Gender-Specific Differences between the Concentrations of Nonvolatile (R)/(S)-3-Methyl-3-Sulfanylhexan-1-Ol and (R)/(S)-3-Hydroxy-3-Methyl-Hexanoic Acid Odor Precursors in Axillary Secretions

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Abstract

The volatile fatty acid, (R)/(S)-3-hydroxy-3-methylhexanoic acid ((R)/(S)-HMHA), and the human specific volatile thiol, (R)/(S)-3-methyl-3-sulfanylhexan-1-ol ((R)/(S)-MSH), were recently identified as major components of human sweat malodor. Their 2 corresponding precursors were subsequently isolated from sterile and odorless axillary secretions. The purpose of this work was to analyze these 2 odor precursors in 49 male and female volunteers over a period of 3 years to elucidate to which extent they are implicated in the gender-specific character of body odor. Surprisingly, the ratio between the acid precursor 1, a glutamine conjugate, and the "sulfur" precursor 2, a cysteinylglycine-S-conjugate, was 3 times higher in men than in women with no correlation with either the sweat volume or the protein concentration. Indeed, women have the potential to liberate significantly more (R)/(S)-MSH, which has a tropical fruit- and onion-like odor than (R)/(S)-HMHA (possibly transformed into (E)/(Z)-3-methyl-2-hexenoic acid) that has a cheesy, rancid odor. Parallel to this work, sensory analysis on sweat incubated with isolated skin bacteria (*Staphylococcus epidermidis Ax3*, *Corynebacterium jeikeium* American Type Culture Collection 43217, or *Staphylococcus haemolyticus Ax4*) confirmed that intrinsic composition of sweat is important for the development of body odors and may be modulated by gender differences in bacterial compositions. Sweat samples having the highest sulfur intensity were also found to be the most intense and the most unpleasant.

Key words: axillary odor, cysteinylglycine-S-conjugate, gender, odor precursor, thiol

Introduction

The generation of malodor on various sites of the human body, for example, foot, mouth, or armpit, is mainly caused by microbial transformation of odorless natural secretions into volatile odorous molecules (Labows et al. 1999). In humans, sebaceous, eccrine, and apocrine sweat glands provide an important source of nutrients for bacterial growth. The strong and unpleasant odor emanating from the underarm region has been mainly correlated to the high number of bacteria, such as *Corynebacteria* and *Staphylococci* species, present in this humid and semioccluded environment (Leyden et al. 1981; Labows et al. 1982; Austin and Ellis 2003; Taylor et al. 2003; James et al. 2004; Rennie et al. 2007).

More recent studies have deepened our understanding of axillary chemistry. In addition to the 2 pheromone-like steroids, androstenone (5-α-androst-16-en-3-one) and androstenol

(5-α-androst-16-en-3-α-ol), the major and specific constituents of human sweat malodor are the volatile fatty acid, (R)/(S)-3-hydroxy-3-methylhexanoic acid ((R)/(S)-HMHA) (Natsch et al. 2006), and the sulphanylalkanol, (R)/(S)-3-methyl-3-sulfanylhexan-1-ol ((R)/(S)-MSH) (Hasegawa et al. 2004; Natsch et al. 2004; Troccaz et al. 2004; Gautschi et al. 2007). (R)/(S)-HMHA is released from a glutamine conjugate by the action of a zinc-dependent aminoacylase from *Corynebacteria* (Natsch et al. 2003), whereas (R)/(S)-MSH is derived from a cysteinylglycine-S-conjugate (possibly via a cysteinyl-S-conjugate) by the action of *Staphylococci* (Starkenmann et al. 2005). In addition to the *Staphylococcus epidermidis* strain, the most abundant microorganisms present in axilla, *Corynebacterium jeikeium*, possessing the aminoacylase (Natsch et al. 2004), and *Staphylococcus haemolyticus*,

possessing C-S lyase activities (Starkenmann et al. 2005), were selected for sensory test in this study.

