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Diurnal rythm of Nampt is gender and weight dependent

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ABSTRACT

Research Aim: Nicotinamide phosphoribosyltransferase (Nampt) is an adipocytokine that is elevated in obesity, type 2 diabetes and increased levels are associated with inflammatory processes. Nampt serum concentrations have been suggested to follow a diurnal rhythm peaking in the afternoon in lean males. However, no data exists regarding the effects of gender and body weight.
Material and Methods: We measured Nampt serum levels over 24 h in a cohort of healthy individuals living with either normal weight or obesity. Furthermore, effects of meals, oral glucose tolerance test and physical exercise on Nampt concentrations were evaluated. Correlation analyses to other hormonal- and lab parameters and anthropometric measurements were performed.
Results: Nampt showed a diurnal rhythm with increased levels at daytime and a peak in the early afternoon. This diurnal rhythm was significant for all groups but obese males. The Nampt amplitude, measured both relatively and absolutely, was significantly higher in females than in males. Meals did not influence Nampt serum levels, whereas physical exercise and an OGTT did significantly influence Nampt serum levels.
Conclusion: In conclusion, we found gender specific differences in Nampt amplitude and coefficient variation with both being higher in females. The circadian rhythm of Nampt was independent of gender in healthy lean individuals, whereas it was disturbed in men with obesity.

Introduction

Nicotinamide phosphoribosyltransferase (Nampt) is a multifunctional protein and adipocytokine, formerly also known as visfatin and pre-B-cell colony-enhancing factor (PBEF) that is produced by adipose tissue as well as skeletal muscle, liver, and immune cells. One of the major sources are lymphocytes [1]. It is known for some years that circulating Nampt concentrations are elevated in type 2 diabetes [2,3] and obesity [4–6]. Furthermore, it was found to be a marker of low-grade inflammation associated with metabolic dysfunction [7,8].

Nicotinamide adenine dinucleotide (NAD^+) is a key biosynthetic substrate, which plays a pivotal role in metabolism, circadian rhythm, inflammation, and aging. Nampt-mediated NAD^+ biosynthesis, together with its key downstream mediator SIRT1, has been demonstrated to regulate glucose and lipid metabolism in a tissue-dependent manner [9–11]. It was found in mice adipocytes and hepatocytes that Nampt shows a rhythmic 24-h pattern [9]. Therefore, Nampt might mediate a circadian feedback loop influencing the integration of energy storage with the rest-activity cycle in rodents [9].

A human study was published by Hayes et al. which was able to show an inverse relationship between sleep duration and serum Nampt levels [12]. Therefore, they speculated that circulating Nampt concentrations might display a diurnal rhythm in humans which might be dependent on the sleep/ wake cycle. Furthermore, Benedict et al. investigated the diurnal profile of serum Nampt levels in healthy males under normal sleep conditions and in addition under sleep deprived conditions. Nampt concentrations followed a diurnal rhythm, peaking in the afternoon. Interestingly, sleep loss induced a Nampt rhythm phase shift that is positively related to the impairment of postprandial glucose metabolism due to sleep deprivation, suggesting a regulatory impact of Nampt

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rhythmicity on glucose homeostasis [13]. Benedict et al. were only investigating young healthy males [13]. No data exists concerning the effects of gender differences and the influence of body weight and obesity on the diurnal or circadian rhythm of Nampt. Therefore, we wanted to close that gap of knowledge and investigate gender differences between males and females and possible differences in the circadian rhythm between individuals living with normal weight and with obesity. Our hypothesis is, that the circadian rhythm of Nampt might be influenced by these factors. In addition, to the circadian rhythm, we investigated the effects of a glucose load and physical exercise on Nampt concentrations depending on gender and obesity.

Subjects and methods

Study population, anthropometrical characterization and laboratory assessment

The study was performed under controlled in-house conditions in 32 young, and apart from obesity, otherwise healthy adults aged 18 to 35 years. All participants where Caucasian white and had sleep-wake cycles within the normal range. We made sure the participants were not working at night and the seven nights before the study they had average sleeping durations of 6–9 h at night. Anthropometrical measurements including height, body weight, and skinfold thickness were always obtained by the same study nurse and physician. Bioimpedance analyses (BIAs) were performed using Nutriguard-MS (Data Input, Germany), and parameters such as fat-free mass or body cell mass (BCM) were calculated using NutriPlus software [14]. Blood parameters were assessed by a certified laboratory (Institute of Laboratory Medicine, University Hospital Leipzig). The study was approved by the ethics committee of the University of Leipzig (registration no. 096–11-07032011, 029–2006). For attaining the ethical vote, leading role for sample size and power calculation was assigned to circadian rhythm of adipocytokines and hormones. Written informed consent was obtained from all subjects that participated in our study.

