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ASSOCIATION OF ACTIVITY STATUS AND PATTERNS WITH SALIVARY CORTISOL: THE POPULATION-BASED COLAUS STUDY

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ABSTRACT

Purpose: Physical activity (PA) has been shown to influence salivary cortisol concentrations in small studies conducted among athletes. We assessed the association of activity status and patterns with salivary cortisol in the general population.

Methods: Cross-sectional study including 1948 adults (54.9% women, 45-86 years). PA and sedentary behaviour (SB) were measured for 14 days by accelerometry. Low PA and high SB status were defined respectively as the lowest and highest tertile of each behaviour. 'Inactive', 'Weekend warrior', and 'Regularly active' patterns were also defined. Four salivary cortisol samples were collected over a single day and the following parameters were calculated: area under the curve to ground (AUCg), awakening response (CAR) and diurnal slope.

Results: After multivariable adjustment, low SB remained associated to steeper slopes relative to high SB (-1.54 ± 0.03 *vs.* -1.44 ± 0.04 nmol/l per hour). Non-significant trends were found for high PA relative to low PA with steeper slopes (-1.54 ± 0.03 *vs.* -1.45 ± 0.04) and lower AUCg (208.7 ± 2.0 *vs.* 215.9 ± 2.9 nmol.hour/l). Relative to 'Inactives', 'Regularly actives' had lower AUCg (205.4 ± 2.4 *vs.* 215.5 ± 2.9) and 'Weekend warriors' had steeper slopes (-1.61 ± 0.05 *vs.* -1.44 ± 0.04). No associations were found for CAR.

Conclusion: Low SB and high PA are related to lower cortisol secretion as measured by different parameters of salivary cortisol, but the effects were only modest.

Abstract word count: 230

Keywords: physical activity; salivary cortisol; pattern; sedentary behaviour; accelerometry; epidemiology.

Abbreviations:

ANOVA: Analysis of variance

AUCg: Area under curve with respect to ground

BMI: Body mass index

CAR: Cortisol awakening response

CVD: Cardiovascular disease

CVRF: Cardiovascular risk factor

MVPA: Moderate-to-vigorous intensity physical activity

PA: Physical activity

SB: Sedentary behaviour

INTRODUCTION

The impact of physical activity (PA) (Li and Siegrist 2012) and sedentary behaviour (SB) (Biswas et al. 2015) on cardiovascular disease (CVD) are well established, but the underlying mechanisms are still incompletely understood. Mora and al. (Mora et al. 2007) suggested that only half of PA-mediated reduction in CVD incidence is explained by known cardiovascular risk factors (CVRF), and recent longitudinal studies found no association between SB and traditional CVRF (Saunders et al. 2013; Shuval et al. 2014).

Psychological stress is increasingly being considered as a potential CVRF (Manenschijn et al. 2013; Winning et al. 2015). Salivary cortisol is commonly used in large-scale epidemiological studies as a marker of psychological stress (Adam and Kumari 2009). Several parameters of salivary cortisol have been proposed to assess stress, namely cortisol awakening response (CAR), diurnal slope, and area under curve with respect to ground (AUCg) (Adam and Kumari 2009). Further, Kumari and al. recently showed that flatter diurnal cortisol slopes were related to increased CVD mortality (Kumari et al. 2011). Hence, it can be speculated that PA and SB might impact CVD by modulating psychological stress and thus salivary cortisol. Nevertheless, little is known on the association of PA or SB with salivary cortisol in the general population. A study reported higher CAR and steeper slopes among physically active participants (Vreeburg et al. 2009) while another study reported no association (Lederbogen et al. 2010). Still, the conclusions of those two studies were limited because they: (i) relied on self-reported PA (Lederbogen et al. 2010; Vreeburg et al. 2009); (ii) did not take into account SB (Lederbogen et al. 2010; Vreeburg et al. 2009); and (iii) used a non-representative sample of the general population (Vreeburg et al. 2009). Further, previous studies only considered PA levels, and it has been shown that PA distribution over time (i.e. PA pattern) also influences CVD. Indeed, exercising 1-2 times per week mostly on weekends, a pattern known as the 'Weekend warrior', has been shown to alter the benefits of high PA on CVD (Lee et al. 2004).

