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#### Review

## Fat taste in humans: Sources of within- and between-subject variability



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#### ABSTRACT

Non-esterified fatty acids (NEFA) are reportedly detectable through taste mechanisms in the human oral cavity. However, wide variability has been observed in NEFA taste sensitivity between and within subjects as well as across research groups. Some of this variability may be due to the hydrophobic nature of the NEFA and the methods used to make stimuli emulsions. As NEFA are poorly soluble in water, emulsification is necessary for delivery of stimuli to taste receptors. However, properties of emulsions may also be detected by somatosensory cues complicating attribution of sensory findings to taste. Additionally, learning (improved test performance) has been observed when using traditional tests for measuring sensitivity to NEFA, which may contribute greatly to within-subject variability if not standardized. Factors such as sex, diet, and BMI have been proposed to affect NEFA taste sensitivity, but the degree to which these individual factors influence NEFA detection thresholds remains to be fully established. Improved knowledge of stimulus properties and individual sensory capabilities will be needed to further evaluate the posited taste component to human oral fat detection. Progress in this area should facilitate the translation of findings on how NEFA taste may contribute to or reflect food choice and chronic disease risk.

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#### 1. Introduction

The number of daily eating occasions has increased markedly over the past four decades [1,2] and most entail oral exposure to dietary fat. The fat content of food contributes sensory properties that guide food choice and energy intake, but oral fat exposure also influences lipid metabolism [3]. While fat exposure results in an

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early peak in circulating triacylglycerol (TAG), preliminary evidence suggests it is not the somatosensory or olfactory cues from fat that mobilize this lipid from enterocytes [4]. This has led to the hypothesis that lipid mobilization is mediated by the gustatory sense (taste), though the view that fat, in the form of non-esterified fatty acids (NEFA), is an effective taste stimulus remains controversial.

The widely accepted "primary" tastes are comprised of sweet, sour, bitter, salty, and, more recently, umami. Somewhat surprisingly, there are no universally accepted definitions regarding the characteristics that qualify a taste quality as a "primary" [5], but

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recently a set of criteria have been proposed by researchers working on both umami [6] and fat taste [7]. Overlap in the proposed criteria include: the stimulus activates taste receptors that generate activity in taste neurons and no mixture of other primary taste stimuli can recreate the quality of the newly proposed primary [6]. Additionally, a primary taste should generate a functional physiological and/or behavioral response [7] that plausibly affords an adaptive advantage [7]. Evidence supporting fat taste as a primary has been reviewed extensively elsewhere [5,7-10]. Briefly, key highlights supporting a taste component for dietary fat include: (a) food itself supplies and/ or lingual lipase generates NEFA, [11-13]; (b) receptors that bind NEFA have been localized to human taste buds [14-16]; (c) humans can detect NEFA in complex stimuli that mask somatosensory and olfactory cues [17]; (d) taste nerves in rodents convey signals from oral fat exposure [16,18], and their sectioning selectively diminishes behavioral [19-22] and digestive [19] responses to fat; (e) aversions to NEFA do not generalize to prototypical stimuli for other taste primaries suggesting their quality is unique [23]; and (f) oral fat exposure may deter ingestion of rancid foods and enhance lipid digestion and intestinal processing among other speculated functions [24-26]. Thus, there is supporting evidence for each of the proposed criteria for a primary. The data are not definitive, but there is also little refuting the findings to-date.

A hallmark of human fat taste research is the large degree of variability seen between and within subjects as well as between research groups. Much of the work conducted in the area of human fat taste has focused on detection threshold testing to determine the lowest concentration of NEFA individuals can distinguish from a background (e.g., water, ice cream) lacking NEFA. Detection threshold testing typically involves presenting a participant with two or three samples, one of which contains emulsified NEFA, and asking them to select the sample that is different from the other(s). Concentrations of the stimulus are altered in successive tastings until the participant can reliably select the correct sample over repeated presentations. Median reported NEFA detection thresholds have spanned more than 4 orders of magnitude between studies [27,28], more than 5 orders of magnitude within a study [29], and as much as 9 orders of magnitude within an individual in a given study [29]. This large variability between individuals has led some to suggest that there are people who are hypo- and hyper-sensitive to fat taste [27,29-31], and that this sensitivity is associated with dietary choices and health outcomes, such as obesity [27,32,33]. However, others have failed to find associations between fat taste sensitivity, diet, and health [9,30,34,35]. Thus, clarification of current knowledge is needed.