Standardized meals and oral glucose tolerance test

Subjects received meals of standardized composition and amount at defined times (8:30 AM, 12:30 PM, 6:30 PM, and 10:00 PM). Blood sampling started at 8:00 AM (with fasting samples) and subsequently continued with hourly samples for 24 h. Additional samples were taken 30 min after meals. During the day, subjects engaged in sedentary activities (e.g. reading or watching videos). On the following day, an oral glucose tolerance test (OGTT) was performed with blood samples collected directly before and 10, 20, 30, 60, 90, and 120 min after an oral glucose intake (1.75 g/kg body weight, with a maximum of 75 g). Samples were immediately processed and stored at − 80 °C. A schema of the daily routine and blood sampling is given in Fig. 1A.

Acute short-term intensive exercise (30 minutes)

The participants (Fig. 1A) underwent an intensive 30-minute physical program (10 min of jogging, 10 min of gymnastics, and a 10-minute sprint). Physical exhaustion was verified by an increase in serum lactate levels. Blood samples were taken immediately before and after exercise and after a 30 min recovery phase.

Quantification of nampt levels

Nampt concentrations were measured in serum samples by ELISA following the manufacturer's protocol (Adipogen, Seoul, South Korea). Assay quality variables including sensitivity and specificity have been validated as published before [15].

Statistical analysis

Data are presented as means ± SEM. Spearman's rho was used for assessing baseline correlations. P values were obtained using Spearman test for baseline correlations, Kruskal-Wallis test for effects after meals, Wilcoxon rank-sum test for paired samples for effects after OGTT and physical activities, F-test within CircWave [16] for circadian effects of Nampt and Wilcoxon rank-sum test otherwise. A p-value < 0.05 was considered significant, < 0.01 as highly significant. F tests were performed to assess the optimal complexity of the best-fit. Key confounding factors like meals, exercise, stress and sleep are considered to be addressed by controlled in-house study design.

Baseline data samples were obtained between 8:00 a.m. and 8:30 a.m. on the first day after an overnight fast. For data analysis of the OGTT effect, the average Nampt between 8:30 a.m. and 10:00 a.m. was compared between the first and second day. For evaluating the effect of physical activity, the average Nampt between 11:30 and 12:00 was compared between the first and second day. For the relative oscillation of Nampt, the quotient of the average Nampt between 4 a.m. and 6 a.m. and the average Nampt between 4 p.m. and 6 p.m. was calculated and compared between both sexes.

Results

Nampt levels are elevated in the obese cohort, independent of gender

We included 32 subjects in our study, 14 females and 18 males. All cohort characteristics are found in Table 1. We found significant differences for Nampt serum levels between the lean and the obese cohort in males and females. The average basal Nampt concentration after an overnight fast was 0.9 + /− 0.11 ng/ml in lean women and 2.7 ng/ml (+/−0.68) in women with obesity and 1.22 ng/ml (+/- 0.15) in men with normal weight and 2.71 ng/ml (+/−0.46) in men with obesity ($p = 0.024$ for the comparison of males). Comparing the cohorts with normal weight to obesity independent of gender, there is a statistically significant difference ($p = 0.003$) (Fig. 2).

Circadian rhythm of Nampt is influenced by weight and gender

We confirmed the typical patterns of cortisol diurnal variation, with a peak at 8:00 AM and a gradual decline over 12 to 16 h, in all subjects

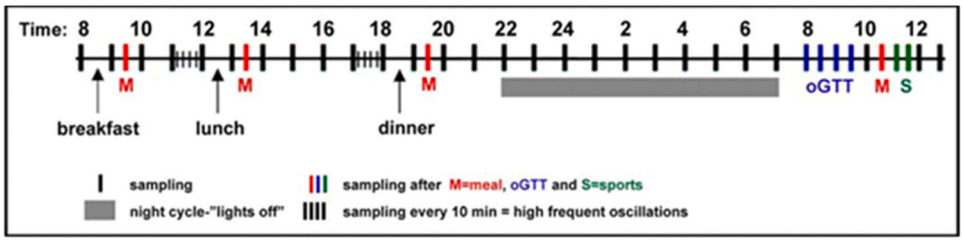

Fig. 1. Sampling schedule for diurnal variation and validation of Nampt detection. Schedule of blood sampling during the 24-hour period including meals (M, red arrows) followed by an OGTT (blue) and an exercise program (S, green) at day 2.

Table 1
Correlation of Serum Nampt levels with Anthropometric, Metabolic, and Cardiovascular Parameters in our cohort. The p-value is given and significant if the p values of Spearman test was < 0.05. columns r^a and p^a refer to correlations adjusted for age, sex and BMI.

