

Olive oil aroma extract modulates cerebral blood flow in gustatory brain areas in humans^{1–3}

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ABSTRACT

Background: Low- and high-fat meals affect homeostatic and gustatory brain areas differentially. In a previous study, we showed that a high-fat meal decreased cerebral blood flow (CBF) in homeostatic brain areas (hypothalamus), whereas a low-fat meal increased CBF in gustatory regions (anterior insula).

Objective: The aim of this study was to investigate the long-lasting effect of fat-free flavor-active compounds of olive oil on the brain and whether those aroma components can trigger fat-associated brain responses in homeostatic and gustatory regions.

Design: Eleven healthy male subjects participated in a functional magnetic resonance imaging study. On 2 measurement days, subjects consumed single-blinded a plain low-fat yogurt or low-fat yogurt mixed with a fat-free aroma extract of olive oil. Resting CBF was measured before and 30 and 120 min after yogurt intake. Hunger was rated before each measurement. Blood samples were collected at 6 time points.

Results: The extract-containing yogurt elicited higher CBF in the frontal operculum 30 and 120 min after a meal. Furthermore, the activity change in the anterior insula after 30 min correlated positively with the glucose change in the extract condition only. No effects were observed in the hypothalamus.

Conclusions: The anterior insula and the frontal operculum are regarded as the primary taste cortex. Modulation of the frontal operculum by the yogurt containing the olive oil extract suggests that it might be possible to simulate fat-triggered sensations in the brain on the gustatory level, possibly by ingredients the body implicitly associates with fat. This trial was registered at clinicaltrials.gov as NCT01716286. *Am J Clin Nutr* 2013;98:1360–6.

INTRODUCTION

As we face the problem of obesity, the market for fat-reduced food achieved strong growth in the past decades. The benefit of low-fat food, however, is still controversially discussed (1). Despite various studies addressing outcome variables for different diets, such as weight loss, body fat reduction, or cardiovascular benefits (2–4), one possible obstacle for the effectiveness of low-fat products may be their specific action in the human brain, which is different from the effect after stimulation with high-fat foods (5, 6). In general, central food-related processes can be separated into 2 main processes: homeostatic control (ie, the hypothalamus, which is mainly responsible for caloric balance) and hedonic control (which is associated with a large range of cortical and subcortical brain networks). Hedonic control can interact with

the homeostatic system, potentially leading to over- or under-consumption of food and is especially influenced by the flavor of ingested food (7–9). Food flavor itself is a multisensory construct that comprises mainly taste, olfactory, and somatosensory inputs (10). Flavor perception, therefore, requires the integration of these different aspects in specific brain networks (11). Besides the taste component at first experienced by the taste buds on the tongue, the aroma of food is also experienced olfactorily via the retronasal route (11–13). Gustatory information for taste perception is relayed through the thalamus to the primary taste cortex (anterior insular cortex and the adjacent frontal operculum) with consecutive projections to the secondary gustatory cortex (orbitofrontal cortex) (14). Because the gustatory cortex is sensitive to food perception (15–18) and hence to different flavors (7), taste valence, and intensity (19, 20), flavor leads to neuronal activations in the right frontal operculum and the orbitofrontal cortex bilaterally (8). These activations are modified by personal experiences, as shown in an fMRI BOLD study (21). From a system theoretic approach, the manipulation of food by changing the multisensory aspect affecting the brain is of special interest. In a previous study, we were able to show that the total fat content influences gustatory (anterior insula) and homeostatic brain regions (hypothalamus)

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within 30 min and up to 2 h after consumption (5). In that particular study, male subjects ingested a high- or a low-fat yogurt on 2 separate measurement days while undergoing resting cerebral blood flow (CBF)⁴ measurements with perfusion MRI. With regard to taste and texture, subjects were not able to distinguish between the low- and high-fat yogurts; nevertheless, decreased CBF in the high-fat condition was observed in the hypothalamus after 30 min, and an increased activity was observed in the anterior insular cortex after 120 min in the low-fat condition. Therefore, fat seems to modulate gustatory and homeostatic brain regions, even when the fat content of the meal is not explicitly known.

