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The effect of complete caloric intake restriction on human body odour quality

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Abstract

Previous studies on various vertebrates have shown that quantity and quality of food intake affect odour attractiveness as perceived by potential mates. In humans, the quality of body odour is similarly affected by ingested foods, such as by variation in meat and garlic intake. Nevertheless, it is not known whether quantity of food has an impact on human body odour attractiveness. Thus, here we tested how 48 hours of complete caloric intake restriction affects the hedonic quality of human axillary odour.

Odour samples (cotton pads fixed in both armpits and worn for 12 hours) were obtained from healthy female donors across three conditions: i) during their habitual food regime; ii) after 48 hours of complete caloric intake restriction (drinking water was provided), and iii) 72 hours after restoration of caloric intake. Axillary samples were assessed by male raters regarding their pleasantness, attractiveness, femininity, and intensity. We also collected blood samples to assess physiological changes due to dietary restriction (e.g., glucose, sodium, albumin, triacylglyceride assays) and anthropometric measurements at the same intervals as body odour samples.

We found no differences in pleasantness, attractiveness and intensity between the odour samples collected at baseline and during complete caloric intake restriction. Interestingly, we found that body odours were rated more pleasant, more attractive and less intense after restoration of food intake as compared to the baseline and during caloric restriction. Our results suggest that restoration of food intake positively influences hedonic quality of human body odour which might thus provide cues to current fitness status and metabolic efficiency.

Keywords

Olfaction, smell, diet, fasting, affective states, BMI

Highlights

The restriction of food intake affects body odour quality in various vertebrates.

We investigated the influence of 48 hours of food restriction on human axillary odour.

Restriction decreased levels of physiological markers (e.g. glucoses, triacylglycerides).

Caloric restoration positively affects odour compared to baseline and restriction phase.

Human body odour may provide cues to fitness status and metabolic efficiency.

1. Introduction

Diet is arguably considered to be one of the most significant environmental factors shaping chemical communication (Fialová, Roberts, & Havlíček, 2013; Havlicek & Lenochova, 2008). It may affect individual recognition, such as mother-offspring and kin recognition (Rajakaruna & Brown, 2006), and diet-related chemical cues are also frequently involved in assessment of the quality of potential mates. Such cues may provide indirect information about the quality of an individual's territory, its foraging efficiency, and the physiological ability to metabolise important nutrients. Thus, one may expect that responses to such cues will be more sensitive to chemicals that originate from food types which are either relatively rare or difficult to obtain, because there will be individual variation in ability to acquire such food types in large quantities. This information may be particularly important in mate assessment because food quantity and quality can impact on fitness through choice of mate and development of resulting offspring. For example, dietary quality can be reflected in weight and dominance status in agonistic encounters, reproductive success (Meikle & Westberg, 2001), and in the attractiveness of offspring, particularly sons (Meikle, Kruper, & Browning, 1995).

A series of experiments in European lizards show how odour may reveal this information. Females prefer areas scent marked by males with experimentally increased vitamin E levels in their femoral secretions. Vitamin E is assumed to be costly, because it is important for calcium metabolism, antioxidative processes and in immunostimulation, and it can only be attained from the diet (Kopena, Martín, López, & Herczeg, 2011). Similarly, Iberian rock lizard females prefer odours from males supplemented by provitamin D (Martín & López, 2010).

An increasing number of studies suggest that diet also affects human body odours. For example, research has demonstrated effects of garlic consumption on the olfactory quality of amniotic fluid and mothers' milk (as reported by adult panellists) (Mennella & Beauchamp, 1991; Mennella, Johnson, Staley, & Beauchamp, 1995), and its corollary effect on nursing behaviour (Mennella & Beauchamp, 1993). Garlic intake also increases the perceived attractiveness of axillary odour in

adults (Fialová, Roberts, & Havlíček, 2016). Although the mechanisms (e.g. metabolic pathways) underlying the influence on body odour are not yet fully understood, it may be that the various positive effects of garlic on human health (e.g. antioxidant and bactericidal properties) could be responsible for the effect. Other studies have focused on the effects of meat consumption. An early study reported that meat consumption has a negative effect on pleasantness of body odour (Havlicek & Lenochova, 2006), while a more recent study found the reverse effect (Zuniga, Stevenson, Mahmut, & Stephen, 2017). The seemingly contradictory findings might be a consequence of differences in the amount of ingested meat between these studies. In the former (Havlicek & Lenochova, 2006), researchers actively provided relatively high amounts of meat to their participants, while the latter study (Zuniga et al., 2017) used self-reports of consumed food, where the quantity of meat ingested was perhaps considerably lower. This may suggest that effect of meat consumption on body odour is curvilinear, with very high quantities having negative effects, while lower or moderate consumption is associated with more pleasant body odour. Zuniga et al. (2017) also found a positive influence of egg, oils, and fat consumption on odour pleasantness, while a negative impact of seafood and carbohydrates was observed.

Apart from these chemical cues related to specific individual components of diet, odour cues may also provide a proxy to more general aspects of an individuals' current nutritional status. It was for instance found that meadow voles of both sexes prefer the odour of opposite-sex individuals fed on a high protein diet compared with the odour of individuals on a low protein diet (Ferkin, Sorokin, Johnston, & Lee, 1997). A similar pattern of preferences was observed in females of red-backed salamander (Chouinard, 2012) and Nile tilapia (Giaquinto et al., 2012). Moreover, the effects of low- and high-quality diet on odour of Australian cashmere goats is detectable even by humans (Walkden-Brown, Restall, Norton, Scaramuzzi, & Martin, 1994).