These last findings confirmed the importance of bacteria, more precisely bacterial enzymes, and sweat odor precursors for the generation of body odors. It is commonly known that gender, along with ethnicity, emotional, physiological, and environmental factors may influence the quantity and the quality of sweat (Akutsu et al. 2006; Havlicek and Lenochova 2006; Rawlings 2006; Dixon et al. 2007; Penn et al. 2007; Savelev et al. 2008), and some studies have led to the conclusion that individuals have a distinct body odor type, which is partially determined by their inherited major histocompatibility complex alleles (Wedekind et al. 1995; Roberts et al. 2005). Unfortunately, our knowledge of the importance of skin bacteria or sweat composition to explain the individual and gender specificity of human body odor is limited, although differences in microflora composition, such as the greater ratio of *Corynebacteria* to *Staphylococci* in male compared with female axilla, have been reported (Labows et al. 1982; Jackman and Noble 1983). In this study, we have chosen to investigate whether there are gender-specific differences between the type of malodor precursors present in axillary sweat secretions and whether sweat sample origins correlate with detectable differences in odor. The first part of this work was therefore to collect enough male and female axillary secretions to quantify the precursors of (*R*)/(*S*)-HMHA and (*R*)/(*S*)-MSH, and the second part was to carry out sensory tests.

Materials and methods

Collection of odorless axillary secretions

Human apocrine and ecrine secretions were collected from the axillae of 49 caucasian volunteers (24 men and 25 women) who used a sauna over 3 winter seasons from November 2004 to April 2007. A small plastic goblet was used to collect the droplets of sweat that develop from underarm while taking a sauna. Secretions were immediately sterilized through a double filter consisting of a 1 μm membrane, followed by a 0.2 μm sterile filter, and frozen separately at –20 °C as reported previously (Troccaz et al. 2004). The summer season was avoided due to changes in diet and activities of the volunteers and higher temperature and humidity variations (Hopwood et al. 2004). Each volunteer provided between a minimum of 1 sample and a maximum of 8 samples per season and was asked to refrain from using any deodorant or perfume both the day before and the day of sweat collection. Individual male or female sweat samples were classified as high protein (HP) if odorless and the protein concentration was superior or equal to 0.15 mg/ml and as low protein (LP) if odorless and the protein concentration was inferior to 0.15 mg/ml. In all, 0.15 mg/ml corresponds to the average protein concentration of male and female sweat samples. One-third of the samples were imme-

diately rejected due to the presence of other descriptors (perfume or human sweat odors).

Biochemical analyses of collected samples

Protein concentration was measured in each collected samples by the micromethod of Bradford with the Coomassie protein assay reagent (Bio-Rad laboratories, Inc., Hercules, CA) and with the bovine albumin 5% (Becton Dickinson and Company, Sparks, MD) as standard (Bradford 1976). The pH of sweat secretions was measured with a Mettler Toledo pH meter (InLab 423 electrode). Glucose concentrations were determined with a glucosimeter (YSI 2700 Select Biochemistry analyzer with a dextrose/lactate membrane).

Quantification of odor precursors

Volatile sweat precursor concentrations in sterile and individual axillary secretion samples were determined by ultra performance liquid chromatography (UPLC) coupled to a mass spectrometer. Separations and quantifications of sweat samples were performed on an Acquity BEH-C18 column (2.1 mm internal diameter × 100 mm; 1.7 μm) (Waters Corporation, Milford, MA). The elution solvents were water with 0.1% formic acid (solvent A) and acetonitrile (CH_3CN) containing 0.1% formic acid (solvent B). The gradient profile started at 8% of solvent B (0.5 min), increased to 30% (8.4 min) and to 90% of solvent B in 2.1 min. The flow rate was 0.3 ml/min and the retention times (t_R) expressed in minutes. The mass spectrometer was a Thermo Finnigan TSQ quantum ultra triple quad spectrometer (Thermo Electron Corporation, Somerset, NJ) with an electrospray ion source operated in positive mode (ESI^+). The spray voltage was fixed at 4.0 kV and the capillary temperature at 349 °C. The sheath gas was nitrogen at a flow rate of 60 (Finnigan arbitrary units). The auxiliary gas was nitrogen at a flow rate of 5 (Finnigan arbitrary units). Analyses were performed in single ion monitoring (SIM) mode according to $[M + 1]^+$ measurements for *N*-α-3-hydroxy-3-methylhexanoyl-(*L*)-glutamine 1 ($C_{12}H_{22}N_2O_5$, MW: 274.32) and *S*-[1-(2-hydroxyethyl)-1-methylbutyl]-(*L*)-cysteinylglycine 2 ($C_{12}H_{24}N_2O_4S$, MW: 292.40). Calibration curves were generated with pure and diluted synthetic compounds in solvent A (Troccaz et al. 2004; Starkenmann et al. 2005). (*R/S* 1:1)-MSH and (*R/S* 1:1)-HMHA were synthesized as reported previously (Hasegawa et al. 2004; Troccaz et al. 2004).