To further elucidate the effect of flavor on food processing with a special emphasis on fat-reduced food, we performed an fMRI study to measure resting CBF before and after intake of a low-fat yogurt and a low-fat yogurt laced with a fat-free extract of olive oil. We hypothesized that the introduction of flavor-active compounds from an olive oil into low-fat yogurt leads to a different activity in gustatory taste areas (anterior insular/frontal operculum) and the hypothalamus compared with plain low-fat yogurt. In addition, we tested whether this flavored yogurt elicits activities similar to those that we found after high-fat yogurt ingestion based on the learned association between flavor and fat content.

SUBJECTS AND METHODS

Subjects

For this study, 11 healthy male subjects were included [age (\pm SEM): 28.82 ± 1.04 y; BMI (\pm SEM) (in kg/m^2): 24.6 ± 0.56], all of whom were right-handed. The same protocol used in the preceding study was applied, beginning with a medical screening (including examination by a physician and blood sampling) and psychological and psychiatric questionnaires and eating behavior questionnaires (5). To address psychiatric disorders, the Patient Health Questionnaire (22) and the Beck Depression Inventory (23) were used. To ensure normal eating behavior by all subjects, the German versions of the Three Factor Eating Questionnaire (24) and the Eating Disorder Examination (25) were applied. None of the subjects showed any kind of physiologic or psychiatric disorder or other diseases, as assured by a physician. The study protocol was approved by the ethics committee of the medical faculty of the University of Tübingen, and all subjects gave their written informed consent.

Study design

Subjects arrived in the morning after an overnight fast of ≥ 10 h. In total, subjects completed 3 pulsed arterial spin labeling (PASL) measurements to determine CBF on each of 2 separate days. Before each PASL measurement, blood samples were collected, and subjects rated their subjective feeling of hunger on a 0–100 (0 = not at all hungry, 100 = very hungry) visual analog scale (VAS). Because it has been shown that the emotional state can have an influence on the processing of fat (26), the VAS also included emotional and physical variables [anxiety, (physical) wellbeing, agitation]. After the first measurement (CBF 1), subjects

were instructed to eat 500 mL of a low-fat ($<0.1\%$) yogurt within 10 min using a spoon. On 1 of the 2 measurement days, yogurt with the fat-free extract of olive oil produced and provided by one of the authors (PS) was given to the subjects. The order of the yogurt was counterbalanced and single-blinded. Thirty minutes after the start of the yogurt intake, the first postyogurt measurement (CBF 2) was made, and the second postyogurt measurement (CBF 3) started 120 min after the meal. We chose these time points because corresponding metabolic and endocrine reactions in response to food intake are well known (27–29). An overview of the study design is given in **Figure 1**.

Blood sampling

At 6 time points (pre- and 30, 60, 90, 120, and 150 min postmeal), blood samples were collected to determine insulin and glucose concentrations. Plasma glucose was measured by using the glucose hexokinase method on an ADVIA 1800 chemistry analyzer (Siemens Health Care Diagnostics). Serum insulin was measured with an immunoassay on an ADVIA Centaur XP Immunoassay System (Siemens Health Care Diagnostics).

