

## G Protein–Coupled Receptors in Human Fat Taste Perception

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### Abstract

In contrast to carbohydrates and proteins, which are detected by specialized taste receptors in the forms of their respective building blocks, sugars, and L-amino acids, the third macronutrient, lipids, has until now not been associated with gustatory receptors. Instead, the recognition of fat stimuli was believed to rely mostly on textural, olfactory, and postingestive cues. During the recent years, however, research done mainly in rodent models revealed an additional gustatory component for the detection of long-chain fatty acids (LCFAs), the main taste-activating component of lipids. Concomitantly, a number of candidate fat taste receptors were proposed to be involved in rodent's gustatory fatty acid perception. Compared with rodent models, much less is known about human fat taste. In order to investigate the ability of the human gustatory system to respond to fat components, we performed sensory experiments with fatty acids of different chain lengths and derivatives thereof. We found that our panelists discriminated a "fatty" and an irritant "scratchy" taste component, with the "fatty" percept restricted to LCFAs. Using functional calcium–imaging experiments with the human orthologs of mouse candidate fat receptors belonging to the G protein–coupled receptor family, we correlated human sensory data with receptor properties characterized *in vitro*. We demonstrated that the pharmacological activation profile of human GPR40 and GPR120, 2 LCFA-specific receptors associated with gustatory fat perception in rodents, is inconsistent with the "scratchy" sensation of human subjects and more consistent with the percept described as "fatty." Expression analysis of GPR40 and GPR120 in human gustatory tissues revealed that, while the GPR40 gene is not expressed, GPR120 is detected in gustatory and nongustatory epithelia. On a cellular level, we found GPR120 mRNA and protein in taste buds as well as in the surrounding epithelial cells. We conclude that GPR120 may indeed participate in human gustatory fatty acid perception.

**Key words:** calcium imaging, fatty acid, G protein–coupled receptors, papillae, taste perception

### Introduction

The sense of taste plays an important role in the evaluation of the nutritional value or potential toxicity of food compounds prior to ingestion. The detection of nutritionally relevant food components is facilitated by specialized taste receptor molecules expressed in sensory cells residing in morphological structures, called taste buds (Miller 1995). Of the 5 basic taste qualities, sweet, sour, salty, umami, and bitter, 2, sweet and umami, are devoted to the detection of macronutrients (Behrens et al. 2011). The sweet taste receptor is activated by mono- and disaccharide carbohydrates (Nelson et al. 2001), whereas the umami receptor recognizes certain L-amino acids (Nelson et al. 2002) representing the

building blocks of proteins. In contrast to the gustatory detection of carbohydrates and protein, the perception of the third macronutrient and second most important energy source for humans, dietary fat, has been attributed mainly to trigeminal (Tepper and Nurse 1997), olfactory (Ramirez 1993), and postingestive mechanisms (Greenberg and Smith 1996). Over the recent years, however, evidence has been accumulating for a possible involvement of gustatory cues in fat perception of rodents and human (Takeda et al. 2001; Fukuwatari et al. 2003; Hiraoka et al. 2003; Laugerette et al. 2005; Chale-Rush et al. 2007b; Cartoni et al. 2010).

When rats furnished with a gastric fistula to prevent metabolic utilization of ingested liquids were sham fed with either nutritive corn oil or nonnutritive mineral oil, they ingested both types of oils with similar preference. Given the choice between corn oil and mineral oil in subsequent 2-bottle preference tests, the cannulated rats unequivocally preferred the triacylglyceride-containing corn oil over mineral oil despite the textural similarity of both stimuli, indicating an orosensory detection mechanism independent of postingestive effects (Mindell et al. 1990).

Because the predominant components of dietary lipids from vegetable and animal sources are triacylglycerides (Lawson 1995), further experiments addressed the question whether these triacylglycerides are the primary stimulus for orosensory fat perception or if rather fatty acids (FAs) liberated from triacylglycerides by lipases may account for this activity. Indeed, lingual lipase activity in the oral cavity of rodents is sufficient to hydrolyze triacylglycerides even during short exposure times. In the presence of orlistat, a potent lipase inhibitor, the preference for triacylglyceride significantly decreases, whereas the preference for free FAs is not affected (Kawai and Fushiki 2003). From a nutritional point of view, the gustatory detection of either essential FAs or the recognition of predominant FA-species found in naturally occurring triacylglycerides or a combination of both would be most favorable. Indeed, an activation of rodent taste cells by essential *cis*-polyunsaturated fatty acids (PUFAs) as well as a particular sensitivity toward long-chain fatty acids (LCFAs), the major component found in triacylglycerides, was observed in various studies (Gilbertson et al. 1997; Tsuruta et al. 1999). Moreover, bilateral sectioning of the glossopharyngeal and chorda tympani nerves innervating gustatory papillae diminishes the preference for a solution of linoleic acid in mice (Gaillard et al. 2008). Mounting evidence for a gustatory component in the perception of free LCFAs has spawned considerable interest in the identification of receptor molecules involved in the detection of these stimuli. To date, 4 main candidate genes implicated in fat perception of rodents were put forward: A delayed-rectifying potassium (DRK) channel sensitive to PUFAs (Gilbertson et al. 1997), the FA transporter CD36/FAT (Fukuwatari et al. 1997; Laugerette et al. 2005) as well as the 2 G protein-coupled receptors, GPR40 and GPR120 (Matsumura et al. 2007, 2009; Cartoni et al. 2010). All of the candidate receptors mentioned above fit into the spectrum of taste-active FAs and were documented to be expressed in rodent's taste buds. Electrophysiological recordings from isolated taste buds of the anterior rat tongue in combination with molecular biological analyses indicated that KCNA5 represents the major functional DRK channel. Because in taste cells, these channels were shown to be predominantly in the closed state at resting membrane potential (Liu et al. 2005) and FAs act as open-channel blockers on KCNA5 (Honore et al. 1994), a modulatory role was proposed

(Gilbertson et al. 1997). Indeed, interactions between various taste stimuli and FAs were observed in behavioral experiments in rodents (Gilbertson et al. 2005; Pittman et al. 2006). The FA transporter CD36 is expressed in lingual epithelium of rodents at the apical side of foliate and vallate papillae, colocalizing with the type II taste cell marker  $\alpha$ -gustducin (Fukuwatari et al. 1997; Laugerette et al. 2005). Analysis of CD36 knockout mice demonstrated perceptual deficits for linoleic acid compared with wild type mice, whereas their sensitivity to stimuli of other taste qualities remained unchanged (Laugerette et al. 2005). Of the 2 G protein-coupled receptors, GPR120 (Hirasawa et al. 2005) was the first detected in gustatory tissue of mice. It was shown to be predominantly expressed in type II taste receptor cells of foliate and vallate papillae (Matsumura et al. 2007, 2009; Cartoni et al. 2010). In contrast to that, GPR40 (Briscoe et al. 2003) is mainly found in type I taste cells of mice (Cartoni et al. 2010) but not found in rat gustatory papillae (Matsumura et al. 2007). Recently, knockout mice for both GPR40 and GPR120 were generated and characterized for their ability to respond to FAs (Cartoni et al. 2010). Intriguingly, both mouse lines, the GPR40-deficient as well as the GPR120-deficient line, exhibited diminished sensitivity toward LCFAs, underscoring the proposed role of these receptors.

In contrast to rodents, much less is known about human fat taste perception. However, sensory experiments revealed that humans perceive FAs in a multimodal fashion (Chale-Rush et al. 2007b) including gustatory cues (Chale-Rush et al. 2007a, 2007b). The thresholds for the detection of free FAs were reported to be roughly in the low millimolar range (Chale-Rush et al. 2007a, 2007b; Mattes 2009a, 2009b; Stewart et al. 2010) and seem not to depend on chain length (Mattes 2009a; Stewart et al. 2010), the degree of saturation (Chale-Rush et al. 2007a, 2007b; Stewart et al. 2010), or the site of application on the tongue (Mattes 2009b). Thus far, only one of the various fat taste receptor candidates proposed for rodent fat taste perception, CD36, has been investigated in humans. The authors used CD36-specific antibodies to localize this molecule in human foliate and circumvallate papillae, which is in agreement with previous data obtained in rodents (Simons et al. 2010). Taken together, it appears that the mechanism of human FA taste perception might be similar but not identical to the pathway that has been proposed in rodents.

In the present study, we investigated human fat taste perception by sensory and molecular analyses. For the molecular analysis, we focused on FA-sensitive G protein-coupled receptors present in human taste tissue and their pharmacological characterization to explore their possible involvement in gustatory fat perception. We reasoned that 1) a putative fat taste receptor should be located in taste buds on the human tongue and 2) its activation spectrum observed by functional heterologous expression *in vitro* should correlate with human sensory data.

## Materials and methods

### Sensory analyses

For sensory experiments, free FAs (C10:0–C20:4), oleyl alcohol, and linoleyl alcohol purchased from Sigma-Aldrich were emulsified in a triacylglyceride-free lipid matrix (TFL-matrix) containing Gum Arabic (Ph Eur, from acacia tree), ethylenediaminetetraacetic acid (EDTA) (bioUltra), highly refined mineral oil (white oil, mouse embryo tested) from Sigma-Aldrich, and whey protein (100% Natural Whey Protein Isolate) from Olimp. FAs, oleyl alcohol, and linoleyl alcohol were purified by anaerobic low temperature recrystallization from acetonitrile and chromatography, respectively, to afford a purity of each stimulus of >99% (HPLC-ELSD, HRGC-MS).

