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ORIGINAL ARTICLE

Effects of a 48-h fast on heart rate variability and cortisol levels in healthy female subjects

N Mazurak[1,2], A Günther[1], FS Grau[1], ER Muth[3], M Pustovoyt[2], SC Bischoff[4], S Zipfel[1] and P Enck[1]

BACKGROUND/OBJECTIVES: The physiological changes that occur during fasting are not completely understood, regardless of the cause for fasting (for example, medical, lifestyle, religious, political or famine). The purpose of this study was to examine the effects of a 48-h fast on heart rate variability (HRV) and cortisol levels in healthy young female volunteers.

SUBJECTS/METHODS: A total of 16 young healthy female volunteers underwent 48 h of total fasting under 24-h medical surveillance. Psychological (subjective feeling of hunger) as well as physiological data (HRV, diurnal cortisol profiles) were measured upon admission (Day 1), and after 24 (Day 2) and 48 h (Day 3) of fasting.

RESULTS: There was a measured weight loss from Day 1 to Day 3 that resulted in significant body mass index (BMI) reduction across all subjects ($P<0.001$). The slope of the diurnal cortisol profile significantly shifted towards lower values from baseline to the end of experiment ($P=0.002$). HRV during resting showed a significant ($P<.001$) decrease in standard deviation of the normal-to-normal interval (SDNN) and root mean square of successive differences (RMSSDs) from Day 1 to Day 3 of the experiment, with a small increase after 24 h that did not reach statistical significance. A 48 h of fasting also induced a significant ($P<.001$) decrease of mean interbeat intervals (IBIs), SDNN, RMSSD and log high-frequency (HF) power during head-up tilt testing.

CONCLUSIONS: An acute (48 h) total fast induced parasympathetic withdrawal with simultaneous sympathetic activation. These changes appear to reflect stress. Further studies are needed to demonstrate the specificity of these changes to fasting.

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INTRODUCTION

Fasting and starvation have accompanied humans since the beginning of history, due to life circumstances (for example, seasonal changes, famine, war, imprisoning), and religious (for example, Ramadan, Lenten season) or political beliefs (for example, hunger strike).[1] Only during the second half of the twentieth century has fasting also become a medical and scientific topic. The first and most prominent investigation of the long-term effects of starvation was the Minnesota Starvation Study in the 1940s.[2] Motivated by the long-term starvation that had occurred in prisoners of war and in NAZI concentration camps, this study aimed to understand the psychological and physiological changes associated with starvation and the ability of the body and mind to survive long-term starvation.[3]

Fasting as a medical and scientific tool came to life during the 1950s and 1960s and was widely recommended for rapid weight loss in obese patients.[1] Studies by Bloom,[4] Bloom *et al.*,[5] Drenick and Smith,[6] Cahill *et al.*[7] and Cahill[8] developed a framework for 'therapeutic starvation'. Later, in the 1990s, a second wave of interest came in connection with the increased incidence and public and scientific awareness of eating disorders such as anorexia nervosa (AN), bulimia and obesity.[9,10] In AN, predominantly young women, 'apparently' voluntarily fast to an extent that poses them at risk for survival.[11,12] However, the relationship between the physiology of dieting in healthy persons and that in AN is insufficiently understood.

The spread of obesity among the population of Western societies[13] has created an environment in which dieting and fasting cycles have almost become an everyday life experience for many.[14] Beside the fact that many patients, and many clinicians as well, believe this procedure to be beneficial for health and its wide use all over the world, the physiological and psychological influence of fasting is incompletely understood.

Numerous research groups have examined metabolic changes and their regulatory mechanisms during different types of food restriction, including: short (1–7days) and long-term (usually up to 28 days) fasting; total fasting; and intermittent fasting.[2,7,15–18] However, experimental studies where volunteer participants of normal or overweight have been asked to maintain a low- or zero-calorie diet during at least 24–72 h are limited.[19–22] Despite the fact that short-term total food withdrawal models do *not* reflect general changes during fasting in clinical settings or 'everyday' dieting experiences, they allow the observation of initial physiologic changes during food deprivation. These type of short-term experimental fasts could be used as a model of fasting due to religious convictions (for example, 24 h of total fast in