An extreme form of nutritional inadequacy is complete food deprivation. Previous studies indicate that deprivation of 24 h significantly decreases odour attractiveness of meadow vole females (Pierce

et al., 2005), although this was restored 48h after refeeding. The negative effect of food deprivation was also shown in swordtail fish, where females preferred odours of well-fed males over those that had been food–deprived for 5 consecutive days (Fisher & Rosenthal, 2006). Importantly, there was no similar difference in preferences among female odours, suggesting that the effect is specific to mate choice rather than a general preference for well-fed individuals.

Based on such evidence from the non-human animal literature, the main aim of the current study was to investigate the effect of complete caloric intake restriction on human axillary odour. We hypothesized that body odour collected during the food deprivation phase would be perceived as less attractive to opposite-sex individuals compared to odours collected either pre-deprivation or after restoration of normal food intake. Furthermore, to investigate possible physiological mechanisms for such effects, we also assessed changes in levels of key metabolic markers: glucose, albumin, bilirubin, uric acid, sodium, potassium, chlorine, cholesterol, and triacylglycerides. Finally, we also collected data on affective states which are expected to be influenced by restricted caloric intake.

2. Methods

2.1. Participants

2.1.1. Odour donors

Fifteen female students were initially recruited as axillary odour donors, but data from 3 women were discarded as these participants did not fully follow the protocol, more specifically they felt nauseated as a consequence of complete caloric restriction. The final sample thus consisted of 12 women (mean age = 22.4 years, SD = 3.3, age range 20 – 31 years). Their mean body weight was 60.3 kg (SD = 4.8, range 54 – 67.3 kg) and mean height was 166.9 cm (SD = 5.8, range = 158.5 – 174.5 cm). As body odour quality fluctuates across the menstrual cycle, we recruited only women using hormonal contraception (Havlíček et al., 2006; Kuukasjarvi et al., 2004). All were healthy, non-smokers and regularly shaved their armpits (this can influence odour intensity: Kohoutová, Rubešová,

& Havlíček, 2011). They were recruited via posters or contacted via e-mail by JF. To compensate for their time and possible inconvenience they received 1000 CZK (approx. 40 EUR).

2.1.2. Odour raters

In total, 56 male students (mean age = 24.1, SD = 4.3, age range 18 – 34 years) participated as odour raters. The raters were recruited via posters, online or personally and were reimbursed by 100 CZK (approx. 4 EUR).

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration. The study was approved by the IRB at Charles University, Faculty of Science. The participants were informed about the goals of the study and provided informed consent.

2.2. Procedure

2.2.1. Body odour sampling

Before participation, the general health status of the odour donors was examined by a physician and by blood and urine tests (serum glucose, sodium, potassium, chlorine, uric acid, bilirubin, total protein, cholesterol, triacylglycerides, albumin) to ensure that none had any condition which might compromise their health during the dietary restriction phase such as diabetes, hypertension and other major illnesses, e.g., gastrointestinal disturbance, liver function and nephropathy.

We used a within-subjects design in which axillary odour samples were collected three times from each donor: 1] during their habitual dietetic regime, 2] during 48h of complete caloric restriction, and 3] 72 h after caloric intake restoration. During the habitual dietetic regime, participants consumed their usual diet. The complete caloric intake restriction phase involved no food intake; drinking (only sugar-free) water was allowed. Following the complete caloric intake restriction, participants received wholegrain bread with fresh cheese and a list of recommended light meals (e.g., wholegrain pastry, rice, pasta, cottage cheese) to prevent any digestive issues and to achieve a gradual return to their habitual food regime.

Each participant received a written list of instructions. They were asked to follow specified hygienic and dietetic restrictions the day before and during the sampling. Specifically, they were asked to refrain from (i) using perfumes, deodorants, antiperspirants, aftershave and shower gels, (ii) eating meals containing garlic, onion, chilli, pepper, vinegar, blue cheese, cabbage, radish, fermented milk products, and marinated fish, (iii) drinking alcoholic beverages, and (iv) smoking or using other drugs. They were further asked to avoid strenuous physical (e. g. jogging, aerobic) and sexual activities, and to not share a bed with their partner or pet during sampling. The day before the sampling procedure began, participants washed without fragranced products (soap, shower gel), and on the day of sampling they washed using provided non-perfumed soap (Neutral, DM-drogeriemarkt, www.dm-drogeriemarkt.cz, Prague). They then affixed a cotton pad (elliptical in shape, approximately 9 x 7cm at the longest axis; Ebelin cosmetic pads, DM-drogeriemarkt, www.dm-drogeriemarkt.cz, Prague) to either armpit using surgical tape (Omnipur, DM-drogeriemarkt, www.dm-drogeriemarkt.cz, Prague). They were also provided with a new, white, 100% cotton t-shirt (pre-washed in non-perfumed washing powder) to wear as a first layer of clothing, to minimize possible odour contamination from their own clothing and the ambient environment. They wore the cotton pads for the next 12h (7am-7pm) before delivering them to the laboratory, sealed in zip-lock plastic bags. The pads were immediately placed in a freezer at -21°C. It has been shown that freezing has no significant effect on hedonic ratings (Lenochova, Roberts, & Havlicek, 2008; Roberts, Gosling, Carter, & Petrie, 2008). Donors' conformity with the instructions was checked by a questionnaire, and no violations were found.