Twenty assessors, who gave the informed consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, participated for at least 2 years in sensory training sessions and were asked to recognize and quantify the taste of aqueous solutions (5 mL each) of the following purified reference compounds dissolved in bottled water (Evian, low mineralization: 500 mg/L) by means of duo and triangle tests, respectively, using a sip-and-spit procedure (Schlutt et al. 2007): sucrose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (12 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, monosodium glutamate (8 mmol/L, pH 5.7) for umami taste, an aqueous emulsion of oleic acid (1 mmol/L, containing 0.02% Emultop) for fatty taste, a water/sunflower oil emulsion (3/6 w/w, containing 0.5% Span 65) for oily mouthfeel, and an emulsion of stearic acid (1 mmol/L, containing 0.02% Emultop) for a grainy powdery mouthfeel. From these trained panelists, a group of 12 assessors (4 males, 8 females, 26–40 years) were selected in a preliminary screening test by verifying their oral fatty acid sensitivity using oleic acid (1.4 mmol/L) as the test stimulus (Stewart et al. 2010).

The sensitive subjects were asked in a qualitative evaluation to freely describe the orosensation of TFL emulsions of oleic acid and linolenic acid, respectively, in concentrations of 1.4 and 14.0 mmol/L. At the lower concentration, the descriptors “fatty,” “more lipid-rich,” and “creamy” were selected, whereas “scratchy,” “raspy,” and “burning” were reported to be predominant at 14.0 mmol/L. By means of a consensus evaluation, the panel agreed on using the terms “fatty” and “scratchy” for one or the other orosensation. The sensory sessions were performed in an air-conditioned room (22 °C) with separate booths in 3 independent sessions. The subjects were instructed to refrain from food, beverages, and oral care products for a minimum of 2 h before the sensory experiments. To prevent cross-modal interactions with odorants, the panelists used nose clips.

For the determination of threshold concentrations, each chemical stimulus was dissolved in the TFL-matrix to

minimize textural cues the stimulus may impart (Chale-Rush et al. 2007a, 2007b). The stimuli capric acid (C10:0), lauric acid (C12:0), oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidonic acid (C20:4), oleyl alcohol, and linoleyl alcohol were added to an aqueous solution containing 5.0% (w/v) Gum Arabic to minimize viscosity cues and 0.01% (w/v) EDTA to prevent free FA oxidation and, after ultrasonification for 20 min at 40 °C under an atmosphere of argon, 0.3% (w/v) whey protein and 5.0% (w/v) highly refined white oil were added as a control for lubricity, followed by homogenization for 20 s. Because capric acid (C10:0) and lauric acid (C12:0) are solid at room temperature, they were presented at 50 °C. All other samples were presented at 22 °C. Each sample solution was prepared daily and kept in sealed bottles under an argon atmosphere in the dark.

The expected sensitivity range for each individual and the 2 main descriptors used for fatty acids and related compounds, “fatty” and “scratchy,” were determined in pretests. By means of an ascending, 2-alternative forced-choice test of 5–6 sample pairs, detection threshold concentrations were determined for the “fatty” and/or the “scratchy” orosensation of each stimulus. Aliquots (2 mL) of sample dilutions differing by 0.25 log units were presented in the order of increasing concentrations to the panelists in 3 different sessions using a sip-and-spit procedure. Between the sample pairs, the panelists were asked to wait for 5 min and to rinse the oral cavity with water. In case of a correct selection by the panelist, the same concentration was presented again aside one blank as a proof for identification. An incorrect identification was followed by presentation of the next higher concentration. The duration of the individual test sessions did not exceed 25–30 min. The individual taste threshold concentration of each panelist was calculated as the geometric mean between the last incorrectly identified sample and the sample solution correctly identified 3 times consecutively. The threshold of the panel was calculated from the geometric means of all individual threshold concentrations ( $P < 0.05$ ). In an additional set of experiments, the oral detection thresholds of oleic acid and linoleic acid were determined in bottled water containing 0.01% (w/v) EDTA using the same protocol.

### Viscosity measurements

Aliquots (15 mL) of emulsions of linoleic acid and linoleyl alcohol (0.001–10.0 mmol/L) in TFL-matrix were each analyzed by means of an LVDV II Pro Extra Brookfield viscosimeter at room temperature using a UL Adapter for low viscosity materials. The system was operated with a shear rate of 50 s<sup>-1</sup> representing the shear rate in the mouth (Wood and Goff 1973). Each concentration step was measured 6 times and the average was used for calculations.

### Reverse transcription-polymerase chain reaction

Reverse transcription-polymerase chain reaction (PCR) was performed as before (Behrens et al. 2006, 2007). Biopsy

materials of human CV, fungiform papillae, and nongustatory lingual epithelium were obtained from patients requiring oral surgery and subjected to RNA extractions using TRIzol reagent (Invitrogen). Total RNAs from human colon and pancreas were purchased from BD Biosciences Clontech. For removal of contaminating genomic DNA, RNAs were subjected to RNase-free DNase I (Invitrogen) digestion for 30 min at room temperature according to the instructions of the manufacturer. cDNA was synthesized using SuperScript II reverse transcriptase and random hexamers (Invitrogen) following the recommendations of the manufacturer. Identical reactions omitting reverse transcriptase were performed to generate negative control templates. Subsequently, the templates were used for amplifications of FA-sensitive molecules, of hTAS2R16 cDNA to control for the presence or absence of taste cells, and of human GAPDH cDNA to demonstrate

cDNA quality. Oligonucleotide sequences are given in Table 1 (oligonucleotides 9–13). PCRs were performed using *Taq*-DNA polymerase (Fermentas) and the following conditions: 30 s at 94 °C for denaturation, 30 s to 1 min at the respective annealing temperature, and 1 min at 68 °C for elongation (40 cycles for fat and bitter sensors, 30 cycles for GAPDH). Amplified products were visualized by agarose gel electrophoresis.

Immunohistochemistry of human circumvallate papillae

Human CVs were embedded in paraffin and 5 µm cross-sections were prepared and stored at 4 °C. The sections were deparaffinized and rehydrated by passing them through toluene and a graded ethanol series. Antigen retrieval was achieved by heating the sections in sodium citrate buffer (1 mM trisodium citrate, pH 6.0) for 20 min at 95–99 °C.

Table 1 Oligonucleotides (Eurofins mwg Operon) used in cloning (#1–8) and RT-PCR (#9–13)

#	Oligonucleotide <sup>a</sup>		Sequence (5'–3')	Annealing (°C)	Amplicon size (bp)
1	hGPR120	fw	GAATTCCACCATGTCCCCTGAATGCGCGCG	60	1086
		rv	GCGGCCGCTTAGCCAGAAATAATCGACAAGTCATTTC		
2	mGPR120	fw	GAATTCACCATGTCCCCTGAGTGTGCACA	66	1086
		rv	GCGGCCGCTTAGCTGGAATAACAGACA		
3	hGPR40	fw	GAATTCACCATGGACCTGCCCCGCAGCTC	60	903
		rv	GCGGCCGCTTACTTCTGGGACTTGCCCCCTTGCG		
4	mGPR40	fw	GAATTCACCATGGACCTGCCCCACAG	60	903
		rv	GCGGCCGCTTACTTCTGAATTGTTCTCTT		
5	hGPR41	fw	TCGAATTCACCATGGATACAGGCCCGACCAG	65	1040
		rv	TCTGCGGCCGCTAGCTTTCAGCACAGGCCACC		
6	hGPR43	fw	TCGAATTCACCATGCTGCCGACTGGAAGAGC	65	993
		rv	TCTGCGGCCGCTTACTCTGTAGTGAAGTCCGAACCTGGC		
7	Exon1/2 GPR120	rv	GTTCCGAGGTCTGTAAAATTTGGAGTAACTGATC	64	707
8	Exon3/4 GPR120	fw	TACAGACCTCGGAACACCTCCTG	61	442
		rv	hGPR120		
9	hGPR120S_RT	fw	CGATTTGCACACTGATTGGC	61	402
		rv	TGCACAGTGCATGTTGTAGA		
10	hGPR120L_RT	fw	GCTGTCGTGACTCACAGTG	61	303
		rv	hGPR120S_RT		
11	hGPR40_RT	fw	CTGCTGCTGACAGTCTCTCTG	60.8	315
		rv	CAGAAGGCTGTGATGGCCAAG		
12	hTAS2R16_RT	fw	CCTGGGAATTTTTAATATCCTTACATTCTGGT	63	449
		rv	GAAGCGCGCTTTCATGCT T		
13	hGAPDH_RT	fw	ACCACAGTCCATGCCATCAC	58	454
		rv	TCCCACCACCCTGTTGCTGTA		

<sup>a</sup>fw: forward primer, rv: reverse primer.

Immunohistochemical staining was performed using the diaminobenzidine (DAB)-staining method. Endogenous peroxidases were blocked for 30 min in 1× phosphate-buffered saline with 3% methanol, 3% H<sub>2</sub>O<sub>2</sub> and sections were incubated for 60 min with blocking solution (0.1% gelatin, 0.2% Triton X-100, 5% normal horse serum in Tris-buffered saline [TBS]). Sections were incubated overnight at 4 °C with primary antibody (LS-A2003, MBL, 1:500) diluted in TBS with 3% normal horse serum. For negative control experiments, the antibody was applied after preabsorption with the antigenic peptide 1:4 (LS-P2003, Lifespan). Next, the sections were washed and biotinylated goat anti-rabbit antibody was applied at a dilution of 1:500 (BA-1000 Vector Laboratories) for 1–2 h at RT. After washing and incubation with streptavidin-horseradish peroxidase (Vectastain ABC Elite kit, Vector Laboratories) for 30 min, sections were stained using DAB as substrate (DAB Substrate Kit for peroxidases, Vector Laboratories) according to the manufacturer's instructions. Following dehydration, the sections were mounted with Vectastain water-free mounting medium (Vector Laboratories). Images were taken with a microscope (Axioplan; Zeiss) connected to a CCD camera (RT slider; Diagnostic Instruments).