[1]University Hospital, Department of Internal Medicine VI: Psychosomatic Medicine and Psychotherapy, Tübingen, Germany; [2]Department of Clinical and General Pathophysiology, Odessa National Medical University, Odessa, Ukraine; [3]Department of Psychology, Clemson University, Clemson, SC, USA and [4]University of Stuttgart-Hohenheim, Institute for Nutritional Medicine, Stuttgart, Germany. Correspondence: Professor P Enck, University Hospital, Department of Internal Medicine VI: Psychosomatic Medicine and Psychotherapy, Frondsbergstrasse 23, Tübingen 72076, Germany.
E-mail: paul.enck@uni-tuebingen.de
Contributors: NM performed the statistical analysis and wrote the manuscript, FSG and AG recruited the volunteers and performed the investigations, ERM helped with statistics and writing the manuscript, MP assisted in writing, SCB and SZ were responsible for the clinical assessments at investigational site and PE had the idea for the study and supervised data recording, statistics and the writing of the paper.
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orthodox states, usually is practiced once a week) or intermittent fasting (24–36 h of total fasting followed by the day of *ad lib* food intake).[23,24]

Most studies have focused on metabolic effects during and after the fasting period, with only a few measuring changes in autonomic balance during total fasting.[20,22,25] Consideration of autonomic changes in conjunction with the neuroendocrine changes is important as the autonomic nervous system (ANS) takes part in the regulation of adaptation to different environmental conditions (for example, food restriction), and regulates energy expenditure and the visceral systems (cardiovascular, digestive, and so on).[26,27]

Analysis of heart rate variability (HRV) is a non-invasive tool for estimation of autonomic activity, including the central regulatory inputs, and allows for the observation of the general state and reactivity of the ANS under different conditions.[28,29] Despite ongoing discussion about its reliability and reproducibility for clinical purposes,[30,31] it provides relatively stable results in controlled experimental conditions[29] and allows evaluation of cardiovascular and metabolic adaptation, as well as estimation of possible risks of regulation failure.[32]

Another important central-to-peripheral control branch that should be evaluated when adaptation is the focus of research is the hypothalamic-pituitary adrenal axis. Besides the role of hypothalamic-pituitary adrenal axis in regulating stress conditions, recent studies have also discussed its role in the regulation of appetite[33] through its influence on leptin mRNA levels and leptin secretion.[34,35] In addition, cortisol plays a role in body weight and appetite regulation by its counter-regulatory effects on insulin secretion.[36,37]

Therefore, the aim of this experiment was to estimate cardiovascular and regulatory (ANS, cortisol) changes during short-time (48 h), voluntary total food deprivation in healthy young female volunteers.

MATERIALS AND METHODS

Subjects

A total of 16 healthy female volunteers (average age: 21.4 ± 2.1 years, range: 18–28 years; body mass index (BMI): 21.6 ± 1.6 kg/m^2) participated in this study. All were recruited through public advertisements and received payment for their participation in the study. All participants gave written informed consent before participation, and the study protocol was approved by the Ethics Committee of the University of Tübingen Medical School.

Before inclusion, all participants were interviewed and underwent physical and psychological examination by an experienced health-care practitioner from the psychosomatic department. To be included in the study, participants were required to have a BMI between 19 and 25 kg/m^2. Participants were excluded based on self-report of substance abuse (alcohol, nicotine, drugs), use of any medication up to 2 weeks before the experiment (excluding contraceptives), any acute or chronic bodily or mental disease, past trauma of the head or face, and a past history of eating disorders, obesity and excessive sport training. Nine volunteers reported regular sport practice (running, swimming, bike), six had irregular training and one reported not engaging in any sport at all. Of the female volunteers, 10 reported having used oral contraceptives.

Study protocol

The study was conducted between 5 March and 11 April 2008 at the Department for Psychosomatic Medicine and Psychotherapy, University Hospital Tübingen, Germany. Volunteers were recruited and investigated pairwise at a metabolic ward. They were kept under strict surveillance day and night, and were escorted to the investigations reported here by study personal (FSG, AG). A 24-h medical observation was employed to control the health state of participants and to react to any possible complications.

The study protocol required a 60-h stay (from 0700 hours on Day 1 to 1700 hours on Day 3) at the metabolic ward and maintenance of a zero-calorie diet during the entire experimental period, with water '*ad lib*'. Water intake was carefully documented. On the day of admission, participants were instructed to consume their usual breakfast at home before coming to the laboratory at 0700 hours. The timeline of the entire experiment is illustrated in Figure 1.