Odor detection threshold of sweat volatiles

Air dilution olfactometers were used to determine the odor detection threshold (ODT) of major sweat volatiles and its corresponding dose–response curve, which corresponds to the plot of the perceived odor intensity (response) versus the gas phase concentration (dose) (Vuilleumier et al. 2008). The body odor volatile was first diluted in propylene glycol and injected continuously into a chamber at 130 °C, where

vaporization occurs instantaneously at the end of the tube. Nitrogen as carrier gas is used to avoid oxidative processes in the concentrated odor flow, which is then diluted with humidified air to obtain the final gas phase concentration. The sniffing outlet delivers a continuous and constant odorized air flow. Sensory evaluations were performed by 30 nontrained Firmenich employees (15 men and 15 nonpregnant women between 17 and 60 years of age) (Cameron 2007). Dose–response curves were determined for each volatile with 8 concentrations varying from its volatility value to 1.10^{-6} µg/l air. The volatility is defined as the gas phase concentration at 22 °C/730 mm Hg (±30) expressed by the concentration of the volatile in the air above the pure liquid or solid at equilibrium.

Bacterial transformation of axillary secretions

Three underarm bacterial isolates, *C. jeikeium* American Type Culture Collection 43217 (Taylor et al. 2003), *S. haemolyticus* Ax4 (Troccaz et al. 2004), and *S. epidermidis* Ax3 (Troccaz et al. 2004), were grown aerobically at 37 °C in liquid brain heart infusion (from Becton Dickinson and Company, Sparks, MD) containing 0.5% between 80 until OD_{600} was equal to 1.0. *Staphylococcus haemolyticus* and *C. jeikeium* were confirmed to be "sulfur" and "acid" malodor producer after incubation with sweat (conversion yield of precursor 2 was between 50% and 80% for *S. haemolyticus* and *C. jeikeium*. Conversion yield of precursor 1 was between 0.1% and 0.5% for *C. jeikeium*, data not shown). Cells were harvested by centrifugation for 10 min at 3000 × *g*, washed once with sterile 0.1 M phosphate buffer (pH 6.0), and suspended in fresh buffer to a final 4-fold concentration. The cell suspension (50 µl) was mixed with 400 µl of male or female, HP or LP sweat odorless axillary secretions, and incubated for 17 h at 37 °C. Axillary secretions comprise a mixture of individual collected odorless samples after carrying out biochemical analyses. A closed glass bottle was used to reproduce a semioccluded environment that minimized the water evaporation and maximized the sweat malodor generation by bacterial transformation.

Sensory evaluation of incubated sweat samples

Twelve external and trained assessors (1 man and 11 nonpregnant women between 25 and 55 years of age) evaluated incubated sweat samples in blind test conditions. The sweat samples were prepared as previously described and consisted of a male sweat sample and a female sweat sample incubated with *S. epidermidis*, *S. haemolyticus*, or *C. jeikeium*. The 6 samples consisted of several pots and each pot was not opened more than twice during the session. Samples were evaluated at 37 °C in a balanced order in a sequential monadic way. Two replicates were carried out with either HP or LP on 2 consecutive days. The assessors evaluated the samples according to several attributes: overall sweat odor intensity, sulfur odor intensity, acid odor inten-

sity, and odor unpleasantness. Attributes were evaluated on an unstructured 0–10 linear scale (0 = not perceptible; 10 = very strong intensity or 0 = not at all unpleasant; 10 = very unpleasant). To evaluate the sulfur odor intensity and the acid odor intensity, the assessors were first given references to smell and then rated the attributes of samples on the basis of these references. The sulfur and acid odor references were glass pots containing 0.002% (*R/S* 1:3)-MSH in dipropylene glycol (DIPG)/mQ water (50/50) (sulfur odor reference) and 0.1% (*E/Z* 3:1)-3-methyl-2-hexenoic acid in DIPG/mQ water (1/10) (acid odor reference; Zeng et al. 1991). The sensory tests were performed using FIZZ 2.30C system (Biosytèmes, Couternon, France). Analyses of variance (ANOVAs) were carried out using XLSTAT 2008 2.03 (Addinsoft, Paris, France) followed by Duncan's post hoc analysis ($\alpha = 0.05$).