#### In situ hybridization of human circumvallate papillae

Probes specific for human GPR40 and GPR120 were prepared from fragments generated by restriction digests of full-length cDNA clones using *Xba* I/*Xho* I and *Pst* I, respectively. The resulting 903 bp (GPR40) and 656 bp (GPR120) fragments were subcloned into the vector pBluescript (Stratagene) and subsequently linearized with *Eco*R I/*Not* I or *Xba* I/*Xho* I. This was followed by in vitro transcription with T3 or T7 RNA polymerase (Roche) for sense and antisense riboprobes, respectively. The probes were labeled using Dig-RNA-labeling mix (Roche), treated with RNase-free DNase I (Invitrogen), and then purified by ethanol precipitation.

In situ hybridizations were performed mainly as before (Behrens et al. 2007). Briefly, 10 µm cross-sections of human CV were processed and thaw mounted onto positively charged glass slides. Prior to hybridization sections were fixed, permeabilized, and acetylated. Prehybridization was done at 58 °C for 5 h, hybridization overnight at 58 °C. After hybridization, the slides were washed several times at low stringency and incubated with RNaseA (Roche). Specimens were then washed under high stringency conditions in 0.4× saline-sodium citrate buffer at 50 °C. Hybridized riboprobes were detected using an anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche Applied Science) and a colorimetric reaction (NBT/BCIP). Images were taken with a microscope (Axioplan; Zeiss) connected to a CCD camera (RT slider; Diagnostic Instruments).

#### Cloning of human and mouse GPR40, GPR41, GPR43, and GPR120

Human (h) GPR41 and GPR43 and human and mouse (m) GPR40 and GPR120 cDNAs were amplified by PCR using

gene-specific primers spanning the entire coding region and subsequently subcloned into the vector pCDNA5/FRT (Invitrogen). mGPR120, hGPR40, hGPR41, hGPR43, and hGPR120 were amplified from commercially available cDNA clones (Imagenes). Mouse GPR40 was amplified from C57/BL6 genomic DNA. Human GPR120 (accession # BC101175) corresponds to a reported splice variant lacking exon 3 (Moore et al. 2009) and is used in all functional experiments unless stated otherwise. The long splice variant of GPR120 (accession # NM\_181745) (Hirasawa et al. 2005) was reconstructed by PCR-mediated recombination (Fang et al. 1999) from colon cDNA and the short GPR120 variant as follows: The cDNA sequence corresponding to exons 1–2 was amplified from the plasmid using a vector-specific forward primer in combination with a GPR120-specific reverse primer. The reverse primer consisted of 25 bases specific to exon 2 at its 3'-end and at its 5'-end an overhang of 10 bases corresponding to exon 3 of the human GPR120 gene to facilitate subsequent recombination with exons 3–4. Exons 3–4 were amplified from human colon cDNA using GPR120-specific forward and reverse primers. The forward primer used for this amplification consisted of 5 bases of the common exon 2 and 18 bases specific to exon 3, which is unique to the long GPR120. The reverse primer was the same used to amplify short GPR120. Next, the 2 fragments were mixed and the overlap of 15 bases between the 2 PCR products allowed to effectively join exons 1–2 with 3–4 in a second PCR (for oligonucleotide sequences 1–8, annealing temperatures, and amplicon sizes, see Table 1). PCRs were performed with *Pfu*-DNA polymerase (Fermentas) using 30 cycles of 30 s, 95 °C for denaturation, 1 min at the respective annealing temperature, and 1 min at 72 °C for elongation. Amplified products were purified from agarose gels using the High Pure PCR product Purification kit (Roche), digested with *Eco*R I and *Not* I (Fermentas) and ligated with the *Eco*R I/*Not* I-digested vector. After transformation into competent cells, the constructs were purified and checked for their integrity by sequencing (Eurofins mwg Operon).

#### Functional calcium-imaging analyses

Sodium salts of the corresponding FAs as well as oleyl and linoleyl alcohol for the in vitro experiments were obtained from Sigma-Aldrich. Stock solutions were prepared fresh before each experiment and then diluted in C1 buffer (130 mM NaCl, 5 mM KCl, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, 2 mM CaCl<sub>2</sub>, 10 mM glucose; pH 7.4) to the working concentrations. The stock solutions were prepared as follows: 100 mM propionic acid (C3), 50 mM capric acid (C10), and 5 mM linoleic acid (C18:2) in C1 buffer; 10 mM oleic acid (C18:1) and 100 mM oleyl alcohol in 100% ethanol; 500 mM linolenic acid (C18:3) in 100% dimethyl sulfoxide (DMSO); 50 mM linoleyl alcohol in 100% ethanol; and 10mM arachidonic acid (C20:4) in 100% methanol. To avoid toxic effects when applying to the cells, the used

solvents did not exceed 0.1% (v/v) DMSO or 0.3% (v/v) ethanol or methanol. Transfection and functional calcium-imaging analyses were performed as before (Brockhoff et al. 2007; Reichling et al. 2008; Slack et al. 2010). Briefly, HEK 293T and HEK 293T cells stably expressing  $G\alpha_{16}$ gust44 were maintained in Dulbecco's Modified Eagle medium (Gibco) containing 10% fetal bovine serum (Biocrom), 4.5 g/L glucose (Invitrogen), 2 mM L-glutamin (Invitrogen), 100 U/mL penicillin (Sigma), and 100 mg/mL streptomycin (Sigma) at 37 °C, 5% CO<sub>2</sub>. The cell line stably expressing the  $G\alpha_{16}$ gust44 was grown in the presence of 400 µg/mL G418 (Calbiochem). For calcium-imaging experiments, cells were seeded into 10 µg/mL poly-D-lysine-coated 96-well black clear-bottom plates (µClear; Greiner) and ~24 h later at ~60% confluency transiently transfected using Lipofectamine 2000 (Invitrogen). For transient transfection of HEK 293T- $G\alpha_{16}$ gust44 cells, 150 ng of the GPR40, GPR41, GPR43, or GPR120 constructs were applied per well. For the transient cotransfection of HEK 293T cells with constructs coding for G protein chimeras and receptor cDNAs, mixtures of equal amounts of chimeric G protein constructs and GPR40 or GPR120 constructs were prepared and transfected. After 22 h, cells were loaded for 1 h with 1 µM of the Ca<sup>2+</sup>-sensitive dye Fluo-4 AM (Invitrogen) in the presence of 2.5 mM probenecid in serum free medium. After washing with C1 buffer, cells were stimulated with the agonists at concentrations referred to in the figures. Receptor responses were measured using a fluorometric imaging plate reader (FLIPR<sup>TETRA</sup> system, Molecular Devices). Calcium traces from 2 independent experiments carried out in duplicates were processed with SigmaPlot 9.0 and presented as the mean ± standard error of the mean. Plots of the amplitudes versus concentrations were fitted by nonlinear regression to the function  $f(x) = 100/[1 + (EC_{50}/x)^{nH}]$ , where  $x$  is the agonist concentration and  $nH$  is the Hill coefficient, allowing us to calculate EC<sub>50</sub> values of activation. The peak fluorescence after compound addition was normalized to background fluorescence ( $\Delta F/F = (F - F_0)/F_0$ ) and baseline noise was subtracted.

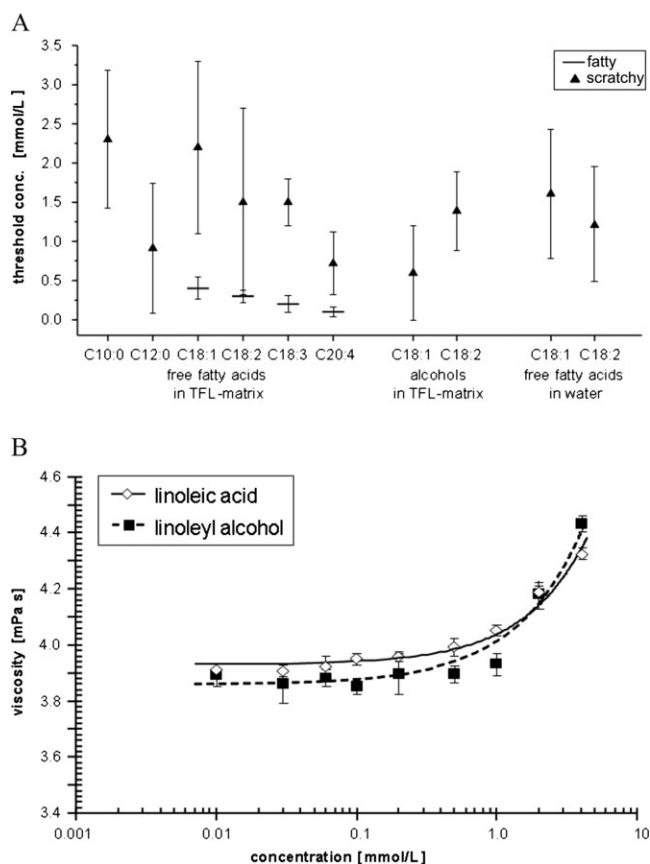
## Results

### FA taste perception in human subjects

Based on the observation that gustatory neurons that respond to fat also respond to texturally similar but tasteless substances such as silicon oil and paraffin oil (Rolls et al. 1999), sensory analysis with test compounds were performed in TFL-matrix containing mineral oil. The masking of the textural component of oral fat sensing in the samples including taste stimulus-free controls allows the investigation of putative gustatory cues provided solely by the test substances. Preliminary sensory experiments revealed that the subjects recognized a concentration-dependent “fatty” sensation and a burning “scratchy” sensation when the free