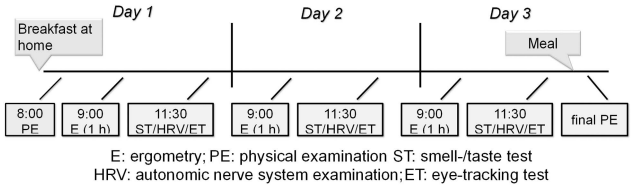

Figure 1. Timeline of the experiment. E, ergometry; ET, eye-tracking test; HRV, autonome nerve system examination; PE, physical examination; ST, smell/taste test.

In this paper, we report data on cardiac autonomic function, salivary cortisol release and psychometric measures. To exclude the influence of the circadian rhythm, all physiologic measures were taken between 0900 hours and 1400 hours.

Assessment

Blood samples for routine clinical laboratory assessment were taken before the fasting period. Blood samples were repeated after 48 h of fasting, as were the medical exam and the psychometric assessment. Participants' weight was taken on admission, and after 24 and 48 h of fasting. The BMI as well as BMI changes were calculated at each time point. Blood glucose levels were assessed every 6 h during the entire experiment to assure compliance and to prevent possible hypoglycemia events due to fasting. Hunger ratings (between 0 = no hunger and 10 = maximum hunger) were recorded at the time of blood glucose measurement.

Salivary cortisol. Cortisol was measured in saliva samples collected by means of commercial Salivettes (Sarstedt, Nuremberg, Germany) at 1-h intervals between 0800 hours and 1100 hours, and a final daily sample at 1700 hours (except on Day 3). These samples were used for the diurnal cortisol profile. Samples were stored at − 20 °C for later analysis. Salivary cortisol levels were determined using commercial LIA kits (IBL, Hamburg, Germany) as described previously.[38] In short, 1000 μl of saliva was pipetted, and centrifuged for 5 min at 2000 r.p.m. before salivary-free cortisol analyses. An unknown amount of cortisol in the sample and a fixed amount of enzyme-labeled cortisol (20 μl per well) compete for the binding sites of the antibodies coated onto the wells of a 96-well plate. After 3-h incubation at room temperature, the wells were washed to stop the competition reaction. A measure of 50 μl of luminescence substrate solution (1:1 luminol enhancer and peroxide solution) was added for 10 min, and a luminometer read the plates.

Heart rate variability. Subjects underwent a standardized research protocol to measure HRV with the TaskForce Monitor (CNSystem, Graz, Austria). The investigation took place in a laboratory with controlled temperature and light conditions at 1130 hours on Days 1–3. During the investigation, all subjects were in the supine position on a tilt table. After fixation of a standard lead II electrocardiogram, all subjects completed the standard clinical ANS function protocol that consisted of a baseline recording (3 min), two subsequent periods of paced breathing to a metronome (1 min each), a modified color–word conflict (Stroop) test presented on a computer screen (< 1 min), two Valsalva tests (for 15 s), a mental arithmetic test (2 min) and passive head-up tilt test at 60° (3 min). Between each stress test and after the final tilt test, a 3-min recovery period was maintained. This protocol was developed for routine clinical testing. With the exception of the tilt test, all stress tasks were too short to be applicable to HRV analysis individually. We therefore restricted the analysis to recording periods of 3 min, which included a baseline recording, the tilt test and a post-tilt resting period.

Heart rate data were recorded at a 1000 Hz sampling rate. Time domain data that reflect general characteristics of the HRV, and frequency domain HRV parameters that measure more specific contribution of each ANS branch, were calculated to assess autonomic regulation[29,39] using the 'Kubios HRV' v. 2.0 software developed by the 'Biosignal Analysis and Medical Imaging Group' (Department of Physics, University of Kuopio, Kuopio, Finland).[40]

A total of 144 data sets (16 participants in 3 different days, 3 sections from each record) were selected and screened for artifacts. In all, 16

intervals needed correction due to either missing or doubled R-spikes. In the time domain, mean interbeat interval (meanIBI), standard deviation of the normal-to-normal (NN) interval (SDNN) and root mean square of successive differences (RMSSD) were assessed. In the frequency domain, low-frequency (LF) and high-frequency (HF) absolute power and normalized units (nu) were calculated, as well as an LF/HF ratio. Spectral domain parameters were obtained using a running fast Fourier transformation algorithm with a window width of 64 s and a window overlap of 75%. The latter transforms the time domain of R–R intervals into a spectral curve in which several 'bands' can be estimated: the HF band (0.15–0.4 Hz) represents exclusively vagal influences, and the LF band (0.03–0.15 Hz) represents baroreflex (including sympathetic and parasympathetic) influences on the heart rhythm. The LF/HF index presumably reflects the relation of the two ANS branches, but the question of its validity and reliability is still widely discussed in the literature.[28] Absolute power values were logarithmically transformed to normalize them for parametric statistical analysis.

