiments were dairy consumers, the possibility of long-term past exposure having an influence on fat odour discrimination cannot be ruled out either. Moreover, the DFFQ utilised in the current study might not have been the optimal approach for assessing habitual dairy consumption: increasing the range of response options, along with the range of dairy products it covers, could improve its accuracy. Furthermore, perhaps instead of looking into dairy consumption habits, information about overall fat consumption, beyond dairy, could help reveal potential effects on discrimination ability. To date, only a handful of studies investigated the relationship between olfactory fat detection and habitual intake, yielding somewhat mixed results. Further research on the nature of olfactory fat detection abilities is therefore warranted.

Despite going beyond vapour-phase fatty acids, utilising actual food as an olfactory stimulus, the ecological validity of the current study should not be overstated. It must be acknowledged that inhalation via containers resulted in a retronasal stimulus transportation path not likely to occur during food consumption. When odours are inhaled orally, in the absence of food, they first travel to the lungs before ultimately reaching the olfactory epithelium. This results in varying degrees of lung retention (mainly depending on the type of odorant), which not only reduces the odour mixture concentration, but can also potentially alter the relative composition of the originally inhaled mixture. This is in contrast to what happens during actual food intake, where swallowing closes the trachea, thereby forcing odorants through the nasopharynx into the olfactory mucosa (Verhagen, 2015). Nevertheless, despite the highly likely occurrence of odorant lung retention in our experiments, odorant intensities were sufficient for the subjects to detect and discriminate between. We speculate that the effect of these odorants is more pronounced in normal eating situations.

Another point that needs to be addressed is the nature of chemical signals that are being perceived when “smelling fat”. Since triglycerides – the most common form of dietary fat (Lichtenstein et al., 1998), are not known to be volatile, it is highly unlikely that they are directly responsible for the smell differences between our samples. However, since triglycerides can act as carriers of flavour compound reservoirs (McSweeney & Sousa, 2000), it is likely that compounds bound to them elicited the smell differences. As demonstrated by Roberts and Pollien (2000) and Roberts, Pollien, and Watzke (2003), the amount of aroma compound retention in dairy milk mainly depends on the fat content, with higher fat samples absorbing more aroma compounds than low fat ones. Furthermore, food matrix manipulations, such as the ones done in our experiments, lead to changes in lipophilicity which can potentially alter flavour release (Roberts, Pollien, Antille, et al., 2003). These factors might have caused qualitative shifts in odour characters between the samples in our experiments and could potentially be the key underlying mechanism by which subjects could discriminate between the samples. Furthermore, fatty acids, which are present in trace quantities in dairy milk (Parodi, 2004) and were demonstrated to be effective olfactory stimuli (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011), could also have caused perceptual differences. Effects of fat oxidation by-products cannot be ruled out either. For a better understanding of the mechanisms behind olfactory fat perception, more work is needed in on identifying the source of fat-odour-related chemical signals.

While orthonasal odours seem to aid in guiding towards potential (fat) food sources during the anticipatory phase of food consumption (de Vries et al., 2020), the behavioural relevance of (discriminating) retronasal odours in fat perception is less evident. Nevertheless, the ability to retronasally detect differences in food fat content points towards retronasal fat odours being behaviourally relevant in the consummatory phase of eating, likely beyond their contribution to flavour. Perhaps they serve to reinforce choice and intake of fat-rich food sources via reward mechanisms. The influence of retronasal odour exposure on food intake has been studied before (Raemaekers, 2014; Ramaekers et al., 2014; Ruijschop et al., 2010; Ruijschop et al., 2008), yet the observed effects were minor. The studies, however, used either non-fat odours or fat-related aromas (Raemaekers, 2014), rather than fat itself. The olfactometer-based delivery method employed in these studies, which can be considered rather unnatural when studying behaviour, possibly affected results as well. Notwithstanding, studying the effects of retronasal odours on behaviour is inherently difficult, mainly due to limited and often invasive options of stimulus delivery, and interactions with other senses involved in flavour perception (Bojanowski & Hummel, 2012; Goldberg et al., 2018). In view of these limitations, perhaps investigating underlying neural responses to olfactory fat exposure could shed light on potential behavioural correlates. Considering that the neural underpinnings of olfactory fat remain unexplored, neuroimaging techniques could be utilised to map involved brain regions and explore activation patterns in response to fat exposure (fat source, concentration, and exposure duration) for both olfaction routes.

To conclude, the current study represents an important step towards understanding olfactory fat perception, as our results clearly demonstrate that humans are capable of not only detecting the presence of fat retronasally, but also discriminating between its levels in a real food product. Additionally, this ability does not appear to be affected by habitual intake. The next important step, besides investigating individual factors that might affect discrimination ability and unravelling if and how retronasal fat perception affects food intake and choice, is to identify which chemical signals are responsible for the smell of fat. Doing so would provide opportunities to reduce fat content in a range of fat-laden foods, while maintaining their pleasurable sensory characteristics via the addition of compounds responsible for the alluring flavour.

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SUPPLEMENTARY MATERIAL A

Table A1. *Experiment 1 sample mixture ingredients, with corresponding nutritional values per 100 g of sample.*

	Ingredients (per 100 g)			**Nutritional values (per 100 g)**		
Sample	Skimmed milk (g)	Cream (g)	0.5% κ carrageenan solution (g)	F (g)	CH (g)	P (g)
0%	90.0	0.0	10.0	0.0	4.1	3.2
1.5%	85.7	4.3	10.0	1.5	4.0	3.1
3.5%	80.0	10.0	10.0	3.5	3.9	3.1

F = dietary fat; CH = carbohydrates; P = protein

Table A2. *Experiment 2 sample mixture ingredients, with corresponding nutritional values per 100 g of sample.*

	Ingredients (per 100 g)			**Nutritional values (per 100 g)**		
Sample	Skimmed milk (g)	Cream (g)	0.5% κ carrageenan solution (g)	F (g)	CH (g)	P (g)
3.5%	90.0	10.0	/	3.5	4.4	3.4
7%	80.0	20.0	/	7.0	4.2	3.3
10.5%	70.0	30.0	/	10.5	4.1	3.2
14%	60.0	40.0	/	14.0	3.9	3.1

F = dietary fat; CH = carbohydrates; P = protein

Table A3. *Experiment 1 frequencies of hits, misses, false alarms and correct rejections, pooled across four tests within each block, along with results of Chi-square analyses.*

Olfaction route	**Fat % Comparison**	**Hits**	**Misses**	**False Alarms**	**Correct Rejections**	χ^2	**p - value**[a]
Orthonasal	$F_{0\text{-}1.5}$	79	53	38	94	9.045	.433
	$F_{0\text{-}3.5}$	76	58	24	106	4.339	.888
	$F_{1.5\text{-}3.5}$	65	67	53	79	12.425	.190
Retronasal	$F_{0\text{-}1.5}$	82	50	36	96	9.270	.413
	$F_{0\text{-}3.5}$	75	57	42	90	2.301	.986
	$F_{1.5\text{-}3.5}$	65	67	55	77	2.526	.980

[a] Possibilities of learning and warm-up effects across the four repetitions within a fat concentration comparison were assessed by comparing frequencies of hits, correct rejections, false alarms and misses with Chi-Square tests.

Table A4. *Experiment 2 frequencies of hits, misses, false alarms and correct rejections, pooled across six tests within a block, along with results of Chi-square analyses.*

Fat % Comparison	Hits	Misses	False Alarms	Correct Rejections	χ^2	p - value[a]
$F_{3.5\text{-}7}$	70	62	61	71	10.42	.792
$F_{3.5\text{-}10.5}$	63	69	57	75	13.89	.534
$F_{3.5\text{-}14}$	85	49	53	77	13.77	.543
$F_{7\text{-}10.5}$	66	66	61	71	10.06	.816
$F_{7\text{-}14}$	71	61	63	69	11.28	.732
$F_{10.5\text{-}14}$	66	66	80	52	18.87	.220

[a] Possibilities of learning and warm-up effects across the four repetitions within a fat concentration comparison were assessed by comparing frequencies of hits, correct rejections, false alarms and misses with Chi-Square tests.

SUPPLEMENTARY MATERIAL B

Table B1. *Experiment 2 perceptual rating means with ± SD.*

Fat %	Odour intensity	Creaminess	Liking
3.5%	26.6 ± 20.6	31.2 ± 21.6	38.1 ± 23.7
7%	26.0 ± 20.7	32.2 ± 23.6	35.9 ± 18.6
10.5%	27.6 ± 20.1	35.6 ± 23.4	41.4 ± 21.8
14%	30.9 ± 20.4	38.4 ± 23.5	45.2 ± 21.9

4

Chapter 4

Neural Correlates of Olfactory Fat Perception

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ABSTRACT

Olfaction is involved in detecting, identifying, and discriminating dietary fat within foods, yet the underlying neural mechanisms remain uncharted. Our fMRI study therefore investigated the neural correlates of olfactory fat perception and their association with discrimination ability in a complex food matrix. We measured brain activation resulting from orthonasal exposure to an ecologically relevant fat-related odour source - dairy milk, manipulated to contain 0%, 3.5% or 14% fat. Twenty-six healthy, non-smoking, normosmic, normal-weight adults underwent olfactory fat content discrimination testing, followed by an fMRI task during which the three odour stimuli were delivered via an olfactometer (25 times/fat level) and rated on perceived intensity and liking. Participants discriminated between all fat levels, with fat level influencing perceived odour intensity and liking. These perceptual differences, however, were not reflected in differential brain activation. Brain activation differences were observed only when comparing odour exposure with no exposure. Specifically, in response to any odour, activation occurred in the anterior part of the supplementary motor area, while deactivating parts of the hippocampus, putamen, superior temporal gyrus, anterior cingulate cortex, insula and posterior part of the supplementary motor area. Exposure to the 0% fat odour also activated the thalamus. No associations were found between perceived intensity and liking and neural responses. Results reaffirm the human ability to distinguish food fat content using solely olfactory cues and reveal a divergence between sensory perception and neural processing. Subsequent research should replicate and extend these findings onto retronasal fat perception, while also examining potential effects of hunger, genetics, and dietary habits.

Keywords: fat perception, odour, discrimination, fMRI, brain activity

1 INTRODUCTION

Dietary fat is an indispensable macronutrient in the human diet, playing a vital role in maintaining and promoting optimal health (Lichtenstein et al., 1998). The nutritional significance of dietary fat is often overshadowed by its pleasure-inducing sensory characteristics (Drewnowski, 1997; Drewnowski & Almiron-Roig, 2009), which can promote overconsumption, thereby contributing towards the development of obesity (Blundell & Macdiarmid, 1997a; Bray et al., 2004; Golay & Bobbioni, 1997). In light of this, gaining a deeper understanding of the sensory perception of dietary fat could offer valuable insights for developing effective public health strategies targeting the reduction of fat intake.

Sensory perception of dietary fat is multimodal (Guichard et al., 2018), with olfaction playing a considerable role. It contributes to its detection, identification and discrimination, even when fat or its constituents are embedded within complex food matrices. Olfactory discrimination of food fat content has been corroborated by our previous work, demonstrating that fat levels in dairy milk can be distinguished using solely ortho- or retronasal cues (see Pirc et al. (2022)). Moreover, fat-related odours have been shown to have the capacity of altering the perception of various olfactory as well as non-olfactory sensory qualities, such as mouthfeel (see review by Pirc et al. (2023b)). These findings affirm the notion that humans possess a functional olfaction-based mechanism for detecting and discriminating food fat content. Nevertheless, despite convincing perceptual evidence, the underlying mechanisms remain to be fully understood, particularly in the neurobiological domain. Considering that orthonasal odours relate to food source detection and appetite arousal prior to eating, with retronasal odours playing a vital role in flavour perception, potentially affecting intake and satiation (Boesveldt & Lundstrom, 2014; Bojanowski & Hummel, 2012), investigating neural mechanisms underpinning olfactory fat perception could further our understanding of feeding behaviour surrounding fatty foods. In the context of curbing excessive fat intakes, exploring differences in brain activation resulting from olfactory exposure to varying food fat levels is particularly relevant.

In contrast to oral fat perception (i.e. taste and/or mouthfeel), which has been investigated in numerous neurobiological studies (Andersen et al., 2020; De Araujo & Rolls, 2004; Eldeghaidy et al., 2011b; Grabenhorst et al., 2010a; Verhagen et al., 2003; Wistehube et al., 2018), to our knowledge, no study investigated how exposure to fat-related odours is processed in the human brain. Notwithstanding the lack of such studies, oral fat perception research has identified several brain areas which might be of relevance in the neural processing of fat-related odours as well. Activation in response to oral fat stimulation has been observed in the insula (Eldeghaidy et al., 2011b), orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), hypothalamus and ventral striatum (VS) (De Araujo & Rolls, 2004). Given the overlap in neural processing between smell and taste perception in regions such as the insula (Lundström et al., 2011; Small et al., 2005; Torske et al., 2022; Veldhuizen et al., 2010; Yeung et al., 2018), OFC and ACC (Small et al., 2004), which are crucial in the processing of flavour perception, investigating brain activation in these regions in response to fat-related odour exposure seems promising. Moreover, it is widely acknowledged that energy-dense foods, including those rich in fat, are potently rewarding (Drewnowski, 1997). A crucial brain system involved in the processing of reward, specifically the generation of desire and pleasure in response to rewarding stimuli such as fat-containing foods, is the mesolimbic dopamine system (Berridge & Kringelbach, 2015; Berridge & Robinson, 2016; Higgs, 2016; Robinson et al., 2016; Volkow et al., 2011). It encompasses overlapping reward networks, which include regions such as the prefrontal cortex, including portions of the OFC, the insula, and ACC, as well as subcortical limbic structures such as the VS (including the nucleus accumbens (NAc) and ventral pallidum (VP)), ventral tegmental area (VTA) and amygdala (Berridge & Kringelbach, 2015; Berthoud, 2002; Higgs, 2016). Taken together, brain areas likely to be involved in the processing of fat-related odours include reward- and oral fat perception-related areas, both contained within the broader reward system. Olfaction-related areas, as per Seubert et al. (2013), Lundström et al. (2011) and Fjaeldstad et al. (2017) might be implicated as well.

The current study aimed to map brain activation in response to olfactory (orthonasal) exposure to varying levels of dietary fat embedded within an ecologically relevant food source (dairy milk), and exploring potential associations between brain activation, olfactory fat content discrimination ability and perceived odour intensity and liking of the utilised samples. We hypothesised that exposure to higher fat levels would lead to increased activation in regions associated with reward and olfactory perception. We also expected to confirm previous findings demonstrating participants' ability to discriminate between sample fat levels using only orthonasal cues. Since high fat foods tend to be perceived as pleasurable (Blundell &

MacDiarmid, 1997b; Guichard et al., 2018), we expected positive correlations between fat content and perceived liking, and between perceived liking and brain activation in various regions of the reward system.

2 MATERIALS AND METHODS

The study was conducted according to the Declaration of Helsinki and approved by the Medical Ethics Committee Oost Nederland (NL78261.091.21). Parameter estimates extracted from relevant brain regions, along with perceptual data are available on the Open Science Framework Repository (Pirc et al., 2023a).

2.1 Participants

Twenty-six volunteers (mean age 24.9 ± 5.4 years; mean BMI 22.6 ± 2.1 kg/m2; 6 males), recruited from Wageningen (The Netherlands) and its surroundings, participated in the study. All were healthy, non-smoking, regular dairy milk consumers (self-reported) and met the following eligibility criteria: being between 16 and 55 years of age (as smell function decreases with age (Doty & Kamath, 2014)); having a BMI between 18.5 and 25 kg/m2 (as BMI may negatively influence olfactory functioning (Peng et al., 2019)); being normosmic; being right-handed (as handedness is associated with differences in odour-related processing (Hummel et al., 1998; Royet et al., 2003)); not dieting currently or in the past two months; not having any dairy-related allergies or intolerances; not being pregnant or lactating (as pregnancy may negatively influence olfactory performance (Ochsenbein-Kolble et al., 2007)); not suffering from claustrophobia; and having no other MRI-related contraindications (e.g. non-removable ferromagnetic implants and piercings, epilepsy). All participants provided written informed consent and received financial compensation for taking part in the study.

2.2 Olfactory stimuli

Dairy milk samples, containing 0%, 3.5% and 14% fat (henceforth referred to as low fat (L), medium fat (M) and high fat (H), respectively) served as olfactory stimuli throughout the study. They were chosen to represent ecologically relevant fat sources with comparable fat content, namely skimmed milk, whole milk, and reduced-fat cooking cream. Fresh, pasteurised skimmed milk (0% fat, AH Magere melk, Albert Heijn B.V.) served as the L sample (containing traces of fat), whereas M and H samples were made from fresh, pasteurised skimmed milk and fresh, pasteurised full-fat cream (35% fat, AH Verse Slagroom, Albert Heijn B.V.), homogenised with a dispersing apparatus (T 25 digital Ultra-Turrax, IKA® - Werke GmbH & Co. KG). All samples were prepared freshly prior to each experimental session. See Table A1 in the supplementary material for sample ingredients and corresponding nutritional composition.

2.3 Study design and procedures

The study was carried out in two sessions, conducted on separate days. During the first session, participants underwent screening and training, along with discrimination testing aimed at assessing their orthonasal fat content discrimination ability. The second session was aimed at measuring brain responses resulting from orthonasal exposure to the three milk samples, by means of functional magnetic resonance imaging (fMRI). Perceived intensity and liking of said odour stimuli were assessed during this session as well. See Figure 1 for an overview of the study design.

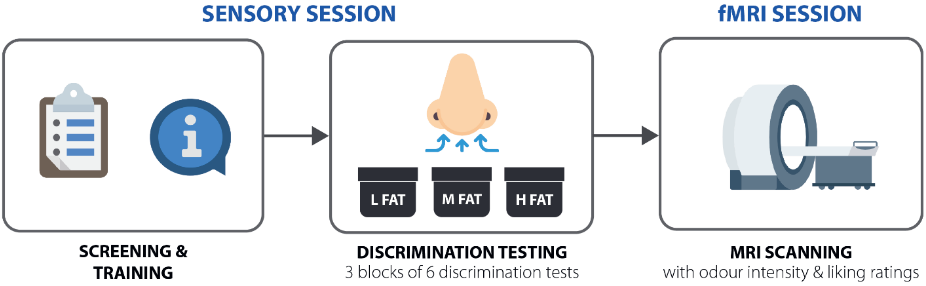

Figure 1. *Study design overview.*

Participants were instructed to refrain from using strong-scented cosmetic products and consuming anything apart from water two hours prior to all sessions.