This review discusses a number of factors proposed to contribute to between-subject, within-subject, and between-study variation in NEFA taste studies. An understanding of this variation will be essential to identify the functions of fat taste in health and disease. Between-subject factors include salivary composition, somatosensory sensitivity, sex, diet, and BMI. Within-subject factors also include consideration of sex and diet, as well as genetic makeup and learning. The reliability of the participant's performance from one test to the next is a known problem in psychophysical testing, with test-retest reliability being relatively low [36]. Between-study variance could be explained by vehicle composition as this changes the background sensory noise level and ability to detect the NEFA signal relative to this background. While there is support for many of these factors, the contribution of others to variation is more theoretical. As the field progresses, other, still unknown, factors that could contribute to variation will likely come to light as well.

#### 2. Oral textural sensations of fats, oils, and emulsions

An understanding of how fat mixtures and emulsions are detected orally is critical for testing and interpreting NEFA taste responses. Much of the argument against the existence of NEFA taste centers around the distinct texture of fat compared to water soluble stimuli. However, fats in the diet are usually emulsions or mixtures. For example, butter is a water in oil emulsion and milk is an oil in water emulsion (often written as w/o or o/w, respectively). Fried food may have oil absorbed into the exterior coat, or pizza may have oil pooled on top of the melted cheese, but such products are still consumed as mixtures, not pure fat. Multiple studies indicate that the bulk viscosity of a fat or emulsion is not the only way fat and oils are detected in the mouth. Sugars, starches, fibers, and/ or non-nutritive oils can be used to match viscosities, and yet the fatty solution is still distinct from the non-fat or non-nutritive solution [37-39]. This knowledge has led to several studies on the somatosensory detection and perception of emulsions.

Orally, the texture of fat and emulsions is detected through their bulk viscosity (flow of food in the mouth detected while swishing) and tribology (thin film rheology, which includes adhering, lubricating, frictional effects) [37,40-42]. Emulsions consist of at least two phases. For oil in water emulsions, the most common emulsion used in NEFA taste evaluation studies, oil droplets are suspended in an aqueous solution. Carbohydrate gums are often added to aid in keeping the oil droplets suspended and separated. Such gums are distinct from true emulsifying agents, which may also be added. These emulsifiers, such as lecithin, whey protein, polysorbate, and the protein component of gum acacia, stabilize the oil droplets through interactions of the hydrophobic portion of the emulsifying agent with the oil phase and the hydrophilic portion with the water phase. If an emulsion is unstable, sensations based on viscosity and tribology can be easily detected or even changed in the oral cavity [43-45]. Several causes of instability are flocculation (when droplets adhere to each other but do not combine into a single drop), coalescence (when small droplets combine to form larger droplets), and creaming (when droplets adhere together and rise to the top of the water phase). Flocculation increases oral perception of thickness [46], and coalescence increases lubricity [43,45]. As a result, the stability of NEFA taste testing emulsions may influence the ability of subjects to detect a difference between the NEFA solution and the "blank" vehicle. Mineral oil is frequently added to the "blank" in NEFA taste experiments, which could theoretically assist in keeping the emulsions' textures similar. However, mineral oil, which is a mixture of alkanes, is less stable in an emulsion than NEFA, which have a hydrophilic carboxylic acid group. The presence of the carboxylic acid moiety allows the NEFA to act as soaps and even form micelles at certain concentrations [47]. Whether or not the oral texture and stability of a mineral oil emulsion is equal to that of a NEFA emulsion is thus uncertain and may differ depending on the type and amount of carbohydrates and emulsifiers used.

The viscosity of a fat emulsion (without carbohydrate thickeners added) may be enhanced by increasing the quantity of oil droplets. Droplets add body to the emulsion, creating the perception of greater thickness in the oral cavity [46]. Addition of carbohydrate gum thickeners typically increases viscosity much more than the oil droplets themselves [48], which is why such gums are used in NEFA taste experiments. Orally perceived viscosity of an emulsion also increases if oil droplets aggregate, as in the case of saliva-induced flocculation of emulsions [48–51]. However, the viscosity of the oil phase itself does not appear to influence oral sensory perception of thickness. In one study, oils ranging in viscosity from 30.4 to 984 mPa s were rated similarly for thickness after incorporation into emulsions at 2% or 20% oil [46]. However, whether the

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