Statistics

Repeated-measures analysis of variances (ANOVAs) with *post hoc t*-tests were applied to compare the results for the three time points: on admission day, after 24 and 48 h of total fast. It has been shown in various studies that the BMI influences HRV data.[41,42] Hence, the BMI difference between Day 1 and Day 3 expressed in percent of BMI change from admission was used as a covariate in statistical tests for HRV parameters. Because HRV parameters are dependent, we accounted for the effect of multiple ANOVAs with dependent variables by setting the level of significance to $P = 0.01$. For all other tests, the significance level was set at 0.05. All data are presented as mean ± s.d.

RESULTS

General results

There was a continued loss of weight from Day 1 to Day 3 that resulted in significant BMI reduction across all subjects (Table 1).

Average hunger ratings for each day increased from admission day to the end of experiment. However, five participants showed the opposite effect: they reported a reduction of feelings of hunger throughout the experiment. Linear regression analysis revealed a significant dependence between BMI on admission day and changes in subjective feeling of hunger during the experiment ($R = 0.597$, $F = 7.752$, $P = 0.015$) (Figure 2), with those individuals having lower BMIs showing smaller increases or even a reduction in hunger ratings.

The correlation of subjective feeling of hunger with other parameters, for example, cortisol levels or percent of body fat, did not show consistent results (data not shown).

Table 1. Individual body weight, BMI and hunger ratings (averaged over the day) (mean ± s.d.) in 16 healthy female subjects on three consecutive days on a zero-calorie diet

Subject no.	*Day 1*	*Day 2*	*Day 3*	*Statistics*
1	53.2	52.4	51.8	
2	54.0	54.0	52.5	
3	52.2	51.0	50.0	
4	66.3	66.5	64.8	
5	59.2	57.8	57.7	
6	60.1	58.8	58.9	
7	61.3	61.1	59.3	
8	73.8	73.0	71.7	
9	58.0	57.2	56.1	
10	61.6	60.3	59.5	
11	61.1	60.1	59.6	
12	57.7	56.5	56.3	
13	75.0	75.0	74.1	
14	61.5	60.7	60.3	
15	50.3	49.4	48.5	
16	60.0	58.9	58.0	
Mean weight (kg)	60.3 ± 6.9	59.5 ± 7.1	58.7 ± 7.0	$P < 0.0001$
Mean BMI	21.62 ± 1.48	21.33 ± 1.59	21.03 ± 1.49	$P < 0.0001$
Rating of hunger	3.09 ± 1.21	4.10 ± 2.66	4.70 ± 3.08	$P = 0.015$

Abbreviations: BMI, body mass index; s.d., standard deviation.

Cortisol

Peak cortisol values did not change after 24 and 48 h of total fasting compared with admission day. However, the diurnal profile showed a shift to the right (Figure 3) that was stronger after 24 h as compared with 48 h, and the slope of the cortisol day profile revealed significant changes, that is, there was a shift towards lower values from baseline to the end of experiment. This change was significant between admission day and 24 h of fasting (3.45 ± 0.92 vs 1.98 ± 0.8, $P = 0.002$), indicating a flatter profile with a slower decrease of cortisol values from morning peak to mid-day levels.

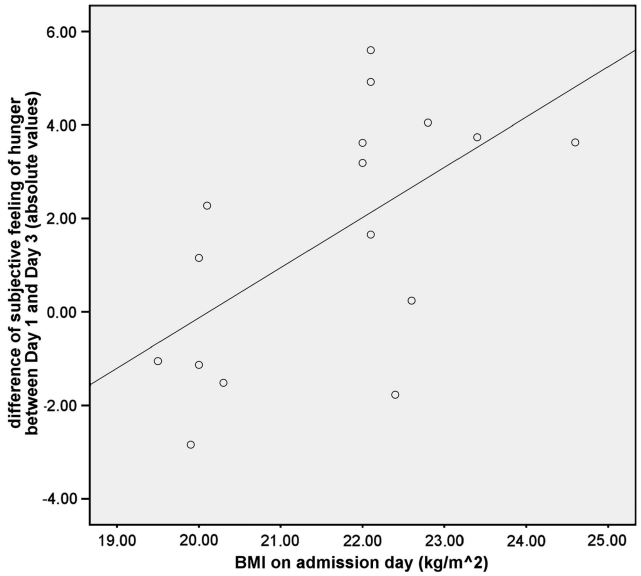

Figure 2. Linear regression between BMI on admission day and difference of subjective feeling of hunger between the last day of the experiment and Day 1. Participants with lower BMI showed no increase or even a decrease in subjective feeling of hunger ($R = 0.597$, $F = 7.752$, $P = 0.015$).