FAs were dissolved in a TFL-matrix. Next, we determined, by means of ascending 2-alternative forced-choice tests, the oral thresholds of these sensations in subjects who tasted the free FAs C10:0, C12:0, C18:1, C18:2, C18:3, and C20:4 (Figure 1). The unsaturated FAs, C18:1–C20:4 induced a “fatty” taste perception in the subjects with threshold concentrations between 0.1 and 0.4 mM, whereas the “scratchy” oral sensation was detected at higher threshold concentrations of up to 2.2 mM (Figure 1A). At least for C18:1, C18:3, and C20:4, the threshold concentrations for the “scratchy” orosensation were significantly above the values found to induce the “fatty” percept. The “scratchy” orosensation also showed a higher interindividual variance relative to the “fatty” sensation (Figure 1A). In contrast, the shorter saturated FAs, C10:0 and C12:0 did not elicit any “fatty” taste impression, whereas they caused the “scratchy” sensation at threshold concentrations that resembled those of the unsaturated LCFA.

In order to investigate the influence of the carboxyl group of free FAs on fat taste perception, the detection thresholds of the fat alcohols oleyl alcohol and linoleyl alcohol were



**Figure 1** Sensory responses of human subjects to various FAs and corresponding alcohols. **(A)** Detection threshold concentrations determined for the “fatty” and “scratchy” oral sensation induced by free FAs and selected alcohols in the triacylglyceride-free lipid-like (TFL)-matrix and in water, respectively. **(B)** Influence of linoleic acid (open diamonds) and linoleyl alcohol (black squares) on the viscosity of the TFL-matrix (95% confidence interval).

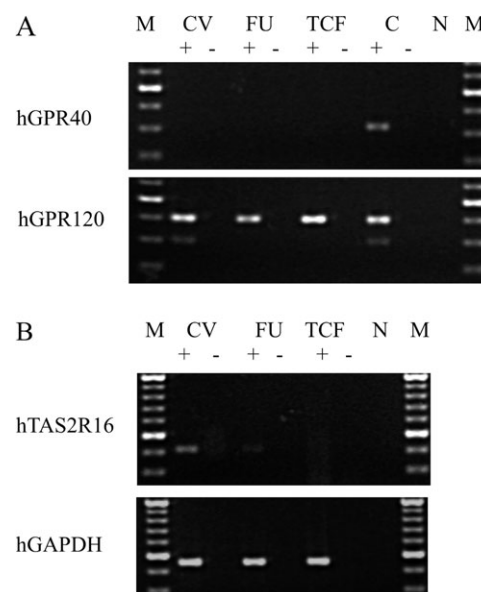
each determined in the TFL-matrix. All panelists described the taste of both substances as “scratchy” only, whereas any “fatty” perception was lacking (Figure 1A).

In order to rule out that the “fatty” taste of linoleic acid in the TFL-matrix is caused by increased viscosity, the TFL-matrix was spiked with linoleic acid or linoleyl alcohol at concentrations ranging from 0.01 to 1.3 mM and the viscosities determined. Figure 1B shows that linoleic acid did not influence the viscosity of the matrix up to a concentration of ~1.0 mM. This concentration is about 4-fold higher than the detection threshold of the “fatty” orosensation elicited by this compound (Figure 1A). The corresponding alcohol also did not significantly change the viscosity of the TFL-matrix in this concentration range. Thus, the data demonstrate that the difference in fat taste perception observed between the TFL-matrix spiked with linoleic acid and the blank control is not due to minor differences in viscosity and suggest that the “fatty” taste of free FAs is generated through chemosensory mechanisms.

As mentioned already at the beginning of this section, fat-sensitive neurons were found to respond also to texturally similar paraffin oil (Rolls et al. 1999). Because the unsaturated LCFAs were shown to induce a “fatty” oral sensation if suspended in the TFL-matrix, the question arose as to whether the “fatty” taste sensation of these compounds is also perceived in the absence of the textural component. Therefore, the detection threshold of oleic acid and linoleic acid was determined in water containing 0.01% EDTA only. Both oleic acid and linoleic acid induced a “scratchy” orosensation at concentrations above 1.2 mM, but any “fatty” taste was lacking in the absence of the TFL-matrix (Figure 1A). These data strengthen the assumption that the perception of fat taste by subjects might be due to a convergence of receptor-mediated FA detection and mechanosensory inputs.

### Expression analysis

In order to study whether the G protein–coupled receptors, GPR40 and GPR120, participate in the transduction mechanism of oral fat detection in humans as shown recently for rodents (Cartoni et al. 2010), we examined, by RT-PCR, their expression in human CV and fungiform papillae as well as in lingual taste cell-free epithelium (Figure 2A). We included RNA preparations from tissues known to express GPR40 (pancreas) and GPR120 (colon) genes (Figure 2A, lanes C) as positive controls to demonstrate that the experimental conditions allow successful amplification of the target sequences. The lack of DNA fragments specific for GPR40 (upper panel) in lanes corresponding to reverse transcribed RNA (+) documents that the corresponding mRNA is absent from CV, fungiform papillae, and nongustatory epithelium. In contrast, GPR120 mRNA is present in CV, fungiform papillae, and taste cell-free lingual epithelium (lower panel). The amplification of GPR120 cDNAs from CV and colon



**Figure 2** Expression analysis of fat taste sensor candidates by RT-PCR. **(A)** cDNAs originating from different human gustatory and nongustatory tissues were used as templates to study the expression of GPR40 and GPR120. **(B)** GAPDH was amplified to control for the presence of cDNA throughout the samples. The presence of gustatory cells in the tissue preparations was assessed by the amplification of cDNA specific for the bitter receptor hTAS2R16. M, molecular weight marker; CV, cDNA from circumvallate papillae; FU, cDNA from fungiform papillae; TCF, cDNA from taste cell-free lingual epithelium; C, cDNAs from human control tissues (GPR40, pancreas; GPR120, colon); N, H<sub>2</sub>O negative control; +, reaction including reverse transcriptase; –, control reaction without reverse transcriptase.

yielded additional PCR products smaller than the expected size of 402 bp. Cloning and sequencing of these minor by-products revealed that in both cases, the fragments represented truncated forms of the GPR120 cDNA lacking 107 bp of the central portion of exon 4. We considered these sequences as PCR artifacts. In order to demonstrate the presence of gustatory cells in CV and fungiform epithelium and the absence of such cells in preparations of surrounding non-sensory epithelium, we amplified cDNA specific for the bitter taste receptor gene hTAS2R16 (Figure 2B, upper panel) whose expression is well documented in taste buds (Bufe et al. 2002; Behrens et al. 2007). As predicted, hTAS2R16-specific cDNA was detectable in CV and fungiform samples but not in samples originating from nonsensory epithelium. Amplification of the housekeeping gene GAPDH was included to demonstrate the presence of cDNA in all samples treated with reverse transcriptase (Figure 2B, lower panel). From these experiments, one can conclude that due to the absence of detectable expression of the GPR40 in gustatory papillae, GPR40 cannot be considered as a candidate fat taste sensor in man. GPR120, on the other hand, is present in gustatory and nongustatory lingual epithelia, which may indicate a role in FA taste perception, although the cellular origin of GPR120 mRNA remains to be determined.

To identify the lingual cell type(s) expressing GPR120, we performed *in situ* hybridization and immunohistochemical experiments. First, we hybridized cross-sections of CV with digoxigenin-labeled riboprobes specific for human GPR120. Signals obtained with the GPR120 antisense probe were visible in taste bud cells (Figure 3A, solid arrowheads) as well as in cells of surrounding lingual epithelium (Figure 3A, open arrowheads). The absence of signals in sections hybridized with the sense probe demonstrated the specificity of the hybridization conditions (Figure 3B). Secondly, to confirm the results obtained by *in situ* hybridization and to localize GPR120 protein we used an antiserum directed against human GPR120 to analyze human CV sections. Figure 3C shows that GPR120-like immunoreactivity was concentrated in the apical part of a subset of taste bud cells (solid arrowheads, Figure 3C) as well as in epithelial cells outside of taste buds (open arrowheads, Figure 3C). Sections incubated with the antibody preabsorbed with antigenic peptide showed no staining (Figure 3D) demonstrating the specificity of the signals. Thus, these data are in agreement with our RT-PCR results and verify the presence of GPR120 in taste bud cells of the CV and nonsensory lingual cells.