Parameter	R	P	r[a]	P[a]
Anthropometric parameters				
Age	0.433	0.021	0.072	0.694
Height	0.019	0.922	-0.356	0.390
Weight	0.510	0.006	0.169	0.742
BMI (kg/m2)	0.522	0.004	0.185	0.749
BCM (kg)	0.378	0.069	-0.030	0.678
Skinfold subs (mm)	0.540	0.003	0.209	0.760
Skinfold biceps (mm)	0.508	0.006	0.166	0.741
Skinfold triceps (mm)	0.442	0.018	0.083	0.700
Skinfold suprailiakal (mm)	0.615	0.001	0.307	0.807
FFM (kg)	0.484	0.017	0.100	0.742
Metabolic parameters				
Fasting blood glucose	0.424	0.025	0.060	0.688
120-minute blood glucose	0.438	0.020	0.078	0.697
Fasting plasma insulin	0.474	0.011	0.123	0.720
Peak insulin	0.285	0.141	-0.098	0.595
HOMA-IR	0.460	0.014	0.105	0.711
QUICKI	-0.460	0.014	-0.711	-0.105
Triglycerides	0.472	0.011	0.120	0.719
HDL cholesterol	-0.606	0.001	-0.798	-0.300
LDL cholesterol	0.286	0.140	-0.098	0.595
Cholesterol	0.103	0.603	-0.281	0.458
Cardiovascular parameters				
Systolic BP (mm Hg)	0.256	0.227	-0.164	0.598
Diastolic BP (mm Hg)	0.328	0.118	-0.087	0.646
RHI	0.152	0.500	-0.288	0.539
Hormonal parameters				
Estradiol	0.282	0.307	-0.269	0.694
Testosterone	-0.154	0.584	-0.617	0.389

Abbreviations: BCM= body cell mass; FFM= free-fat mass; HOMA-IR= homeostatic model assessment of insulin resistance; QUICKI= quantitative insulin sensitivity check index; HDL= high-density lipoprotein; LDL= low-density lipoprotein; BP= blood pressure; RHI= reactive hyperaemia index

(Fig. 3A, Table 2).

Furthermore, in our cohort we found a circadian rhythm of Nampt in both lean men and women with increased levels at daytime, peaking in the early afternoon. A similar circadian rhythm occurred in women with obesity, but no clear pattern was detectable in men with obesity, as this is the only group where we did not find significant results for the circadian rhythm. For statistical characteristics, we refer to Table 2.

An additional parameter which can be evaluated is the amplitude in Nampt concentrations. The amplitude was higher in women (26.5 %–28.8 %) compared to men (14.1 %–17.2 %). This finding can be confirmed when analyzing the Nampt ratio (Nampt concentration at early morning compared to Nampt concentration at late afternoon): with a significant higher Nampt ratio in women of 2.27 + /- 0.55 compared to men 1.23 + /- 0.10 ($p = 0.01758$) independent of body weight (Fig. 4). Therefore, we found gender-specific differences in the amplitude and ratio for the circadian rhythm of Nampt.

Nampt serum levels are positively correlated with anthropometrical data and parameters of the lipid and glucose metabolism

For anthropometric data, we found a positive correlation of Nampt levels for weight, amount of body fat in % and kg, body cell mass in kg and free-fat mass in kg and skinfold thickness. For lab values, we found a positive correlation of Nampt with triglycerides (Table 1). As expected for metabolic values, we found a positive correlation of Nampt with basal Glucose, basal Insulin and HOMA-IR. A negative correlation was found for HDL and QUICKI.

Meals and caloric intake did not affect Nampt serum concentrations

We measured, if meals and therefore caloric intake directly influence Nampt serum levels. Therefore, we measured Nampt serum concentrations 30, 60 and 90 min after breakfast, lunch and dinner. After standardized meals, we did not find a common pattern or significant changes of Nampt serum levels in general and between the genders and also between lean and obese subjects (Supplemental Figure 1).

An oral glucose load significantly changed Nampt serum levels

We measured changes in Nampt levels during a glucose load in an oral glucose tolerance test. As expected there was a positive correlation