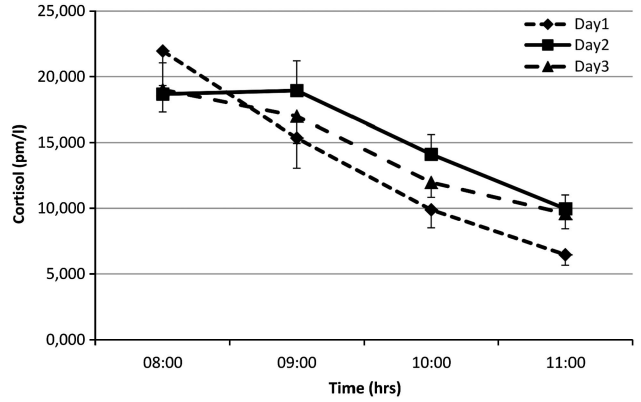

Figure 3. Diurnal profiles of cortisol on admission day and for two consecutive days of fasting. The slope of the cortisol profile shows a significant shift to the right from Day 1 to Day 2 (for details see text). Data are presented as mean ± s.e.m.

HRV during baseline

As is shown in Table 2, some baseline (Day 1) HRV measures increased after 24 h of fasting (Day 2) and then decreased below baseline on Day 3 (48 h). While the moderate increases from Day 1 to Day 2 did not reach statistical significance for any of the measures, the subsequent fall on Day 3 was significant for SDNN and RMSSD. Normalized HF and LF values did not change across the 3 days.

HRV during tilt testing

As can be seen in Table 3, mean IBI, SDNN, RMSSD and logHF power showed a significant and steady decline from Day 1 to Day 3. LFnu and the LF/HF index showed a steady increase from Day 1 to Day 3, but the values did not reach statistical significance owing to the statistical correction for multiple testing.

All HRV measures returned to their pretilt baseline values on all three days, with no difference between days (data not shown).

DISCUSSION

We report the influence of 48 h of total fasting on autonomic regulation of heart rhythm measured by HRV and on salivary cortisol levels in healthy non-obese women. We found a decrease of most measures during resting after 48 h as compared with admission, but more so for the response to a cardiac load during tilt testing.

The data reported suggest a decrease in total HRV as well as a decrease in parasympathetic and baroreceptor regulation of heart rhythm (vagal withdrawal) under baseline conditions as a result of fasting. The moderate increases in SDNN and RMSSD from Day 1 to Day 2 may represent a short-term compensatory response. Evidently, decrease in HRV and vagal withdrawal associated with fasting are more pronounced with a stress challenge to the cardiac regulatory system during (tilt) testing than during resting activity. It appears that the normal compensatory mechanism to tilt testing is no longer effective after 24 h of food deprivation.

The moderate increase in LFnu (significance is lost owing to correction for multiple comparisons), especially during tilting, may be explained either as increased vagal withdrawal, and/or as sympathetic activation. The latter option is further supported by the shift of the diurnal cortisol profile towards higher levels during the day and a slower decline indicating stress-induced growth of cortisol release and sympathetic activation. As with resting HRV measures, the cortisol values tend to return to baseline on Day 3 (after 48 h of zero-calorie diet) as compared with Day 2 (24 h), indicating that sympathetic activation may be responsible for the compensatory response (Days 1–2).

Our data are in partial agreement with previously published studies. Chan *et al.*[20] observed a reduction in mean IBI, SDNN, RMSDD, LF and HF powers from Day 1 to Day 4 of 72 h of zero-calorie fasting under both placebo and leptin administration conditions. In addition to vagal withdrawal, they found support for sympathetic activation in response to fasting, by observing increased level of urine catecholamines (dopamine and norepinephrine). Vagal withdrawal and sympathetic activation were also found in another study by our group.[21] In our previous study, we observed a reduction in HFnu and shortening of pre-ejection period as a measure of sympathetic activity after 24 h of zero-calorie fasting in a different sample of healthy female volunteers.