#### Characterization of the agonist selectivity of human FA-sensitive GPRs

An important prerequisite for candidate fat taste receptors is that the pharmacological response profile of the receptor(s) in question should match data obtained by sensory experiments. Our human sensory data demonstrated that FA emulsions described as “fatty” by our panelists (Figure 1A) contained FAs with more than 12 carbon atoms. Therefore, if the oral

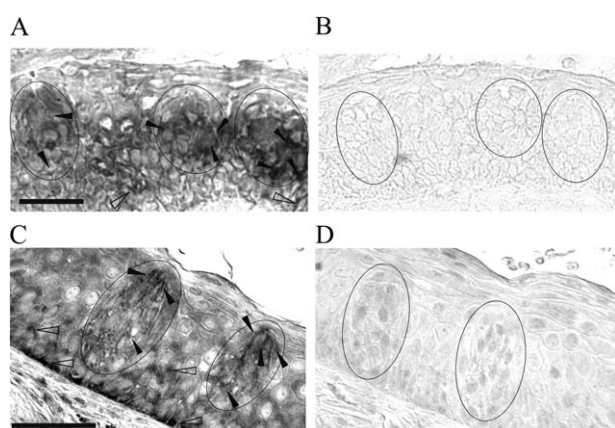
detection of dietary lipids would rely on FA-sensitive GPCRs, receptors preferentially activated by FAs of longer chain lengths are more likely candidates for gustatory FA-sensing molecules than receptors responding to FAs of shorter chain lengths.

We therefore performed functional calcium-imaging experiments with cDNA of GPR120 transiently expressed in HEK 293T-G $\alpha_{16}$ gust44 cells and various FAs of different chain lengths as stimuli. Although the lack of expression in human gustatory tissue argues against an involvement of GPR40 in human fat taste perception, we included GPR40 cDNA in the functional experiments. Moreover, to evaluate the specificity of heterologously expressed FA receptors, we also included 2 additional receptors, reported to respond to short-chain fatty acids (SCFAs), GPR41 and GPR43, for an initial screening experiment (Figure 4A).

In agreement with published pharmacological data, human GPR41 and GPR43 (Brown et al. 2003; Le Poul et al. 2003) responded exclusively to the SCFA propionic acid (C3:0) (Figure 4A). In marked contrast, GPR40 (Itoh et al. 2003) and GPR120 (Hirasawa et al. 2005) are not activated by SCFA but by both medium-chain fatty acids (MCFAs) and LCFA. Both receptors responded to the application of decanoic acid (C10:0) representing MCFAs. Their activation was even more pronounced when the unsaturated LCFA linoleic acid (C18:2) was applied. Whereas GPR120 exhibited a clear preference for linoleic acid versus decanoic acid, a similar preference was not observed for GPR40. Hence, whereas GPR41 and GPR43 are, as predicted, not activated by FAs of “fatty” taste-relevant chain length, the response profiles of both GPR40 and GPR120 would qualify them as fat taste sensors.

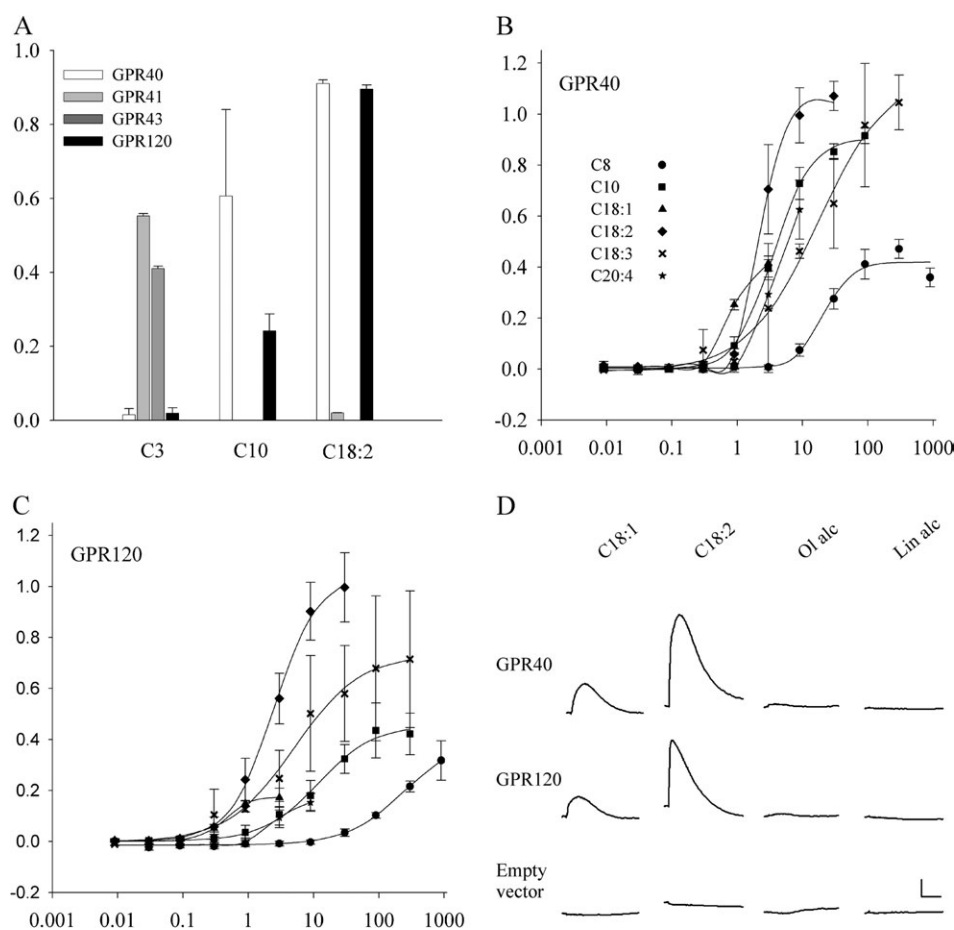
In order to address the question whether the pharmacological properties of FA-sensitive GPCRs match the sensory data shown in Figure 1 in more detail, we now focused on GPR40 and GPR120 and monitored concentration–response curves for both receptors with an almost identical set of substances *in vitro* as we used for the *in vivo* analyses (Figure 4B,C). Due to solubility limitations, we substituted the FA C12:0 used for sensory analyses for an additional shorter FA, namely C8:0. Nevertheless, we observed that not all concentration–response curves reached saturation (C8:0 and C18:2 on GPR120, C18:1 and C18:3 on GPR40, and C20:4 on both receptors).

Both MCFA and LCFA activated GPR40 as well as GPR120 in the micromolar concentration range (Figure 4B,C). Surprisingly, C18:1 and C20:4 exhibited apparently low efficacies despite being potent agonists for GPR40 and GPR120. Although the low efficacy of C18:1 would be consistent with previous observations done in rats, which demonstrated that oleic acid is a weaker orosensory stimulus than linoleic and linolenic acid (Tsuruta et al. 1999), it should be noted that the applicable maximal concentrations for both substances, C18:1 and C20:4, were limited because of artifacts occurring at higher concentrations in cells that were



**Figure 3** Cellular expression pattern of GPR120 in human circumvallate papillae. (A) Cryostat cross-section hybridized with digoxigenin-labeled antisense riboprobe specific for GPR120 mRNA. (B) Control experiment using a sense riboprobe specific for GPR120. (C) Immunoreactivity in a subset of intragemmal cells obtained by indirect staining of the section with anti-GPR120 antiserum. (D) Control experiment conducted with anti-GPR120 antiserum preabsorbed with immunogenic peptide. Taste buds are indicated by circles. Solid arrowheads point to selected positive taste bud cells. Open arrowheads point to positive mucosal cells. Scale bars = 50  $\mu$ M.





**Figure 4** Pharmacological characterization of human GPR40 and GPR120. HEK 293T cells stably expressing  $G\alpha_{16}$ Gust44 were transiently transfected with GPRs as indicated. **(A)** Stimulation with a single concentration (10  $\mu$ M) of the FAs: C3, C10, and C18:2. White bars, GPR40; light gray bar, GPR41; dark gray bar, GPR43; black bar, GPR120. y axis, changes in fluorescence relative to basal fluorescence. **(B)** Concentration–response curves of hGPR40 and **(C)** GPR120 using FAs increasing in number of C-atoms and double bonds. Circles, C8; squares, C10; triangles, C18:1; diamonds, C18:2; crosses, C18:3; stars, C20:4. y axis, changes in fluorescence relative to basal fluorescence; x axis, concentration in  $\mu$ M. **(D)** Stimulation of GPR40 and GPR120 with oleic acid (C18:1), linoleic acid (C18:2), or the corresponding fat alcohols, oleyl alcohol (Ol alc) and linoleyl alcohol (Lin alc). Changes in fluorescence after substance application were monitored. Scaling of traces is indicated by the insert at the bottom right of the figure: y axis, changes in fluorescence relative to basal fluorescence = 200 relative light units; x axis, time = 100 s.

not transfected with receptor constructs (mock transfection, negative controls). C18:2 and C18:3 are good agonists of both receptors. Whereas the EC<sub>50</sub> value obtained for C18:2 was lower for GPR40 ( $1.37 \pm 0.12 \mu$ M) than for GPR120 ( $\geq 4.3 \mu$ M), GPR120 ( $5.03 \pm 1.8 \mu$ M) was more sensitive to C18:3 than GPR40 ( $\geq 17.5 \mu$ M). Although in general, the 2 receptors responded rather similar to the various agonists, GPR40 is clearly more sensitive to C8:0 and C10:0, reaching higher amplitudes and having lower EC<sub>50</sub> values. Whereas GPR40 responded to C8:0 and C10:0 with EC<sub>50</sub> values of  $25.23 \pm 1.08$  and  $3.56 \pm 0.14 \mu$ M, respectively, the signals of GPR120 challenged with C8:0 did not reach saturation, and the EC<sub>50</sub> value observed for C10:0 was  $\sim 3$  times higher ( $11.93 \pm 2.05 \mu$ M). Moreover, the maximal response amplitude of GPR120 challenged with C10:0 reached only half of the maximal signal observed for GPR40 suggest-

ing that C10:0 is a full agonist for GPR40 but only a partial agonist for GPR120.