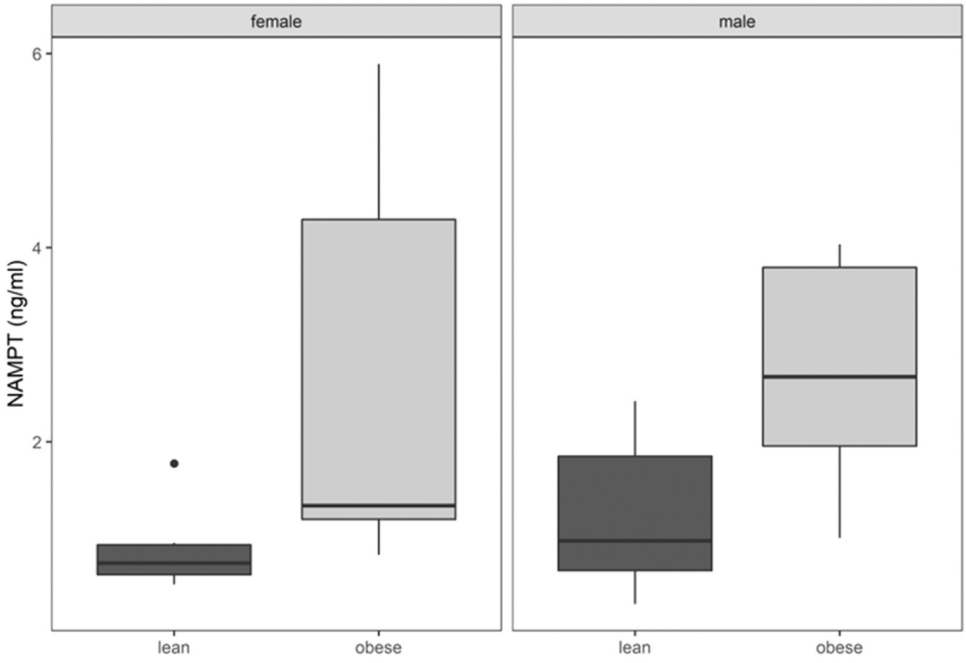

Fig. 2. Serum Nampt level in ng/ml in both genders. If you compare individuals with normal weight and obesity independet of gender we found a significant difference of ($p = 0.003$). For statistical analysis a Wilcoxon rank sum test and U-Test was used.

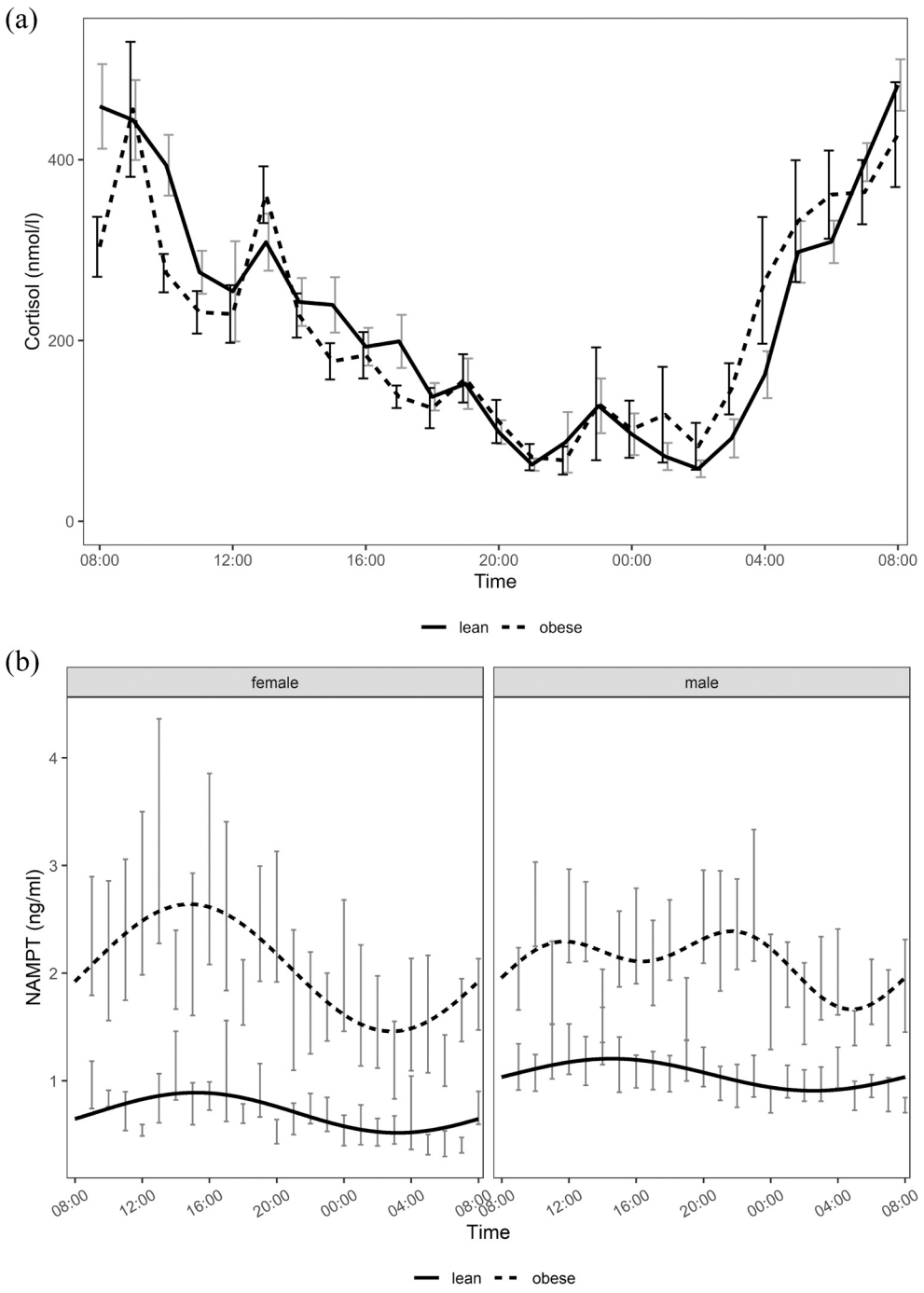

Fig. 3. A: Cortisol serum levels + /- SEM in nmol/l have been measured in parallel to Nampt every hour for a period of 24 h. Cortisol showed the expected diurnal fluctuations in individuals with normal weight compared to patients living with obesity. B: Diurnal course of serum Nampt levels in ng/ml. Data are presented as CircWave estimate together with group average of all participants at every measured time point (every full hour) + /-SEM over a period of 24 h.