Yogurt production

Low-fat yogurt was produced at the Institute of Food Science and Biotechnology (University of Hohenheim). Bovine raw milk was obtained freshly from the Dairy Research Station Meiereihof (University of Hohenheim), separated (fat 0.1% wt:wt; wt, weight percentage), and pasteurized at 74°C for 30 s. Dry matter of skim milk was set to $12 \pm 0.1\%$ (wt:wt) by adding low-heat skim milk powder (type Instant C, 37% wt:wt total protein; Schwarzwaldmilch GmbH). Standardized milk was heated (95°C for 4.3 min) and subsequently cooled to 35°C in the tubular heating equipment of a pilot plant (Asepto GmbH) as described in a previous study (5). A fat-free Italian virgin olive oil extract consisting of volatile compounds was added (0.025%, wt:wt) to the cooled yogurt milk. For detailed information about the macronutrients, see Supplementary Table S1 under “Supplemental data” in the online issue. A control without the olive oil extract was produced accordingly. Both milk types were inoculated with 0.02% (wt:wt) YoFlex 812, prepared as a freeze-dried mixture of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. The milk was acidified at 35°C over a period of 14 h to a pH of 4.4 ± 0.1 . After fermentation, the milk gel was manually broken with a stainless steel-bored disk by up-and-down movements for 60 s, pumped and sheared with a needle valve, and filled into 500-g plastic containers.

Data acquisition

Scanning was performed on a 3T scanner (Tim Trio; Siemens) equipped with a 12-channel trans-receiver head coil. PASL images were obtained with a PICORE-Q2TIPS (proximal inversion with control for off-resonance effects—quantitative imaging of perfusion by using a single subtraction) sequence by using a frequency offset corrected inversion pulse and echo planar imaging readout for acquisition. A total of 12 axial slices with a slice thickness of 5 mm (1.25-mm gap) were acquired in ascending order. Each measurement consisted of 100 alternating tag and control images with the following imaging parameters: inversion time (TI), $\text{TI}_1 = 700$ ms, $\text{TI}_2 = 1800$ ms, repetition time (TR) = 2500 ms,

⁴ Abbreviations used: CBF, cerebral blood flow; PASL, pulsed arterial spin labeling; TE, echo time; TI, inversion time; TR, repetition time; VAS, visual analog scale.

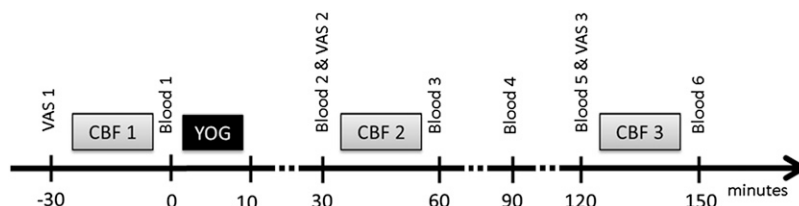


FIGURE 1. Study design. CBF, cerebral blood flow; VAS, visual analog scale; YOG, yogurt.

echo time (TE) = 13 ms, inplane resolution = $4 \times 4 \text{ mm}^2$, field of view = 256 mm, and flip angle = 90° . On the basis of the limited number of slices, upper parts of the motor area were not included in the acquisition. The same sequence was used to estimate the equilibrium magnetization of the blood (M_{0B}) for absolute CBF quantification with the same parameters as mentioned above, except that TR and TI_2 were chosen to be 10 and 4 s, respectively, to allow for T_1 (spin-lattice relaxation) recovery and to avoid saturation effects (30).

In addition, a high-resolution T_1 -weighted anatomical image was acquired (magnetization-prepared rapid gradient echo, matrix size = 256×256 , 176 slices, $1 \times 1 \times 1 \text{ mm}$ isotropic voxels, TR = 1900 ms, TE = 2.26 ms, TI = 900 ms).