2.3.1 Screening & Training

Screening involved questionnaires aimed at assessing study eligibility criteria, body height and weight measurements and olfactory function assessment. The latter was carried out using the Sniffin' Sticks 16-item odour identification test, with a score of ≥12 indicating normosmia (Hummel et al., 2007; Oleszkiewicz et al., 2019).

If deemed eligible, participants underwent a short training procedure, to get accustomed to handling odour delivery containers used during discrimination testing. Additionally, a full-size, non-functioning, MRI scanner replica was used to familiarize them with the scanning environment and fMRI task-related procedures. While in the replica scanner, participants performed a short practice sequence, similar to the experimental one, which involved seeing visual stimuli displayed on a screen through a head coil-mounted mirror, experiencing the odour delivery system (cream odour, delivered via an olfactometer (Lundstrom et al., 2010)), hearing pre-recorded MRI noises, and learning how to use a response box to answer visual analogue scale (VAS) questions in the scanner.

2.3.2 Discrimination Testing

Discrimination testing took place in sensory booths and followed the dual reminder A-not A (DR A-not A) (see Mun et al. (2019)), pairwise design (Hautus et al., 2018). In this version of the test, participants are first required to smell a reference stimulus twice, before smelling the test stimulus once, and deciding whether the test stimulus is the reference or not. The following response options were available: “test sample is the reference – I am sure”, “test sample is the reference – I am unsure”, “test sample is the reference – I am guessing”, “test sample is not the reference – I am guessing”, “test sample is not the reference – I am unsure”, “test sample is not the reference – I am sure”. Testing was carried out in 3 blocks of 6 tests, with 30-s and 3-min breaks implemented in-between tests and blocks, respectively. Two fat content levels were compared within a block: either 0% and 3.5% (L-M); 0% and 14% (L-H); or 3.5% and 14% (M-H), with the lower fat level of each pair always serving as a reference. Two possible sample presentation sequences were utilised: SA – SA – SA or SA – SA – S not A. Block and test orders were randomised across participants and each test block started with a sample familiarisation procedure, to stabilise participants’ cognitive decision criteria (Lee et al., 2007) (see Pirc et al. (2022) for details on familiarisation). Olfactory stimuli were presented in 60-ml amounts at 20 ± 1 °C, using specialised odour delivery containers, as described by Pirc et al. (2022).

2.3.3 MRI Scanning

Upon arrival to the MRI facility, participants’ adherence to experimental and MRI safety protocols were assessed. If necessary, MRI-safe clothing and glasses were provided. Scanning commenced with a 5-min anatomical scan, followed by a brief practice procedure, to familiarise participants with the use of the response box, before concluding with the olfactory fMRI task which spanned three consecutive 14-min functional runs.

A scanner-mounted computer display, receiving input from a computer running E-Prime 3.0 (Psychology Software Tools, Sharpsburg, USA) stimulus presentation software, was used to present instructions, questions, and other visual stimuli to participants in the scanner. Specifically, an orange fixation crosshair indicated imminent odour release, a green fixation crosshair indicated odour release and prompted participants to inhale, while a white fixation crosshair indicated the waiting period. All visual stimuli were presented on a dark grey background.

Throughout the fMRI task, odours were delivered by means of an 8-channel olfactometer (Burghart Messtechnik GmbH, Holm, Germany) and presented orthonasally to both nostrils using a small nasal canula. During scanning, the odours were embedded in a constant non-odorous airflow (8 L/min without odour, 4 L/min with odour) with a relative humidity of 80% and a temperature of 36°C. Odour release was controlled using E-Prime 3.0 stimulus presentation software.

Participants were randomly assigned to one of two odour presentation sequences to avoid potential order effects. In both sequences, each odour (L, M or H) was presented 25-times for 2 s, with the inter-stimulus interval varying between 27 and 32 s, to avoid expectation effects. Each of the presentation sequences included 100-unit VAS questions about perceived odour intensity and liking (three of each per odour stimulus level, left anchor = "not at all"; right anchor = "extremely"). Participants were allotted 11 s to provide their responses using the response box. In total, the fMRI task consisted of 75 odour trials. See Figure 2 for an overview of the fMRI task.

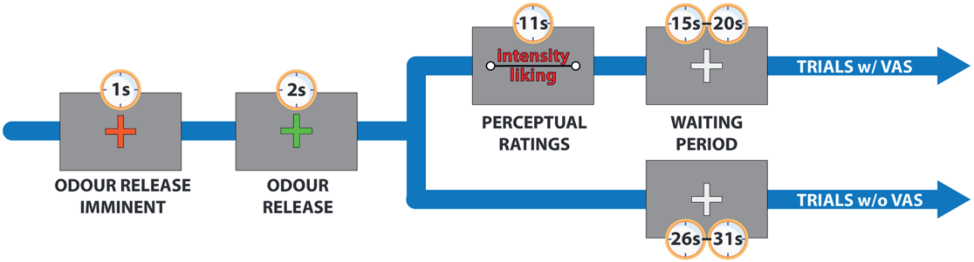

***Figure 2.*.** *fMRI task overview.*

MRI image acquisition was performed using a 3-Tesla MRI scanner (Elition X, Koninklijke Philips N.V., Amsterdam, the Netherlands) using a 32-channel head coil. A high-resolution T_1-weighted 3D TFE anatomical scan was conducted with the following parameters: repetition time (TR) of 10 ms, echo time (TE) of 4.6 ms, flip angle of 8°, field of view (FOV) of 256 x 243 x 180 mm, acquisition of 450 sagittal slices, scanning voxel size of 0.8 x 0.8 x 0.8 mm, and reconstructed voxel size of 0.4 x 0.4 x 0.4 mm. Functional scans were conducted using a T_2-weighted gradient echo 2D-EPI sequence. The following parameters were used: TR = 1152 ms, TE = 25 ms, a flip angle of 57°, SENSE factor = 2.2 (AP), multiband factor = 3, FOV = 230 x 230 x 139 mm, acquisition = 63 axial slices in an ascending order, scanning voxel size = 2.2 x 2.2 x 2.2 mm, reconstructed voxel size = 1.8 x 1.8 x 2.2 mm.

2.4 Data analysis

2.4.1 Perceptual data

Discrimination ability was assessed with R-index analyses carried out in accordance with the protocols described by Lee and van Hout (2009). To account for replicated testing, R-indices were computed based on weighted means of individual R-index values (derived from 6 signal / noise tests per participant) (Bi, 2015). Statistical significance was established by calculating the R-index critical value, using R statistical software (R-Core Team, 2020) and the code provided by Bi and O'Mahony (2020). The R-index critical value for a one-sided test, involving 78 control and 78 test samples, at significance levels of .05 and 0.001, amounts to 57.52 and 63.67, respectively.

Effects of odour fat levels on ratings of perceived intensity and liking were analysed using linear mixed model analyses (LMM) in SPSS Statistics version 29 (IBM Corp.), using intensity or liking as dependent variables, odours as a fixed factor, and subjects as a random factor. Post-hoc pairwise comparisons with Bonferroni corrections were applied to compare ratings between the odours. Potential effects of olfactory adaptation due to repeated exposure to the odours throughout the three functional runs were assessed by adding intensity as dependent variables to the model, fMRI task progress (functional runs) as a fixed factor, and subjects as a random factor. Statistical significance was set at $p < .05$.

2.4.2 fMRI data

Pre-processing and analysis of the imaging data were performed using the SPM12 (revision version 7771) software package (Wellcome Department of Imaging Neuroscience, London, UK) implemented in MATLAB R2021a (The Mathworks, Inc., Natick, MA, USA).

Pre-processing involved realigning and slice-time correcting functional images before co-registering anatomical images with the mean functional image. Following co-registration, both the anatomical and functional images were normalized to the standard MNI (Montreal Neurological Institute) space. After this, functional images underwent smoothing, using a 3-D Gaussian smoothing kernel ([3.6, 3.6, 4.4] mm full width at half-maximum). To assess motion-related artifacts, a volume-wise check was performed using ArtRepair software (Mazaika et al., 2009). Data from all participants were deemed suitable for analysis, as none of the participants exceeded the motion exclusion threshold (more than 20% of the total volumes exceeding 0.5 mm / TR).

Statistical parametric maps were generated per participant as part of subject level analyses. This was done by fitting a boxcar function to the time series and convolving it with the canonical hemodynamic response (HRF) function. To remove low-frequency noise, data were filtered using a high-pass filter (128 s cut-off). Individual general linear models (GLM) included five conditions per functional run: (1) “Prepare to smell” (orange fixation crosshair); (2) L odour; (3) M odour; (4) H odour; and (5) subjective ratings (VAS). Motion-related variance was accounted for by adding the realignment parameters as regressors to the model. Despite odour presentation lasting 2 s, it was modelled as a 3-s event, due to the potential lingering nature of the utilised odours, which were expected to have an impact beyond the duration of the direct exposure. Ultimately, contrast images comparing various conditions were created.

Contrasts compared odours with each other (L - M, M - H, and L – H) and with the “REST” condition (L – REST; M – REST and H – REST), during which participants viewed a white crosshair in the absence of odour stimulation, subjective ratings, or preparatory cues. As further exploration, comparisons were also made between exposure to samples containing no fat and samples containing fat (L – MH). Parametric modulation, using odour stimulus levels as modulators was performed as well. However, as the obtained results closely replicated those of the other analysis approach, further discussion of these findings is omitted.

The different contrast images were analysed on a group level using one sample-t tests. Specifically, whole-brain and region of interest (ROI) analyses were performed. ROI analyses were performed using masks encompassing broader reward-related areas, as described by Smeets and de Graaf (2019)), as well as olfaction-related brain areas, as described by Seubert et al. (2013) and Fjaeldstad et al. (2017)). ROI masks were created using the Automated Anatomic Labelling (AAL) atlas (Tzourio-Mazoyer et al., 2002) in the WFU PickAtlas toolbox (Maldjian et al., 2003). They included the insula, anterior and middle cingulate cortex, supplementary motor area, OFC (including the inferior, middle, and superior frontal gyri), thalamus, striatum (caudate, putamen, pallidum), amygdala, hippocampus, piriform cortex, entorhinal cortex, parahippocampal gyrus, gyrus rectus, and the temporal lobe. Statistical significance for ROI analyses was determined using a cluster-forming threshold of $p = 0.001$ (uncorrected), with a cluster voxel extent (k) of $k > 19$. Clusters were deemed significant when the cluster-level quantitative false-discovery rate (qFDR) was less than .05. Whole-brain analyses were executed using an FWE-corrected threshold of $p = 0.05$, with a cluster voxel extent threshold of $k > 5$. The rationale for choosing a less stringent multiple comparison correction for ROI analysis was to avoid missing potentially relevant neural landmarks (Lieberman & Cunningham, 2009).

Potential associations between brain activation in response to utilised odours and perceptual ratings were investigated by correlating mean parameter estimates from significant clusters identified during ROI analyses with mean perceived odour intensity and liking ratings, using Spearman correlations (in SPSS Statistics version 29 (IBM Corp.)). Mean parameter estimates were extracted from significant clusters using the MarsBar toolbox (http://marsbar.sourceforge.net/) run in MATLAB R2021a. Correlation analyses were carried out per contrast and corresponding odour ratings. Due to the absence of significant correlations within the significant ROI clusters and the lack of theoretical rationale, we did not extend the correlation analysis to include broader brain regions outside of these clusters.

Brain activation clusters were overlaid onto a mean anatomical image of all participants and identified with a combination of the use of the AAL brain atlas in MRIcron, version 1.40, build 1 (https://www.nitrc.org/projects/mricron) and Neuromorphometrics in SPM.

3 RESULTS

3.1 Discrimination testing

Results of R-index analyses (Figure 3) indicate that all three odours could be discriminated orthonasally: L-M ($M_{R\text{-index}} = 82.4 \pm 23.7$, $p < .001$), L-H ($M_{R\text{-index}} = 84.7 \pm 24.2$, $p < .001$) and M-H ($M_{R\text{-index}} = 69.6 \pm 21.4$, $p < .001$).

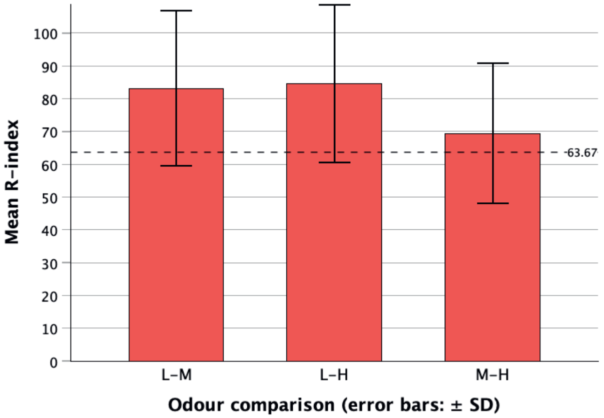

Figure 3. *R-index analysis results. Dashed line indicates the cut-off for discrimination at p < .001. L-M = low fat compared to medium fat; L-H = low fat compared to high fat; M-H = medium fat compared to high fat.*

3.2 Perceptual ratings

LMM analyses indicate that odour stimulus fat level had a main effect on perceived odour intensity ($F(2, 206) = 3.86$, $p = .023$) and liking ($F(2, 206) = 14.29$, $p < .001$) (see Figure 4).

Intensity of the L odour ($M_L = 42.9 \pm 25.8$) was rated significantly lower ($p = .020$) than that of the M odour ($M_M = 51.5 \pm 21.1$). No significant differences in intensity were observed between L and H odours ($M_H = 48.4 \pm 18.8$) ($p = .244$), nor between M and H odours ($p = .963$).

Odour liking ratings differed significantly between all the three odours ($p_{L\text{-}M} = .029$; $p_{L\text{-}H} < .001$; $p_{M\text{-}H} = .021$), and increased with fat level ($M_L = 44.4 \pm 20.3$; $M_M = 50.8 \pm 18.0$; $M_H = 57.4 \pm 16.6$).

No effects of repeated exposure to the three odours were observed on intensity ratings ($F(2, 206) = 2.103$, $p = .125$), indicating that olfactory adaptation had not occurred with repeated exposure to the three odours during the fMRI task.

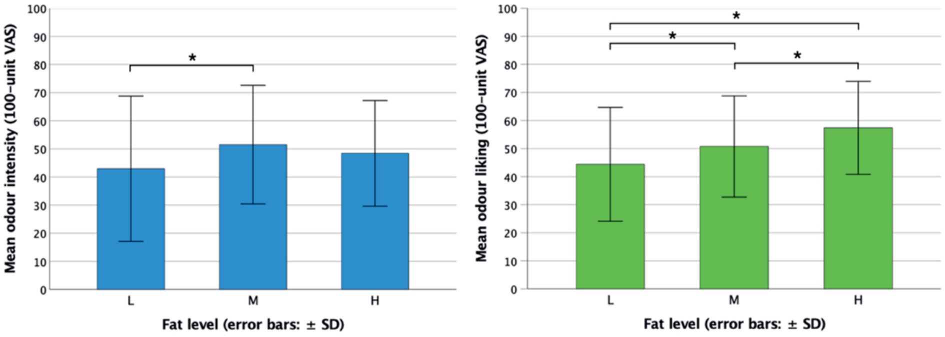

Figure 4. *Mean odour intensity (left) and liking ratings (right) for the three odours. Asterisks denote statistically significant (p < .05) differences between odours (L = low fat, M = medium fat, H = high fat).*

3.3 Neuroimaging results

Significantly differentially activated ROIs per stimulus are presented in Figure 5, with corresponding mean parameter estimates being presented in Figure 6.

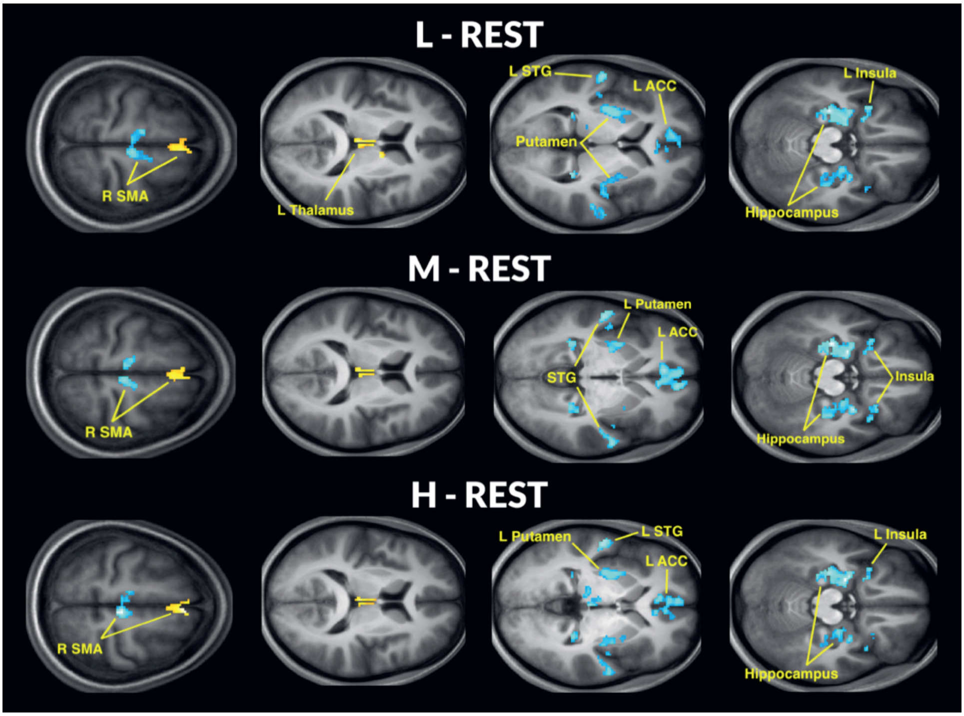

Figure 5. *Colour-coded T-maps of differential ROI activation per odour condition compared to REST, overlaid onto the mean anatomical image at T = 3.45 (p = 0.001). Named ROIs denote significant clusters (q_{FDR} < .05).*