Results

Biochemical analysis of sterile axillary secretions

Results of sweat sampling are summarized in Table 1. Average analytical results on mixed samples and variations in individual samples per gender per year and per protein concentrations are presented. In all, 191 male and 113 female samples, which correspond to 2252 and 265 ml of sweat, respectively, were collected over a period of 3 winter seasons from 2004 to 2007 following a 15-min sauna session. As previously noticed, the average sweat yield was 5 times greater for males at 11.8 ± 1.2 ml (mean ± standard error of the mean [SEM]) versus females at 2.4 ± 0.5 ml, whereas the average protein content was similar (0.22 ± 0.06 vs. 0.16 ± 0.03 *g*/l for male and female, respectively). The glucose content was 2–5 times higher for males than for females (3.6 ± 0.9 *g*/l and less than 1.0 ± 0.1 mg/l in male and female secretions, respectively), which is an important source of carbon for Gram-positive bacterial growth. The average pH was lower in females (7.5 ± 0.3 vs. 8.0 ± 0.3) which is consistent with results of previous reports (Herrmann and Mandol 1955; Ehlers et al. 2001).

### Odor precursor analysis in sterile axillary secretions

The analytical method was optimized with the 2 major sweat precursors present in sweat samples. Thus, racemic precursors 1 and 2 (Figure 1) were prepared and injected on UPLC in SIM mode. The diastereoisomers were not separated by UPLC and thus the peak area corresponds to the quantification of both diastereoisomers. The odor precursor quantification was very sensitive and precise using this method. The lowest detected concentration of 2 in individual sweat samples was 0.02 µg/ml after 4 repetitions with only a 2% variation (0.02 ± 0.0004 µg/ml), whereas the lowest concentration of 1 was 25 µg/ml with a 0.3% variation.

The mean quantity (±SEM) of 1 in male sweat samples was 1021 (±121) µg with a maximum of 2790 µg, whereas in the

Table 1 Chemical analysis of odorless human sweat samples collected over 3 winter seasons from November 2004 to April 2007

Gender	Year	Protein level	N	Volume (ml)	Protein (mg/ml) (SD)	pH (SD)	Glucose (mg/l) (SD)	Acid precursor 1 (μg/sample)	Sulfur precursor 2 (μg/sample)	Acid precursor 1 (mg/l)	Sulfur precursor 2 (mg/l)	Ratio acid precursor 1/sulfur precursor 2 (1)/(2)
Male	2004–2005	LP	67	750.4	0.09 (0.01)	9 (0.14)	3.0 (0.5)	212.8	2.2	19	0.2	95
	2005–2006	LP	66	844.8	0.146 (0.02)	8 (0.20)	2.0 (0.8)	1100.8	6.0	86	0.47	182.98
	2006–2007	LP	17	265.2	0.08 (0.01)	7 (0.15)	1.5 (0.5)	1185.6	7.5	76	0.48	158.33
	2004–2005	HP	2	17.0	0.53 (0.03)	8 (0.07)	6.0 (1.0)	136.0	1.5	16	0.18	91.43
	2005–2006	HP	21	226.8	0.202 (0.01)	8 (0.20)	6.0 (0.5)	1144.8	9.1	106	0.84	126.19
	2006–2007	HP	18	147.6	0.27 (0.02)	8 (0.17)	3.0 (0.7)	984.0	4.8	120	0.59	203.39
Average (male samples)				11.8	0.22	8	3.6	794.0	5.2	70.5	0.46	142.89
SEM				1.24	0.06	0.24	0.9	198.1	1.2	8.77	0.05	20.71
Female	2004–2005	LP	15	48.0 (15)	0.08 (0.02)	9 (0.16)	<1	179.2	3.3	56	1.02	54.9
	2005–2006	LP	22	63.8 (22)	0.06 (0.02)	7 (0.20)	<1	174.0	4.3	60	1.47	40.82
	2006–2007	LP	20	74.0 (20)	0.13 (0.01)	7 (0.14)	<1	791.8	13.6	214	3.66	58.4
	2004–2005	HP	10	13.0 (10)	0.19 (0.03)	8 (0.15)	<1	72.8	2.7	56	2.1	26.67
	2005–2006	HP	25	22.5 (25)	0.24 (0.02)	7 (0.14)	1.0 (0.5)	139.5	5.8	155	6.43	24.11
	2006–2007	HP	21	44.0 (21)	0.28 (0.03)	7 (0.12)	1.0 (0.3)	833.9	13.6	398	6.49	61.36
Average (female samples)				2.4	0.16	7.5	<1.0	365.2	7.2	156.5	3.53	44.38
SEM				0.49	0.03	0.31	0.1	156.1	2.3	27	0.49	7.3