2.2.2. Laboratory assays

As a further check on whether participants' compliance with food restriction, they were asked to take urine samples 3 times a day and perform a ketone strip test that measures urine ketone concentrations. Ketone levels are biomarker for tracking the effectiveness of fasting. The end of the strip was passed through the urine stream and the results of the strip test (colour of the strip) were

photographed using their mobile phone and the image sent to the researchers. The test showed increase in ketones levels in majority of participants during the complete caloric restriction.

To assess physiological changes induced by complete caloric intake restriction, which could be responsible for any measurable changes in body odour, we collected blood samples at the same intervals as the body odour samples. The blood samples were subsequently shipped to the Prevedig laboratory to analyse levels of glucose (saccharide metabolism), sodium, potassium, chlorine (all electrolyte metabolism), uric acid (protein metabolism), total and conjugated bilirubin, cholesterol, triacylglyceride (lipid metabolism), and albumin levels (marker of food deficiency, Bharadwaj et al., 2016). As a consequence of diet restriction, we expected an increase in levels of bilirubin and uric acid, and a decrease in levels of glucose, sodium, potassium, chlorine, cholesterol, albumin, and triacylglycerides.

2.2.3. Questionnaires

Each evening, participants completed a validated 16-item questionnaire developed to assess mood (Bensafi, Brown, Khan, Levenson, & Sobel, 2004; Bensafi et al., 2003). The questionnaire consists of adjectives loading onto two scales: negative affective score (afraid, angry, annoyed, anxious, bored, contemptuous, disgusted, embarrassed, sad, stressed) and positive affective score (amused, calm, confident, content, happy, interested). Participants rated how strongly they were currently experiencing each of the 16 items, using a 7-point scale (1 – “not at all” to 7 – “very strongly”). Following the procedure of previous studies, the scores of the individual scales were calculated as a sum of answers provided to the respective items. Moreover, we obtained detailed information on participants’ food intake and the amount of food consumed (Havlicek & Lenochova, 2006), in order to estimate their caloric intake, see Supplementary material S4.

Participants completed these questionnaires assessing mood and food consumption every evening for one week before and until the end of the experiment (7 days before the first odour collection, 1 day before and after collecting the first odour sample, 2 days of complete caloric restriction, 3 days

of caloric restoration and 1 day after collecting the last body odour sample, 15 days in total). Into analysis, we included responses on mood and food intake obtained at the same time points as body odour and anthropometric measurements were collected.

2.2.4. Anthropometric measurements

To investigate whether the dietary restrictions have an impact on selected physical parameters, we measured body height and weight, the circumferences of breast, waist and hips, and finally body composition (body fat, muscle, water levels). Body weight and composition was measured using a Tanita UM-076 bioelectrical impedance scale. Breast size was measured as the largest circumference at the level of the chest, while waist and hips were measured as the smallest circumference of the waist and largest circumference of the hips. Subsequently, we calculated both body mass index (BMI: weight in kg divided by height in m squared) and waist-to-hip ratio (WHR: circumference of the waist divided by circumference of the hips).

2.2.5. Odour rating session

Both to avoid possible effects of olfactory adaptation in raters and in order to accommodate a large number of raters, we split the rating sessions over two days. On each day, we used one half of the samples, randomly selected. The ratings took place in a quiet, ventilated room with temperature ranging between 18°C – 20.5°C and humidity 35% - 37 %. The odour samples were removed from the freezer approximately 1 h before the rating session started. The stimuli were enclosed in opaque 250 ml glass jars, marked by a code. The samples were rated using verbally anchored 7-point scales for their (i) pleasantness, (ii) attractiveness, (iii) masculinity and (iv) intensity. In the event that raters found any of the samples too weak to assess, they were asked to select “I cannot smell the sample” instead of using the rating scales (this occurred in 5.28% of cases, rate similar to previous studies, e.g., Fialová, Sorokowska, Roberts, Kubicová, & Havlíček, 2018); these ratings were not included in further analysis. Participants recorded their rating immediately after sniffing each stimulus, but the time spent sniffing was not restricted.

2.3. Statistical analysis

To compare odour changes related to diet restriction, we used repeated measures ANOVAs with baseline, restriction phase and restoration phase as the within-subjects factor and odour ratings (e.g., intensity) as dependent variables. Similarly, we used repeated measures ANOVA to assess changes in physiological markers and affective states. In the case of uric acid, we observed that the assumption of sphericity was violated (assessed by the Mauchly's test), so we report results with Greenhouse-Geisser corrections. The Bonferroni test was used for the post-hoc comparisons. To avoid pseudoreplication and inflation of degrees of freedom, we first computed mean values of the individual ratings (e.g., attractiveness) for each body odour rater separately for each condition (i.e., baseline, restriction and restoration phases) and these values were entered in the subsequent repeated measures ANOVAs (i.e., rater is the unit of analysis). To investigate the relationship between body odour quality and physiological measurements, we used Kendall's Tau correlation. We opted for Kendall's tau test due to violation of assumptions for parametric tests (non-normally distributed data) and as it is recommended for small data sets and more accurate generalizations (Field, 2013). For descriptive statistics and post-hoc tests, see Supplementary materials S1 and S2, respectively.