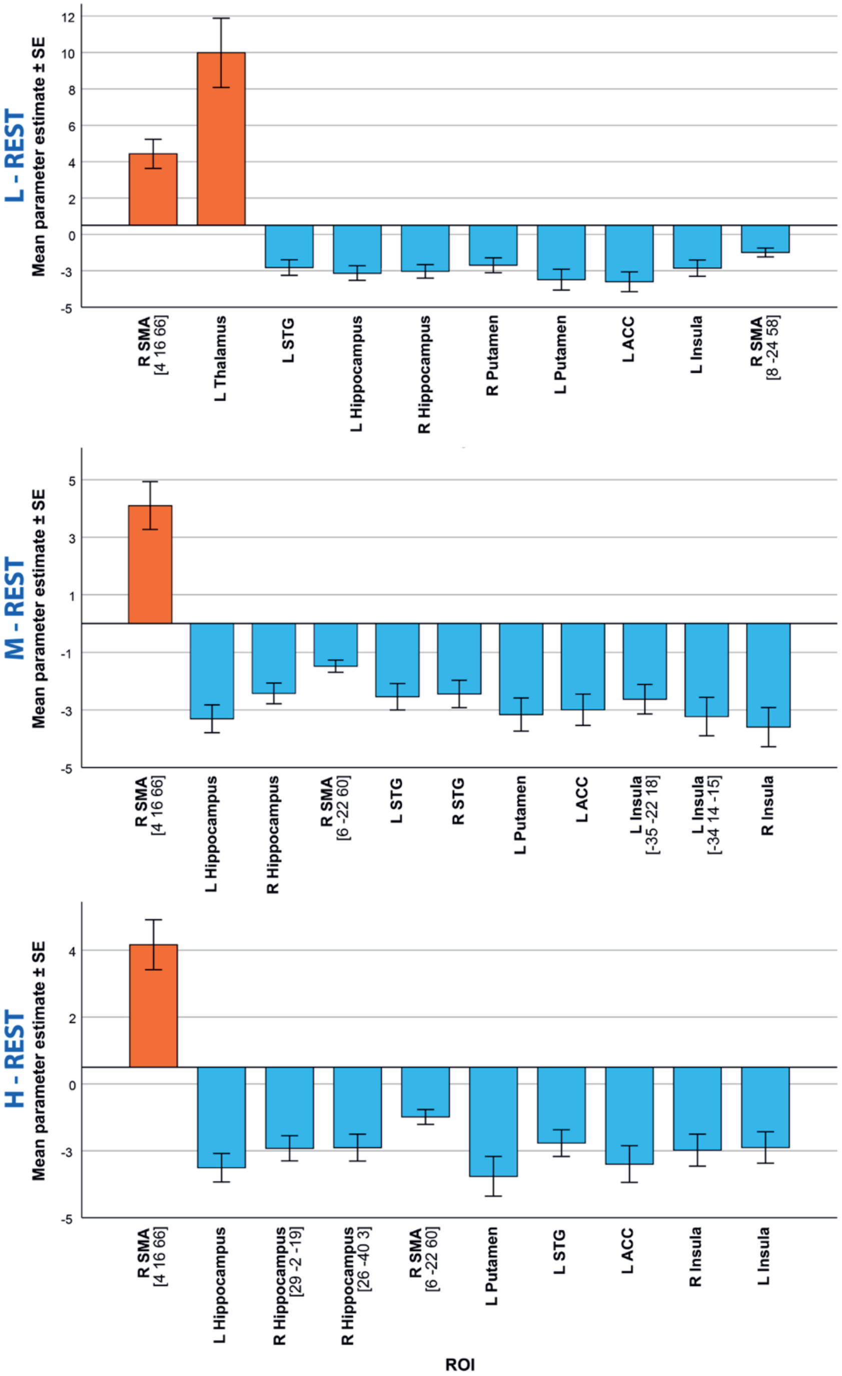

Figure 6. *Parameter estimates (mean ± SE) associated with differential ROI brain activation per odour condition compared to REST.*

An overview of the differentially activated ROIs in the L-REST comparison is presented in Table 1. ROI analyses comparing exposure to the L odour versus the REST condition revealed greater activation in the right supplementary motor area (SMA) and left thalamus. Conversely, deactivation was observed in the left superior temporal gyrus (STG), bilateral hippocampus, bilateral putamen, left ACC, left insula, and right SMA.

Table 1. *ROI brain activation in response to the L odour, compared to REST.*

Contrast[a]	Brain Region	Side	k	Peak voxel coordinate (MNI)			Z-score
				X	Y	Z	
L > REST	*SMA*	R	157	4	16	66	5.03
	Thalamus	L	83	-1	-22	11	4.52
L < REST	*STG*	L	256	-57	-15	0	5.49
	Hippocampus	L	701	-30	-22	-17	5.27
		R	404	26	-42	0	4.99
	Putamen	R	897	28	-2	14	5.12
		L	411	-28	-9	5	4.60
	ACC	L	1055	-8	39	7	4.82
	Insula	L	136	-35	-22	18	4.67
	SMA	R	245	8	-24	58	4.65

[a] *Significant at cluster-level* $q_{FDR} < 0.05$*; L = left, R = right; k = cluster extent*

An overview of the differentially activated ROIs in the M-REST comparison is presented in Table 2. ROI analyses comparing exposure to the M odour versus the REST condition showed greater activation in the right SMA, and deactivation in the bilateral STG, bilateral hippocampus, left putamen, left ACC, bilateral insula, and right SMA.

Table 2. *Brain activation (ROI) in response to the M odour, compared to REST.*

Contrast[a]	**Brain Region**	**Side**	**k**	*Peak voxel coordinate (MNI)* X	Y	Z	**Z-score**
M > REST	*SMA*	R	175	4	16	66	4.87
M < REST	*Hippocampus*	L	722	-17	-8	-22	5.24
		R	523	28	-2	-19	5.11
	SMA	R	230	6	-22	60	5.20
	STG	L	276	-59	-13	0	5.13
		R	703	49	-9	-13	4.59
	Putamen	L	445	-28	-9	5	5.12
	ACC	L	920	-5	41	-4	4.72
	Insula	L	159	-35	-22	18	4.45
		L	95	-34	14	-15	3.98
		R	101	38	14	-15	4.25

[a] *Significant at cluster-level $q_{FDR} < 0.05$; L = left, R = right; k = cluster extent*

An overview of the differentially activated ROIs in the M-REST comparison is presented in Table 3. ROI analyses comparing exposure to the H odour versus the REST condition showed greater activation in the right SMA, and deactivation in the left STG, bilateral hippocampus, left putamen, left ACC, bilateral insula, and right SMA.

Table 3. *Brain activation (ROI) in response to the H odour odour, compared to rest.*

Contrast[a]	**Brain Region**	**Side**	**k**	*Peak voxel coordinate (MNI)* X	Y	Z	**Z-score**
H > REST	*SMA*	R	172	4	16	66	4.80
H < REST	*Hippocampus*	L	672	-23	-8	-17	5.38
		R	243	29	-2	-19	4.60
		R	196	26	-40	3	4.78
	SMA	R	200	6	-22	60	5.30
	Putamen	L	418	-30	-9	3	5.20
	STG	L	245	-55	-13	-2	4.91
	ACC	L	645	-7	39	-4	4.72
	Insula	R	595	42	-17	5	4.56
		L	150	-39	-22	20	4.41

[a] *Significant at cluster-level $q_{FDR} < 0.05$; L = left, R = right; k = cluster extent*

An overview of brain regions differentially activated in various comparisons during exploratory whole-brain analyses is presented in Table 4. Whole brain analyses showed activation in the left Cerebellum Crus1 region and deactivation in the left SMA in the L odours versus REST comparison; activation in the left Cerebellum Crus1 region and deactivation in the left Fusiform gyrus in the M versus REST comparison; and activation in the left Lingual gyrus, with deactivation in the left Putamen and right Cerebellum IX region in the H versus REST comparison.

Table 4. *Identified brain activation (whole-brain analysis) in response to the three odours, compared to REST.*

Contrast[a]	**Brain Region**	**Side**	**k**	*Peak voxel coordinate (MNI)*			**Z-score**
				X	**Y**	**Z**	
L > REST	*Cerebellum Crus1*	L	10	-7	-83	-15	5.56
L < REST	*STG*	L	14	-57	-15	0	5.49
M > REST	*Cerebellum Crus1*	L	7	-7	-83	-15	5.42
M < REST	Fusiform gyrus	L	13	-32	-49	-15	5.81
H > REST	Lingual gyrus	L	6	-5	-78	-11	5.25
H < REST	Putamen	L	12	-30	-9	3	5.20
	Cerebellum IX	R	7	4	-49	-37	5.46

[a] *Significant at cluster level $p_{FWE\text{-}corr} < 0.05$; L = left, R = right; k = cluster extent*

No significant correlations between significant ROI clusters and corresponding perceptual ratings of odour intensity and liking were observed in any condition (see Appendix A, Table A2 for correlation analysis outputs).

ROI, as well as whole-brain analyses comparing brain activation resulting from exposure to the different odour stimuli (L-M, L-H, M-H and L-MH), did not show any significant differences. Consequently, investigation into potential relationships between discrimination ability for these odour comparisons and brain activation was not pursued.

4 DISCUSSION

The current study investigated brain responses resulting from olfactory exposure to varying fat concentrations embedded within an ecologically relevant food source – dairy milk. In addition to mapping neural correlates of olfactory fat perception, it also aimed at exploring potential associations between brain activation, olfactory fat content discrimination ability and perceived odour intensity and liking of the odour stimuli. Despite samples being perceptually distinguishable, there was no differential brain activation between the odours differing in fat content. Perceived intensity and liking differences between the odours could not be linked to specific neural responses either.

In line with previous work on olfactory fat perception (Boesveldt & Lundstrom, 2014; Pirc et al., 2022), all three odour stimulus fat levels could be discriminated using solely olfactory cues. Whereas discrimination between 3.5% and 14% fat was previously only reported for retronasal cues (Pirc et al., 2022), the current study also confirmed it for orthonasal ones. Considering that orthonasal detection thresholds tend to be lower than retronasal ones (Chale-Rush et al., 2007; Goldberg et al., 2018), this is an expected finding. Perceptual differences between the three odours were also reflected in perceived odour intensity and liking. Notably, while the M odour was perceived as more intense compared to both L and H odours, liking consistently increased with fat concentration. This aligns with observations from our earlier work (Boesveldt & Lundstrom, 2014; Pirc et al., 2022). The consistent discrepancy between discrimination ability and perceived odour intensity observed across our experiments reaffirms the notion that olfactory fat content discrimination is underpinned by factors other than intensity differences. It is likely that odour quality differences between fat levels play a more relevant role when it comes to olfactory fat content discrimination. Several studies support the notion that even minute odour stimulus concentration alterations may affect perceived odour quality (Gross-Isseroff & Lancet, 1988; Le Berre et al., 2008; Stevenson, 2011).

Contrary to expectations, and despite perceptual differences, no differential ROI brain activation was observed when comparing exposure to the three odours. Likewise, no differential activation was observed when comparing exposure to non-fat odours from the lower fat range to exposure to odours from the higher fat range (L – MH). Odour exposure resulted in changes in brain activity only when compared to the rest condition (no odour exposure). Specifically, exposure to either odour activated the supplementary motor area (SMA), while exposure to the L odour also activated the thalamus. Additionally, all three odours led to deactivation in the superior temporal gyrus (STG), hippocampus, SMA, putamen, anterior cingulate cortex (ACC), and insula.

The thalamus, hippocampus and insula are all involved in olfactory processing (Lundström et al., 2011; Roy-Côté et al., 2021; Seubert et al., 2013). The thalamus, while traditionally not considered as an olfactory relay (Kay & Sherman, 2007), receives input from primary olfactory sensory areas (Lundström et al., 2011). It has also been implicated in modulating odour-related attention (Plailly et al., 2008; Sabri et al., 2005; Tham et al., 2009). Considering the latter, thalamic activation in response to the L odour suggests that its relatively low intensity might have required more attention from participants anticipating a percept during odour release. However, given the absence of intensity differences between L and H odours, one might also anticipate a thalamic response during H odour exposure. Like the thalamus, the hippocampus plays a role in integrating information from various sensory inputs, including olfaction (Zhou et al., 2021). Moreover, this area is known for its role in the formation of odour-related memories (Eichenbaum, 1998; Eichenbaum & Otto, 1992) and has been shown to be activated by orthonasally presented food odours, such as chocolate (Small et al., 2005). While we can speculate that the hippocampus's role in this study relates to exposure to food odours, the absence of a non-food modality and the lack of comparable studies hinders definitive conclusions regarding its deactivation. Lastly, the insula acts as a junction for chemosensory inputs integral to food flavour perception (Roy-Côté et al., 2021; Seubert et al., 2013), responding to various food odours (Small et al., 2005; Sorokowska et al., 2016). A pivotal factor in insular activation appears to be stimulus valence (Roy-Côté et al., 2021), with the right insula purportedly responding to pleasant odours (Fulbright et al., 1998; Heining et al., 2003), and the left to unpleasant ones (Bensafi et al., 2012; Lombion et al., 2009; Sorokowska et al., 2016). The right insula has also been found to be more activated by food odours than non-food ones (Sorokowska et al., 2017). While these insights into insular function provide valuable context, they do not directly elucidate the insular deactivation observed in our study. The bilateral insular response to M and H odours challenges the typical lateralization based on valence. Furthermore, the notion that the left insula primarily responds to unpleasant odours is at odds with the liking ratings for the L odour, which suggest it was perceived as neutral rather than negative. The reasons for insular deactivation in this study therefore remain unclear.

Two distinct clusters of activation were observed in the supplementary motor cortex. Specifically, in response to all odours, activation occurred in the anterior SMA also referred to as pre-SMA, with concurrent deactivation in the posterior SMA. The SMA is known for its role in motor planning and execution (Makoshi et al., 2011; Nachev et al., 2008). This region also overlaps with reward-related regions, enabling approach and avoidance behaviours (Hollmann et al., 2012). The anterior SMA tends to engage during the planning phase of movement, while the posterior portion becomes active during movement execution (Lee et al., 1999; Nachev et al., 2008). Our analysis approach modelled odour release as a 3-second event, with actual odour release lasting 2 seconds. This was done to account for any potential lingering of the odours beyond the duration of direct exposure. Although odour release was preceded by an orange crosshair indicating an imminent requirement to sniff an odour, part of the preparatory stage of the act of sniffing might have carried over into odour release. We can therefore speculate that observed anterior SMA activation was due to participants preparing to respond (i.e. sniff the odour) in the initial stages of odour release, while posterior SMA deactivation reflected sniffing inhibition once the odour release stage concluded and participants received the cue that sniffing was no longer required (white cross).

In addition to being involved in the processing of food rewards (Weltens et al., 2014), the ACC and putamen are also both involved in odour processing (Seubert et al., 2013). Both regions tend to exhibit greater activation in response to food odours compared to non-food ones (Sorokowska et al., 2017). Moreover, while the putamen has been associated with encoding odour pleasantness (Torske et al., 2022), the ACC is likely involved in mediating odour-taste interactions in flavour perception (Small et al., 2004). Specifically, when a taste is perceived simultaneously with a retronasal odour, the ACC has been shown to activate, whereas when presented alongside an orthonasal odour, deactivation occurs (Small et al., 2004). This observation lends support to the idea that ACC involvement in our task might have been the result of orthonasal exposure to food-related odours. Given that fat odour-flavour associations can be learned (Sundqvist et al., 2006) and that the odours utilised here are typically experienced retronasally in combination with other modalities involved in dairy milk flavour perception such as taste, ACC deactivation might stem from learned cross-modal associations. The reason behind the putamen's deactivation, as that of the STG – a region with no apparent relevance in the context of our study, remains to be elucidated.

All in all, brain activation within the ROIs does not directly reflect perceptual findings. Not only are there no activation differences between the odours, but odour exposure led to decreased activity in most identified regions. This contrasts with studies on oral fat perception (De Araujo & Rolls, 2004; Eldeghaidy et al., 2011a; Grabenhorst et al., 2010b), as well as those on neural processing of odour intensity and valence. Specifically, Anderson et al. (2003) observed that perceived intensity correlates with amygdala and piriform cortex activation; Rolls et al. (2003) observed correlations between perceived pleasantness and activation in the orbitofrontal cortex and ACC; while Winston et al. (2005) showed that the amygdala reflects perceived intensity of pleasant or unpleasant odours, but not neutral ones. However, these studies not only employed relatively small sample sizes and artificial odorants (e.g., anisole, citral acid, valeric acid, geranyl acetate), they were also not replicated.

Whole brain analyses identified four additional potentially relevant brain activation clusters: in the fusiform gyrus, in the crus-1 region of the cerebellum, in the IX region of the cerebellum and in the lingual gyrus. Both the lingual and fusiform gyri are visual areas known to respond to high calorie food cues, as highlighted by the meta-analysis of Yang et al. (2021). With repeated exposure, our participants might have formed associations between the visual cue signifying odour delivery (green crosshair) and M and H milk odours as high-calorie food cues. Similarly, although the cerebellum has traditionally been associated with motor and coordination control, the crus-1 region has been shown to respond to visual food cues (such as the green crosshair in our study) (Berman et al., 2013; Iosif et al., 2023). Lastly, the IX cerebellar region is part of the so-called default mode network (Stephen et al., 2018). Since default mode network areas deactivate during externally focused tasks, the observed deactivation in this region might have resulted from active engagement with the fMRI task (Menon, 2023).

Assuming that discrimination ability in our experiment was underpinned by quality, rather than intensity differences between the samples, as suggested above, then it is reasonable to expect differential activation within the posterior piriform cortex, as shown by Howard et al. (2009), Gottfried et al. (2006) and Li et al. (2010). One could argue, however, that our analysis approaches were not fine-grained enough to detect differential brain activation resulting from subtle odour quality differences. An approach like the one employed by Howard et al. (2009), who utilised multivariate analysis techniques which focus on patterns of voxel activity across regions rather than individual voxel activations, might be more suitable. Including additional perceptual ratings, especially those relating to fat-related odour quality differences (as in Howard et al. (2009) and Gottfried et al. (2006)) would further refine the fMRI task.

Within the context of olfactory fat perception, the current study is the first of its kind. It included a substantial number of participants and employed ecologically relevant odour stimuli. The lack of benchmark studies, however, makes the interpretation of our findings challenging. Nevertheless, the study serves as a foundation for subsequent work on the topic and yields several relevant considerations. To deepen our understanding of olfactory fat perception, it is essential to not only corroborate our findings but to explore fat perception using other olfactometer-compatible fat-related food sources. For instance, it might be interesting to compare vegetable oil emulsions varying in fat or oils/fats varying in origin (e.g., olive oil, sunflower oil, lard). Considering that fatty acids can be discriminated using solely olfactory cues (Bolton & Halpern, 2010; Kallas & Halpern, 2011), it seems relevant to explore the neural correlates of olfactory perception of food sources with distinct fatty acid profiles. Moreover, given that retronasal olfaction plays a central role in food perception (Boesveldt & de Graaf, 2017) and is arguably more ecologically relevant for food odours than orthonasal olfaction, future studies should consider an experimental design primarily focused on retronasal fat perception. Such an approach could reveal distinct neural pathways associated with retronasal fatty odour processing. The influence of hunger state on olfactory fat perception and discrimination and associated neural activity should be assessed as well. It is plausible that brain activation differences between food odours varying in fat might become more apparent in a depleted state, such as with protein (Griffioen-Roose et al., 2014). Lastly, it might be worthwhile exploring potential genetic predispositions or dietary influences on olfactory fat perception. The former appears especially intriguing. Not only were genetics identified as a factor in fat taste sensitivity (Running & Mattes, 2016), but our own observations across different experiments hint that genetics might play a role in olfactory fat perception. Specifically, a subset of participants could not distinguish between any fat levels, while the majority could - raw discrimination data from the current and past experiments is available on the Open Science Framework Repository (Pirc et al., 2023a; Pirc et al., 2021).