Image processing

Image preprocessing was performed by using SPM8 (Wellcome Trust Centre for Neuroimaging). Functional data were analyzed as described previously, including perfusion quantification by using FSL software (31) and Linux shell script routines. Images were realigned and resliced by using the mean volume of the corresponding session as reference. The M_0 images of each session and day were coregistered separately to the mean image of the corresponding session by using a 6-parameter rigid body transformation and sinc interpolation. In addition, the functional images were coregistered to the individual anatomical image and smoothed with a 3-dimensional isotropic Gaussian kernel (full width at half maximum: 8 mm). A brain mask was created to exclude extracranial voxels. Time series of all functional sessions were high-pass filtered (cutoff: 128 s) to remove low-frequency baseline drifts potentially caused by scanner instability, subject motion, and physiologic noise. Perfusion images were generated by calculating the control-tag differences by using surround subtraction. We used the identical parameters for absolute perfusion quantification based on the general kinetic model, as described before (5). For accurate CBF quantification, we used an M_{0B} map instead of a global value to quantify the perfusion on each voxel. The high-resolution T_1 -weighted image was normalized in Montreal Neurological Institute space ($1 \times 1 \times 1 \text{ mm}$), and the resulting parameter file was used with the individual coregistered CBF maps in normalized space ($3 \times 3 \times 3 \text{ mm}$). Baseline-corrected relative CBF maps were computed to quantify the CBF changes after 30 and 120 min during the 2 visits.

Statistical analyses

Whole-brain analyses were performed by using a voxel wise approach (32). To investigate differences due to the yogurt types, a full factorial design was conducted including the factors yogurt (plain compared with extract) and time (postmeasurements corrected for the premeasurement: CBF 2-CBF 1, CBF 3-CBF 1)

and the covariates hunger and BMI. In addition, correlation analyses of the CBF change with the change in glucose and insulin were performed. A family wise error-corrected P_{FWE} value < 0.05 on cluster level was considered statistically significant. Blood data were analyzed by using SPSS 18. Repeated-measures ANOVA was performed to analyze the factors yogurt (plain compared with extract) and time (0, 30, and 120 min).

RESULTS

Subject characteristics

The scores from the eating behavior questionnaires resulted in low scores for restraint eating, disinhibition during eating, and generally experienced hunger assuring normal eating behavior in our subjects. In addition, subjects reported low eating-related, weight, or shape concerns (see Supplementary Table S2 under “Supplemental data” in the online issue). Yogurt intake led to a significant time effect for subjective hunger ($F_{(2,40)} = 7.364$, $P = 0.002$). No difference between the plain and the extract yogurt or any interaction effect was observed. Neither the emotional nor physical parameters showed differences between the 2 yogurt conditions ($P > 0.05$).

Blood samples

Glucose and insulin concentrations increased 30 min after yogurt intake and decreased again after 60 min: time course glucose ($F_{(5,80)} = 17.231$, $P < 0.001$) and time course insulin ($F_{(5,90)} = 41.461$, $P < 0.001$). No significant difference in the course of glucose and insulin was found for the 2 conditions (plain compared with extract) ($P_{\text{glucose}} = 0.932$, $P_{\text{insulin}} = 0.258$; see Supplementary Figure S1 under “Supplemental data” in the online issue).

Evaluation of the yogurt

Subjects were asked to report which of the 2 yogurts they “liked more” and were considered to have more fat. Seven subjects liked the plain yogurt more, whereas one liked the extract yogurt more. The other 3 subjects were undecided. Concerning the fat content of the yogurt, 5 subjects thought of the extract yogurt to be higher in fat, 3 of the plain yogurt without extract, and 3 were undecided.

CBF

The main effect of yogurt elicited a higher activation at both time points after food intake in the frontal operculum (Figure 2, Table 1). In addition, after intake of the yogurt including the olive oil extract, the bilateral anterior insular cortex showed significant positive correlations with the glucose change at 30 min after food intake (Figure 3, Table 1) but not at 120 min. No significant correlation with the insulin change was observed.