Table 2
CircWave statistics for the circadian rythm of Nampt stratified by sex and obesity with descriptive characteristics and F test statistics of the approximated circadian function.

groups	CW F	CW p	CW r2	mean	min.	max.	amplitude	H min.	H max.
Lean female	5.341	0.006	0.063	0.704	0.518	0.891	26.5 %	3.18	15.18
Obese female	6.649	0.002	0.076	2.051	1.460	2.641	28.8 %	2.82	14.82
Lean male	4.095	0.018	0.031	1.057	0.908	1.206	14.1 %	2.54	14.54
Obese male	1.906	0.111	0.045	2.101	1.665	2.389	17.2 %	4.82	21.68

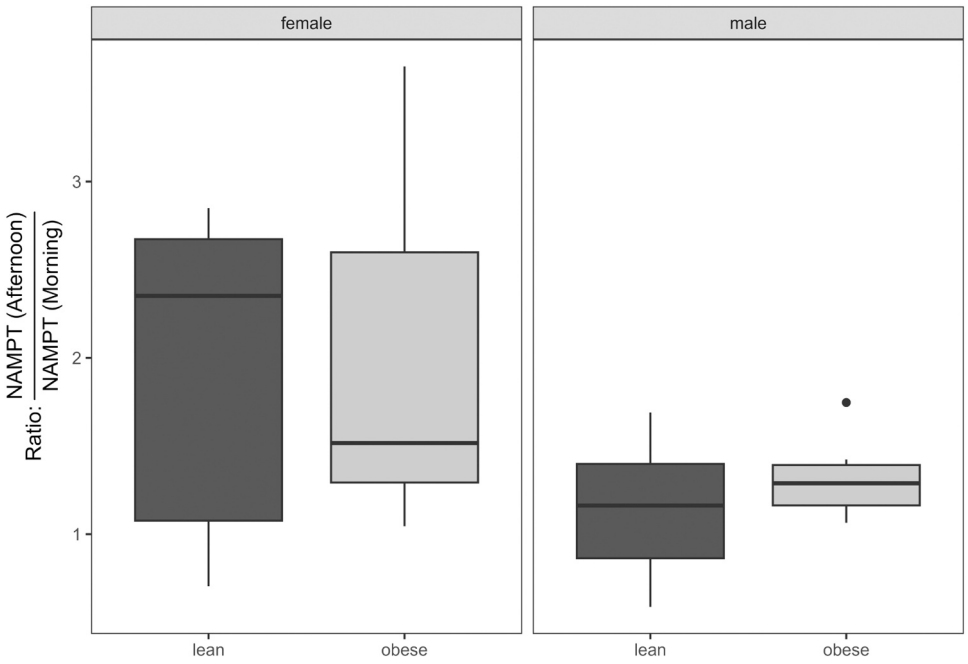

Fig. 4. Relative changes of Nampt levels during the day between both genders. For the relative oscillation in Nampt, the quotient of the average Nampt between 4 a.m. and 6 a.m. and the average Nampt serum concentrations between 4 p.m. and 6 p.m. was calculated and compared between both sexes. The differences between males and females is significant with a p-value of 0.01758 * .

between basal glucose levels and the insulin increase during the OGTT (spearman-correlation: 0.29 with a p-value=0.02039). Comparing Nampt levels from the first day with the Nampt levels during an OGTT on the next day, we found a significant decrease of Nampt serum levels in normal weight women by 34.5 % (p-value = 0.00851), in women living with obesity by 34.8 % (p-value = 0.00007319) and men with obesity by 26 % (p-value = 0.01301) (Fig. 5). We first found a reduction of Nampt levels 30 min after glucose load and then an increase to normalization of Nampt levels again. Comparing individuals with normal weight and obesity independently of gender, we also found a significant effect of an oral glucose load (p-value = 0.000000645). In addition, we found a significant difference of Nampt concentrations between women with normal weight and obesity at 120 min during the OGTT (p = 0.006).

Acute short-term exercise increases Nampt serum levels

The second day our participants had to undergo an extensive exercise program for 30 min. In each individual group, we found an increase of Nampt serum concentrations directly after the 30 min strenuous exercise. Comparing individuals with normal weight and obesity, we found a significant difference and increase of Nampt serum concentrations in the cohort with normal weight by 29.8 % and in the cohort living with obesity by 33.3 % (p-value = 0.005812).