We excluded one body odour donor from analysis of changes in bilirubin, due to her being in the hormone free interval with withdrawal bleeding, which would compromise validity of measurement. Another woman was not included into analysis of triacylglycerides as the levels were outliers (higher than 3 SD).

3. Results

3.1. Physiological changes

Across sampling phase, we found statistically significant changes in glucose levels ($F_{2,11} = 11.07$, $P < 0.001$). Post-hoc tests showed that levels of glucose were significantly lower during deprivation compared to both baseline and after restoration, with no significant difference between baseline and restoration phases. We also found significant changes in electrolyte levels of sodium ($F_{2,11} = 35.39$, P

< 0.001) and chlorine ($F_{2,11}$ = 24.07, P < 0.001), and triacylglycerides ($F_{2,10}$ = 7.789, P = 0.003), with post-hoc tests showing the same pattern across phases as for glucose.

The opposite pattern of changes was found in levels of uric acid ($F_{1.3,11}$ = 21.0, P < 0.001), bilirubin ($F_{2,10}$ = 77.4, P < 0.001), and albumin ($F_{2,11}$ = 12.06, P < 0.001). Post-hoc tests showed significantly higher levels of uric acid, bilirubin, and albumin during caloric restriction compared to baseline levels and after caloric intake restoration. Further, levels of both uric acid and bilirubin after intake restoration were significantly lower than baseline levels. No significant changes were detected in the levels of potassium ($F_{2,11}$ = 0.034, P = 0.966).

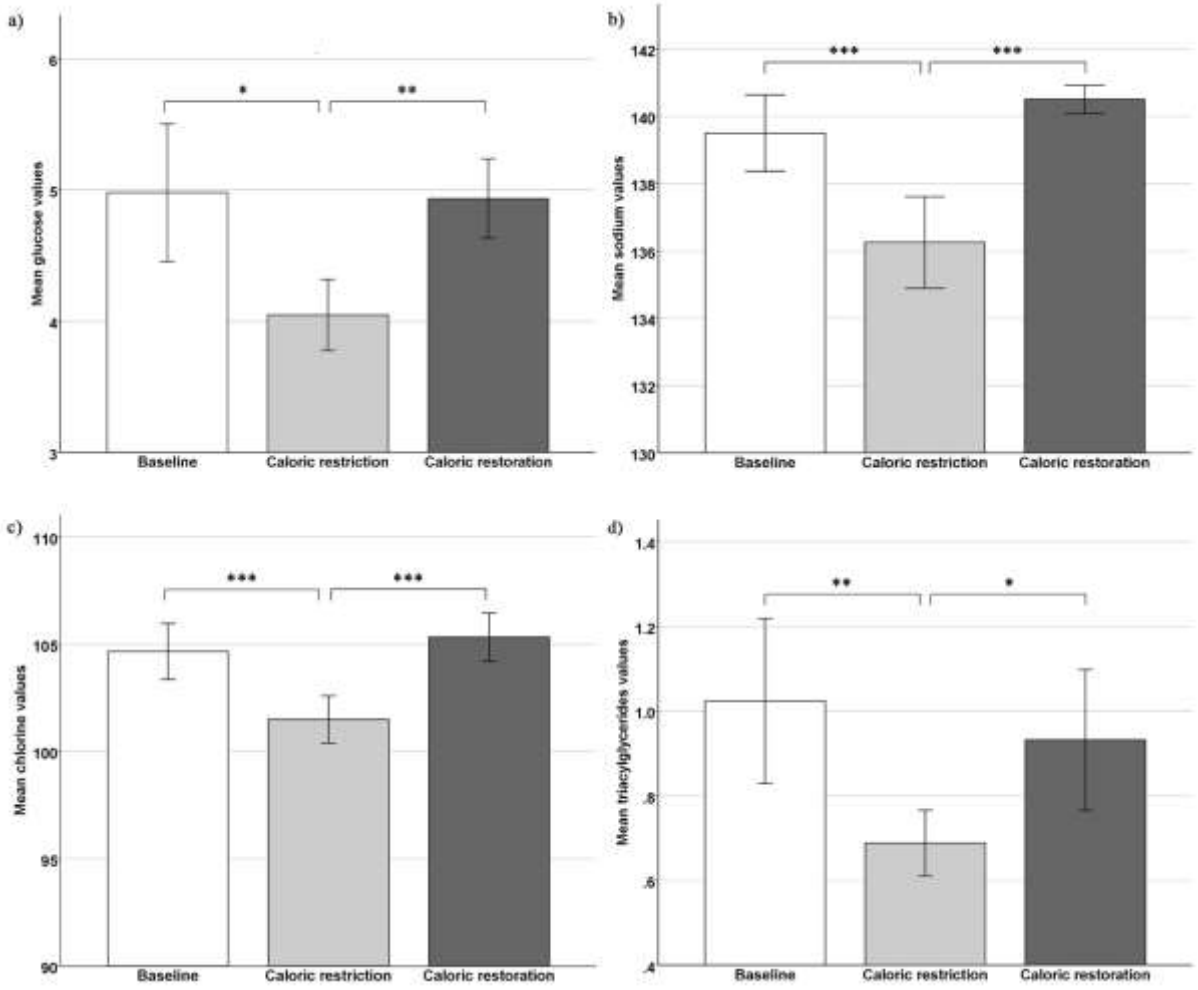

Figure 1 Mean values (± 95% CI) of a) glucose, b) sodium, c) chlorine, and d) triacylglycerides levels during habitual food regime (white bars), caloric restriction (light grey bars), and caloric intake

restoration (dark grey bars). Asterisks indicates level of significance; *p < 0.05 level, **p < 0.01 level, ***p < 0.001 level.