The fact that GPR120 in vitro shows a more pronounced selectivity for LCFA compared with MCFA is somewhat more consistent with our sensory data than the pharmacological profile obtained for GPR40. However, the finding that our human panelists associated a “fatty” taste perception strictly with LCFA and not with MCFA that were associated with a “scratchy” taste (Figure 1A), raised concerns whether GPRs may rather be involved in the detection of the “scratchy” sensation. We therefore performed additional experiments to test whether GPR40 and GPR120 might be involved in the “fatty” or “scratchy” taste sensations or both. To this end, we challenged the receptors with the FA C18:1 and C18:2, which elicited both “fatty” and “scratchy” sensations and their respective alcohols, oleyl and linoleyl

alcohol, which were described only as tasting “scratchy” but not “fatty.” Both receptors, GPR40 and GPR120, responded exclusively to the FA C18:1 and C18:2, whereas they were insensitive to the corresponding alcohols (Figure 4D).

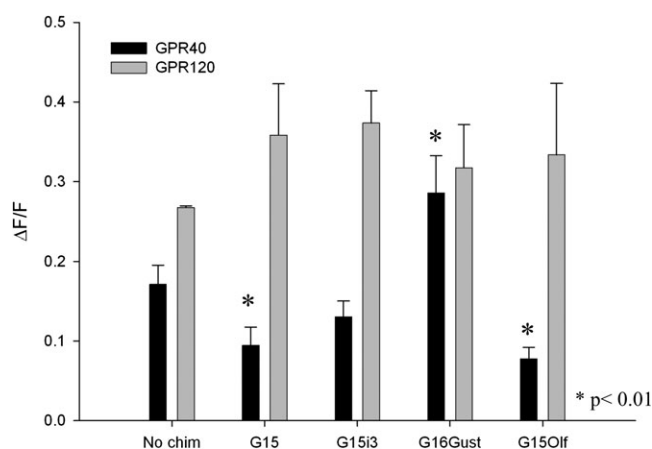
### Analysis of the G protein selectivity of GPR40 and GPR120

The functional experiments performed so far were done using HEK 293T cells stably expressing the chimeric G protein  $G\alpha_{16}\text{gust44}$ . However, whether the robust responses observed for GPR40 and GPR120 in the previous experiments may indicate preferential coupling to  $\alpha$ -gustducin, the “gustatory G protein” present in type II taste receptor cells (McLaughlin et al. 1992; Wong et al. 1996; Ming et al. 1999; Ruiz-Avila et al. 2001), thereby providing circumstantial evidence for a gustatory function of both receptors, remained to be determined. To examine the specificity of GPR40 and GPR120 in G protein coupling, we transiently coexpressed each receptor with different chimeric  $G\alpha$  subunits and determined their functional responses upon stimulation with linoleic acid. The G protein chimeras consisted of N-terminal parts originating from the promiscuous  $G\alpha_{15}$  or  $G\alpha_{16}$  subunits (Offermanns and Simon 1995; Zhu and Birnbaumer 1996) to activate the phosphatidylinositol/calcium pathway, whereas the C-terminal parts responsible for receptor coupling (Conklin and Bourne 1993; Conklin et al. 1993; Lichtarge et al. 1996; Bourne 1997; Ueda et al. 2003) were derived from various G protein  $\alpha$  subunits reflecting  $G_q$  ( $G\alpha_{15}$ ),  $G_i$  ( $G\alpha_{15} - G\alpha_{i3}$ ),  $G_s$  ( $G\alpha_{15} - G\alpha_{s/Olf}$ )—types of  $G\alpha$  subunits in addition to  $G\alpha_{16}\text{gust44}$ .

HEK 293T cells transiently transfected with either GPR40 or GPR120 robustly responded to stimulation with an  $EC_{50}$  concentration of C18:2 (calculated from the dose–response curves in Figure 4B,C) even in the absence of any exogenous G protein chimera indicating efficient coupling to endogenous G proteins (Figure 5). Whereas the responses of cells coexpressing GPR120 (gray bars) with any of the G protein chimeras showed only minor deviations from responses of cells that were only transfected with the GPR120 construct, stimulation of cells expressing GPR40 (black bars) revealed larger differences. While the cotransfection of GPR40 with  $G\alpha_{16}\text{gust44}$  resulted in moderately elevated receptor activation,  $G\alpha_{15}$ ,  $G\alpha_{15} - G\alpha_{i3}$ , and  $G\alpha_{s/Olf}$  cotransfection had the opposite effect.

### Expression and function of GPR120 splice variants

Thus far, we performed the functional characterization of GPR120 with a cDNA coding for a 362 amino acid protein (BC101175), which is 1 of 2 splice variants differing by 16 amino acids from a larger protein (NM\_181745) which was originally characterized by Hirasawa et al. (2005). The 16 amino acids present in the larger variant are coded by 48 bp of the alternative exon 3 of the human GPR120 gene (Moore et al. 2009). Since taste system-specific receptor

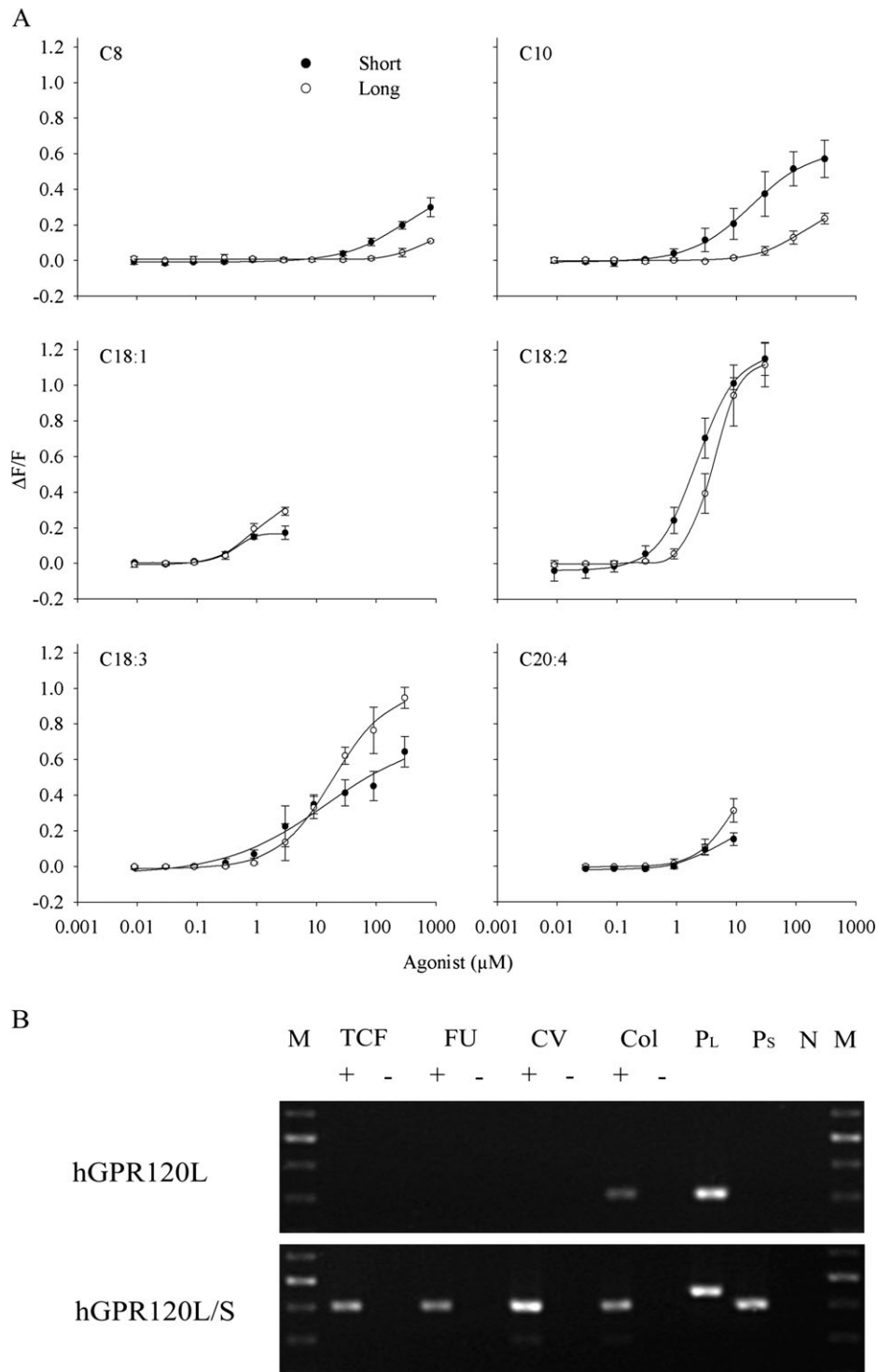


**Figure 5** Analysis of the G protein specificity of GPR40 and GPR120. The receptors GPR40 (black bars) and GPR120 (gray bars) were transiently coexpressed in HEK 293T cells with different G protein chimeras. To measure responses mediated by endogenous G proteins, the receptor constructs were cotransfected with empty vector. Cells were stimulated with C18:2 at a concentration corresponding to previously determined  $EC_{50}$  values (2  $\mu$ M for GPR40, 3  $\mu$ M for GPR120). Significance: \* $P < 0.01$ .

variants were identified in the past, for example, a truncated form of mGluR4 (Chaudhari et al. 2000), a putative additional umami receptor, and alternative splicing of human GPR120 have been reported (Moore et al. 2009), we next screened for possible functional differences of alternatively spliced variants of GPR120 and investigated their presence in human lingual epithelium.