If the groups were analyzed individually, we observed an increase of Nampt serum concentrations after 30 min and directly after maximal workout in men with obesity by 63 % (p = 0.003) and women with obesity by 50 % (p = 0.003) and after a 30-minute recovery phase in men with obesity by 61 % (p = 0.026) and women with obesity by 49 % (p = 0.05). Differences in Nampt serum concentrations were measurable between lean individuals and patients living with obesity after 30 min by 75.14 % (p = 0.002) and after 60 min in males by 83.23 % (p = 0.014) and an extreme difference by 60 min by 355.7 % (p = 0.008) in females (Fig. 6).

Discussion

As demonstrated by others, Nampt serum levels are significant higher in individuals with obesity compared to individuals with normal weight, which was independent of gender [2, 4, 5, 17]. We did not observe significant changes in Nampt serum concentrations after meal intakes, but found reduction of Nampt immediately after an oral glucose load. There are no data on the effect of caloric intake on Nampt serum levels in individuals with different body weights published so far. It seems that the composition of a meal might be important. In contrast, we measured changes in Nampt serum concentrations after a high glucose

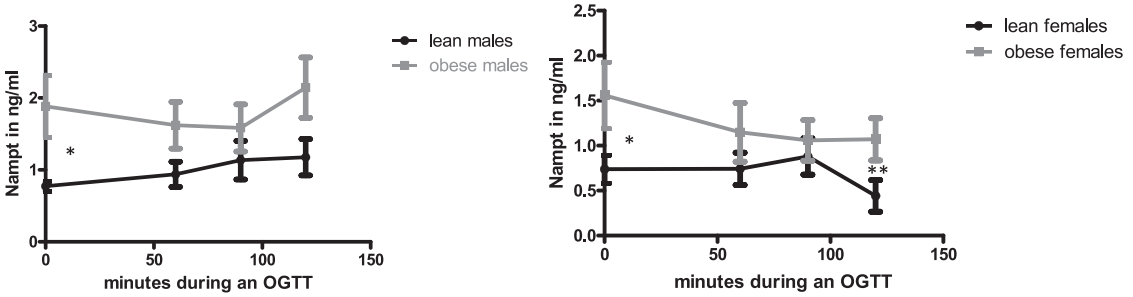

Fig. 5. Nampt levels in ng/ml have been measured the next day during an oral glucose tolerance test after an glucose load of 75 mg. Differences have been calculated between subjects with normal weight and obesity and during an OGTT. p = *< 0.05 was significant and p = ** <0.01 was highly significant.

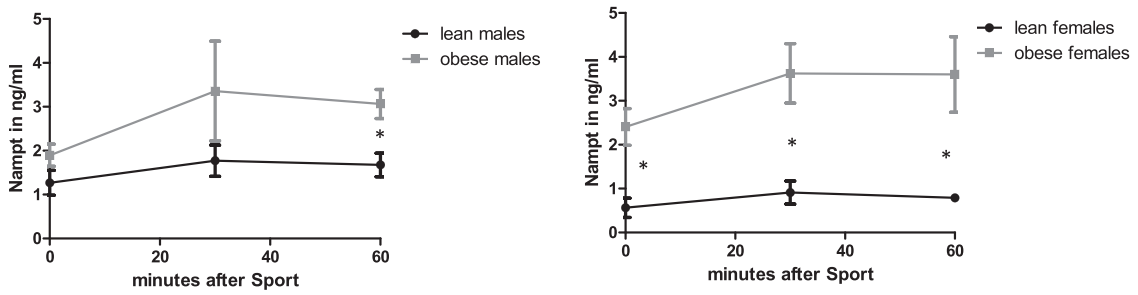

Fig. 6. Nampt levels in ng/ml after excessive exercise for a 30 min duration. The serum Nampt levels were measured 30 and 60 min after the exercise and differences were measured between subjects with normal weight and subjects with obesity in males and females. p = *< 0.05 was significant.