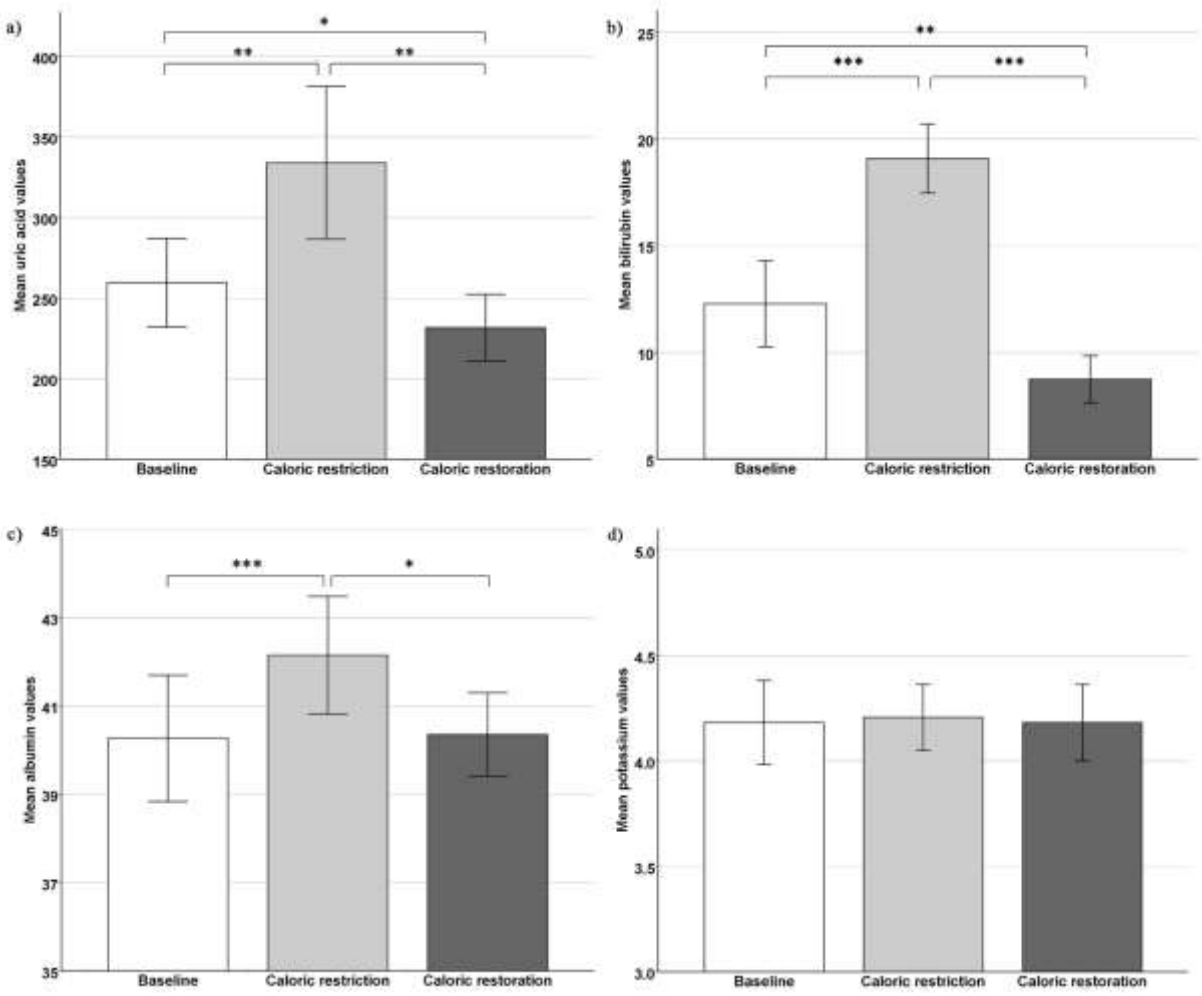

Figure 2 Mean values (± 95% CI) of a) uric acid, b) bilirubin, c) albumin, and d) potassium levels during habitual food regime (white bars), caloric restriction (light grey bars), and caloric intake restoration (dark grey bars). Asterisks indicates level of significance; *p < 0.05 level, **p < 0.01 level, ***p < 0.001 level.

3.2. Changes in affective states

There was also a statistically significant change in positive mood scores across sampling phases ($F_{2,11}$ = 3.57, *P* = 0.045). Scores of positive mood were lower during the restriction phase compared to

baseline and restoration phases, although these differences were not formally significant in post-hoc tests. There were no significant differences in negative mood scores ($F_{2,11} = 1.6$, $P = 0.225$).

3.3. Differences in caloric intake

Statistically significant differences were found in rates of caloric intake between baseline and restoration phases, as assessed by participants' dietary self-reports ($t_{11} = 2.424$, $P < 0.05$). Participants consumed less food after caloric intake restoration compared to baseline.