Average data on sterile odorless sweat secretions (HP or LP) per year and per gender are represented. In all, 24 male and 25 female volunteers participated in this study. All the volunteers gave at least 1 sample. Precursors concentrations and precursor ratios were average by individual sweat donor and per protein concentrations before mean calculation. LP = low protein sweat (<0.15 mg/ml); HP = high protein sweat (≥0.15 mg/ml sweat); and N = number of samples. Acid precursor 1 = N-α-3-hydroxy-3-methylhexanoyl-(L)-glutamine; sulfur precursor 2 = S-[1-(2-hydroxyethyl)-1-methylbutyl]-(L)-cysteinylglycine. SEM = (SD/$\sqrt{Nr-1}$); Nr (number of repetitions) = 6; and SD = standard deviation.
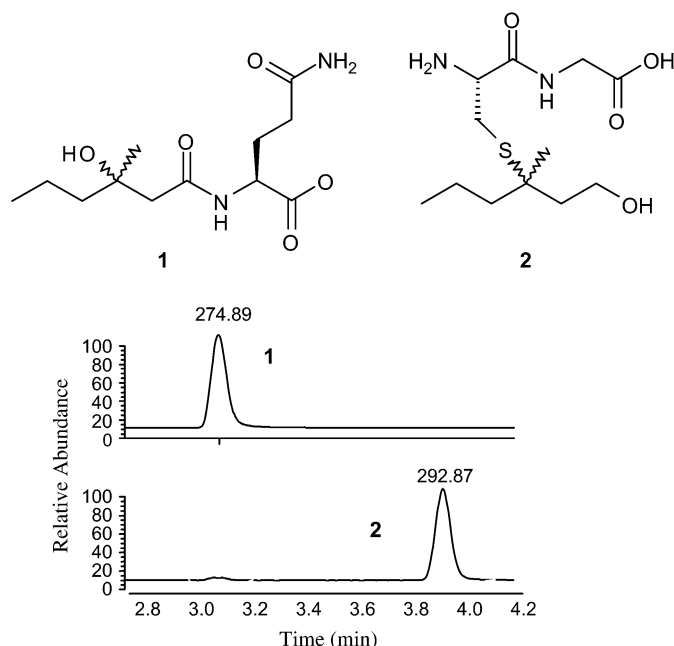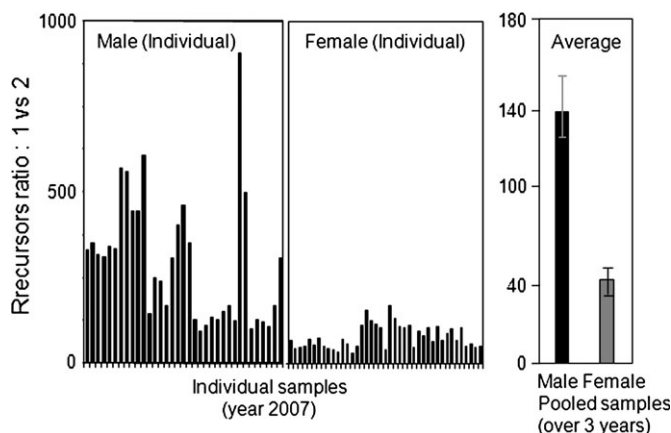
Figure 1 Structure of major sweat precursors 1 (N-α-3-hydroxy-3-methylhexanoyl-(L)-glutamine) and 2 (S-[1-(2-hydroxyethyl)-1-methylbutyl]-(L)-cysteinylglycine) and the UPLC trace (UPLC coupled to a Thermo Finnigan TSQ quantum in SIM mode with electrospray ion source operated in positive mode [ESI⁺]) of a standard solution containing 1 µg/ml of each precursor.

female sweat sample, the average quantity was 606 (±86) µg of 1 with a maximum of 2290 µg per sample. The mean quantities (±SEM) of 2 were 5 (±1) and 9 (±1) µg in males and females, respectively, with a maximum of 17 and 22 µg. Although huge variations were observed between individuals, for each individual, the ratio of 1 and 2 was consistent over the 3 years. To our surprise, this ratio was 3 times higher in men (value of 143) than in women (value of 44) and independent of the sweat volume or the protein concentration (see Table 1, Figure 2).