load in an OGTT. Those findings are difficult to interpret because it can of course be influenced by many other factors, but it seems that high glucose levels influence Nampt excretion and circulating levels of Nampt. This is in line with data from Haider et. al. They showed that human islets can secrete extracellular nicotinamide phosphoribosyltransferase (eNampt), which has an enzymatic activity, and the secretion is regulated by glucose [18]. If you stimulate human primary adipocytes in culture with glucose, they showed an increase of 50 % of eNampt secretion. Haider et al. speculated that eNampt may have a compensatory role in supporting insulin secretion when beta cell dysfunction is present. eNampt is important in supporting insulin secretion by generating nicotinamide monocleotide (NMN). This mechanism might be negatively affected when beta cells are under metabolic stress or deficient in Nampt [18,19] as found in obesity and hence in our cohort. In addition to glucose, insulin may act as an autocrine factor regulating eNampt release [19]. Therefore, one can speculate that changes of body weight and body metabolism which can induce a high insulin response might influence Nampt serum levels. In individuals with obesity the Nampt levels decrease, whereas in the cohort with normal body weight the differences are less pronounced [20–22]. Nampt levels were also measured in healthy normal weight men during an OGTT by Haider et al. They found that circulating Nampt concentrations increase by hyperglycaemia. This effect was suppressed by an exogenous hyperinsulinaemia or somatostatin infusion [20]. Nampt is also secreted from adipocytes in an active form and is one of the putative regulators of insulin secretion [22]. Furthermore, they were able to show that glucose signaling for Nampt release in adipocytes involves the PI3-kinase/AKT pathway. Haider studied the effects of an OGTT on Nampt release in a male cohort and Unlutürk et al. studied the effect of an oral glucose challenge on Nampt levels in women with normal weight compared to women with obesity. The Nampt levels increased and this was linked to insulin levels and adiposity [22], which is in line with our data. They speculate that counter-regulatory adaptations in adiponectin and Nampt might have an impact on a suggested adipoinsular axis, contributing to maintenance of normal glucose tolerance [22]. Nampt levels during an OGTT can also be driven by insulin. It is suggested that Nampt has a blood glucose lowering effect and in vitro studies have shown that it can induce the phosphorylation of the insulin receptor and its related substrates (IRS) 1 and 2 [23]. We just found an increase of Nampt levels at the end of the OGTT in males with obesity, but interestingly a slight decrease at the beginning and during the OGTT of Nampt concentrations. The decrease of Nampt concentration directly after the glucose load was not reported by others. But it remains unclear if they have not measured at those early time-points or if they did not see differences. The increase at the end of the OGTT is in line with data published by Haider et al. and Unlutürk et al. Furthermore, in obesity patients usually present with hyperinsulinism and insulin resistance which could also influence Nampt serum levels. Another possible link is glucose metabolism and the process of inflammation which are both disturbed in obesity. Besides visceral adipose tissue, leukocytes are the major source of Nampt [7]. A positive correlation between serum Nampt levels and body fat content and a decrease of its circulating levels after weight reduction have been described in adults [6].

However, we did not detect a response in Nampt levels given exposure to more balanced Glucose level profiles observed after intake of meals. This might be depending on the meals composition and can also be influenced by the protein, fat and carbohydrate content of the meal and would of course need further investigation.

In line with other adipocytokines like irisin, the study showed that acute exercise intervention increases Nampt levels directly [14]. To our surprise, this effect was much more pronounced in individuals living with obesity. Therefore, acute exercise activates Nampt excretion. If the source during acute exercise is mainly the adipose tissue or the leucocytes remain unclear. Furthermore, it remains to be elucidated, if Nampt concentrations increase after long-term exercise or duration exercise [24]. It is well known that exercise training exerts beneficial effects on metabolic and vascular risk factors in patients with type 1 diabetes mellitus (T1DM) and obesity. There was one study by Haider et al. that examined the long-term effects of regular physical exercise after 2, 4 and 8 months on Nampt serum levels in a cohort of patients with type 1 diabetes. Haider et al. concluded that elevated Nampt concentrations in patients with T1DM can be lowered by regular physical exercise [24]. But to our knowledge effects of excessive short-term exercise on Nampt serum concentrations have not been analysed so far. As in our cohort shown for Nampt, Löffler et al. observed a transient exercise-induced increase in the adipocytokine Irisin immediately after acute strenuous exercise, what would make sense so that energy can be provided. Whereas Löffler et al. did not find significant changes after long-term exercise [14]. Of course, the pathomechanisms are completely different after short- and long-term exercise. So far little is known about the signaling of Nampt and whether a small transient increase upon exercise is of physiological relevance remains to be elucidated. It is known that Irisin and other myokines are released acutely by muscle exercise, and this increase is not a result of muscle damage. Furthermore, inflammatory markers like IL-6 increase rapidly in response to exercise then quickly decline in the postexercise period. The magnitude is related to exercise duration and intensity. In addition, prolonged exercise (e.g. 1 year) does not alter IL-6 [25].

As Benedict et al., we also observed a diurnal variation of Nampt with a Nampt peak in the morning and afternoon. Interestingly, in all subjects we found lower Nampt concentrations at night and during the sleep period. This suggests that sleep actively down-regulates circulating Nampt levels in humans. This conclusion is in accordance with recent cross-sectional examinations of more than 500 adults of the Cleveland Family Study showing that each hour of total sleep time reduction was associated with a 14 % increase in serum Nampt concentrations [12]. In healthy young adults under normal sleep-wake cycles Benedict et al. displayed a pronounced diurnal rhythm of Nampt, peaking during early afternoon, which was inverse to leptin profiles peaking in the early night. The circulating Nampt concentrations that peaked during the wake phase were associated with periods of high metabolic activity.