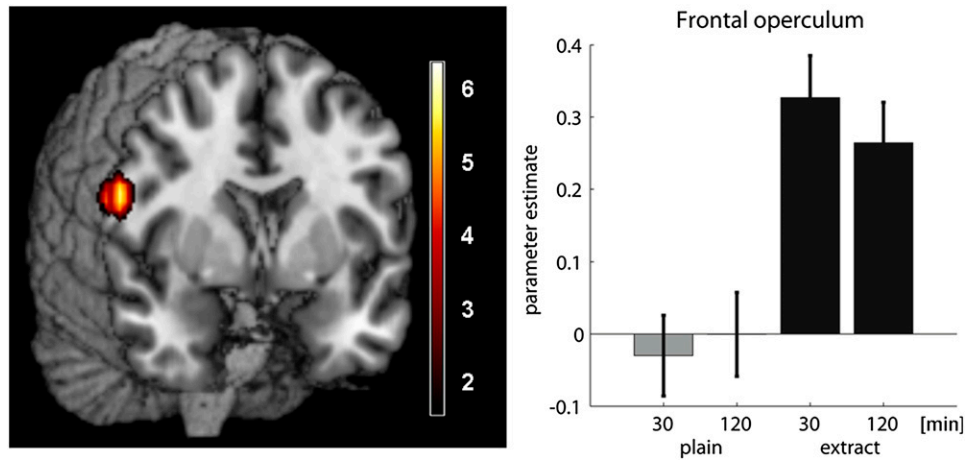


FIGURE 2. Coronal view of the activation difference in the frontal operculum for the differences between the 2 yogurt conditions and corresponding bar graph. Data were analyzed with a full factorial repeated-measurement design with the covariates hunger and BMI; a *t* test showed higher activation in the frontal operculum in the extract condition as compared with the plain yogurt ($n = 11$). The color bar represents *T* values. The bar plots represent baseline-corrected (ie, corrected for the “pre” measurement) parameter estimates \pm SEMs. $P_{\text{FWE}} < 0.05$ (corrected for multiple comparison on cluster level). FWE, family wise error.

DISCUSSION

Notwithstanding that the yogurts used for this study had the same amount of fat, protein, and carbohydrates, we found an increase in frontal opercular CBF in the extract condition. Besides the sensitivity of the frontal operculum to food intake, the presented results are, thus, not based on the energy value of the yogurts. In task-related studies, the frontal operculum as part of the primary taste cortex, has shown pronounced activation to visual food cues (33, 34) and anticipation of food intake (21, 35) and is crucially involved in visual-gustatory interaction (36) and odor-taste integration (37). In addition, activity in this area in response to food-specific stimuli was positively correlated with BMI (38, 39). Earlier fMRI studies showed that oral delivery of a drop of fat leads to an immediate increase in insular and frontal opercular activity (40, 41). Those results are not based on the calorie content of food but rather provide information about flavor processing in the brain (11). The odor-taste integration is especially interesting, because we used aroma components to induce a fat-associated experience. Besides the taste component, such olfactory aroma components influence the brain via the olfactory retronasal route. In general, odors can be processed orthonasally (via the nose) and retronasally (via the mouth and pharynx) (12, 13). In our study, retronasal sensing is essential, because the aroma used for the yogurt contains olfactorial

components. An odor sensed retronasally is known to activate the frontal operculum (12). Such retronasal experiences are dependent on the specific aroma and on the subjects (42). One study even claimed that a part of the frontal operculum is a unimodal taste area, not responsive to olfactory stimuli (43). Other regions in the frontal operculum, however, respond to both taste and olfactory stimuli. The peak activation in the frontal operculum found by de Araujo et al (43) for the combination of a taste and olfactory stimulus corresponds with the peak activation found in our study. Therefore, we can assume that both taste and olfaction are integrated in the differential processing of the plain and the extract yogurt.