5 CONCLUSION

Our work reaffirms the notion that food fat content can be distinguished using solely olfactory cues. This ability and underlying perceptual differences, however, were not reflected by brain activation in the current experiment. While this study has paved the way in understanding the neural underpinnings of olfactory fat perception, it also highlights the complex dynamics between olfaction and brain responses. Moreover, it underscores the need for further neuroimaging studies on food odour processing, especially those focusing on olfactory fat perception.

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SUPPLEMENTARY MATERIAL A

Table A1. *Experiment 1 sample mixture ingredients, with corresponding nutritional composition per 100 g of odour sample.*

	Ingredients (per 100 g)		**Nutritional values (per 100 g)**		
Sample	Skimmed milk (g)	Cream (g)	F (g)	CH (g)	P (g)
0% (L)	100.0	0.0	0.0	4.7	3.7
3.5% (M)	90.0	10.0	3.5	4.5	3.6
14% (H)	60.0	40.0	14.0	4.0	3.7

F = dietary fat; CH = carbohydrates; P = protein

Table A2. *Correlation analysis output. Mean parameter estimates from significant clusters identified in L versus REST, M versus REST and H versus REST contrasts were correlated with average perceived odour intensity and liking ratings of the corresponding odour.*

Brain Region	**Side**	**k**	*Peak voxel coordinate (MNI)*			**Z-score**	**Odour intensity**		**Odour liking**	
			X	**Y**	**Z**		**ρ**	**p-value**	**ρ**	**p-value**
L versus REST										
SMA	R	157	4	16	66	5.03	-.051	*.805*	.168	*.411*
	R	245	8	-24	58	4.65	.078	*.704*	.316	*.116*
Thalamus	L	83	-1	-22	11	4.52	-.294	*.144*	.113	*.584*
STG	L	256	-57	-15	0	5.49	-.129	*.530*	.258	*.204*
Hippocampus	L	701	-30	-22	-17	5.27	.015	*.943*	.036	*.860*
	R	404	26	-42	0	4.99	.141	*.493*	.202	*.321*
Putamen	R	897	28	-2	14	5.12	-.135	*.511*	.111	*.590*
	L	411	-28	-9	5	4.60	.164	*.423*	.133	*.518*
ACC	L	1055	-8	39	7	4.82	.074	*.720*	.081	*.695*
Insula	L	136	-35	-22	18	4.67	.215	*.291*	.309	*.116*
M versus REST										
SMA	R	175	4	16	66	4.87	.143	*.486*	-.139	*.498*
	R	230	6	-22	60	5.20	-.200	*.328*	.113	*.372*
Hippocampus	L	722	-17	-8	-22	5.24	-.089	*.666*	.137	*.505*
	R	523	28	-2	-19	5.11	-.028	*.890*	.113	*.582*
STG	L	276	-59	-13	0	5.13	-.149	*.469*	.124	*.546*
	R	703	49	-9	-13	4.59	-.100	*.626*	.096	*.640*
Putamen	L	445	-28	-9	5	5.12	.100	*.626*	.091	*.660*
ACC	L	920	-5	41	-4	4.72	.062	*.765*	-.097	*636*
Insula	L	159	-35	-22	18	4.45	.006	*.978*	-.014	*.944*
	L	95	-34	14	-15	3.98	-.156	*.447*	.249	*.220*
	R	101	38	14	-15	4.25	-.027	*.897*	.177	*.387*
H versus REST										
SMA	R	172	4	16	66	4.80	.172	*.400*	.032	*.877*
	R	200	6	-22	60	5.30	-.126	*.540*	.023	*.910*
Hippocampus	L	672	-23	-8	-17	5.38	-.059	*.774*	-.002	*.992*
	R	243	29	-2	-19	4.60	-.191	*.351*	-.148	*.470*
	R	196	26	-40	3	4.78	-.079	*.701*	.187	*.361*
Putamen	L	418	-30	-9	3	5.20	.106	*.606*	.127	*.538*
STG	L	245	-55	-13	-2	4.91	-.136	*.508*	-.177	*.387*
ACC	L	645	-7	39	-4	4.72	.140	*.494*	.001	*.997*
Insula	R	595	42	-17	5	4.56	.043	*.836*	-.016	*.937*
	L	150	-39	-22	20	4.41	.172	*.401*	.014	*.946*

L = left, R = right; k = cluster extent; ρ = Spearman correlation coefficient (N = 26)

5

Chapter 5

Retronasal Fatty Odours Produce Limited Effects on Food Perception and Intake

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ABSTRACT

Reduction of food fat content often comes at the cost of sensory appeal. Given that odours can enhance various fat-related sensory characteristics, their use as fat substitutes seems promising. This cross-over study investigated whether sensory characteristics of a low-fat product (dairy milk) can be enhanced by the addition of a fat-related aroma (cream) and whether this influences subsequent eating behaviour within an ecologically valid scenario. Fifty-six consumers evaluated 0% fat milk without aroma (skim), 0% fat milk infused with cream aroma (skim+) and 3.5% fat milk without aroma (full) on perceived flavour intensity, creamy mouthfeel, aftertaste and liking (ranking rating scale - 100-unit VAS). In three separate subsequent sessions, 54 of the participants consumed the samples above as fixed preloads (300ml) after which they were provided an ad-libitum breakfast consisting of granola and yogurt. Appetite ratings (hunger, fullness, desire to eat, prospective consumption) were collected pre- and post-preload and post-breakfast. While skim+ samples were more liked compared to skim ones, there was no difference in flavour intensity, creamy mouthfeel, or aftertaste. Full fat samples were rated higher than both skim and skim+ ones on all attributes. Despite the added aroma (skim+) enhancing liking, it did not influence subsequent intake amounts or eating rate. In fact, there were no differences in intake or appetite between any of the preloads. This demonstrates that odours can be used to enhance liking of reduced-fat food products, yet the broader implication of such an approach on food intake behaviour remains to elucidated.

Keywords: fat perception, retronasal olfaction, eating behaviour, intake, fat substitutio

1 INTRODUCTION

Excessive dietary fat consumption remains a concern in Western countries (European Commission, 2021; Eilander et al., 2015; Van Rossum et al., 2020), contributing to obesity and related comorbidities (Blundell & MacDiarmid, 1997). Despite initiatives aimed at curbing dietary fat consumption, both fat intake and obesity rates continue to rise (Shen et al., 2017; Vadiveloo et al., 2014; WHO, 2018). Dietary fat is the most energy dense nutrient with a low effect on satiation (Blundell & MacDiarmid, 1997; Bolhuis et al., 2016; Gerstein et al., 2004). Its overconsumption is further exacerbated by its flavour, texture, and aroma-enhancing qualities, all of which considerably contribute towards the enjoyment of eating (Drewnowski, 1997a, 1997b; Drewnowski & Almiron-Roig, 2009). Addressing this public health issue necessitates effective strategies that curtail fat content in foods while maintaining their sensory appeal.

Fat perception is a complex multisensory experience, involving taste, mouthfeel, and principally, olfaction (Drewnowski & Almiron-Roig, 2009; Pirc et al., 2023b; Zhou et al., 2016). Humans have been shown to be able to smell dietary fat and its constituents in isolation (Bolton & Halpern, 2010; Chale-Rush et al., 2007; Chukir et al., 2013; Kallas & Halpern, 2011; Kindleysides et al., 2017), or as part of complex food matrices (Boesveldt & Lundstrom, 2014; Glumac & Chen, 2020; Mu et al., 2022; Pirc et al., 2022). As integral components of flavour, retronasal odours seem particularly relevant to fat perception.

The relevance of retronasal odours in fat perception is highlighted by findings showing that, using solely retronasal cues, humans can detect 18-carbon fatty acids (Chale-Rush et al., 2007), discriminate them from blanks (Bolton & Halpern, 2010) and each other (Kallas & Halpern, 2011), and identify them from blanks and each other (Chukir et al., 2013). Retronasal cues in isolation have also been found sufficient for discriminating fat content differences in dairy milk (Pirc et al., 2022). Furthermore, the presence of retronasal cues seems crucial for fat content discrimination in milk and yogurt (Le Calvé et al., 2015), with their absence limiting discrimination in cottage cheese (Schoumacker et al., 2017). Lastly, the addition of fat-related retronasal odours to foods has been shown to enhance the perception of various fat-related sensory qualities, such as fattiness (Frøst et al., 2001; Yackinous & Guinard, 2000), creaminess (Bult et al., 2007; Han et al., 2019), thickness (Bult et al., 2007) and even fat texture (Han et al., 2019; Syarifuddin et al., 2016). Collectively, the available evidence confirms that retronasal odours are a crucial component of dietary fat perception and can enhance the perception of fat-related qualities in foods. This implies that incorporating fat-related odours as substitutes in foods could offer a feasible method to lower fat content while retaining the appealing sensory attributes associated with fat. While retronasal odours have been found to influence perceived satiation (Bojanowski & Hummel, 2012; Ramaekers, 2014; Ruijschop et al., 2010; Ruijschop et al., 2008), it remains uncertain whether this strategy would lead to observable changes in food intake behaviour. Specifically, the duration (Ruijschop et al., 2008) and complexity of strawberry aroma (Ruijschop et al., 2010) were found not to effect ad libitum intake of yogurt drinks, while duration and intensity of tomato aroma had no effect on intake of tomato soup (Ramaekers et al., 2014b). Additionally, the presence of a cream aroma had no effect on intake of tomato soup (Ramaekers, 2014) and dairy products (Hogenkamp et al., 2011). However, apart from Hogenkamp et al. (2011), other studies delivered retronasal odours by means of an olfactometer, which is a relatively invasive approach, not representative of a typical eating occasion and could have obscured the effects on eating behaviour. Moreover, none of these studies utilised actual fat sources as odour stimuli. A more ecologically relevant approach to studying effects of fat-related retronasal odours on eating behaviour would be to utilise actual fat sources as stimuli in a setting resembling a typical eating occasion.

Notably, the broader influence of food odours on eating behaviour remains unclear, with inconsistent and sparce findings (Boesveldt & de Graaf, 2017), particularly when it comes to behavioural outcomes of exposure to fat-related odours. Although there is limited circumstantial evidence regarding the impact of fat-related food odours on hedonics (Boesveldt & Lundstrom, 2014; Han et al., 2019; Jervis et al., 2014; Running et al., 2017; Syarifuddin et al., 2016; Yackinous & Guinard, 2000), with our own findings suggesting that retronasal odours associated with a higher fat content are more liked (Pirc et al., 2022), the understanding of how fat-related odours affect eating behaviour within ecologically valid settings remains limited. The present study therefore aimed to investigate (1) whether fat reduction-induced sensory alterations in dairy milk can be compensated by the addition of fatty odours, and (2) whether fat-related retronasal odours have the capacity to influence eating behaviour within an ecologically valid scenario. To attain the aim, we carried out a study wherein participants consumed dairy milk samples with fat-related odours infused into the beverage. Odour-infused low-fat dairy milk samples were compared to low-fat and full-fat samples without odour infusion. The cross-over study comprised of two parts: (1) A single-session sensory evaluation to assess the impact of an added retronasal fat-related odour on sensory characteristics and liking of the milk samples, and (2) a three-session fixed preload-based intake experiment to investigate the potential influence of retronasal exposure to fat-related odours on subsequent eating behaviour. In addition to collecting appetite ratings and measuring ad libitum intake, we also measured eating rate, which is considered to reflect palatability and satiety (Bobroff & Kissileff, 1986; Drewnowski, 1998; Yeomans, 1996).

2 MATERIALS AND METHODS

Before enrolling, participants received detailed information on experimental procedures and provided written informed consent, in accordance with the Declaration of Helsinki. All aspects of the study were approved by the Wageningen University Social Science Ethics Review Board. Data supporting the findings are available on the Open Science Framework Repository with the identifier DOI 10.17605/OSF.IO/KZX4W (Pirc et al., 2023a).

2.1 Participants

Sixty naïve consumers were recruited from Wageningen (The Netherlands) and its surroundings. Sensory evaluation ultimately included 56 participants (MAge = 24.0 ± 3.1 years; MBMI = 22.4 ± 2.2 kg/m2; 16 males), while the intake experiment included 54 (MAge = 23.8 ± 3.2 years; MBMI = 22.4 ± 2.2 kg/m2; 15 males) - due to either non-adherence to experimental procedures or dropping out, some participants had to be excluded. All participants were self-reported dairy product consumers and met the following eligibility criteria: being between 16 and 55 years of age; normosmic, as determined using the Sniffin' Sticks 16-item odour identification test (Hummel et al., 2007); self-reported as healthy, non-smoking, not dieting at the time or in the past two months, having no relevant allergies or intolerances, being willing to consume yogurt and granola (provided as part of the intake experiment), and not pregnant or lactating.

2.2 Olfactory stimuli & stimulus delivery

All experimental procedures utilised so-called “air up” drinking bottles (air up GmbH, Munich, Germany) as stimulus delivery vehicles. Air up® bottles consist of a beverage container and an odour pod affixed to a silicone mouthpiece, which holds a drinking straw. The odour pod connects with the straw through a small opening within the mouthpiece, allowing odour release as liquid is drawn through it – effectively delivering retronasal odours during beverage consumption. All bottles utilised in the current study were semi-opaque black and labelled with random 3-digit codes, to eliminate the potential impact of visual cues on perception and conceal sample identities. See Figure 1 for a schematic illustration of an air up® bottle and its components.

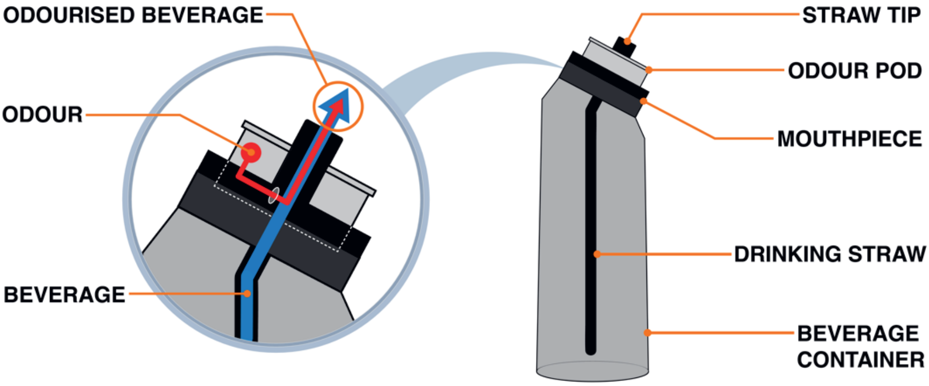

Figure 1. *Air up® bottle schematic.*

Two types of odour pods were utilised in the experiment: (1) blanks, containing no odorous substances and (2) odour pods containing a 13.2% fat mixture, made by dissolving 1 part of heavy cream powder (66.5% fat; Hoosier Hill Farm, Middleton, Wisconsin, US) in 4 parts of skimmed milk (0% fat - AH Magere Melk, Albert Heijn B.V.). Odour pods were then coupled with either a skim milk – water solution (5% water, 95% skim milk - AH Magere melk, Albert Heijn B.V.) or full fat milk (3.5% fat - AH Volle Melk, Albert Heijn B.V.). The purpose of diluting skim milk with water was to balance the carbohydrate and protein nutrient content between skim and full-fat milk, while only altering the fat content (see Appendix A, Table A1 for nutritional compositions of the dairy milk samples). Three odour pod – milk combinations were ultimately utilized in the study: (1) Skim milk with 5% water combined with a blank odour pod (hereafter referred to as 'skim'); (2) skim milk with 5% water combined with the fat-containing odour pod (hereafter referred to as 'skim+'); and (3) full-fat milk combined with a blank odour pod (hereafter referred to as 'full'). All samples were freshly made no more than 15 minutes prior to testing and were served at room temperature (ranging between 20 and 22 °C). Sensory evaluation involved servings of 150 ml, while 300-ml servings were provided during intake experiment sessions.

2.3 Study procedures

All study sessions were conducted on separate days. Prior to attending the sensory evaluation session, participants filled out an online screening questionnaire, which included questions needed to assess various eligibility criteria. Additionally, on-location screening involved measurements of height and weight (needed to establish BMI), as well as assessment of olfactory function, using the Sniffin' Sticks 16-item odour identification test (Hummel et al., 2007).

2.3.1 Sensory evaluation session

Participants were instructed to refrain from eating or drinking anything except water for two hours before the session. They were also instructed not to chew gum or brush their teeth in the 45 minutes prior. The experiment took place in sensory booths equipped with a computer running EyeQuestion software (Logic8 B.V., Elst, Nederlands), which was used to collect data. Prior to sensory evaluation, participants underwent a short familiarisation procedure, intended to make them acquainted with the stimulus delivery bottles and the proper way of handling them.

Samples during sensory evaluation were presented in two blocks, each separated by a 5-minute break to minimize sensory fatigue. Each block allowed for one sample comparison: (1) skim versus skim+; or (2) skim+ versus full. The order of samples within and between blocks was randomized and counterbalanced across participants. The following presentation orders were possible: (1) skim, skim+ & skim+, full; (2) skim+, skim & skim+, full; (3) skim, skim+ & full, skim+; (4) skim+, skim & full, skim+; (5) skim+, full & skim, skim+; (6) skim+, full & skim+, skim; (7) full, skim+ & skim, skim+; (8) full, skim+ & skim+, skim. A full factorial comparison design was not implemented due to concerns about sensory fatigue and satiation arising from multiple tastings. See Figure 2 for an overview of the sensory evaluation session timeline.

Samples were presented dyadically and evaluated with the ranking on a scale procedure (Heymann & Ebeler, 2017; Kim & O'Mahony, 1998), which entails rating both samples on a shared 100-unit visual analogue scale (VAS). VAS were anchored with "Not at all" and "Extremely" at the extreme ends and accompanied with questions (e.g. "How intense is the overall flavour of the presented samples?"). Samples were assessed in terms of perceived overall flavour intensity, creamy mouthfeel (defined as "a smooth, fatty, rich (full) texture that coats the tongue and mouth"), aftertaste (defined as "intensity of the flavour that remains in the mouth after swallowing the sample") and liking.