Therefore, one could speculate that Nampt/visfatin likewise affects food intake [20, 21, 26, 27]. Thus, the inverse 24-h patterns of Nampt and leptin, respectively, might bear some functional significance for the regulation of ingestive behavior. Interestingly, Benedict et al. were able to show, that when subjects stayed awake, the Nampt rhythm was preserved but phase advanced by about 2 h. Benedict et al. also evaluated the effects of sleep loss. Sleep loss induces a Nampt rhythm phase shift that is positively related to the impairment of postprandial glucose metabolism due to sleep deprivation, suggesting a regulatory impact of Nampt rhythmicity on glucose homeostasis [13]. Therefore, one can summarize that the circadian rhythm of Nampt might be influenced by many external factors, including sleep deprivation.

Benedict et al. were only investigating young healthy men with normal weight. Therefore, we wanted to investigate gender differences between men and women and possible differences in the circadian or diurnal rhythm between individuals with normal weight or obesity. Our hypothesis was, that the diurnal rhythm of Nampt might be disturbed by obesity. In men living with obesity, this diurnal rhythm of Nampt was not seen as clearly as in individuals with normal weight. One could speculate that patients with obesity might have a deranged diurnal rhythm of Nampt which negatively influences the whole metabolism and might be associated with inflammation. The question is why this effect was not seen in females. We can just speculate that Nampt levels are also dependent on other adipocytokines and hormones and Nampt concentrations might vary during the menstrual cycle. Our sample size was too small and the females that were included in the study were during different phases of the menstrual cycle. Therefore, it could be possible that there is also a deranged diurnal rhythm in females living with obesity but our sample size was too small to detect that. Therefore, we would need a study to investigate the effects of hormones and the menstrual cycle of Nampt concentrations and the circadian rhythm of Nampt in a larger cohort of females. One other possible explanation is the difference of fat distribution and metabolism between the two genders, which could also have influenced the Nampt concentration and especially the effects of the diurnal rythm. And of course, many other factors as inflammation and glucose -/ insulin metabolism could influence Nampt concentrations and be explanations for gender-specific differences.

In addition, we found that the amplitude in Nampt concentrations was significant higher in women compared to men, which we confirmed when analyzing the Nampt ratio. Therefore, we can summarize that we found gender-specific differences in the amplitude and ratio for the diurnal rhythm of Nampt. But the physiological relevance of the gender-specific differences remains unclear and needs further investigation with a larger sample size. But possible effects for the gender-specific differences are of course, as mentioned above for the differences in diurnal rhythm, effects of other hormones and adipocytokines and again might be dependent on the menstrual cycle. And again the fat distribution and metabolism is gender-specific between males and females. Hormonal differences like hyperandrogenism in females might also influence adipocytokine concentrations. For example, for Irisin Löffler et al. showed a higher variability in patients with obesity but no gender-specific differences [14]. Our cohort was analyzed for 30 h but not under everyday-life conditions. In everyday-life they would probably be more active. The sample size was quite small. Unfortunately, we had to test the effects of extensive exercise only an hour later after the OGTT. This might also have influenced our results. And as mentioned to confirm data, it would be beneficial to increase the study population with a larger sample size, and in different age groups. Other factors, like stage of the menstrual cycle have been known but not taken into consideration for evaluation. All females that were included in the study had regular menstrual cycles and were not on an oral contraceptive pill. Nevertheless, our cohort and sample size was large enough to see statistically significant differences.

In summary, we found a diurnal rhythm on Nampt, which was not unambiguously evident in men with obesity. In women, we found a higher variability and amplitude of diurnal Nampt concentrations compared to males. We found differences in Nampt serum levels between lean individuals and patients living with obesity. Therefore, we found gender- and weight specific differences in Nampt serum concentrations. The relevance for metabolism and obesity related complications remains to be elucidated.

Conclusion

In our cohort, we were able to measure different Nampt levels between individuals with normal weight and obesity. Furthermore, an oral glucose load significantly influenced Nampt serum concentrations. Acute physical exercise increased Nampt serum concentrations for a short period, but long-term effects, need further investigations We found a diurnal rhythm for Nampt and a slight disruption of the diurnal rhythm for men with obesity. The amplitude and ratio of Nampt was gender-specific and higher in women than in men. The physiological relevance, the influence on metabolism and most importantly the influence of those gender- and body weight-specific differences of Nampt concentrations regarding the metabolic and cardiovascular long-term risk remain to be elucidated. Our data provide evidence that it is important to take the circadian rhythm of Nampt into consideration when measuring and interpreting Nampt serum levels.

Ethical Statement

The performed study and experiments conform to the ethical standards of Obesity Research and Clinical Practice:

- The authors declare that all experiments on human subjects were conducted in accordance with the Declaration of Helsinki, and that all procedures were carried out with the adequate understanding and written consent of the subjects.
- The authors certify that formal approval to conduct the experiments described has been obtained from the human subjects review board of their institution and could be provided upon request.

Declaration of Competing Interest

none.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.orcp.2024.06.005.

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