To identify putative pharmacological differences between long and short GPR120 splice variants, the corresponding cDNAs were expressed in HEK 293T- $G\alpha_{16}\text{gust44}$  cells and tested for function. Concentration–response relationships were determined with various MCFAs and LCFAs (Figure 6A). Overall, the responses of both receptor variants to the tested FAs are rather similar to each other. The best agonists showing the highest amplitudes are C18:2 and C18:3. The most pronounced differences between the 2 variants were seen using MCFA as stimuli. Both compounds, C8:0 and C10:0, elicited signals with larger amplitudes in cells transfected with the smaller GPR120 variant.

We also investigated the occurrence of the 2 GPR120 variants in human lingual epithelium. Therefore, we examined by RT-PCR human CV, fungiform papillae and taste cell-free lingual epithelium as well as colon, a tissue reported to express the long GPR120 variant (Hirasawa et al. 2005) (Figure 6B). DNase I-digested total RNA without the following reverse transcription was used to demonstrate the absence of contaminating genomic DNA. Parallel PCR reactions with cloned splice variants (lanes  $P_L$  and  $P_S$ ) were performed for exact size determination of the amplified fragments. Whereas the long splice variant of GPR120 was detected exclusively in colon (upper panel), the short variant was present in all tissues investigated including lingual tissues (lower panel).



**Figure 6** Characterization of the short and long splice variant of GPR120. **(A)** Each variant was transiently expressed in HEK 293T-G $\alpha_{16}$ Gust44 cells and challenged with various MCFA and LCFAs. Graphs represent concentration–response curves of the short (solid circles) and the long variant (open circles). **(B)** RT-PCR analysis of the expression of the splice variants in gustatory and nongustatory tissues. Upper panel: Specific amplification of the long splice variant (hGPR120L). Lower panel: RT-PCR analysis with oligonucleotides amplifying both GPR120 variants (hGPR120L/S). M, molecular weight marker; TCF, cDNA from taste cell-free lingual epithelium; FU, cDNA from fungiform papillae; CV, cDNA from circumvallate papillae; Col, cDNA from colon serving as positive control; P<sub>L</sub> and P<sub>S</sub>, control amplifications of cloned cDNAs encoding the long and short splice variants, respectively; N, H<sub>2</sub>O negative control; +, reactions including reverse transcriptase; –, reactions without reverse transcriptase.

Comparison between mouse and human GPR40 and GPR120

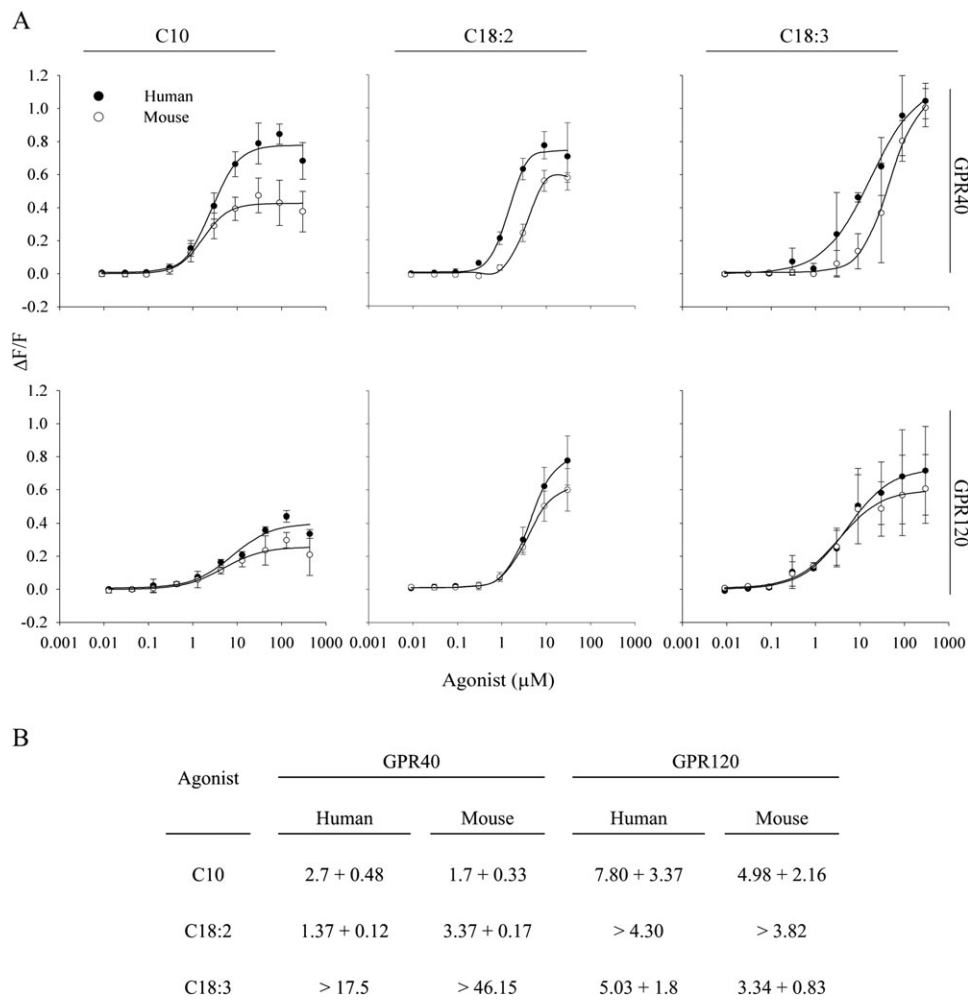
Although the designs of various psychophysical studies performed in rodents and man differ considerably, making a direct comparison difficult, it seems that rodents are more sensitive to the taste of LCFA than human subjects (rat, low- to midmicromolar range (Smith et al. 2000; McCormack et al. 2006; Stratford et al. 2006; Pittman et al. 2007); human, high-micromolar (this study) to low-millimolar range (Chale-Rush et al. 2007a)). Moreover, whereas rats show a higher sensitivity for linoleic acid compared with oleic acid (Tsuruta et al. 1999), human thresholds for these 2 compounds did not deviate substantially (this study and Chale-Rush et al. 2007a). In order to investigate whether the apparent differences in the perception of FAs correlate with pharmacological differences between human and mouse GPR40 and GPR120, we directly compared the functional characteristics of the human and mouse receptors. The amino

acid sequence identity between human (BC120944) and mouse (NT\_039413) GPR40 is 83% and the amino acid sequence identity of human (BC101175) and mouse (BC053698) GPR120 is 82%.

We administered various known agonists to our functional receptor assay and analyzed the calculated EC<sub>50</sub> values and the maximal response amplitudes. The data revealed a trend for mouse GPR40 and GPR120 of lower signal amplitudes and slightly right-shifted dose–response curves relative to their human counterparts (Figure 7). However, the calculated EC<sub>50</sub> values were not significantly different.

Discussion

In the present study, we examined whether free FAs of different chain lengths and derivatives thereof are fat-tasting stimuli for humans. Furthermore, we investigated if FA-sensitive G protein–coupled receptors are expressed in human taste cells



**Figure 7** Functional properties of human and mouse GPR40 and GPR120. **(A)** Each receptor was transiently expressed in HEK 293T-Gα<sub>16</sub>Gust44 cells. After 22 h, cells were stimulated with C10, C18:2, or C18:3. Graphs represent concentration–response curves obtained with mouse (open circles) and human (solid circles) GPR40 (upper panels) or GPR120 (lower panels). **(B)** EC<sub>50</sub> values obtained from the dose–response curves shown in A.

and, by functional heterologous expression assays, we determined the agonist activation patterns of the receptors in question to correlate data from our sensory experiments with *in vitro* results.

Our sensory analysis demonstrated that trained human panelists characterized the stimuli, we have used to induce either of the 2 attributes “fatty” or “scratchy.” In contrast to the “scratchy” perception, which was observed for all stimuli, including MCFA and LCFA dissolved in TFL-matrix or in water, as well as fat alcohols, the “fatty” perception was limited to LCFA in TFL-matrix. Moreover, the threshold concentrations determined for the “scratchy” sensation were in general higher than for the “fatty” sensation and showed larger interindividual variations. These data support 2 main conclusions: First, all test substances are being perceived by the panelists. However, even for the LCFA it depends on the concentrations used for sensory tests, whether they represent a “fatty” or a “scratchy” sensation or a mixture of both. Second, in contrast to the general notion that the texture of fat-rich food items provides a separate stimulus in addition to potential gustatory cues, it appears that the “fatty” taste of LCFA is, in fact, dependent on the presence of the TFL-matrix.