At this point, the question might be raised whether we just measured the aroma component of the extract yogurt instead of the association with fat. Worthy of mention here are the results by Eldeghaidy et al (41), who showed increased BOLD activity in the frontal operculum in response to drops of fat emulsions with increasing fat content. In contrast with the event-related fMRI studies describing flavor processing, we performed CBF measurements after 30 and 120 min. Considering that acute aroma responses decrease after 20 s (44), we do not assume that the pure aroma would have had an effect after 30 min and even after 2 h. Thus, we assume that the effect in the frontal operculum is not necessarily based on the acute response but rather on a signal that

TABLE 1
Results of the fMRI measurements¹

Data	Analysis	Effect	Brain region	MNI coordinates			Cluster size (in voxels)	Z value
				x	y	z		
CBF	ANOVA	Extract > plain	Frontal operculum	60	14	16	45	4.38
CBF	Regression (extract)	Glucose change (pre to 30 min)	Insula left	-33	23	-5	82	4.48
			Insula right	36	17	-5	50	4.10

¹The results were derived by using the MNI coordinate system. Data were analyzed with a full factorial repeated-measurement design with the covariates hunger and BMI; a *t* test showed higher activation in the frontal operculum in the extract as compared with the plain condition. Correlation analyses showed a significant positive correlation of the glucose change with the change in CBF in the insular cortex bilaterally. Results are significant at $P_{\text{FWE}} < 0.05$, family wise error corrected on cluster level. $n = 11$. CBF, cerebral blood flow; FWE, family wise error; MNI, Montreal Neurological Institute; pre, before the meal.

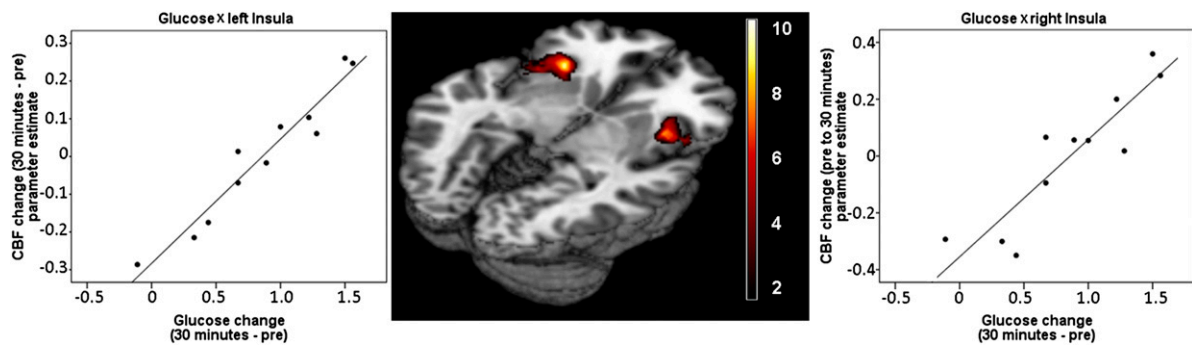


FIGURE 3. Transversal view of the regression analysis of the glucose change (30 min – pre) and the CBF change in the left and right insular cortex after intake of the extract yogurt with corresponding scattergrams. The color bar represents T values. $P_{FWE} < 0.05$ (corrected for multiple comparisons on cluster level). Glucose was measured in nmol/L. $n = 11$. CBF, cerebral blood flow; FWE, family wise error; pre, before the meal.

modifies later responses to achieve an appropriate sensory control. We did not observe differences in the hunger rating in the 2 yogurt conditions. However, evidence indicates that food laced with different flavors can also have an effect on hunger, food intake, and satiation (45, 46). In accordance with earlier findings, we found a lateralization, which suggests that dietary flavor processing is predominant in the right hemisphere (8). Small et al (21) also showed that the frontal operculum is highly influenced by experiences. Taken together, our results indicate that the flavor of olive oil comprises a learned association with a fat-rich component of a meal eliciting a pronounced effect in the frontal operculum.