Nowadays, light and wearable accelerometers allows an easy and objective assessment of PA and SB in large samples (Troiano et al. 2014). Given the importance of exploring PA patterns, we assessed the association of objectively measured PA and SB levels and patterns with parameters of salivary cortisol in a population-based sample from the city of Lausanne, Switzerland.

MATERIALS AND METHODS

Recruitment of participants

The detailed description of the recruitment of the CoLaus study and the follow-up procedures has been described previously (Firmann et al. 2008; Marques-Vidal et al. 2011). Briefly, the CoLaus study is a population-based cohort exploring the biological, genetic and environmental determinants of CVD. A non-stratified, representative sample of the population of Lausanne (Switzerland) was recruited between 2003 and 2006 based on the following inclusion criteria: (i) age 35-75 years and (ii) willingness to participate. The second follow-up occurred ten years after the baseline survey and included an optional module assessing the participant's PA and salivary cortisol.

Physical activity measurement

PA was assessed using a wrist-worn triaxial accelerometer (*GENEActiv*, Activinsights Ltd, United Kingdom). The accelerometers were pre-programmed with a 50 Hz sampling frequency and subsequently attached to the participants' right wrist. Participants were requested to wear the device continuously for 14 days in their free-living conditions.

Accelerometry data were downloaded using the *GENEActiv* software version 2.9 (*GENEActiv*, Activinsights Ltd, United Kingdom) and transformed into 60-second epoch files. Data were analyzed using the *GENEActiv macro file* 'General physical activity' version 1.9 (GENEActiv 2014) which had been previously validated (Esliger et al. 2011). A valid day was defined as ≥10 h (i.e. 600 min) and ≥8 h (i.e. 480 min) of wear-time on week days and weekend days, respectively. For each participant, the proportion of time (in percentage) spent in moderate-to-vigorous intensity PA (MVPA) and in SB was averaged for all valid days and separately for valid week and weekend days.

For PA status, participants were split into tertiles of average proportion of time spent in MVPA and classified as 'low PA' if they were in the first tertile and as 'high PA' otherwise. For SB status, participants were split into tertiles of average proportion of time spent in SB and classified as 'high SB' if they were in the highest tertile and as 'low SB' otherwise.

Activity patterns were defined according to PA status and its distribution throughout the week (see **Figure 1**). For the distribution of PA, average proportion of time spent in MVPA on weekend days was divided by average proportion of time spent in MVPA on week days and split into tertiles. Participants were categorized as 'PA mainly on weekends' if they were in the highest tertile and as 'PA throughout the week' otherwise. This classification allowed creating three mutually exclusive activity patterns as described by O'Donovan and al. (O'Donovan et al. 2017): 1) 'Inactive': low PA; 2) 'Weekend warrior': high PA & PA mainly on weekends; and 3) 'Regularly active': high PA & PA throughout the week.

Salivary cortisol

Salivary cortisol has been established as a reliable indicator of circulating cortisol concentrations and hypothalamus-pituitary-adrenal axis function (Hellhammer et al. 2009). Saliva samples were collected using cotton swabs ('Salivette', Sarstedt, Germany). Based upon another study (Ouanes et al. 2017), four salivary samples were obtained from each participant: (T1) on waking (before getting out of bed); (T2) 30 minutes after T1; (T3) at 11 am; and (T4) at 20 pm. Saliva sampling was to be done on any week day, but waking time was not specified as it could disrupt the participants' daily routine. Participants were instructed not to eat, drink, smoke, brush their teeth or engage in PA for at least 30 minutes before saliva sampling. An instruction booklet was used to record adherence to the protocol including exact time of saliva collections. The sampling material was returned by mail to the investigators and subsequently frozen at -20°C before being sent to the laboratory. Samples were sent at -20°C to the laboratory of the Department of Psychology at the Technische Universität Dresden, Germany. Upon arrival, samples centrifuged at 3,000 rpm for 5 min, and salivary cortisol was measured using a commercially available chemiluminescence immunoassay (IBL International, Hamburg, Germany), with intra- and interassay coefficients of variation <8%.

Three salivary cortisol markers were assessed based upon previous studies (Lederbogen et al. 2010; Vreeburg et al. 2009). Activation of cortisol secretion was defined by CAR, which was calculated by subtracting the T1 from the T2 value (Clow et al. 2004). Diurnal cortisol slope was calculated by subtracting the T1 value from the T4 value and dividing the result by the number of hours separating both samples (Adam and Kumari 2009; Fekedulegn et al. 2007). The total output of cortisol was estimated by AUCg and calculated using the trapezoid formula (Pruessner et al. 2003). Data cleaning was performed by replacing parameters of cortisol as missing values if they were lower than percentile 2.5 or higher than percentile 97.5.