Previous studies on human FA taste perception demonstrated that the threshold concentrations determined for FAs of different chain lengths did not show pronounced differences (Mattes 2009a, 2009b), exhibited rather large variances (Mattes 2009a, 2009b; Stewart et al. 2010), and were not correlated to the degree of saturation (Chale-Rush et al. 2007a, 2007b; Mattes 2009a; Stewart et al. 2010). This is in good agreement with the perceived “scratchy” taste described by our human panelists. For example, for oleic acid, we determined an average detection threshold of 2.2 mM which is identical to the threshold found by Stewart et al. (2010) and in the same concentration range reported in other studies (Chale-Rush et al. 2007a, 2007b). Also the concentration ranges for the detection of lauric acid (C12:0) as reported here and by other studies (Mattes 2009a, 2009b) show a clear overlap although the average threshold values differ somewhat. Hence, at first glance, it appears as if FA detection by human relies on a mechanism that is not distinguishing between FA of different chain length and linked to a rather aversive percept. Indeed, some of the main descriptors used to characterize the taste of the nutritionally most relevant LCFA indicate, apart from “fatty,” a rather unpleasant mouthfeel, such as “pungent,” “soapy,” etc. (Schiffman and Dackis 1975). However, the lack of correlation between the individual detection thresholds for LCFA on the one hand and MCFA on the other hand (Stewart et al. 2010) indicates already that, despite resulting in similar average threshold concentrations, not a single detection mechanism may underlie the perception of FAs of different chain lengths. This in turn fits to our observation that only the LCFAs, if presented in TFL-matrix, exhibit, in addition to their “scratchy” taste, a “fatty” taste component. In fact,

the subset of “fatty” tasting FAs, namely the LCFAs, were reported in a number of rodent studies to elicit pancreatic responses, whereas MCFA did not show this effect (Hiraoka et al. 2003; Laugerette et al. 2005; Gaillard et al. 2008). That not only the chain length but also the intact carboxyl function of FA is important for being a preferred stimulus was shown by Tsuruta et al. (1999). In a series of preference tests using methylated LCFA and long-chain fat alcohols along with their unmodified counterparts, a clear preference for unmodified oleic acid and linoleic acid was demonstrated. Moreover, the MCFA caprylic acid (C:8) even induced avoidance. Again, the above data are in agreement with our observations that fat alcohols and MCFA were recognized as “scratchy” but not as “fatty.”

Taken together, our sensory data point to at least 2 FA-sensing mechanisms in humans: One is a rather broadly tuned detection of FAs of various chain length and derivatives thereof, eliciting a “scratchy” sensation at high concentrations and the other is specifically tuned and highly sensitive in detecting LCFA as “fatty.” Tentatively, one could speculate that the “scratchy” sensation indicates an involvement of the trigeminal system, however, as shown by Chale-Rush and colleagues, capsaicin treatment used to desensitize trigeminal nerve fibers did not affect the linoleic acid detection threshold in human sensory tests. This indicates minimal involvement of this pathway in linoleic acid detection (Chale-Rush et al. 2007a). Our observation that the LCFAs, C18:1 and C18:2, dissolved in water only tasted “scratchy” but not “fatty” indicates a complex interplay between the sensing of FAs and the TFL-matrix. Whether this interaction occurs at the site of the putative receptor on the tongue or represents a function of the interaction of taste and textural cues within the brain remains to be determined in future experiments.

In order to investigate which of the candidate fat taste receptors proposed from rodent studies may also play a role in human FA perception, we focused first on the candidates belonging to the GPCR gene family. We reasoned that a putative FA taste receptor should match the following criteria: 1) It should be expressed in human taste buds. 2) It should respond to stimuli that are detected by the human test panel or, at least, a subset thereof in case multiple receptors transmit FA taste information. 3) The rank order of potency observed in the sensory analysis should correlate with the activation of the receptor in question. 4) Although, depending on the assay system and the receptors investigated, the sensitivities obtained by heterologous expression can deviate substantially from *in vivo* data (e.g., odorant receptors cf. Mombaerts 2004), the sensitivity for FAs observed *in vivo* should at least roughly match the receptor responsiveness *in vitro*.

It has been shown before that human GPR41 and GPR43 are preferentially activated by SCFA (Brown et al. 2003; Le Poul et al. 2003). However, depending on the assay system chosen for the pharmacological characterization, residual

responses with FAs of chain lengths beyond C7:0 were noticed. Our own functional experiments (Figure 4A) confirmed the preferential activation by SCFA and did not reveal any activation by C10:0 or C18:2, which were not tested in previous functional characterizations. Thus, human GPR41 and GPR43 can be excluded as receptors underlying the perception of “fatty” tasting LCFAs. As we have not determined a lower limit for fatty acid chain lengths eliciting a “scratchy” perception or other additional taste attributes that might be associated with FAs of shorter chain length, we can, at present, not exclude GPR41 and GPR43 from the oral detection of FAs *per se*. In contrast to the 2 SCFA receptors, the human counterparts of the 2 receptors shown to participate in mouse FA taste perception, GPR40 and GPR120 (Cartoni et al. 2010), both respond to stimuli characterized as “fatty” by our human sensory panel. However, based on the chain length of compounds activating GPR40 and GPR120, the pharmacological properties of both receptors would also be consistent with a role in the “scratchy” taste perception. The lack of response upon stimulation of GPR40 as well as GPR120 transfected cells with “scratchy” tasting fat alcohols excludes an involvement in this type of percept. With respect to the “fatty” perception, the pharmacological profiling of human GPR40 and GPR120 is not as conclusive as for the “scratchy” sensation. Both receptors responded rather similarly to MCFA and LCFA and hence to “scratchy” as well as to “fatty” stimuli. The fact, however, that GPR40 responded almost equally well to C10:0 (“scratchy”) and C18:2 (“scratchy” and “fatty”), may indicate that this receptor is a rather unlikely candidate for the “fatty” taste in humans. On the other hand, GPR120 clearly exhibits a pronounced preference for C18:2 over C10:0, which would be more consistent with a role as a receptor for the “fatty” tasting subset of stimuli. Comparative analysis of the 2 splice variants of human GPR120 revealed that the longer variant is slightly more selective for LCFA than the short variant (Figure 6) and therefore would match the requirements for a candidate receptor for the “fatty” taste better, however, the lack of detectable expression in human lingual epithelium precludes such speculations.

As discussed already, fat taste perception in humans is, even after reducing potentially confounding factors such as texture, olfaction, etc. as much as possible, a rather complex sensation. On the one hand, this may account for some variability among the results of human sensory studies and, moreover, between human sensory and rodent behavioral studies. On the other hand, the frequent observation of a higher sensitivity of rodents for LCFA, the apparent higher selectivity of rodents for LCFA compared with MCFA, might truly reflect species-specific differences. If the latter is the case, then rodents might either possess a different composition of molecules involved in sensing FAs or the orthologous FA sensors of humans and rodents may respond pharmacologically different. The direct comparison between human and mouse GPR40 and GPR120 receptors (Figure 7)

suggests that the properties of both receptors are highly similar and hence cannot account for species-specific differences. Our analysis of GPR expression in human lingual epithelium, however, indeed points toward a different expression pattern in human and mice. As shown recently in mice, both GPR40 and GPR120 are expressed in taste bud cells (Cartoni et al. 2010). Whereas GPR40 was found mostly in type I cells, GPR120 was colocalized predominantly with a subpopulation of type II cells.

Our data support the existence of the receptor GPR120 also in human lingual epithelium. Contrary to mice (Cartoni et al. 2010), but in agreement with data presented for rats (Matsumura et al. 2007), GPR40 was not detected in human lingual epithelium (as we had no access to human foliate papillae, we cannot exclude the presence of GPR40 completely). As GPR40 responds somewhat more to stimulation with MCFAs than GPR120, its absence from taste cells would predict that FA taste perception should be more narrowly tuned toward LCFAs which is, in fact, observed in human and rodent sensory studies. The expression of GPR120 in humans is not restricted only to taste buds but extends into the surrounding epithelium. Both mRNA and protein were detected in lingual taste and nontaste epithelium (Figures 2 and 3). An extragemmal expression of GPR120 has not been reported until now, and it remains to be elucidated if its presence outside of taste buds is also related to taste perception or other orosensory cues. As the tissues used for the RT-PCR, the *in situ* hybridization experiment and the immunohistochemical staining procedure originated from 3 different individuals who underwent oral surgery, and the results are in good agreement with each other, we have no reason to believe that the expression pattern of the GPR120 gene is related to specific pathological conditions of the donors.

Beside GPRs, other molecular sensors have been proposed to play a role in rodents' oral fat perception. The delayed-rectifying K<sup>+</sup> channels are inhibited in presence of polyunsaturated FAs and because dietary lipids are proposed to modify the palatability of other tastants (Pittman et al. 2006; Mattes 2007), these channels could participate in modulating the perception of a primary stimulus depolarizing the taste receptor cell (Gilbertson et al. 1997, 2005; McKay and Worley 2001). In addition, CD36 participates in the translocation of LCFA through the plasma membrane (Coort et al. 2002). In lingual epithelium, this molecule is thought to be directly involved in fat taste or indirectly in the perception cascade (Laugerette et al. 2005; Gaillard et al. 2008).

This raises the question, which of the above molecules is/are involved in human FA taste perception? From rodent studies it is known that PUFAs act on KCNA5 as open-channel blockers. Therefore, activation of this potassium channel is unlikely the primary event during fat detection. For each of the other candidate receptors, GPR40, GPR120, and CD36, knockout mouse models were generated showing perceptual deficits for oral FA stimulations. On the one hand, this hints to an involvement of all 3 genes

in fat perception, however, on the other hand raises the question of why any one of the knockout models showed only a diminished reaction upon FA stimulation although the other 2 fat sensors should still be intact? This would argue in favor of a signaling cascade that involves more than one of the above candidates. Within such a cascade in humans, GPR120 could fulfill several requirements of an FA receptor: 1) As a GPCR it could initiate a signaling cascade implemented in taste receptor cells. 2) Its pharmacological profile fits into the spectrum of actually detected FAs best, though not perfectly. Therefore, future studies probing possible interactions between GPR120, CD36, and KCNA5 should be undertaken to investigate this hypothesis.

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