ODT of major axillary components

The ODT, that is, the lowest detectable odor level expressed in liquid or gaseous concentration units, was calculated for 3 sweat odor volatiles in both male and female subjects (E)/(Z)-3-methyl-2-hexenoic acid, its hydrated form (R)/(S)-HMHA, and (R)/(S)-MSH. The ODT reflects the olfactive impact of the body odor volatiles. The average ODT and the corresponding dose–response curves calculated from the data generated from 15 males and 15 females are shown in Figure 3 and Table 2. The most common descriptor for synthetic (R)/(S)-HMHA was animalic, cheesy, and rancid, whereas the sulfur molecule (R)/(S)-MSH was judged to be fruity and onion like. (R)/(S)-MSH was perceived at a concentration 100× lower than (R)/(S)-HMHA, a fact that may explain its important organoleptic contribution in human malodor (ODTs of 2.27×10^{-6} µg/l air and 1.80×10^{-4} µg/l air for the

Figure 2 Ratio between the acid precursor 1 (N-α-3-hydroxy-3-methyl-hexanoyl-(L)-glutamine) and the sulfur precursor 2 (S-[1-(2-hydroxyethyl)-1-methylbutyl]-(L)-cysteinylglycine) in sterile male and female sweat secretions (collected from the axillae of 24 men and 25 women). Ratios were calculated on individual samples collected during the season 2007 (76 samples) and on 304 samples collected between November 2004 and April 2007 (mean ± SEM). Dotted lines represent the average results per gender in 2007. Precursors were quantified by direct injection on UPLC on a water acquity system coupled to a Thermo Finnigan TSQ quantum in SIM mode.
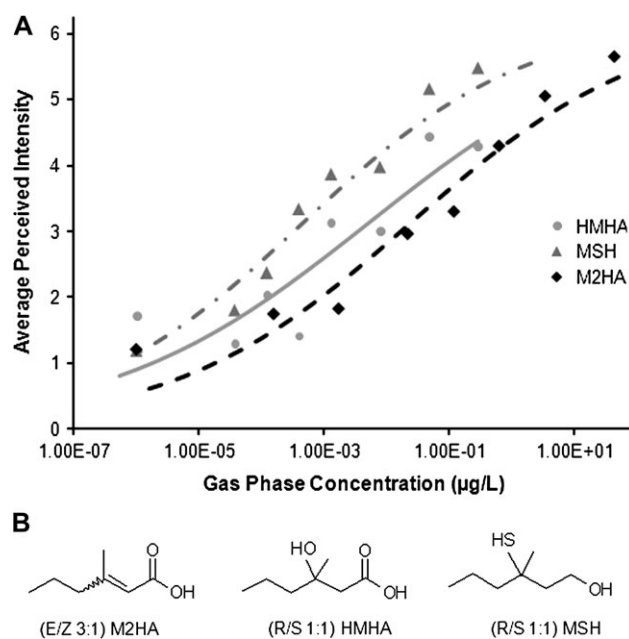
Figure 3 Dose–response curve of the major sweat volatiles: (R)/(S)-HMHA, (E)/(Z)-3-methyl-2-hexenoic acid ((E)/(Z)-M2HA), and (R)/(S)-MSH. Olfactive evaluations were performed by 30 Firmenich employees (15 women and 15 men).

2 molecules, respectively). Similar results were found for both male and female assessors. The low ODT of sulfur-containing molecules has already been reported in several studies and summarized in the review of Vermeulen et al. (2005).

Table 2 ODTs of the major sweat volatiles: (*R/S* 1:1)-HMHA, (*E/Z 3:1*)-3-methyl-2-hexenoic acid, and (*R/S* 1:1)-MSH

Chemical name	Volatility (µg/l air)	ODT (µg/l air)		Descriptors
		Men (*N* = 15)	Women (*N* = 15)	
(*E*)/(*Z*)-3-methyl-2-hexenoic acid	40.00	2.59×10^{-4}	1.44×10^{-4}	Perspiration, acidic, rancid, and cheese
(*R*)/(*S*)-HMHA	0.29	1.49×10^{-4}	2.00×10^{-4}	Perspiration, animal, cheese, and rancid
(*R*)/(*S*)-MSH	132.00	2.31×10^{-6}	1.53×10^{-6}	Perspiration, sulfur, onion, and grape fruit

Olfactive evaluations were performed by 30 Firmenich employees (15 women and 15 men). ODTs were determined for women and men subjects together and separately. ODTs of (*R*) and (*S*) isomers and (*E*) and (*Z*)-3-methyl-2-hexenoic acids were not significantly different (data not shown).