3.4. Anthropometric measurements changes

The repeated measures ANOVA showed statistically significant changes in BMI ($F_{2,22} = 33.79$, $P < 0.001$), body fat mass ($F_{2,22} = 5.389$, $P < 0.05$), body water content ($F_{2,22} = 4.53$, $P < 0.05$), muscle mass ($F_{2,22} = 7.26$, $P < 0.01$), breast circumference ($F_{2,22} = 6.231$, $P < 0.05$), and WHR ($F_{2,22} = 7.858$, $P < 0.01$). We found that BMI was significantly lower during the restriction phase compared to baseline levels and after caloric intake restoration, and was lower after caloric intake restoration than at baseline. Levels of body fat mass were lower during caloric restriction and restoration than baseline levels. The post-hoc comparisons did not reveal any differences in body water levels. Muscle mass was significantly lower after caloric restoration compared to baseline and the restriction phase. Furthermore, breast circumference and WHR were significantly lower during the restriction phase compared to baseline.

3.5. Hedonic ratings of the body odours

The repeated measures ANOVA revealed a significant main effect of sampling phase in odour pleasantness ($F_{2,56} = 16.26$, $P < 0.001$), attractiveness ($F_{2,56} = 17.72$, $P < 0.001$) and intensity ($F_{2,56} = 22.19$, $P < 0.001$). However, in contrast to our expectation, post-hoc tests revealed that this variation was driven more by differences in the restoration phase than during caloric restriction. There were no significant differences between baseline and restriction phases in any of the ratings, but odour samples following caloric intake restoration were rated significantly (all *P's* < 0.001) more pleasant and attractive, and less intense, compared to both baseline and the restriction phase. There was no significant phase difference in ratings of odour femininity (see Figure 3).

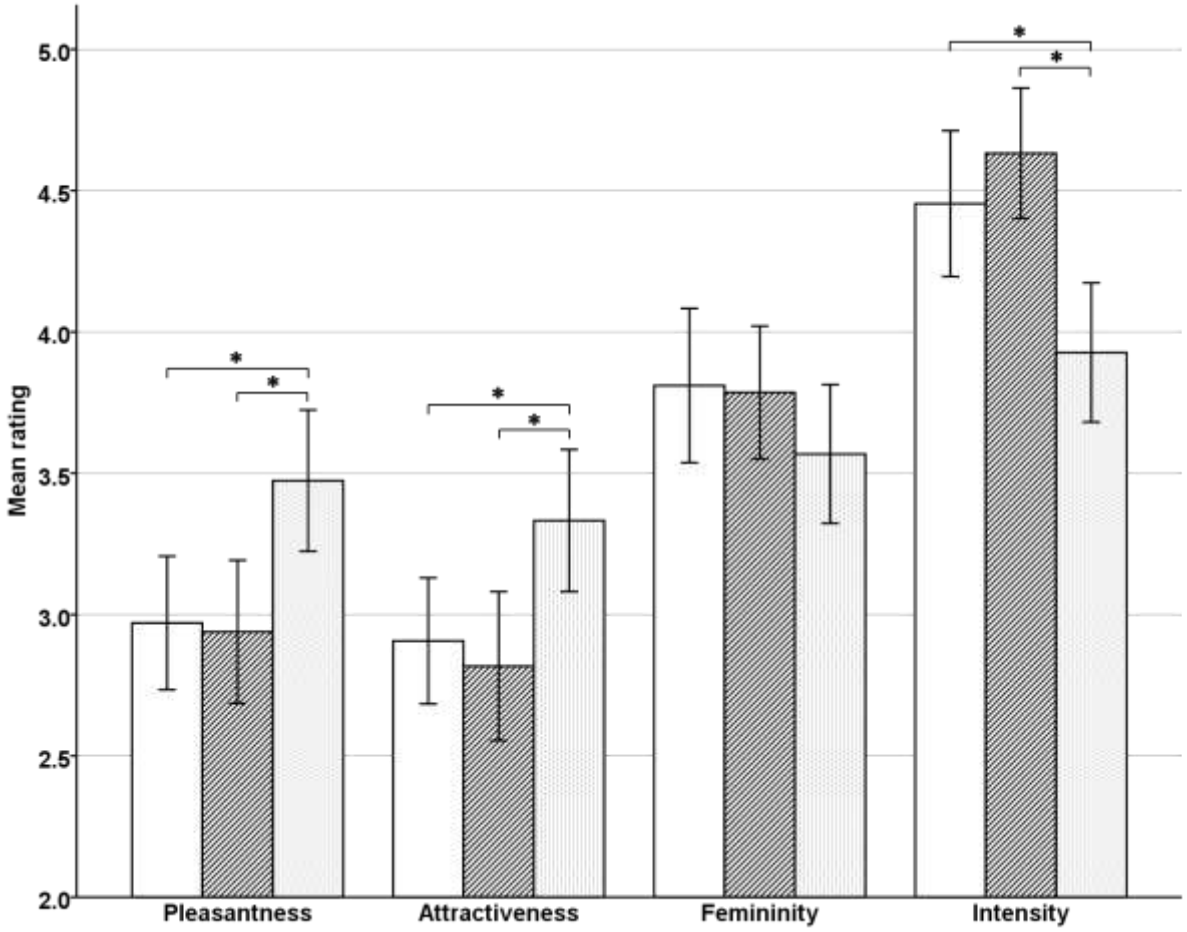

Figure 3 Mean ratings of female axillary odours on pleasantness, attractiveness, femininity, and intensity during habitual food regime (white bars), complete caloric intake restriction (dashed bars), and restoration of caloric intake (dotted bars) on a 7-point scale (e.g., 1 - very unattractive and 7 - very attractive). Asterisk indicates level of significance $p < 0.001$.