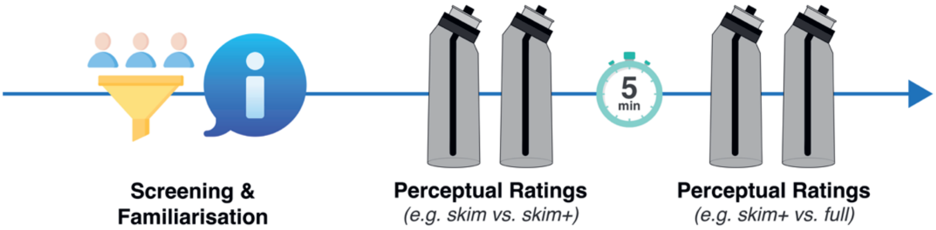

Figure 2. *Sensory evaluation session timeline overview.*

Participants were instructed to always taste and rate samples in the order of presentation (from left to right) and rate them one-by-one. Attributes were rated one at a time, which encouraged participants to take at least one sip of sample per attribute. They were also instructed to take sufficiently sized sips and rinse their mouth with provided water between samples. Samples were presented in 150 ml amounts, while water was provided in 200 ml amounts per block. Sample retasting was permitted, but only in the order of presentation. To assess compliance with experimental procedures, returned sample and water amounts were measured. If the consumed amount of sample or water was less than 15% of the presented amount, ratings data for that specific sample comparison were excluded.

2.3.2 Intake experiment sessions

Intake experiment sessions took place in the morning at either 8:00, 9:00 or 10:00. Participants were required to arrive in a fasted state, having consumed no calorie-containing foods or drinks after midnight, and to refrain from chewing gum and brushing their teeth for 45 minutes before their session. Sessions were scheduled a minimum of 72 hours apart and took place in a dining room environment. Measures were taken to eliminate any visible time cues (e.g. wall clocks, easy access to mobile phones), ensuring that they would not influence eating behaviour. Interaction between study participants was prohibited throughout the experimental part of the intake sessions.

Upon arrival, participants were allocated to their respective dining tables, each equipped with a laptop running the EyeQuestion data collection software. After completing an appetite questionnaire (adapted from Blundell et al. (2010)), which included VAS with questions about hunger, fullness, desire to eat and prospective consumption, participants were presented with one of the three samples (either skim, skim+ or full – presentation order randomised and counterbalanced across sessions and participants). They were instructed to consume all the milk from the bottle, at their own pace. Consumption duration was recorded to account for potential sensory exposure differences during data analyses. Immediately after consuming the milk, they completed the same appetite questionnaire again. After a short break (2min), they received breakfast.

Breakfast consisted of 300 g of granola cereal (Quaker Cruesly 4 Nuts, PepsiCo, Inc) and 900 g of yogurt (Jumbo Milde Halfvolle Yoghurt, Van Eerd Beheer B.V.) in a large cereal bowl. It was served on a tray along with 200 ml of water provided in a cup (which could be refilled if requested), a napkin, a spoon, and a stopwatch with the screen covered, to hide time cues. Participants had the liberty to consume breakfast ad libitum, until comfortably full, starting the stopwatch when they began eating and stopping it when they chose to halt or take a pause. If they decided to continue eating, they were instructed to resume the timing process. This breakfast timing procedure was implemented to allow for the calculation of eating rate. A 20-minute period was designated for breakfast consumption, and participants were instructed not to leave before this time was up, even if they finished eating earlier (a separate timer implemented in EyeQuestion was used to measure the allocated breakfast time and notify participants when 20 minutes elapsed). Following this, participants completed a final appetite questionnaire before departing the session. The last intake session additionally included the Dutch Eating Behaviour Questionnaire (DEBQ) (van Strien et al., 1986) before concluding. See Figure 3 for an overview of the intake experiment session timeline.

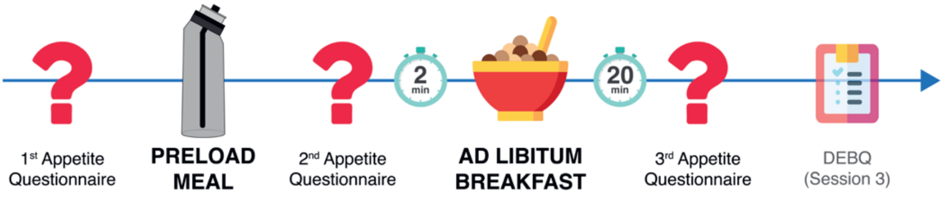

Figure 3. *An overview of the intake experiment timeline.*

2.4 Data analysis

Data analysis procedures were executed using SPSS Statistical Software (Version 29. Armonk, NY: IBM Corp.) Statistical significance for all procedures was set at p < .05.

Effects of samples on perceptual ratings of overall flavour, creamy mouthfeel, aftertaste, and liking were analysed using linear mixed models (LMM). Attribute ratings were defined as dependent variables, samples (skim, skim+ or full) as fixed factors and subjects as random ones. Each of the two comparison blocks (skim versus skim+; skim+ versus full) were analysed independently. Potential presentation order effects on attribute ratings were assessed by defining each of the ratings as dependent variables, presentation orders as fixed factors and subjects as random ones in separate LMM.

Breakfast ad libitum intake quantities were calculated by subtracting empty cereal bowl weights from the total weight of each participant's returned bowl. Amounts of consumed water were calculated by subtracting empty cup weights from the weight of returned cups. Eating rate (in g/min) was calculated by dividing consumed amounts with breakfast consumption times. Effects of preloads on intake parameters, namely breakfast consumption amounts (in g) and eating rate were then analysed with separate LMM. The two intake parameters were individually defined as dependent variables, preload types (skim, skim+ or full) as fixed factors and subjects as random ones. Consumed water and preload exposure time were initially included as covariates in both models, however, since the covariates and their interactions were not found to be significant, the models were simplified to only include the aforementioned factors. LMM were also utilised to assess differences in appetite ratings (hunger, fullness, desire, prospective consumption) across the intake experiment. Ratings were defined as dependent variables, time points (pre-preload, post-preload, post-breakfast) as fixed factors and subjects as random ones. The interaction between preload type and rating timepoint was also added to the model.

Given that restrained eating may impact ad libitum intake behaviour, we reran ad libitum LMM analyses after removing 19 participants (12 females, 7 males) classified as restrained eaters (DEBQ restraint domain score > 3.2 for females; > 2.4 for males) (van Strien, 2005; van Strien et al., 1986), to assess whether their inclusion might have affected intake differences between the preloads.

Potential effects of odour addition on preload consumption time (s) were explored using LMM as well. Preload exposure time was defined as a dependent variable, preload type as a fixed factor and subjects as a random factor. Additionally, we evaluated potential effects of preload consumption times on changes in appetite ratings between the pre-preload and post-preload time points. Differences in appetite ratings, calculated by subtracting the pre-preload values from the post-preload ones, were defined as dependent variables, preload types as fixed factors, and subjects as random factors. Preload exposure time was also added to the model as a covariate to account for its potential effects on appetite rating shifts.

When significant main effects of fixed factors were observed on dependent variables in any of the analyses, Bonferroni post-hoc tests were applied to identify mean differences.

3 RESULTS

Sensory evaluation results are presented in Figure 4.

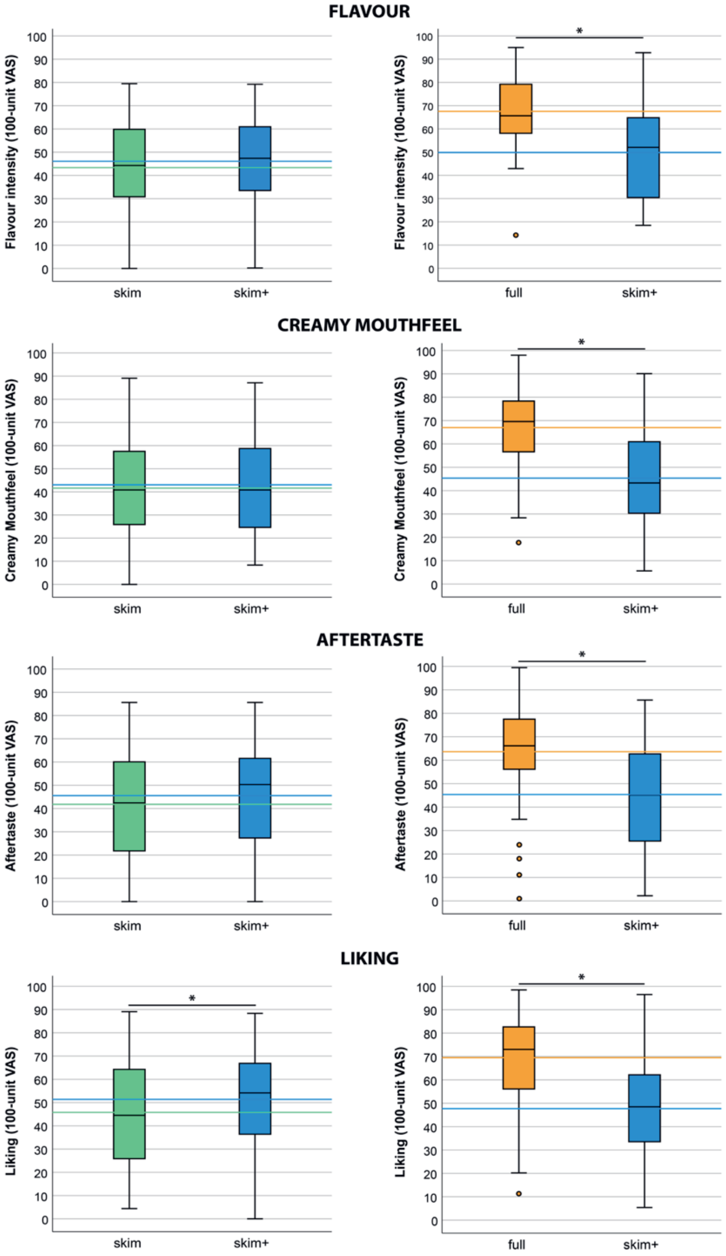

Figure 4. *Boxplots of sensory evaluation results per perceptual rating. Coloured horizontal lines represent sample means, with mean differences being denoted by an asterisk.*

No differences in perceived overall flavour intensity (M_{skim} = 43 ± 18; M_{skim+} = 46 ± 19; F(1, 57) = 1.28, p = .26), creamy mouthfeel (M_{skim} = 42 ± 22; M_{skim+} = 43 ± 21; F(1, 57) = 0.22, p = .64) and aftertaste (M_{skim} = 42 ± 22; M_{skim}+ = 46 ± 22; F(1, 57) = 2.02, p = .16) were observed between skim and skim+ samples. A significant difference between the samples was only observed in terms of liking (F(1, 55) = , p = .027)), with the skim+ sample being more liked compared to the skim one (M_{skim} = 46 ± 25; M_{skim+} = 51 ± 23). Compared to full samples, skim+ samples were rated lower in terms of flavour intensity (M_{full} = 68 ± 16; M_{skim+} = 50 ± 18; F(1, 55) = 44.40, p < .001), creamy mouthfeel (M_{full} = 67 ± 16; M_{skim+} = 45 ± 22; F(1, 57) = 46.49, p < .001), aftertaste (M_{full} = 63.6 ± 21.5; M_{skim+} = 45.4 ± 22.4; F(1, 57) = 28.744, p < .001) and liking (M_{full} = 70 ± 19; M_{skim+} = 48 ± 23; F(1, 55) = 35.44, p < .001). No presentation order effects were observed on any of the perceptual ratings.

Effects of preload type on breakfast intake and eating rate are presented in Figure 5.

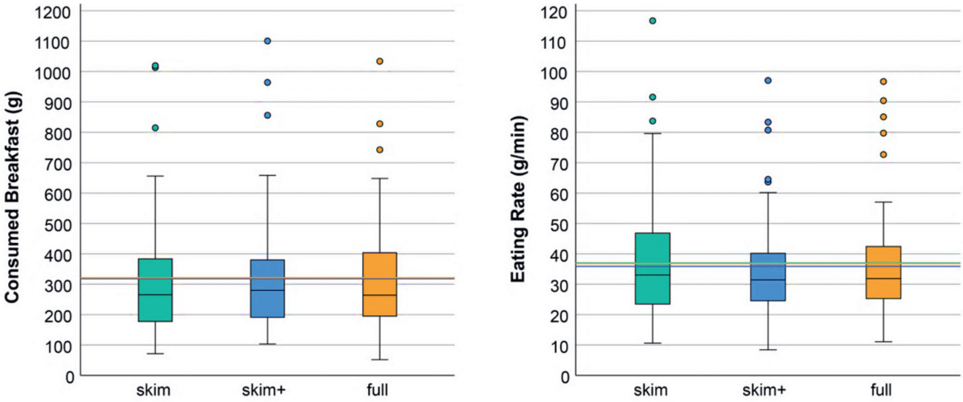

Figure 5. *Boxplots of intake parameters. Coloured horizontal lines represent intake parameter means per preload.*

No effects of preload type were observed on ad libitum intake amounts (F(2, 106) = .01, p = .99; M_{skim} = 320, M_{skim}+ = 318, M_{full} = 321) nor eating rates (F(2, 106) = 1.02, p = .36; M_{skim} = 37, M_{skim}+ = 36, M_{full} = 37). LMM excluding restrained participants yielded similar results, showing no effects of preload type on ad libitum intake (F(2, 68) = .00, p = 1.00; M_{skim} = 318, M_{skim+} = 316, M_{full} = 317) or eating rate (F(2, 68) = .09, p = .91; M_{skim} = 36, M_{skim+} = 35, M_{full} = 36).

Effects of preload type and timepoint on perceptual ratings of hunger, fullness, desire to eat and prospective consumption are presented in Figure 6.

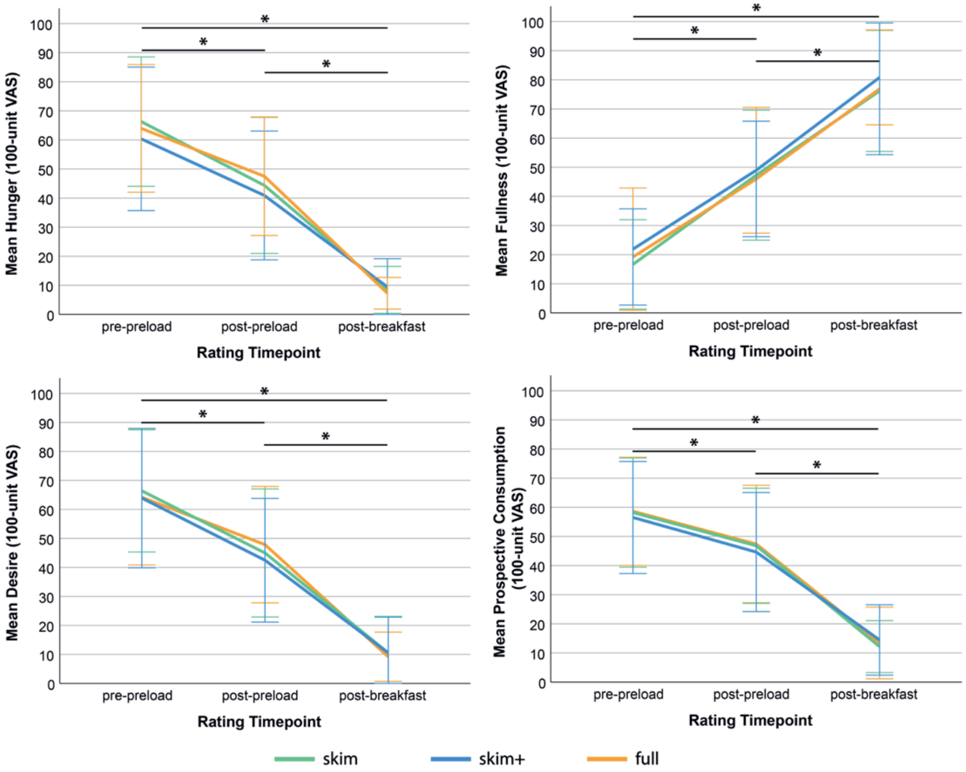

__Figure 6.__ Line graphs of perceptual rating means per preload type, across rating timepoints. Error bars represent ± SD.

No interactions between preload type and rating timepoint were observed for any of the perceptual ratings ($F_{hunger}(4, 424) = 1.55$, $p_{hunger} = .19$; $F_{fullness}(4, 424) = .23$, $p_{fullness} = .92$; $F_{desire}(4, 440) = .53$, $p_{desire} = .71$; $F_{prospective}(4, 440) = .53$, $p_{prospective} = .71$). No effect of preload type was observed on perceived hunger ($F(2, 424) = 1.72$, $p = .18$; $M_{skim} = 40$, $M_{skim+} = 37$, $M_{full} = 40$), fullness ($F(2, 424) = 2.29$, $p = .10$; $M_{skim} = 47$, $M_{skim+} = 51$, $M_{full} = 47$), desire to eat ($F(2, 440) = .56$, $p = .57$; $M_{skim} = 41$, $M_{skim+} = 39$, $M_{full} = 40$) or prospective consumption ($F(2, 440) = .38$, $p = .68$; $M_{skim} = 39$, $M_{skim+} = 39$, $M_{full} = 40$). There was, however, a main effect of rating timepoint observed on all perceptual ratings ($F_{hunger}(2, 424) = 538.03$, $p_{hunger} < .001$; $F_{fullness}(2, 424) = 464.27$, $p_{fullness} < .001$; $F_{desire}(2, 440) = 536.24$, $p_{desire} < .001$; $F_{prospective}(2, 440) = 492.30$, $p_{prospective} < .001$). Specifically, hunger ($M_{pre\text{-}preload} = 63$, $M_{post\text{-}preload} = 44$, $M_{post\text{-}breakfast} = 8$), desire to eat ($M_{pre\text{-}preload} = 66$, $M_{post\text{-}preload} = 46$, $M_{post\text{-}breakfast} = 10$) and prospective consumption ($M_{pre\text{-}preload} = 57$, $M_{post\text{-}preload} = 47$, $M_{post\text{-}breakfast} = 14$) decreased with time, while fullness increased ($M_{pre\text{-}preload} = 18$, $M_{post\text{-}preload} = 48$, $M_{post\text{-}breakfast} = 78$).