Other data

Demographic data, medicine use, smoking status and professional occupation were collected by questionnaire. Participants were considered as smokers if they reported current smoking and as non-smokers otherwise. Educational level was collected at baseline by questionnaire and categorized as low (obligatory school or apprenticeship), medium (high school), or high (university degree).

Body weight and height were measured to the nearest 0.1 kg and 5 mm (Seca® scale, Seca® height gauge, Hamburg, Germany), with participants in light indoor clothes standing without shoes. Body mass index (BMI) was computed as weight/height^2. Obesity was defined as a BMI ≥30 kg/m^2.

Exclusion criteria

Participants were excluded if they: (i) did not participate in accelerometry; (ii) had less than 5 week days or 2 weekend days of valid accelerometry data, (iii) did not participate in salivary sampling, (iv) had collected saliva after getting out of bed or on weekends, (v) had systemic corticosteroid medication, or (vi) had any missing data in smoking status, BMI, awakening time, professional occupation or educational level.

Statistical analysis

Statistical analyses were conducted using Stata version 14.1 for windows (Stata Corp, College Station, Texas, USA). In bivariable analyses, categorical variables were expressed as percentage and between-group comparisons were performed using chi-square. Continuous variables were expressed as average ± standard deviation and between-group comparisons were performed using Student t-test and one-way analysis of variance (ANOVA). For ANOVA, post-hoc pairwise comparisons were performed using the method of Scheffe.

Multivariable analyses were conducted using ANOVA. Results were expressed as multivariable-adjusted average ± standard error. Post-hoc pairwise comparisons were performed using the method of Scheffe. All multivariable models were adjusted for age (continuous), gender (male/female), smoking status (no/yes), BMI (continuous), awakening time (continuous), professional occupation (no/yes) and educational level (high/medium/low), as performed by others (Adam and Kumari 2009; Clow et al. 2004). Additional adjustments were performed for PA level during the day of sampling (continuous), or the week day of saliva sampling (categorical). Statistical significance was assessed for a two-sided test with $p<0.05$.

Ethical statement and consent

The institutional Ethics Committee of the University of Lausanne, which afterwards became the Ethics Commission of Canton Vaud approved the baseline CoLaus study (reference 16/03, decisions of 13th January and 10th February 2003); the approval was renewed for the first (reference 33/09, decision of 23rd February 2009) and the second (reference 26/14, decision of 11th March

2014) follow-up. The full decisions can be obtained from the authors upon request. The study was performed in agreement with the Helsinki declaration and in accordance with the applicable Swiss legislation. All participants gave their signed informed consent before entering the study.

RESULTS

Low SB status was associated to steeper diurnal cortisol slopes. Non-significant trends were observed for high PA status with lower values in AUCg and steeper slopes. For PA patterns, the 'Regularly actives' and 'Weekend warriors' had respectively lower values in AUCg and steeper slopes in comparison to the 'Inactives'.

Selection procedure and characteristics of participants

Of the initial 4882 participants, 1948 (39.9%) were retained for the analysis. The selection procedure is indicated in **Figure 2.** Included and excluded participants' characteristics are presented in **Supplementary table 1**. Included participants were younger, more professionally active, less likely to be smokers, and had lower BMI levels and lower prevalence of obesity than excluded ones.

Participants' characteristics per activity status are presented in **Supplementary table 2**. Younger age, female gender, adequate BMI level, and being professionally active were associated with high PA and low SB status, non-smoking status with high PA only. Participants' characteristics per activity patterns are presented in **Supplementary table 3**. Younger age, female gender, non-smoking status, adequate BMI level, being professionally active or having higher education were associated with the 'Weekend warrior' pattern.

Association of activity status with salivary cortisol

The associations between PA and SB status and salivary cortisol markers are described in **Table 1**. In bivariate analysis, high PA status was associated to lower values in AUCg. Participants in the high PA and low SB groups had steeper diurnal slopes, while no differences were found for CAR (**Table 1**). After multivariable adjustment, the association between low SB and steeper cortisol slopes persisted (**Table 1**). Trends remained for high PA status with lower values in AUCg (p=0.05) and steeper slopes (p=0.06). Adjusting for PA during the day of saliva sampling lead to similar findings (**Supplementary table 4**).