3.6. Associations between physiological measures and hedonic ratings of body odour

Correlational analysis showed no associations between changes in body odour quality and changes in any physiological measurements, when we compared parameters recorded at baseline and during the restriction phase (all $p > 0.05$). In comparisons of change between restriction and restoration phases, however, we found a positive correlation between change in sodium levels and change in both odour pleasantness ($\tau = 0.550$, $p < 0.05$) and attractiveness ($\tau = 0.614$, $p < 0.01$). There was also

a negative association between change in sodium level and change in odour intensity ($\tau = -0.453$, $p < 0.05$). For further details, see Supplementary Materials S3.

4. Discussion

The main aim of the current study was to test whether complete dietary deprivation for 48h affects body odour quality as judged by an independent panel of raters. In contrast to our predictions, we found no differences between pleasantness, attractiveness and intensity of the odour samples collected at baseline and during complete caloric intake restriction. However, we observed that odour samples collected 48h after diet restoration were rated significantly more pleasant and attractive, and of lower intensity, compared to both baseline and diet deprivation phases.

Previous studies have shown effects of different diet on social odours in humans (Fialová et al., 2016; Havlicek & Lenochova, 2006) and in other animals (e.g., Ferkin et al., 1997; Giaquinto, da Silva Berbert, & Delicio, 2010; Walls, Mathis, Jaeger, & Gergits, 1989). It is thus possible that the positive effect we observed here in the restoration phase might be a factor of dietary quality, as we recommended that odour donors should consume more “light and healthy” foods when they recommenced eating, in order to prevent possible digestive perturbation. Indeed, entries in their dietary diaries show slightly different food choices during the restoration phase compared with baseline. Moreover, if we consider many health benefits of alternate-day fasting regimes (e.g., decrease of body weight, body fat, total cholesterol, LDL cholesterol, triacylglycerol concentrations, systolic blood pressure; see Bhutani, Klempel, Kroeger, Trepanowski, & Varady, 2013; Heilbronn, Smith, Martin, Anton, & Ravussin, 2005; Varady, Bhutani, Church, & Klempel, 2009), similar health effects could be expected in our participants, which could be consequently reflected in their body odour. Body odour quality could be further affected by the absolute rate of caloric intake, as we found that participants consumed significantly fewer calories during the restoration phase compared to baseline (their habitual food regime). Correlations between change in sodium levels and changes in body odour quality suggest possible differences in fluid intake between phases which could in turn

affect participants' sweat. Unfortunately, we did not collect detailed data regarding fluid intake from all participants, but inspection of diet diaries did not reveal any unusual pattern during the restoration phase. If anything, it seems that fluid intake may have been higher during the complete caloric restriction phase, probably as a compensation for food deprivation. However, as we did not measure this precisely, we cannot here make stronger conclusions.

Although this is the first study to test the effects of food deprivation on quality of body odour in humans, one may expect that underlying physiological mechanisms will be at least partly shared across various mammal species. Our study is conceptually similar to a study on meadow voles (Pierce & Ferkin, 2005) which showed that 24h of food deprivation significantly decreased odour attractiveness of female voles, and a return to the original values two days after being refed. Nevertheless, it must be pointed out that 24h food deprivation in a small rodent, with a higher rate of metabolism, is likely to be a greater physiological challenge compared to 48h in humans. It is thus possible that, in order to achieve a comparable challenge and perhaps to detect a corollary decline in body odour quality, we would need to have our volunteers fast for a considerably longer period. Of course, for ethical reasons this may not be an achievable or desirable task. Even with the design we used here, three of our odour donors did not complete the complete caloric intake restriction procedure because they felt unwell: even a 48h period was demanding for some participants. Were a similar study to be undertaken in the future, one may therefore need to consider recruiting participants who routinely undergo either partial or complete fasting, such as combat athletes.

Another reason for the discrepancy in effect between our study and existing rodent studies might be a consequence of the stimuli type. The rodent studies employed urine samples, as urine comprises a common mode of olfactory communication. Urine quality is likely to be particularly sensitive to current nutritional status, as it includes molecules (some of them being volatile) involved in catabolic processes typical for metabolism during diet restriction. In the course of fasting, urine markers such as urea, creatinine, methionine, hydroxylysin, arginine, citruline, phenylalanine, alanine, tryptophan,

malondialdehyde, and 8-isoprostaglandin F 2α show considerable decrease (Lee et al., 2006; Rubio-Aliaga et al., 2011). It is thus not incidental that many medical screening assays on digestive metabolism or nutritional status are based on urine markers. In our study, however, we used axillary odour samples because armpit odour is arguably the most salient in adult humans and some scholars suggest that its production is at least partly the result of sexual selection (Comfort, 1971). It is possible that axillary odour might not be as useful at communicating cues of dietary quality as urine odour, although this speculation contrasts with a growing body of evidence suggesting that various aromatic chemicals derived from diet, such as red meat or garlic, do affect the perceived quality of human axillary odour (Fialová et al., 2016; Havlicek & Lenochova, 2006).