Table 2. Resting HRV parameters (mean ± s.d.) in 16 healthy female subjects on three consecutive days on a zero-calorie diet

Parameter[a]	*On admission, Day 1*	*After 24 h, Day 2*	*After 48 h, Day 3*	*Repeated-measures ANOVA, Days 1–3*
IBI	986.76 ± 265.95	1007.45 ± 257.07	982.37 ± 251.02	$F = 0.492$, $P = 0.617$
SDNN	87.40 ± 53.31	89.66 ± 38.76	72.97 ± 40.84	$\mathbf{F = 5.511}$, $\mathbf{P = 0.01}$[b]
RMSSD	96.85 ± 79.11	99.62 ± 54.34	80.41 ± 64.46	$\mathbf{F = 6.222}$, $\mathbf{P = 0.006}$
LogLF power	3.10 ± 0.53	3.07 ± 0.34	2.89 ± 0.40	$F = 4.868$, $P = 0.015$
LogHF power	3.20 ± 0.78	3.31 ± 0.64	3.14 ± 0.60	$F = 3.895$, $P = 0.032$
LFnu	44.38 ± 21.42	36.89 ± 22.23	37.41 ± 22.04	$F = 1.5$, $P = 0.24$
HFnu	55.62 ± 21.42	63.11 ± 22.23	62.59 ± 22.04	$F = 1.5$, $P = 0.24$
LF/HF index	1.40 ± 1.99	1.59 ± 3.87	1.79 ± 4.80	$F = 0.443$, $P = 0.646$

Abbreviations: ANOVA, analysis of variance; HF, high frequency; HRV, heart rate variability; IBI, interbeat interval; LF, low frequency; nu, normalized units; RMSSD, root mean square of successive difference; SDNN, standard deviation of the normal-to-normal interval. Significant differences of data between Days 1, 2 and 3 are printed in bold. *Note*: ANOVA results are corrected for multiple comparisons, and *P*-value was set to 0.01 to assess significance. *Post hoc t*-tests compared the single days in case of significance. [a]See text and Berntson *et al.*[28] and Taskforce[29] for details. [b]*Post hoc t*-test $P < 0.001$, for Day 1 vs Day 3.

Table 3. HRV parameters (mean ± s.d.) during tilt testing in 16 healthy female subjects on three consecutive days on a zero-calorie diet

Parameter[a]	*On admission, Day 1*	*After 24 h, Day 2*	*After 48 h, Day 3*	*Repeated-measures ANOVA, Days 1–3*
IBI	778.98 ± 186.05	732.68 ± 168.97	692.39 ± 144.02	$\mathbf{F = 9.23}$, $\mathbf{P = 0.001}$[b,c,d]
SDNN	77.53 ± 43.58	63.32 ± 28.73	54.83 ± 32.38	$\mathbf{F = 16.662}$, $\mathbf{P < 0.001}$[b,c]
RMSSD	40.05 ± 32.30	30.41 ± 26.42	26.49 ± 27.46	$\mathbf{F = 14.316}$, $\mathbf{P < 0.001}$[c]
LogLF power	3.02 ± 0.42	2.97 ± 0.50	2.89 ± 0.51	$F = 3.103$, $P = 0.061$
LogHF power	2.49 ± 0.79	2.25 ± 0.74	2.15 ± 0.72	$\mathbf{F = 10.853}$, $\mathbf{P < 0.001}$[b,c]
LFnu	72.48 ± 18.87	81.08 ± 15.35	81.57 ± 15.49	$F = 3.513$, $P = 0.044$
HFnu	27.52 ± 18.87	18.92 ± 15.35	18.43 ± 15.49	$F = 3.513$, $P = 0.044$
LF/HF index	6.13 ± 7.23	7.37 ± 5.47	7.82 ± 6.49	$F = 2.353$, $P = 0.114$

Abbreviations: ANOVA, analysis of variance; HF, high frequency; HRV, heart rate variability; IBI, interbeat interval; LF, low frequency; nu, normalized units; RMSSD, root mean square of successive difference; SDNN, standard deviation of the normal-to-normal interval. Significant differences of data between Days 1, 2 and 3 are printed in bold. *Note*: ANOVA results are corrected for multiple comparisons, and *P*-value was set to 0.01 for indication of significance. *Post hoc t*-tests compared the single days in case of significance. [a]See text andBerntson *et al.*[28] and Taskforce[29] for details. [b]*Post hoc t*-test $P < 0.001$: Day 1 vs Day 2. [c]*Post hoc t*-test $P < 0.001$: Day 1 vs Day 3 [d]*Post hoc t*-test $P < 0.001$: Day 2 vs Day 3.