No effects of preload type were observed on preload consumption times ($F(2, 106) = .56$, $p = .56$; $M_{skim} = 130$, $M_{skim+} = 127$, $M_{full} = 122$). Likewise, preload consumption times had no influence on changes in appetite ratings (Δ) between pre- and post-preload rating timepoints, regardless of the preload type. Specifically, preload type was found to have no effect on appetite rating changes between the two timepoints ($F_{\Delta hunger}(2, 430) = 1.33$, $p_{\Delta hunger} = .26$; $F_{\Delta fullness}(2, 430) = 2.52$, $p_{\Delta fullness} = .082$; $F_{\Delta desire}(2, 430) = 2.64$, $p_{\Delta desire} = .073$; $F_{\Delta prospective}(2, 430) = 1.65$, $p_{\Delta prospective} = .19$), and neither did preload exposure time (as a covariate) ($F_{\Delta hunger}(1, 310) = .85$, $p_{\Delta hunger} = .37$; $F_{\Delta fullness}(1, 308) = 1.48$, $p_{\Delta fullness} = .23$; $F_{\Delta desire}(1, 296) = 1.41$, $p_{\Delta desire} = .24$; $F_{\Delta prospective}(1, 312) = 1.03$, $p_{\Delta prospective} = .31$).

4 DISCUSSION

The study aimed to determine whether mimicking a high-fat product by adding fat-related odours to its low-fat counterpart can compensate for the sensory appeal diminished by the absence of fat. Additionally, we evaluated the impact of such an approach on food intake behaviour within a setting reflective of a realistic eating occasion. We found that, although the addition of a fat-related (retronasal) odour enhanced liking of a low-fat food product, it did not affect other sensory attributes or food intake behaviour.

Perceptually, no differences in flavour intensity, creamy mouthfeel, or aftertaste were observed between skim and skim+ samples. There was however a difference in perceived liking, with the skim+ sample being more liked than its skim counterpart. When compared with the full milk sample, the skim+ sample was rated lower across all ratings, including liking. It is noteworthy that sample presentation orders had no effect on perceptual ratings, underscoring the reliability of the observed outcomes. These observations resonate with prior studies indicating that introducing fatty odours to foods can selectively enhance certain perceptual attributes, including liking (Bult et al., 2007; Frøst et al., 2001; Han et al., 2019; Syarifuddin et al., 2016; Yackinous & Guinard, 2000). For instance, while cream aroma was found to enhance fattiness, creaminess and thickness in skim milk, it did not influence its flavour intensity (Bult et al., 2007; Frøst et al., 2001). Similarly, butter aroma enhanced fattiness in mashed potatoes (Yackinous & Guinard, 2000), while in cheese it enhanced creaminess and fat texture without affecting saltiness (Han et al., 2019; Syarifuddin et al., 2016). Akin to our findings on liking, Han et al. (2019) and

Syarifuddin et al. (2016) demonstrated that adding butter aroma to cheese enhances perceived texture pleasantness and overall liking, respectively.

Going beyond fat perception, it is well established that odours have the capacity to enhance intensities of basic taste properties – an effect termed odour-induced taste enhancement (OITE) (Ai & Han, 2022). OITE has been reported for various food matrices, with odours intensifying saltiness in a sodium solution (Chokumnoyporn et al., 2015) and cheese (Thomas-Danguin et al., 2015), umami in an umami solution (He et al., 2020) and sweetness in a sucrose solution (Labbe et al., 2006). It is important to note, however, that the presence of odours does not invariably lead to taste enhancement. As Linscott and Lim (2016) highlight, OITE is influenced by several factors, including the congruency between odour and taste, their temporal proximity, and odorant concentration (Ai & Han, 2022). Drawing insights from OITE studies, the inclusion of taste-related attributes (e.g. sweetness) might have provided a clearer distinction between our samples. Additionally, given the known interactions between odours and mouthfeel (Guichard et al., 2018; Pirc et al., 2023b), diversifying the mouthfeel attributes might have offered more insights. Our pilot experiments pointed towards mouthfeel being the distinguishing factor between skim and skim+ samples, yet current results suggest otherwise. Including other mouthfeel-related attributes (e.g. thickness, smoothness, heaviness) might have been more relevant in capturing the sensory nuances underpinning liking. Nevertheless, despite the absence of sensory differences, the odour-infused low-fat milk sample was preferred to the non-infused low-fat one. However, it still fell short of the sensory appeal of full fat milk. These findings indicate that while not fully making up for the lack of fat, adding fatty odours to low fat foods can enhance their hedonic perception via retronasal exposure.

While the added odour influenced liking during sensory evaluation, it did not affect subsequent intake. In fact, none of the preloads (skim, skim+ or full) had a differentiating effect on subsequent ad libitum intake parameters (intake amount or eating rate). The lack of preload effects remained even after removing restrained eaters from the analysis. Appetite ratings further underscore these findings, as preload type did not differentially influence hunger, desire to eat, prospective consumption and fullness throughout the intake experiment. Additionally, consumption times of the preloads themselves remained unaffected by preload type. Our findings resonate with the broader literature on the topic suggesting a lack of substantial impact of retronasal odours on eating behaviour (McCrickerd et al., 2014; Ramaekers, 2014; Ramaekers et al., 2014b; Ruijschop et al., 2010; Ruijschop et al., 2008). However, drawing direct comparisons within the context of fat perception is challenging due to the glaring absence of similar studies specifically focusing on fat-related odours (Pirc et al., 2023b). This emphasizes the pioneering nature of our work and highlights the pressing need for further research in this specific area.

Considering that consumption of the full sample also had no effect on subsequent intake, our observations align with the notion of fat being a poorly sensed nutrient (Blundell & MacDiarmid, 1997; Hulshof et al., 1993; Rolls et al., 1994; Warrilow et al., 2018). However, it must be acknowledged that our experiment was specifically designed to investigate the oro-sensory effects of fat on immediate intake regulation. Given that these effects occur in the early stages of the so-called satiety cascade (Blundell et al., 2010), we employed a relatively short interval (approximately 3 min) between the preload and the subsequent ad-libitum breakfast. This was done to minimise the chance of potential absorptive effects, which usually start occurring approximately 30 minutes after preload ingestion (Blundell et al., 2010; Rolls et al., 1991). This might explain why fat in the full sample had no effect on intake parameters and appetite ratings. An alternative speculation is that participants might have been able to gauge the fat content of the utilised preloads (e.g., via mouthfeel differences), and adjusted subsequent intake accordingly, as demonstrated by (Viskaal-van Dongen et al., 2009). It is also worth considering that if the full sample had a higher fat content, the outcomes might have been different. Future research could benefit from a prolonged experimental design, focusing on the delayed effects of fat consumption. This would allow for the exploration of potential compensatory eating behaviours at subsequent meals, providing a more comprehensive understanding of the long-term impact of fat content on intake regulation.

The lack of notable odour effects on intake could potentially be attributed to release parameters of our odour stimulus. As demonstrated by Ramaekers et al. (2014b), intake following retronasal odour exposure can be influenced by aroma exposure time and concentration. In the current study, we prioritised maintaining an ecologically valid odour delivery, which limited our ability to standardise exposure time. To account for variations in preload exposure, we monitored the duration each participant took to consume each preload. Notably, we were only able to measure the total duration of preload consumption, not exact odour exposure per sip.

Conversely, Ramaekers et al. (2014b) had the advantage of precisely controlling odour exposure time, albeit in a less natural setting - utilizing a more invasive, olfactometer-based method. Similarly, Ruijschop et al. (2008) found that prolonged exposure to retronasal aromas delivered via an olfactometer increased subjective satiation, though this did not translate to changes in subsequent food intake. In the current study though, preload exposure time was found to have no influence on appetite ratings, irrespective of the preload type. Lastly, we cannot rule out the possibility that our methodological choices underlie the discrepancy between subjective liking and behavioural measures in our experiment. By presenting samples dyadically, where they act as each other's references, we might have accentuated certain distinctions affecting liking that would have remained less noticeable in a monadic presentation (such as during the intake experiment).

In addition to our odour delivery approach, ecological validity was also central to the choice of the odour stimulus. Unlike previous studies that primarily utilised artificial aromas (McCrickerd et al., 2014; Ramaekers et al., 2014b; Ruijschop et al., 2010; Ruijschop et al., 2008), we opted to couple actual cream with a complex matrix. This decision was driven by our objective to determine if the mere odour of fat could emulate the sensory experience of actual fat. Given that cream is the primary fat source in dairy milk, it seemed a more authentic choice than artificial aromas. While the exact compounds responsible for the 'smell of fat' in dairy products remain debated (Mu et al., 2022), using cream likely brought us closer to replicating aroma compounds commonly found in dairy milk. Albeit, it must be acknowledged that the utilised cream aroma was relatively subtle compared to odour intensities typically associated with artificial aromas.

Despite the potential shortcomings, we consider our striving for ecological validity as the main strength of the study. Our approach reflects the real-world relevance of using fatty odours to mimic fat content in foods and expands on previous findings conducted in less natural settings (Ramaekers et al., 2014a; Ramaekers et al., 2014b; Ruijschop et al., 2010; Ruijschop et al., 2008). We encourage future studies on the topic to adopt odour delivery techniques similar to the one utilised in the current study. It offers the advantage of delivering aromas covertly, in a manner that closely mirrors natural ingestion, thus ensuring minimal interference with the eating process. While such an approach is tailored for beverages, it is worth noting that there is a plethora of liquid products that can be paired with a diverse range of odours. This provides ample opportunity to further explore the impact of retronasal odours on eating behaviour in a more naturalistic setting. Furthermore, while the comparison of the full sample with an odourised full sample ('full+') was considered beyond the scope of this study, expanding the current setup with the addition of such a comparison might help elucidate potential synergistic effects of fat content and fat-related odours. We also suggest that future studies consider setups other than the fixed preload paradigm we employed, perhaps focusing on the effect of retronasal odours on the beverage being consumed in real-time. This would offer a more immediate understanding of how retronasal odours influence short-term intake. Furthermore, as it is suggested that odours (orthonasal ones in particular) are more relevant when it comes to choice, rather than intake (Boesveldt & de Graaf, 2017), it might be worth employing the odour delivery method described here within a food choice paradigm. An intriguing approach would be to have participants sample the odour-infused beverages before making decisions on corresponding food products to consume or chose. Such a design could provide valuable insights into how retronasal aromas influence food preferences and selections in a real-world context.

5 CONCLUSION

While we show that retronasal fat-related odours can elevate low fat product liking, the broader implications on eating behaviour remain to be fully understood and warrant further investigation. If future research demonstrates the ability of fat-related odours to guide food choice and/or intake, their incorporation as fat substitutes might help preserve the sensory allure foods, without compromising on health. It is therefore imperative for future research to delve deeper into the potential of retronasal odours, not just as sensory enhancers, but as potential tools for fostering healthier food choices and consumption patterns.

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SUPPLEMENTARY MATERIAL A

Table A1. *Dairy milk sample nutritional compositions per 100 g of sample.*

	Nutritional composition (per 100 g)		
Sample	**F (g)**	**CH (g)**	**P (g)**
Skim	0.0	4.5	3.5
Skim+	0.0	4.5	3.5
Full	3.5	4.5	3.5

F = dietary fat; CH = carbohydrates; P = protein

6

Chapter 6

General discussion

THESIS OVERVIEW

This thesis aimed at expanding our understanding of the olfactory system's contribution to the sensory perception of dietary fat. **Chapter 2** established a basis for the experiments described in this thesis by identifying and summarising existing evidence on the contribution of olfaction to fat perception in humans and rodents and highlighting key knowledge gaps. **Chapter 3** therefore explored the human ability of olfactory food fat content discrimination and potential effects of habitual intake on this ability. **Chapter 4** delved deeper into olfactory fat discrimination by focusing on the neural pathways underlying this ability. It examined brain activation in response to olfactory exposure to varying fat levels within a complex food matrix and explored associations between brain activation, olfactory fat content discrimination and perceived odour intensity and liking. Lastly, **chapter 5** sought to integrate findings from previous studies and bridge the gap between sensory perception and eating behaviour. It assessed whether fat-related odours can enhance sensory characteristics of low-fat food products and influence subsequent food intake and appetite.

An overview of main findings, along with underlying aims, methodology and outcomes per chapter is presented in Table 1. Comprehensive discussions on the findings of this thesis can be found in individual chapters (2, 3, 4 and 5). The following section will discuss the broader implications of our findings within the context of the overarching topic of the thesis: the role of olfaction in dietary fat perception.

Table 1. Summary of aims, methodology, outcomes and main findings per thesis chapter.

Aim	Methodology	Main Outcomes	Main findings
Chapter 2			
To systematically identify and summarise relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents and highlight relevant knowledge gaps.	Systematic scoping literature review based on the PRISMA methodology. Three academic databases searched (Scopus, Web of Science, PubMed) via strings related to olfaction, dietary fat, and perception.	42 articles reporting on the contribution of olfaction to fat perception in humans and rodents.	Olfaction contributes to dietary fat perception in both rodents and humans: rodents can perceive dietary fat via olfaction, which can affect their preference for fatty feed; humans can detect, discriminate, and identify fat solely using olfaction, even within complex food matrices. Food fat content influences perception of various fat- and non-fat olfactory qualities, depending on the food matrix and physio-chemical properties of odorants. Fat-related odours can influence the perception of olfactory and non-olfactory perception (e.g. mouthfeel). Identified knowledge gaps include the role of fat-related odours in eating behaviour (food choice, intake), underlying chemical signals and factors governing sensitivity to fat-related odours.
Chapter 3			
To investigate whether humans can discriminate fat content in dairy milk using solely orthonasal and/or retronasal cues and assess whether this ability depends on habitual dairy intake.	Stimuli: Dairy milk samples varying in fat content (0%, 1.5%, 3.5%, 7%, 10.5%, 14%). Dual reminder (DR) A-not A discrimination testing. Perceptual odour intensity, creaminess and liking ratings (100-unit visual analogue scales (VAS)). Dairy food frequency questionnaire (DFFQ).	Orthonasal and retronasal fat content discrimination ability (derived from R-index values). Perceptual ratings of odour intensity, creaminess and liking. Habitual dairy product intake (g/day), total dairy fat intake (g/day) and dairy product consumption frequency (times/day).	Participants could discriminate food fat content using solely orthonasal or retronasal cues. Odours of fat-containing samples were perceived as more intense and more liked than those of non-fat containing samples. Perceived odour creaminess was not affected by fat content. Perceived odour intensity and creaminess did not relate to discrimination ability. Olfactory fat content discrimination was unaffected by habitual dairy intake.

Chapter 4			
To map brain activation in response to olfactory (orthonasal) exposure to varying levels of dietary fat embedded within an ecologically relevant food source. Exploring potential associations between brain activation, olfactory fat content discrimination and perceived odour intensity and liking.	Stimuli: Dairy milk samples varying in fat (0%, 3.5%, 14%). Functional magnetic resonance imaging (fMRI) employing olfactometer orthonasal odour delivery. DR A-not A discrimination testing. Perceptual odour intensity and liking ratings (100-unit VAS).	Brain activation in response to orthonasal odour exposure (derived from blood-oxygen-dependent (BOLD) signal). Orthonasal fat content discrimination ability (derived from R-index values). Perceptual ratings of odour intensity and liking.	Participants could discriminate food fat content using solely orthonasal cues. Perceived liking increased with fat content, while perceived odour intensity only increased between 0% and 3.5% fat. No differential brain activation was observed between fat levels; activation differences occurred only when comparing odour exposure with no exposure (rest) - exposure to any odour activated the anterior part of the supplementary motor area, while deactivating parts of the hippocampus, putamen, superior temporal gyrus, anterior cingulate cortex, insula and posterior part of the supplementary motor area. Exposure to the 0% odour also activated the thalamus. No associations were observed between perceived intensity, liking, and neural responses.
Chapter 5			
To investigate whether sensory characteristics of a low-fat food product (dairy milk) can be enhanced by the addition of a fat-related aroma (cream) and assess the influence of exposure to retronasal fat-related odours on subsequent ad-libitum consumption.	Stimuli: Dairy milk samples varying in fat content and cream aroma: skim (0%, no aroma), skim+ (0%, with cream aroma), and full (3.5%, no aroma). Perceptual ratings of flavour intensity, creamy mouthfeel, aftertaste intensity and liking (100-unit VAS). Fixed preload-based ad-libitum intake experiment (dairy milk preload followed by ad-libitum breakfast). Appetite ratings (100-unit VAS) pre-preload, post-preload, post-breakfast.	Perceptual ratings of flavour intensity, creamy mouthfeel, aftertaste intensity and liking Ratings of hunger, fullness, desire to eat, prospective consumption over time. Intake of ad-libitum breakfast meal.	Skim+ samples were more liked compared to skim ones; there was no difference in flavour intensity, creamy mouthfeel, or aftertaste between skim and skim+ samples; full samples were rated higher than skim and skim+ samples on all attributes. Subsequent intake and appetite ratings did not differ between any of the preloads.

DISCUSSION OF MAIN FINDINGS

Olfactory fat content discrimination

As established in **Chapter 2**, fatty acids are effective odour stimuli for humans. However, most dietary fat in food is not present in the form of isolated free fatty acids, but rather in the form of triglycerides (Lichtenstein et al., 1998), which are usually accompanied by other odorous substances present in the food matrix. Moreover, fatty acids seem to evoke sensations not typically associated with food odours, such as plastic, rubbery, or oily (Chukir et al., 2013), none of which can be considered pleasant and likely to contribute to the sensory appeal of foods. It is therefore imperative to explore olfactory fat perception in ecologically relevant, complex food matrices, typically encountered in everyday life. One of the fundamental questions when it comes to olfactory fat perception is whether odour cues can serve as reliable indicators of food fat content. The notable lack of studies addressing this question in complex food matrices, as evident from the systematic review (**Chapter 2**), was therefore a primary focus of the research presented in **Chapters 3** and **4**.

Chapters 3 and **4** consistently demonstrated the human ability of food fat content discrimination using solely olfactory cues – orthonasal or retronasal. Not only was discrimination possible between non-fat and fat-containing food samples, but also between varying levels of fat, regardless of olfaction route. Our findings align with evidence on olfactory fatty acid discrimination (Bolton & Halpern, 2010; Kallas & Halpern, 2011) and expand upon studies on orthonasal food fat content discrimination (Boesveldt & Lundstrom, 2014; Glumac & Chen, 2020; Mu et al., 2023; Mu et al., 2022). A notable contribution of our work is the demonstration that retronasal cues alone effectively enable fat content discrimination in complex food matrices. Additionally, we confirmed that olfactory fat content discrimination is possible across a relatively broad spectrum of fat levels and minute fat content differences, irrespective of olfaction route.