A further possibility for discrepancy between rodent studies and ours is that experimenters in the former have absolute control over the diet of the experimental subjects. Clearly this was not the case here, leading to the possibility that some of our participants contravened the instructions regarding fasting. If this happened, it could potentially account for our finding that there was no change in odour ratings across the baseline and the caloric restriction phase. However, we think this is unlikely on the basis of our physiological tests. To control the effectiveness of our experimental diet restriction, we collected venous blood and urine samples in parallel with collection of the body odour samples. The individual assays were selected to encompass individual components of digestive metabolism, such as metabolism of saccharides, proteins, lipids, and electrolytes. During the complete restriction of caloric intake, we found significantly lower levels of glucose, sodium, chlorine, and triacylglycerides, coupled with significantly higher levels of uric acid bilirubin, and albumin. These are all commonly used markers of poor nutrition or acute starvation in assessments by medical practitioners (Bharadwaj et al., 2016; Noakes, Keogh, Foster, & Clifton, 2005), and the results provide confirmation that participants complied with the restrictions. This is further evidenced by changes observed for the anthropometric measurements, specifically decreases in body weight, fat mass, breast circumference and WHR during caloric restriction. Thus, results of the physiological tests

convincingly indicate that the experimental paradigm of 48h complete food deprivation was sufficient to show significant metabolic changes in an expected manner.

We further assessed affective changes due to the complete caloric intake restriction by using a standard measure of positive and negative mood. We found a non-significant decrease in positive mood during complete caloric intake restriction as compared to the baseline and food restoration periods and no significant changes in negative mood score. Subsequently, we also tested whether the size and direction of change in body odour quality across sampling phases was correlated with change in either positive or negative mood scores, but in neither case were there any statistically significant associations. These null findings may either indicate that affective changes do not mediate changes in body odour quality or that we have only moderate power to detect such associations. We think that the latter interpretation may be more likely as there is robust evidence that affective states, such as stress or fear, have significant impact on body odour and can be perceived by others (de Groot & Smeets, 2017; Fialová & Havlíček, 2012).

On similar lines, one might argue that the lack of a detectable decrease in body odour quality during complete caloric intake restriction is due to a relatively small sample size in our study. However, our sample was large enough to detect a statistically significant increase in three measures of perceived odour quality from the restriction to restoration phase. Furthermore, our sample size is comparable to previous olfaction-related studies (e.g., Adolph, Schlösser, Hawighorst, Pause, & Schlosser, 2010; Chen & Haviland-Jones, 2000; Kohoutová et al., 2011; Lenochová, Roberts, & Havlíček, 2008) and importantly, our within-subjects experimental design is sensitive to detect subtle changes.

Nonetheless, it is worth noting that recruitment for this study was unusually challenging. Despite our best efforts, we were unable to find more volunteers who were willing to follow the experimental protocol, because of the fasting requirement. In this regard, we experienced particular and considerable difficulty in recruiting men as body odour donors. On average, men have larger bodies and a higher basal metabolic rate, and thus the influence of a 48h fast could represent a more severe

physiological and psychological challenge. Had we been able to test men as well as women, however, we might expect that these sex differences could lead to more profound effects on their body odour. Furthermore, from a functional perspective, it may be that detection by women of cues of men's nutritional state is more advantageous than the reverse, because of its potential to inform women about a potential mate's ability to provide direct benefits. Indeed, this is a leading theoretical explanation for higher average olfactory function in women than men (Brand & Millot, 2001; Doty & Cameron, 2009; Havlíček et al., 2008). These considerations raise the possibility that our sample may be subject to selection bias, as those participants who volunteered (or even considered) to take part in this specific study might be healthier and fitter than average. If so, then the fasting period might not represent such a different challenge and experience compared to less fit people, who might therefore respond differently. Indeed, participants in our study were healthy (as examined by a physician) and within normal BMI range (values between 19.6 – 23.6). Nonetheless, at least in this sample, the physiological and anthropometrical measurements clearly show that the fasting period considerably affected our participants.

As mentioned above, the lack of a direct effect of food deprivation for 48h on perceived odour quality may arise because it was a relatively short period of deprivation compared to effects on other species with smaller body size. However, there are ethical and procedural barriers to further extending this period of acute deprivation. Thus, an alternative approach would be to assess longer-term effect of chronic caloric restriction. This could be further investigated, for example, if participants were to follow protocols with different levels of caloric intake (e.g., half or quarter of recommended daily rate) over a longer period, thus allowing comparison across various dietary regimes. Furthermore, several previous studies have shown effects of certain foods on body odour (Fialová et al., 2016; Havlicek & Lenochova, 2006; Zuniga et al., 2017), opening up a broad field of research. While numerous food components could be investigated in a similar fashion, future studies

might rather concentrate on manipulation of major nutrient groups, such as proteins, fat, or carbohydrates, as has been done similarly in some rodent studies (Pierce & Ferkin, 2005).

In summary, our study brings new evidence concerning the effect of nutritional quality on human semiochemistry. In general, humans fit well into the emerging picture which illustrates that diet is an important contributor to vertebrate social chemoperception. Until recently, this was frequently considered more as a confounding variable to be controlled for, rather than a primary focus of behavioural research. On the other hand, our study also shows that scholars should carefully consider specificities of the species under investigation, including both attention to the prevalent sources of social odours and differences in size and metabolic rates. More specifically, the close link between the source of chemical cues employed (i.e. urine) and digestive metabolism (Singh et al., 1990) will likely mean that species relying primarily on urinary odour cues might be more responsive to diet-related cues than other species, including humans.

5. Declarations of interest

None.

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