In contrast to our conclusion of sympathetic activation during acute total fasting, Webber and Macdonald[25] found the levels of noradrenaline to be stable after 36 h, but increased after 72 h of fasting. Their data were confirmed by Patel *et al.*,[22] who found the level of noradrenaline in human plasma unchanged after 72 h of total fasting, but it was significantly increased locally in adipose tissue. Andersson *et al.*[19] observed a significant reduction in blood pressure and an increase in sympathetic activity, but no changes in blood and urine levels of noradrenaline. We speculate about a general decrease in sympathetic activity as a result of fasting, but the nonsignificant increases of noradrenaline may also be explained through its accumulation in adipose tissue, as discussed by Patel *et al.*[22] On the basis of these findings one could hypothesize that no signs of general adaptation reaction to food withdrawal occurs, but rather more specific changes (for example, in adipose tissue) occur in response to the fasting. The difference could also be due to estimation of ANS activity using different parameters, that is, adrenaline, noradrenaline and their metabolites vs HRV and cortisol.

Some animal studies[43,44] have shown sympathetic deactivation during short-term fasting, as measured by noradrenaline turnover in the heart. Because these studies were conducted in rats and the data were taken after at least 48 h of starvation, one can only speculate if sympathetic activation could have been found during earlier stages of fasting because of a more intense metabolism in these animals. The adaptation processes may take less time in rats than in humans.

In contrast to short-term studies, experiments using long-term models of calorie reduction have shown opposite results. These studies conducted in human volunteers with normal weight and in obese volunteers, in general, showed homogeneous outcomes. Overall they showed a decrease in sympathetically modulated and an increase in vagally modulated parameters of HRV.[17,45–47]

The apparent discrepancies between results obtained in short- and long-term fasting studies with different calorie reduction models suggest that the changes seen during the early phase (24–72 h) of a low- or zero-calorie diet may be the result of a severe stressor rather than reflecting specific changes in autonomic regulation and cortisol levels to fasting. This opinion is supported by a study by Tomiyama *et al.*[48] Similar to our findings, they found an increase in cortisol release during fasting, mainly due to an increase in evening cortisol levels. The authors proposed fasting as a stressful procedure that, besides being a somatic stressor, may also evoke many aversive feelings and act as psychological stressor. This hypothesis is further supported by animal data. A 10-day moderate (25%) diet restriction in mice led to reprogramming of stress and orexigenic signal pathways. After the diet stress exposure, the animals exhibited binge behavior and rapid weight regain, and the authors[49] suggested that with respect to human weight loss programs, stress management during and after dieting may be essential for successful maintenance of the weight reduction.

Further studies are needed to answer a number of questions. First, does long-term, zero-calorie dieting change cardiac regulation (HRV) similar to short-term total food withdrawal (increased vagal withdrawal, sympathetic activation). In this case, the data would suggest the changes to be predominantly stress-mediated. Second, whether changes in long-term models of total food deprivation reflect modification of vagal activity only and not sympathetic activation. In this case long-term dieting would mimic changes of HRV as seen in AN,[50] and would shed light on the increased cardiac risk not only in anorexia patients[51] but also in healthy volunteers undergoing zero-calorie diets for presumed health reasons.[52] It would also be of interest to know whether, and to what degree, the gastrointestinal tract and its autonomic regulation is involved in such diet-induced changes as it is primarily involved in the processing of the (restricted) food offer. Recordings of the electrogastrogram and other specific measures of intestinal activity have, however, not yet been performed during short- and long-term fasting experiments.

Our study has some limitations that need to be acknowledged. First the number of participants is rather small, and this could have resulted in the loss of significance for some parameters tested, for example, the 'area under the curve' for cortisol. Second, we have studied a rather homogeneous group of young women only with overall normal weight in the lower range (see Table 1). This may have caused us to overlook important variation in those with higher normal weight or moderately overweight volunteers and in male subjects. Finally, we did not control for the menstrual cycle, which could have been of importance because of its known impact on some of our research parameters.[53]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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