The ability to distinguish fat content in foods using solely orthonasal cues (**Chapters 3** and **4**) is consistent with the theory that orthonasal olfaction detects environmental food sources (Boesveldt & de Graaf, 2017). It also implies that fat-related orthonasal cues might act as indirect indicators of food energy content, which would have been advantageous in ancestral environments where food availability fluctuated, as suggested by de Vries et al. (2020). Moreover, since retronasal odours are considered to play a role in the consummatory phase of eating by potentially influencing intake (Boesveldt & de Graaf, 2017), the ability to retronasally discriminate food fat content (**Chapter 3**) might help reinforce the choice and intake of fat-containing food sources, possibly via food reward-related mechanisms (as illustrated by Sclafani (2004)) - this rationale also underpinned the study described in **Chapter 5**. Ultimately, the role of retronasal olfaction in fat perception potentially extends to facilitating associative learning processes (e.g., flavour-nutrient or flavour-flavour learning) (Havermans & Jansen, 2007; Prescott, 2012; Yeomans, 2012), thus playing a role in promoting the ingestion of nutrients. For example, the pleasant sensations elicited by fat-related odours might help promote the ingestion of fat-containing foods.

Our findings underscore the role of olfactory cues in fat flavour perception and raise questions about factors underpinning olfactory fat discrimination abilities. Notably, in the experiments described in **Chapter 3**, we observed a striking variation in discrimination abilities among participants. While some were unable to discriminate at all, others could do so with remarkable ease. Moreover, when asked which perceptual differences informed their answers during discrimination testing, participants often used mouthfeel-related terms such as thickness, heaviness, and creaminess to describe sensations stemming solely from olfactory exposure. This anecdotal evidence not only raises questions about the perceptual differences that drive such discrimination but also warrants exploration of broader factors that might influence individuals' sensitivity to these differences.

Factors Underpinning Olfactory Fat Content Discrimination

Olfactory discrimination is influenced by a variety of factors including cognitive abilities (Hedner et al., 2010), odour intensity (Duchamp-Viret et al., 1990), odour quality (Ravia et al., 2020), as well as memory and past exposure to the odorants (Wilson & Stevenson, 2003). Genetic variations may influence odour perception as well, potentially influencing discrimination abilities of odorants in specific subsets of the population (Logan, 2014). As highlighted in **Chapter 2**, factors governing olfactory fat perception (including discrimination) remain a major knowledge gap.

Potential influences of habitual exposure to fatty odorant sources on olfactory fat content discrimination were therefore investigated in **Chapter 3**. We found that long-term habitual dairy intake parameters had no effect on dairy milk fat content discrimination. This observation aligns with Boesveldt and Lundstrom (2014) and Mu et al. (2022), both reporting no effects of habitual dairy consumption on olfactory fat content discrimination ability in dairy milk. Additionally, as described in **Chapter 3**, no learning effects were observed throughout discrimination testing. In other words, discrimination ability did not improve with repeated exposure to the utilised dairy milk samples in our experiments. The consistency of these findings suggests that olfactory fat discrimination is independent of long-term or short-term past exposure, and aligns with the notion of evolutionary relevance. However, further research on diverse food matrices in different populations are needed for concrete conclusions. The need for further investigation is underscored by Kindleysides et al. (2017), who, in contrast to our results, found a link between the intake of non-dairy fatty foods (i.e., nuts, nut spreads and seeds) and increased oleic acid sensitivity. Similarly, there is conflicting evidence on the influence of habitual consumption on oral fat perception. While most research supports a dietary influence on fat taste perception, there are exceptions (Chmurzynska et al., 2020), and the directionality of effects remains unclear (Running & Mattes, 2016). Additionally, fat taste discrimination has been linked to genetic factors, while fat taste sensitivity appears to be independent of BMI (Chmurzynska et al., 2020; Running & Mattes, 2016).

Despite the robustness of our findings on olfactory fat discrimination, perceptual factors underpinning this ability remain unclarified. Although fat-containing samples were generally perceived as having a more intense odour than non-fat ones in our studies, odour intensity differences were not found to be pivotal to discrimination for both olfaction routes. To illustrate, all fat level comparisons could be orthonasally discriminated in **Chapters 3** and **4**, yet odour intensity differences were observed only between some of the comparisons. Likewise, despite participants being able to retronasally discriminate between two of the six fat level comparisons in **Chapter 3**, no differences in odour intensity were observed between any of the utilised fat levels. Our findings resonate with those of Mu et al. (2022), who employed dairy milk samples with fat levels comparable to ours and found no differences in odour intensity despite participants being able to discriminate using solely orthonasal cues. Boesveldt and Lundstrom (2014) also reported a disconnect between perceived intensity and fat content discrimination in dairy milk samples varying in fat. Intensity not underpinning olfactory fat discrimination in our samples was further corroborated by our neuroimaging findings, as discussed in **Chapter 4**. Specifically, although participants were able to discriminate between all utilised fat levels (0%, 3.5% and 14%), with fat level influencing perceived odour intensity, no associations were observed between perceived intensity and neural responses. Taken together, our findings suggest that odour quality rather than intensity differences likely underpin fat content discrimination ability in certain food matrices, such as dairy milk. Discrimination might for example hinge on differences in terms of perceived fatty, dairy, creamy, sweet, rich, or buttery odour. This line of thinking is supported not only by Gross-Isseroff and Lancet (1988) and Le Berre et al. (2008), both demonstrating that even minute odorant concentration changes cause perceptual odour quality shifts, but also by Mu et al. (2022) and Mu et al. (2023), who identified headspace composition differences likely responsible for odour quality nuances underpinning olfactory fat discrimination in dairy milk and meat, respectively. However, it is important to note that exploration of the influence of odour quality differences on olfactory discrimination was limited in our studies. We only assessed perceived odour creaminess in the second of two experiments described in **Chapter 3**. It was incorporated as a descriptor after numerous participants from the initial experiment indicated that their decisions during discrimination testing were influenced by perceived variations in odour creaminess between the samples. Ultimately, odour creaminess was found not to influence retronasal fat discrimination in our samples. Future research should therefore aim to identify relevant descriptors for fat-related odour qualities and assess how they relate to olfactory fat discrimination.

Effects of fat-related odours on hedonics and intake

Sensory properties of foods influence palatability, which is defined as the positive hedonic evaluation of a food's sensory characteristics (Yeomans, 1998) and considered a key driver of food intake. Higher palatability enhances the likelihood of selecting a specific food and can translate into higher energy intake (Blundell et al., 2010; Forde & de Graaf, 2022; McCrickerd & Forde, 2016). Nevertheless, it must be considered that palatability is merely one among a myriad of complex factors influencing food choice and intake (de Graaf et al., 2004; Forde & de Graaf, 2022). Given that olfaction, particularly retronasal olfaction, is a crucial component of flavour perception and thus contributes to food palatability, it is necessary to investigate the specific impact of fat-related odours on hedonics and food intake. This relationship remains a glaring knowledge gap, as identified in **Chapter 2**. Addressing it could contribute to a more comprehensive understanding of dietary choices and eating behaviour. As such it might provide insights into strategies for modulating food intake, especially in the context of fat consumption.

All experimental studies in this thesis (**Chapters 3**, **4** and **5**) included a measure of odour hedonics (i.e., perceived odour liking). They demonstrated that odour liking tends to increase with increasing fat levels in dairy milk (**Chapters 3**, **4**). This contrasts with other studies utilising dairy milk as a fat-related odour source. Specifically, Mu et al. (2022) observed no effect of fat content on liking, while Boesveldt and Lundstrom (2014) reported a decrease in liking with increasing fat content in one of their experiments, but not in the other two. Discrepancies between findings might stem from varying sample preparation methods. Our approach, using skim milk as a base and manipulating fat using cream, standardised nutrients except for fat. In contrast, Mu et al. (2022) used commercial milks, while Boesveldt and Lundstrom (2014) opted for water and milk powder mixtures. Such approaches might have introduced variations in nutrients and milk processing by-products, potentially obscuring fat-specific sensory characteristics that influence odour liking.

Considering increases in perceived liking for higher fat levels in experiments of **Chapter 3**, we anticipated that exposure to higher fat levels would correspondingly lead to activation in reward processing-related brain regions, as suggested by Grabenhorst et al. (2010) and explored in **Chapter 4**. This, however, was not the case. Perceived liking differences between the utilised odour sources varying in fat could not be linked to specific relevant neural responses. The discrepancy made us question whether observed subjective liking for higher fat odours translates into eating behaviour.

In **Chapter 5**, we therefore coupled a retronasal natural cream aroma with low-fat dairy milk and explored whether its addition influences sensory and hedonic perception and subsequent intake. We confirmed that the fatty odour addition enhanced liking of low-fat dairy milk without altering the evaluated sensory characteristics (flavour intensity, creamy mouthfeel, aftertaste). Odourising low-fat milk (0% fat) with a fatty aroma therefore made it more appealing than non-odourised low-fat milk, but it was still less liked than non-odourised full fat milk (3.5% fat). This odour-induced shift in liking, however, did not translate into intake behaviour. Specifically, consuming a preload of odourised low-fat milk had no effect on subsequent ad-libitum intake parameters and subjective appetite ratings, however, neither did consuming a preload of non-odourised low-fat or full-fat milk. Lastly, there were no differences observed in consumption rates of the odourised and non-odourised preloads themselves. This reinforces the notion that fat is a poorly sensed nutrient (Rolls et al., 1994), potentially leading to passive overconsumption of dietary energy (Cotton et al., 1994; Rolls et al., 1994), which seems to hold true even when foods varying in fat are standardised for texture and composition of other macronutrients (Rolls et al., 1992).

Our observations highlight the role of odours in determining the explicit hedonic value of fatty foods. We consistently demonstrated that exposure to fat-related odours resulted in increased liking (**Chapters 3, 4** and **5**). Nevertheless, the relevance of this effect remains debatable in the context of real-world consumption occasions. Moreover, not only are subjective ratings unreliable predictors of eating behaviour (Giacalone et al., 2022), observed effects also appear too subtle to induce meaningful behavioural alterations, at least in the short-term, as demonstrated in **Chapter 5**. Our findings therefore cast doubt on the potential of fat-related odours to exert meaningful effects on eating behaviour.

METHODOLOGICAL CONSIDERATIONS

This thesis represents a multidisciplinary exploration of the fundamental aspects of olfactory fat perception. It is based on findings of a systematic scoping review, various sensory experiments, a neuroimaging task, and a behavioural study. All experiments utilized a cross-over design, allowing participants to serve as their own controls, effectively accounting for individual differences that could influence the measured outcomes. Additionally, where relevant, all experimental conditions were randomized and counterbalanced to minimize the potential for order effects or familiarization.

A variety of methodological approaches were utilised. The systematic scoping review in **Chapter 2** was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses protocol (PRISMA), which is considered the gold standard for conducting such reviews (Page et al., 2021). Fat content discrimination testing in **Chapters 3 and 4** revolved around the dual reminder (DR) A-not A methodology (Hautus et al., 2018; Mun et al., 2019) with a familiarisation procedure. Not only does the A-not A test outperform other discrimination, such as the triangle test (Bi & Ennis, 2001; Lee et al., 2007a; Mun et al., 2019), the implementation of the familiarisation procedure also help stabilise participants' cognitive decision criteria, thereby improving the consistency of results (Lee et al., 2007b). Moreover, discrimination testing performance was analysed using the so-called R-index, which is a signal detection measure free of response bias or criterion variation (Lee & van Hout, 2009). It provides a nuanced measure of the degree of difference between two samples, offering more depth than a simple binary assessment of discrimination ability. In addition to discrimination testing, **Chapter 4** included functional magnetic resonance imaging (fMRI) to map neural correlates of olfactory fat perception. This technique is one of the most reliable, non-invasive and widely-used methods for studying neural underpinnings in nutrition research (Francis & Eldeghaidy, 2014). Lastly, **Chapter 5** utilised a combination of sensory evaluation and behavioural experiments. The latter was based on a fixed preload intake paradigm striving for ecological validity. Preload-based designs are one of the benchmark approaches for studying short-term regulation of food intake. They are considered to have high external validity, although are prone towards type 2 errors, requiring large sample sizes and sensitive measures (Blundell et al., 2010). It must be noted that our approach, which focused on the effects of exposure to fat-related aromas on subsequent intake, involved altering both energy density (skim and full-fat milk) and sensory properties (non-odourised and odourised samples). This deviates from the typical preload paradigm, which usually involves varying energy or macronutrients while keeping sensory properties constant.

Study population

Our experiments (**Chapters 3, 4, 5**) employed healthy, non-smoking, normal weight young adult men and women of various nationalities. All were self-reported regular dairy consumers, ensuring that unfamiliarity or dislike of the utilised food odour sources did not affect outcomes. During recruitment, we paid close attention to factors which might influence olfactory perception, such as age (Doty & Kamath, 2014), BMI (Peng et al., 2019), pregnancy (Albaugh et al., 2022) and smoking (Ajmani et al., 2017). Additional factors which could affect odour perception and other outcome measures within the context of neuroimaging were considered in **Chapter**

4. These included handedness (Hummel et al., 1998; Royet et al., 2003), the presence of psychiatric or neurological disorders (Keren et al., 2018; Stopyra et al., 2019; Weygandt et al., 2012), claustrophobia, non-removable ferromagnetic implants or piercings, and epilepsy. Prior to inclusion, each participant also underwent smell function assessment, using the Sniffin' Sticks 16-item odour identification test, a widely used and validated tool (Hummel et al., 2007), to ensure they were normosmic. Additionally, at the beginning of each testing session, we assessed whether participants were experiencing any transient conditions that could potentially affect their olfactory performance, such as a blocked nose or a cold.

Although the precautions helped minimise variation and increased the internal validity of our research, they resulted in relatively homogeneous participant groups. This limits the generalisability of our results to other segments of the general population, such as overweight, elderly or people who do not consume dairy products.

Stimuli

Dairy milk was chosen as an odour stimulus in all experiments (**Chapters 3, 4, and 5**) for several reasons. First, dairy milk is an ecologically-relevant fat-related odour source, commonly employed in studies investigating olfactory fat perception (Boesveldt & Lundstrom, 2014; Bult et al., 2007; Chen & Eaton, 2012; Frøst et al., 2001; Le Calvé et al., 2015; Mela, 1988; Miettinen et al., 2004; Miettinen et al., 2003; Roberts et al., 2003a; Zhou et al., 2016), as identified in **Chapter 2**. Utilising dairy milk therefore allowed for direct comparison of our findings with various other studies on the matter. Secondly, being a liquid, it facilitates odorant release from the food matrix more effectively than semi-solid or solid alternatives, as highlighted by Seuvre et al. (2000). This makes it a perfect candidate for examining the concepts outlined by the current thesis. Lastly, manipulating the fat content of dairy milk while keeping other macronutrients constant is a straightforward process. By adding cream, a dairy milk derivative containing all the fats typically present in milk (Deosarkar et al., 2016), we could easily adjust the fat content while preserving the characteristic fat-related volatile profile typical of the product. To ensure consistency and comparability of findings across the thesis chapters, we meticulously aimed at minimising variation between dairy milk samples within and across experiments. To achieve this, we implemented several measures:

- **Macronutrient consistency:** We aimed to only vary fat content of the samples, while keeping other macronutrients as constant as possible. This was essential to ensure that any odour differences were attributable to fat and no other nutrients.
- **Production practices:** To minimise the impact of varying production practices on the sensory profiles of the utilized ingredients, we sourced dairy milk and cream from the same production facility, as indicated by their European Commission (EC) approval numbers.
- **Seasonality effects:** We selected dairy milk and cream products with similar best before dates within our studies. This helped standardise seasonal influences on dairy milk quality, which can be affected by environmental factors such as cow feed and environmental temperature (Ehtesham et al., 2015).
- **Sample freshness:** All samples were prepared fresh and kept in closed containers until presentation, to prevent oxidation-induced sensory profile alterations due to air or light exposure (Brothersen et al., 2016; Mestdagh et al., 2005).
- **Temperature control:** Sample serving temperatures were standardized to 20-22°C to minimize sensory alterations induced by thermal variation (Cardello & Maller, 1982; Ryynänen et al., 2001). Notably, requirements of olfactometer-based odour delivery (utilised in **Chapter 4**) necessitated a serving temperature of 37°C.

Stimulus delivery

We adopted varied approaches to odour delivery in our studies, but as we were mainly interested in the individual contribution of olfaction to fat perception, our approaches aimed at minimising sensory inputs from potentially confounding modalities involved in fat perception (taste, mouthfeel).

As part of discrimination experiments described in **Chapter 3**, we devised odorant delivery containers (adapted from (Bolton & Halpern, 2010) that enabled us to completely isolate olfactory inputs (ortho- and retronasal) from taste, mouthfeel, and vision. These containers served as means of odour delivery during discrimination testing in **Chapter 4** as well. To ensure consistent odour delivery, participants received training on how to handle the odour delivery containers. They were instructed to handle the containers gently to minimize the release of volatiles from the sample matrix into the headspace due to movement. Additionally, participants were advised not to lift the containers off the table surface and to produce uniform, moderately intense inhalations across samples. While our odour delivery method effectively isolated olfaction from other modalities (as described in **Chapters 3 and 4**), it is important to note that it does not reflect the typical retronasal odorant path experienced during food intake. Normally, food-related odorants from the mouth are directed through the nasopharynx to the olfactory mucosa. However, in our setup, orally inhaled odours in the absence of food tend to pass to the lungs before reaching the olfactory epithelium, potentially altering the odour mixture due to lung retention (Verhagen, 2015). Despite this limitation, the odorant intensities in our experiments were adequate for subjects to detect and discriminate, suggesting a potentially more pronounced effect in regular consumption scenarios.

The fMRI experiment in **Chapter 4** utilized an olfactometer for precise control of odour delivery, while the experiment in **Chapter 5** employed specially designed bottles enabling us to covertly manipulate the presence of a fat-related odour during food intake. Although these methods, along with the odour delivery containers used in **Chapters 3 and 4**, enabled a relatively high level of control, they inherently lack the dynamic interplay of sensory inputs present during typical eating occasions, where food matrices are experienced as a whole. Considering that fat perception involves multiple sensory modalities, the impact of odours during regular eating situations might be either enhanced or diminished, depending on the complex interaction with other sensory inputs and food matrices in question.

IMPLICATIONS AND FUTURE RESEARCH DIRECTIONS

This thesis offers a new perspective on the understanding of how fat is perceived via the olfactory system. Our findings show that olfactory cues facilitate fat content perception, with fat-related odours affecting consumer liking. Given that sensory cues generally have an influence on food choice and intake (Forde & de Graaf, 2022; McCrickerd & Forde, 2016), insights from this thesis could have potential implications for food reformulation and public health. As recently proposed by Forde and de Graaf (2022), there is a need to explore the general potential of odour cues in stimulating consumer appeal and reinforcing the positive aspects of healthy food choice and intake.