Sensory analysis

Sensory tests were performed with incubated sweat samples: equal volumes of sterile sweat from males and females were separately fed with a fixed number of *S. haemolyticus*, *C. jeikeium*, or *S. epidermidis* (Gram-positive skin bacteria) and incubated at 37 °C overnight prior to analysis. Samples were rated according to their sulfur, acid, sweat odor intensity, and their unpleasantness on a linear 0–10 scale. The significant influence of bacterial species, gender, and sweat protein concentration is represented by the probability value associated to Fisher's *F* value (Table 3). Indeed, the protein concentration in sweat had no significant effect on the perceived sweat intensity, sulfur, and acid character ($P > 0.1$). However, our results confirmed previous findings that *C. jeikeium* and *S. haemolyticus* generate the most sulfurous and intense odors in comparison to *S. epidermidis* (Troccaz et al. 2004). This effect was stronger after incubation with female rather than with male axillary secretions. Regarding the sulfur and sweat odor intensity, the Fisher *F* value of the analysis of variance indicates a much higher effect of gender compared with the bacterial type (*F* value of 8.6 vs. 3.1 for the sulfur odor and 11.0 vs. 4.3 for the sweat odor intensity). Female sweat samples generated significantly higher sweat and sulfur odor intensity (99% confidence level) (Tables 3 and 4) In addition, samples having the highest sulfur intensity, such as female sweat samples, were also found to be the most intense and the most unpleasant. Although sweat volatiles are somewhat unpleasant, the assessors (92% female) found the male odors to be the least unpleasant ($P < 0.05$).

Discussion

Most of the studies to elucidate gender and individual differences in axillary sweat odor, its chemistry and odor intensity, have confirmed the key role of skin bacteria, more particularly bacterial enzymes, in the sweat transformation process (Leyden et al. 1981; Taylor et al. 2003; Zeng et al. 2006). Consequently, the vast majority of deodorants utilize antimicrobial agents that are also often enzyme inhibitors; the rationale is that, by decreasing the bacterial numbers present in the axilla, the biogenesis of odor can be generally

Table 3 ANOVA with Duncan's post hoc analysis (α = 0.05) on sensory data

Descriptors	Variables	df	*F*	Pr > *F*
Sweat odor intensity	Protein level	1	1.8	0.182
	Sweat gender	1	11.0	0.001***
	Bacterial type	2	4.3	0.006**
Acid odor intensity	Protein level	1	0.5	0.479
	Sweat gender	1	4.9	0.028*
	Bacterial type	2	0.6	0.647
Sulfur odor intensity	Protein level	1	0.1	0.740
	Sweat gender	1	8.6	0.004**
	Bacterial type	2	3.1	0.028*
Overall unpleasantness	Protein level	1	2	0.157
	Sweat gender	1	11.1	0.001***
	Bacterial type	2	0.8	0.504

Sensory analysis was performed with incubated pooled sweat samples collected from November 2006 to April 2007 from male or female (35 male samples and 41 female samples) with various microorganisms (*Corynebacterium jeikeium*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis*). df: degrees of freedom. *F*: Fisher's F. Pr > *F*: probability associated to the Fisher's *F* value. *Differences are significant when $P < 0.05$ (*95% confidence level [CI], **99% CI, and ***99.9% CI). Olfactive evaluation was performed by 12 trained assessors (11 women and 1 man). Sweat descriptors are sulfur, acid, and overall sweat odor intensity and overall unpleasantness. Variables are protein level in sweat secretions (HP or LP sweat), bacterial type (*C. jeikeium*, *S. haemolyticus*, *S. epidermidis*), and sweat gender of the samples (female or male).

decreased (Makin and Lowry 1999; Dayan et al. 2007). Individuals were classified as to have intense, acrid, or acid body odor, with respect to the composition of their microflora (Labows et al. 1982). In this study, we have found that the same microflora may generate distinctively different body odors with respect to the composition of underarm secretions (see Table 3). After incubation with the axillary bacteria *S. haemolyticus*, female sweat has a much more intense and sulfur odor intensity than male sweat. Indeed,