Association of activity patterns with salivary cortisol

The associations between activity patterns and salivary cortisol markers are presented in **Table 2**. In bivariate analysis, the 'Weekend warriors' had steeper cortisol slopes than the 'Inactives' while the 'Regularly actives' stood in between (**Table 2**). The 'Regularly actives' had lower values in AUCg than the 'Inactives' while no differences were found for CAR (**Table 2**). All the associations persisted after multivariable adjustment (**Table 2**). Results did not change after additional adjustment for PA during the day of sampling (**Supplementary table 4**), or the week day of saliva sampling (**Supplementary table 5**).

DISCUSSION

To our knowledge, this is the first study assessing the association between objectively measured PA and cortisol secretion. Our results show for the first time that PA levels, either evenly distributed over the week or concentrated on weekends, are associated to a lower cortisol secretion. Nevertheless, the effects were small, suggesting that the effect of PA and SB on CVD might be only weakly mediated by cortisol secretion.

Association of activity status with salivary cortisol

Low SB status was significantly related to steeper slopes and a similar trend was observed for high PA. These findings are in agreement with a Dutch cohort study (Vreeburg et al. 2009), which showed steeper slopes among physically active participants. Conversely, a German population-based study (Lederbogen et al. 2010) and an interventional study (Corey et al. 2014) failed to find such association. Possible explanations for the discordant findings are that in the German study (i) PA was self-reported and thus prone to recall bias and (ii) it relied on a smaller sample (N=990), thus having lower statistical power. Also, the interventional study was conducted among metabolic syndrome individuals rather than in a general population setting. Our findings suggest that individuals performing high PA or low SB levels have an optimal diurnal decrease in cortisol secretion. Interestingly, high PA and low SB have been reported to be related to lower psychological stress (Hamer et al. 2010), and flatter salivary cortisol slopes have been related to stress (Adam et al. 2017) and CVD (Kumari et al. 2011; Matthews et al. 2006). Nevertheless, the effect of PA on cortisol dynamics could also be explained by changes in social support rather than by changes in stress (Corey et al. 2014). It would be important to confirm our findings in longitudinal studies exploring the role of stress in the association of PA with incident CVD.

No significant associations were found between activity status and the other markers of salivary cortisol (AUCg and CAR) although a trend was observed between high PA and lower values in AUCg. This finding is in agreement with the German study (Lederbogen et al. 2010) but not with the Dutch study (Vreeburg et al. 2009) and another study conducted among the elderly (Sousa et al. 2017), where a positive association between PA and CAR was found. Possible explanations are that: (i) the study on elderly focused on physical fitness instead of PA levels (Sousa et al. 2017), and (ii) the Dutch study used a different definition of CAR than our study (Vreeburg et al. 2009). PA has been shown to acutely increase salivary cortisol concentrations, but most studies were performed among athletes and after high-intensity PA (Hayes et al. 2015; Hayes et al. 2016); hence, the results might not be applicable to our setting. Overall, our findings suggest that, in community-dwelling subjects, common PA levels do not seem to significantly impact total and awakening cortisol secretion as measured by AUCg and CAR.

Association of activity patterns with salivary cortisol

In comparison to the 'Inactive' pattern, the 'Regularly actives' had lower values in AUCg and the 'Weekend warriors' had steeper slopes. We failed to find any study to which we could compare our results. The previous studies conducted in the community focused on PA levels but not on its distribution over time (Lederbogen et al. 2010; Vreeburg et al. 2009). Our findings suggest that either distributing evenly PA throughout the week or concentrating it on weekends decreases cortisol secretion as measured by AUCg or slope, respectively. Therefore, PA distribution does not seem to impact the positive effect of PA on stress but further studies are needed to confirm this hypothesis.

Study strengths and limitations

As far as we know, this is the largest study exploring the association between activity levels and salivary cortisol. Further, and contrary to other studies (Lederbogen et al. 2010; Vreeburg et al. 2009), both PA and SB were taken into account as high PA levels can be associated either with high or low SE levels, and reciprocally (Sugiyama et al. 2008).