Perceptual and hedonic effects reported in this thesis, however, did not translate into tangible behavioural consequences, which are arguably the key measure when considering the use of odours in food reformulation. Nevertheless, absence of a direct link to eating behaviour in our (single) study should not lead us to hastily dismiss the potential behavioural effects of fat-related odours - our investigation represents just one piece of a much larger and immensely complex puzzle.

The broader research landscape, as reviewed by Boesveldt and de Graaf (2017) suggests that the overall influence of food odours on food choice and eating behaviour is limited, with most studies focusing on the effects of orthonasal olfaction. These studies often find that while ambient food odours can stimulate specific food appetites, this rarely translates into food choice or eating behaviour alterations. Yet, it is necessary to recognize that the general influence of retronasal odours on these aspects has been studied to a considerably lesser extent. Although the handful of studies on the topic show minimal effects of retronasal food odours on eating behaviour (McCrickerd et al., 2014; Ramaekers, 2014; Ramaekers et al., 2014; Ruijschop et al., 2010; Ruijschop et al., 2008), one cannot ignore their limitations. Most employed invasive procedures not reflective of real -life situations and were carried out in a relatively narrow range of foods, using non-fat-related aromas. In fact, to our knowledge, apart from **Chapter 5**, no other study investigated the specific impact of fat-related retronasal odours on eating behaviour. Therefore, future research should aim to investigate both the immediate and the long-term effects of retronasal olfactory cues to conclusively determine their role, or lack thereof, in shaping dietary choices and intake patterns. Accordingly, should future research confirm that fat-related olfactory cues, particularly retronasal ones, have the capacity to influence food hedonics, appetite, steer food choice and/or eating behaviour, the prospect of using fat-related odours as sensory optimisers in various foods, might emerge as a viable option. Fat-related odours could then potentially be applied to enhance the sensory appeal and acceptance of low-fat foods and plant-based alternatives to animal foods (e.g., dairy, meat) - either on their own, or in combination with modulations related to other sensory inputs (e.g. taste, texture, trigeminal). Such approaches could promote health by facilitating the development of appealing, healthy foods while simultaneously fostering environmental well-being through reduced reliance on animal-derived foods.

Whether fat-related olfactory cues and their potential applications can ultimately bring us a small step closer towards tackling fat overconsumption and other diet-related pressing societal issues, largely depends on further exploration. As outlined in **Chapters 2-5**, there are several areas warranting consideration. In addition to exploring the yet untapped research possibilities related to the behavioural consequences of retronasal fat-related odours, the following areas appear worthy of attention:

- **Effects of various food matrices on olfactory fat perception:** It is imperative to explore olfactory fat perception – encompassing fat content discrimination ability, perceptual attributes, and effects of fat on odour liking – across a broad spectrum of food products. This includes, but is not limited to, dairy products, meat, fish, oils, and plant-based butters and drinks. Understanding how variations in fat content and distinct processing practices influence olfactory fat perception within these categories is also essential. Such investigations would facilitate establishing a comprehensive knowledge base, helping us identify foods where olfaction plays a relevant role in fat perception.
- **Chemical signals underlying olfactory fat perception:** As discussed in **Chapter 3**, triglycerides, which are the most prevalent form of dietary fat, are non-volatile and thus unlikely to be effective odour stimuli. Nevertheless, they are notorious for being carriers of various other odorous compounds (McSweeney & Sousa, 2000), which highlights the necessity of identifying the compounds indirectly signalling fat content and examining their impact on sensory perception within different food matrices. Yet, the complexity of factors affecting the composition and release of fat-related odorants within different food matrices presents considerable challenges (Roberts et al., 2003b). Although characterisation of volatiles underpinning olfactory fat perception has been carried out before (see Mu et al. (2022) and Mu et al. (2023)), comprehensive exploration in various foods is required to broaden our fundamental understanding of the field and facilitate product reformulation.
- **Neural mechanisms underlying olfactory fat perception:** While we attempted to map the neural underpinnings of olfactory fat perception, conclusive findings remain elusive. Future research should therefore not only aim to replicate the experiment described in **Chapter 4**, but also pivot towards exploring the neural processing of retronasal fat-related odours – which are arguably more relevant when it comes to flavour perception. Studies on the topic could further delineate how hunger state, genetic predispositions, and dietary exposure influence neural processing of fat-related olfactory inputs.
- **Determinants of olfactory fat perception:** Assessment of the determinants influencing olfactory fat perception is warranted to both mitigate individual variability that may obscure research outcomes and to identify the factors that underlie sensitivity to fat-related odours, which may subsequently impact

behaviour. Specifically, it is unclear how sex, BMI, genetics, and dietary exposure modulate olfactory fat perception. While some studies, including **Chapter 3**, present conflicting evidence on the impact of dietary exposure on olfactory fat perception (Boesveldt & Lundstrom, 2014; Kindleysides et al., 2017; Mu et al., 2022) there is a clear need for further research. Future investigations should broaden their scope to assess the effects of overall dietary fat exposure rather than focusing on a narrow selection of food sources.

If it turns out that the impact of fat-related odour cues is inconsequential in terms of food choice and eating behaviour, olfactory inputs still interact with other relevant sensory cues comprising flavour perception. After all, food is usually not perceived through a single sensory modality. Therefore, in addition to the research gaps proposed above, it is imperative to also move beyond olfaction. As highlighted in chapter 2, fat-related odours tend to influence the perception of non-olfactory food qualities (e.g., mouthfeel), while a growing body of evidence underscores the existence of odour-taste synergies (Ai & Han, 2022). The interplay between olfactory cues and others involved in fat perception, across various food matrices, however, remains largely unexplored. Future research should therefore aim to elucidate these relationships, as they might uncover valuable insights for food product reformulation.

It must be acknowledged that fat perception is still largely influenced by textural properties of fat, which can have a profound effect on eating behaviour (Appleton et al., 2021; Forde & Bolhuis, 2022). Leveraging textural properties of foods could prove more effective in addressing fat overconsumption compared to modifying odours. For instance, creating low-fat products that emulate the texture of higher-fat versions (which tend to be thicker and denser) could prove as a promising approach for reducing fat consumption while preserving the desirable sensory attributes associated with fat. In this context, odours could serve as an additional enhancement, further elevating the appeal of these products. Notwithstanding, modulations of gustatory and trigeminal aspects should also be considered in comprehensive strategies aimed at tackling fat overconsumption. In fact, future studies should aim to integrate findings from research on all relevant modalities involved in fat perception into a common, standardised sensory database. Such a database might enable the development of machine-learning models adept at analysing a broad spectrum of effects and intricate interactions among relevant sensory inputs, hedonic outcomes, and the underlying chemical and rheological signals. Ultimately, these models could be instrumental in pinpointing key factors in fat perception across different foods and consumer subgroups, thereby informing product-specific reformulation strategies aimed at reducing fat content while retaining enjoyable sensory characteristics.

CONCLUSION

This thesis explored the role of olfaction in the perception of dietary fat in complex food matrices. We found that humans can discriminate food fat content using solely orthonasal or retronasal olfactory cues. This ability does not appear to be facilitated by related odour intensity differences, nor was it reflected in underlying brain activation. Moreover, olfactory fat content discrimination seems to be independent of past exposure to dietary fat. Lastly, fat-related odours were found to impact food liking, but our results do not support their effect on eating behaviour.

Although these findings underscore the individual contribution of olfaction to fat perception, odours are just one component of the synergistic interplay that culminates in the complex experience of flavour. Accordingly, a comprehensive understanding of fat perception demands a multidisciplinary approach, encompassing not only relevant sensory modalities and their underlying mechanisms, but also post-ingestive and post-absorptive physiological signals associated with dietary fat, along with contextual factors.

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Summary

Obesity is driven by the overconsumption of high-calorie foods, particularly those rich in dietary fat, which poses a significant public health challenge. It is well established that dietary fat contributes to the palatability and energy density of foods. However, it is less clear how sensory characteristics of fat influence food choice and eating behaviour. The role of olfaction in this context is a particularly underexplored area. This thesis therefore explores the contribution of olfaction to the perception of dietary fat, factors underpinning olfactory fat perception, along with olfaction's role in food hedonics and eating behaviour.

In **Chapter 2**, we systematically identify and summarise relevant evidence on the contribution of olfaction to dietary fat perception in humans and rodents and highlight relevant knowledge gaps. Our systematic scoping PRISMA-based literature review yielded evidence showing that rodents can perceive dietary fat via olfaction, which might affect their preference for fatty feed. It also confirmed that humans can detect, discriminate, and identify fat solely using olfaction, even within complex food matrices. Food fat content can modulate the perception of various fat- and non-fat olfactory qualities. Conversely, the presence of fat-related odours can modify the perception of olfactory and non-olfactory sensory qualities, such as mouthfeel. Findings underscored the need for further research on the role of fat-related odours in eating behaviour (e.g., food choice, intake), chemical signals underlying olfactory fat perception and factors governing sensitivity to fat-related odours.

Chapter 3 investigated the human ability to discriminate fat content in complex food matrices and effects of habitual exposure on this ability. Following a series of sensory experiments revolving around the dual reminder A-not A discrimination testing methodology coupled with perceptual ratings and food frequency questionnaires, we confirmed that odour cues in isolation are sufficient for fat content discrimination in dairy milk samples spanning a wide range of fat. Specifically, participants could discriminate between non-fat and fat-containing samples, as well as samples with varying degrees of fat, using either orthonasal or retronasal cues. Odours of fat-containing samples were perceived as more intense and more liked than those of non-fat containing samples. Perceived odour intensity and creaminess were found not to underpin discrimination ability, and neither did habitual intake of dairy.

Factors underpinning olfactory fat perception were further explored in **Chapter 4**, where we employed fMRI to map brain activation in response to orthonasal exposure

to varying levels of dietary fat embedded within dairy milk and explore associations between brain activation, olfactory fat content discrimination and perceived odour intensity and liking. We confirmed findings on orthonasal fat content discrimination from **Chapter 3**, showing that orthonasal cues in isolation are sufficient to allow for fat content discrimination in a complex food matrix. Nevertheless, we observed no, differential brain activation resulting from olfactory exposure to varying fat levels. Activation differences occurred only when comparing odour exposure with no exposure. Moreover, despite fat content influencing perceived odour intensity and liking, no associations were observed between perceived intensity, liking, and neural responses.

The final experiment, described in **Chapter 5**, integrated findings from previous chapters by investigating whether the addition of a fat-related odour (cream) can enhance sensory characteristics of a low-fat food product (dairy milk) and assessing the potential influence of exposure to retronasal fat-related odours on subsequent ad-libitum consumption and appetite. Using a combination of sensory and preload-based ad-libitum intake experiments, we observed that the addition of a fat-related (retronasal) odour enhanced liking of the low-fat food product without affecting other sensory attributes (flavour intensity, aftertaste, creamy mouthfeel). Retronasal exposure to a fat-related odour was also found not to affect subsequent intake or subjective appetite.

In conclusion, this thesis advances our understanding of dietary fat perception through the lens of olfaction. It shows that olfactory cues serve as indicators of fat content in foods, with fat-related odours affecting consumer liking. This however was not found to affect eating behaviour. Although our findings underscore the role olfaction plays in fat perception, more studies are needed to assess whether fat-related odours can steer food choice and intake.

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Wageningen’s very first MINI drivers club. You are one of the most honest, trustworthy, curious, and playful people I have ever met. Also, your signature interjections (”ayoooo”) always bring a smile to my face. **Ina**, my female counterpart, fellow overthinker and one of the few people who hate awkward silences more than me – unless there is food on the table, then there should absolutely be silence. You are the author of one of the best presentation introductions I have ever witnessed and one of the most courageous people I know. **Max**, my brother in fat. I could not have wished for better colleague to share a project with. Your hilarious anecdotes never ceased to amaze me, and I only wish I could get out of awkward situations as effortlessly as you do. You also know how to make a mean hot pot. I am absolutely honoured to have you as my paranymph. **Parvaneh**, your creativity and selflessness know no bounds. You are a true hedonist and a walking, talking flavour lexicon, never hesitating to experiment with even the most exotic food ingredient combinations - somehow, though, I always end up as the taste tester for your mint pairings. You were part of some of my most joyful moments, and I know we will share many more.

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About the author

Matjaž Pirc was born on September 15, 1989, in Ljubljana, Slovenia, and spent his early years in the scenic town of Kamnik, nestled at the foothills of the Alps. His childhood weight struggles spurred a keen interest in nutrition and its impact on health. After high school, he spent two years as a part-time accountant before enrolling in the Dietetics-Nutritional Counselling programme at the Faculty of Health Sciences (Izola, Slovenia). He graduated in 2013 and proceeded with the master's programme in Dietetics & Clinical Nutrition at the same faculty. Despite completing all coursework, he never completed the programme, choosing instead to pursue an opportunity more closely aligned with his aspirations – a MSc programme in Nutrition & Health at Wageningen University (the Netherlands).

During his MSc studies, he devised a novel method of measuring eating motivation dynamics and examined the impact of kokumi compounds on sensory and hedonic perception of food. He also assisted with the master-level 'Psychobiology of Food Choice and Eating Behaviour' course and an online 'Principles of Sensory Science' course. Immediately after obtaining his MSc degree in 2019, he embarked on a PhD journey at the Division of Human Nutrition and Health within the Sensory Science and Eating Behaviour department of Wageningen University.

His PhD project explored the contribution of olfaction to dietary fat perception, blending his affinity for challenges requiring creative approaches and his enthusiasm for scientific exploration. Throughout his PhD trajectory, Matjaž assisted with several MSc-level courses, supervised numerous BSc and MSc students, and participated in various extra-curricular activities. He served as a member of the VLAG graduate school PhD Committee monitoring group; assisted with the execution of the Global Consortium for Chemosensory Research (GCCR) global study on the effects of COVID-19 on smell and taste; acted as a scientific consultant for a start-up food company and was member of the quarterly divisional PhD newsletter editorial board.

Upon completing his PhD trajectory, Matjaž assumed a new role as a Sensory and Consumer Science Manager, applying his expertise in a dynamic industry setting. He remains dedicated to enhancing health and well-being, while aiding the global shift towards sustainable yet palatable food choices. You can reach him via email: matjaz.pirc@gmail.com

List Of Publications

- **Pirc M.**, Mu S., Frissen G., Stieger M., S., Boesveldt S. (2023). Smells like fat: A systematic scoping review on the contribution of olfaction to fat perception in humans and rodents. *Food Quality and Preference*, 104847.
- **Pirc, M.**, Maas, P., De Graaf, K., Lee, H. S., & Boesveldt, S. (2022). Humans possess the ability to discriminate food fat content solely based on retronasal olfaction. *Food Quality and Preference*, *96*, 104449.
- **Pirc, M.**, Čad, E. M., Jager, G., & Smeets, P. A. (2019). Grab to eat! Eating motivation dynamics measured by effort exertion depend on hunger state. *Food Quality and Preference*, *78*, 103741.

Expected Publications

- **Pirc M.**, Krale C., Smeets P.A., Boesveldt S. Neural Correlates of Olfactory Fat Perception.
- **Pirc M.**, Joosten L., Pietersma K.F., Hageman C., Bolhuis D., Boesveldt S. Retronasal Fatty Odours Product Limited Effects on Food Perception and Intake.

Awards

GIRACT European Flavour Research Award for 1st Year PhD Students (2019)

Media Interviews

Resource (2021, Issue 7). Een neus voor vet eten. https://www.resource-online.nl/app/uploads/2021/12/NL_1-32p_resource_1607.pdf

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline-specific courses and activities

Name	Organiser and location	Year
SSIB 27th Annual Meeting - Poster Presentation	Society for the Study of Ingestive Behaviour, Utrecht, NL	2019
AChemS 41st Annual Meeting	Association for Chemoreception Sciences, Bonita Springs, US (FL)	2019
Summer School on Human Olfaction	Smell & Taste Clinic, Dept. of Otorhinolaryngology, University of Dresden Medical, Dresden, DE	2019
Women in Olfactory Science (WIOS) 2nd Conference	WIOS, Wageningen, NL	2019
Eurosense 2020: 9th European Conference on Sensory and Consumer Research – Poster Presentation	Elsevier, Rotterdam, NL	2020
Weurman 16th Flavour Research Symposium 2020 – Poster Presentation	Dr. Elisabeth Guichard & Dr. Jean-Luc Le Quere, Dijon, FR	2020
Sensory Perception & Food Preference: Into the Future! Course	VLAG, Wageningen, NL	2021
AChemS 43rd Annual Meeting – Poster Presentation	Association for Chemoreception Sciences, Bonita Springs, US (FL)	2022
AChemS 44th Annual Meeting – Poster Presentation	Association for Chemoreception Sciences, Bonita Springs, US (FL)	2023
ECRO XXXIII Meeting – Symposium Organiser	European Chemoreception Research Organisation, Nijmegen, NL	2023

General courses

Name	Organiser and location	Year
Introduction to R	VLAG, Wageningen, NL	2020
Philosophy and Ethics of Food Science and Technology	WGS, Wageningen, NL	2021
Scientific Artwork, Data visualisation and Infographics with Adobe Illustrator	WGS, Wageningen, NL	2021
Supervising BSc and MSc students	Educational Staff Development (WUR), Wageningen, NL	2021
Career Perspectives	WGS, Wageningen, NL	2022
European Nutritional Leadership Programme	European Nutrition Leadership Platform (ENLP), Doorn, NL	2023

Assistance with teaching and supervision activities

Name	Organiser and location	Year
Psychobiology of Food Choice and Eating Behaviour (HNH-30306)	WUR, Wageningen, NL	2019-2022
Principles of Sensory Science (HNH-30506)	WUR, Wageningen, NL	2019-2022
Nutritional Neurosciences (HNH-51306)	WUR, Wageningen, NL	2023
Student Supervision (2 BSc, 7 MSc)	WUR, Wageningen, NL	2019-2023

Other activities

Name	Organiser and location	Year
PhD Proposal Writing	VLAG, Wageningen, NL	2019
Human Nutrition and Health Department Meetings	WUR, Wagenignen, NL	2019-2023
Newtrition (Quarterly PhD Newsletter) Editorial Board Member	WUR, Wageningen, NL	2019-2022
GCCR COVID-19 Questionnaire Implementation	Global Consortium for Chemosensory Research (GCCR)	2020
PhD Tour 2023 to Switzerland and Italy	WUR, Wagenignen, NL	2023
VLAG PhD Council HNH Representative (Monitoring Group)	VLAG, Wageningen, NL	2022-2023

COLOPHON

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