Fat-induced effects on the central nervous system are accompanied by various hormonal factors. Only few fMRI studies have investigated the pure long-term effect of fat infusion. Two studies examined exogenous lipid infusion in combination with the infusion of ghrelin or a cholecystokinin antagonist. In general, lipid infusion leads to delayed gastric emptying, an effect that was shown to be reversed by ghrelin infusion (47). In addition, pharmacologic MRI showed increased BOLD activity in homeostatic and limbic areas after lipid infusion. This effect was suppressed by exogenous ghrelin (47), an effect supposedly mediated by cholecystokinin pathways (48). In our previous study, we showed that changes in insulin concentrations correlate with the fat-induced CBF pattern in the hypothalamus. Here, a lower increase in insulin coincided with a greater reduction in hypothalamic activity. This effect was mainly driven by the significant reduction of CBF in the high-fat condition. Because the fat contents of the yogurts were the same in the current study and no significant differences in insulin concentrations between the 2 conditions were observed, we did not see such responses. In the current study, however, positive correlations of the insular cortex activity with the glucose change in the extract condition, but not in the plain condition, were seen. Here, another part of the primary taste cortex showed differential activation pattern as a result of the 2 yogurt types associated with changes in peripheral parameters. No differences in glucose or carbohydrate contents were found between the yogurts, but, the increase in blood glucose after ingestion was higher in the extract condition. The increase in glucose concentration in the extract condition correlated with activity changes in the anterior insular cortex. A previous study showed that BOLD activation in the insular cortex is positively correlated with taster status (41), which is described as a genetic trait to define bitter, sweet, and fat components (49). Therefore, it might be possible that this effect

is also triggered by the taster state, which was not assessed in our study. One other explanation might be the interoceptive character of the anterior insular cortex (50), in terms of a cephalic phase response (51). Learned cephalic phase responses can lead to an anticipation of the challenge of a specific food, which could lead to central and peripheral responses, even without ingestion of the actual calories. Thus, higher CBF increases correlating with higher glucose increases could be based on implicit memories of an interoceptive state associated with intake of flavored high-fat food. In this sense, it appears that dietary flavor is the driving factor for this correlation.

Taking into account the results of our previous study (5), we have to address the fact that we did not find any differential effect of the low-fat yogurts on the homeostatic system. Consumption of the high-fat yogurt elicited a pronounced decrease in hypothalamic CBF similar to well-documented reduced BOLD activity patterns after glucose intake (52). Nutrient-sensing per se influences the hypothalamus in various ways and is generated by hormones such as glucose and insulin (53). Also, direct infusion of lipids in the stomach induced strong responses in the hypothalamus (47, 48). Therefore, we assumed that the hypothalamus might also be sensitive to the fat-associated aroma, triggering an according response. In contrast with our hypothesis, however, we did not observe any changes in the hypothalamus. Earlier studies have shown that, for fat sensing, the feeling in the mouth is crucial for the central response (54, 55). Because of the characteristics of a homeostatic brain area (56), it is conclusive that the hypothalamus is not responsive to an aroma alone without the actual caloric energy. In contrast with the frontal operculum (ie, the gustatory system), the homeostatic system is not primarily affected by flavor or rewarding aspects of food but reacts predominantly to the actual energy value of food (56). This opposes the assumption that low-fat food can mimic fat-enriched foods on the homeostatic level.

A limiting factor of this study was the small sample size. A bigger sample would be useful for further evidence. In addition, we did not include habitual olive oil consumption or liking ratings as possible confounds. Furthermore, another aroma condition could be included in the design to control for pure aroma processing.

In summary, we showed that an aroma extract of olive oil in yogurt affects the gustatory system. Taking into account our previous study, the CBF effects in response to the extract-laced yogurt were not the same as the previously reported effects of the high-fat yogurt (hypothalamus, insula). Although we found an effect in the primary taste cortex, the homeostatic system (in contrast with our



hypothesis) seemed unaffected by the different yogurts. Modulation of the frontal operculum by the extract of olive oil, however, indicates the possibility of a simulation of fat for the brain on the gustatory level by taste and olfactory ingredients the body implicitly associates with fat. Thus, it might be possible to optimize the recipe of the extract and to induce brain activation more similar to that induced by the high-fat yogurt.

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