This study also has several limitations. First, its cross-sectional design precludes the assessment of any causal effect of activity levels and patterns on salivary cortisol; it is expected that the next follow-up of the CoLaus cohort will solve this issue. Second, the accelerometer was worn on the right wrist, which might overestimate PA as it is the dominant side for most people. Still, previous findings found no impact of device location on PA assessment (Dieu et al. 2016; Esliger et al. 2011). Thirdly, as GENEActiv accelerometers have been suggested to over-report MVPA levels (Rosenberger et al. 2016), PA was categorized into tertiles of MVPA but not according to recommendations (World 2010). Finally, the analyses were not controlled for smokeless (chewable) tobacco. However, the prevalence of chewable tobacco in Switzerland is very low (Fischer et al. 2014), so we believe this might not significantly impact our results.

Conclusion

In a population-based sample, low SB and high PA were related to lower cortisol secretion as measured by different parameters of salivary cortisol. Nevertheless, the effects were only modest.

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AUTHORS' CONTRIBUTIONS

CG was involved in data collection, made the statistical analyses and wrote the article; CK and PV revised the article for important intellectual content; PMV revised the statistical analysis and the writing.

CONFLICT OF INTEREST

The authors report no conflict of interest.

FIGURE LEGENDS

Figure 1: Mutually exclusive activity patterns. [1] tertile 1 and [2] tertiles 2 or 3 of average proportion of time spent in moderate-to-vigorous physical activity; [3] tertiles 1 or 2 and [4] tertile 3 of the ratio between average proportion of time spent in moderate-to-vigorous physical activity on weekend days and average proportion of time spent in moderate-to-vigorous physical activity on week days.