Table 4 Sensory analysis performed with incubated sweat samples from male or female

	Female sweat samples				Male sweat samples			
	C.j	S.h	S.e	Average	C.j	S.h	S.e	Average
Sweat odor (CI)	6.60 (0.74)	6.53 (0.68)	5.72 (0.69)	6.28 (0.71)	6.18 (0.77)	4.75 (0.76)	4.90 (0.66)	5.35 (0.75)
Acid odor (CI)	4.00 (1.02)	4.90 (0.95)	4.87 (0.94)	4.62 (0.97)	4.17 (0.90)	4.10 (0.92)	3.70 (1.06)	3.99 (0.95)
Sulfur odor (CI)	5.72 (0.69)	5.86 (0.94)	4.10 (0.92)	5.22 (0.99)	4.62 (1.00)	4.01 (0.97)	3.47 (1.08)	4.03 (1.01)
Unpleasantness (CI)	6.96 (1.00)	7.57 (2.11)	6.55 (0.89)	7.02 (0.91)	6.35 (0.86)	6.00 (0.88)	5.81 (0.88)	6.06 (0.78)

Pooled sweat samples collected from November 2006 to April 2007 (Table 1) were used for the sensory test. Olfactive evaluation was performed in duplicate by 12 trained assessors. Values are average data obtained with HP and LP sweat samples and with various microorganisms (*Corynebacterium jeikeium*, *Staphylococcus haemolyticus*, and *Staphylococcus epidermidis*). CI = 95% confidence interval. Attributes were evaluated on an unstructured 0–10 linear scale: 0 = not perceptible; 10 = very strong intensity or 0 = not at all unpleasant; 10 = very unpleasant. *C.j* = *C. jeikeium*; *S.e* = *S. haemolyticus*; and *S.e* = *S. epidermidis*.

analysis of individual differences in the composition of sweat may help us find new strategies to design individualized products to compete with body odors.

The content of the major acid and sulfur odor precursors, 1 and 2, were analyzed by ultrahigh pressure liquid chromatography, coupled to a Thermo Finnigan TSQ quantum, in limited amount of freshly secreted filter-sterilized sweat samples from 49 male and female volunteers. In previous reports, the sulfur precursor 2 had been shown to be transformed into (*R*)/(*S*)-MSH by *S. haemolyticus* and *Corynebacteria* (Starkenmann et al. 2005; Emter and Natsch 2008). For the first time, we have shown that this precursor was found in higher amounts in female underarm secretions compared with males (5.1 mg/ml of 2 in females vs. 0.5 mg/ml of 2 in males). As expected, huge variations were found between individuals, but to our surprise, the ratio of 2 to 1 was constant in males and females over the 3-year period of the study. This ratio was equal to 143 ± 21 (mean ± SEM) in male axillary samples and 44 ± 21 in female samples. These results indicate that females have the potential to liberate significantly more sulfur volatiles, such as (*R*)/(*S*)-MSH, which has a tropical fruit- and onion-like odor in underarm, relative to (*R*)/(*S*)-HMHA (possibly converted into (*E*)/(*Z*)-3-methyl-2-hexenoic acid), which possesses a cheesy, rancid odor. Similarly to the results of Kuhn and Natsch (2008), our results indicate that the most probable hypothesis for these gender differences may be genetic or hormonal (Emter and Natsch 2008). Savelev et al. (2008) have showed recently that both genes and environmental factors determine characteristic odor of an individual.

Sensory tests have shown a higher sulfur odor intensity of female compared with male sweat after incubation with individual skin bacteria. The sulfur odor induces negative emotions (unpleasantness) toward female sweat samples, suggesting the repulsive effect of (R)/(S)-MSH in sweat. More intense odors tend to be more unpleasant for women as previously reported (Wedekind et al. 1995). Olfactive evaluation of synthesized body odor volatiles confirmed the low ODT of thiols and (*R*)/(*S*)-MSH was perceived at a concentration 100× lower than (*R*)/(*S*)-HMHA, with the same sen-

sitivity for males and females (similar odor thresholds), which explains its important contribution in body odors.

In conclusion, chemical analysis of freshly secreted male and female sweat samples and statistical analysis on data generated by sensory tests, with individual bacteria, have clearly shown that male and female excrete thiol and acid precursors 1 and 2 in different concentration ratio and that intrinsic composition of sweat is important for the development of body odors and may be modulated by gender differences in bacterial compositions.

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