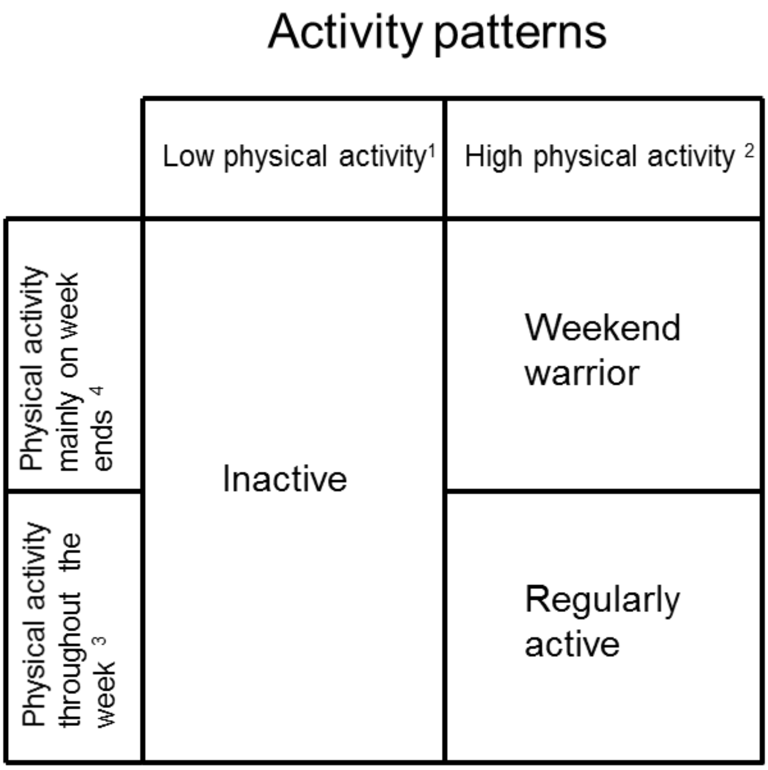
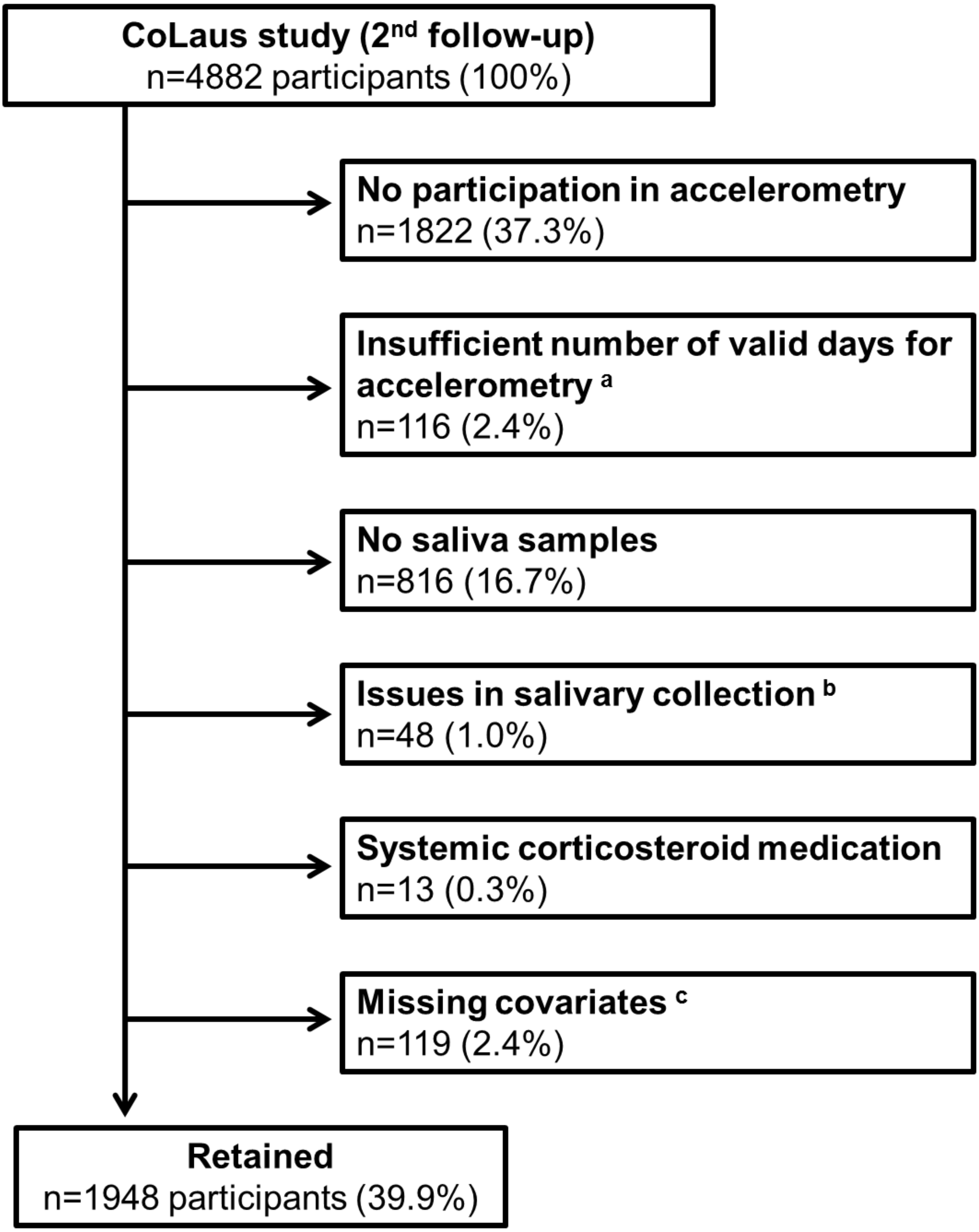


Figure 2: Selection procedure. [a], less than 5 week days with minimum 10 h of diurnal wearing time or less than 2 weekend days with minimum 8 h of diurnal wearing time. [b], Collection after getting out of bed or on weekends. [c], smoking status, body mass index, awakening time, professional occupation or educational level. Percentages were calculated using the total sample size as denominator.



TABLES

Table 1: Association of physical activity and sedentary behaviour status with salivary cortisol parameters. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			**Sedentary behaviour**		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Sample size	625	1323		617	1331	
AUCg [nmol.hour/l]						
Bivariate	215.8 ± 71.9	208.7 ± 67.5	0.05	213.5 ± 69.9	209.8 ± 68.6	0.30
Multivariable-adjusted	215.9 ± 2.9	208.7 ± 2.0	0.05	212.6 ± 2.9	210.3 ± 2.0	0.52
Awakening response [nmol/l]						
Bivariate	12.8 ± 14.1	14.3 ± 15.3	0.05	13.1 ± 14.6	14.1 ± 15.1	0.21
Multivariable-adjusted	13.4 ± 0.7	13.9 ± 0.4	0.53	13.9 ± 0.7	13.8 ± 0.4	0.89
Slope [nmol/l per hour]						
Bivariate	-1.44 ± 0.87	-1.54 ± 0.91	0.04	-1.44 ± 0.87	-1.54 ± 0.91	0.03
Multivariable-adjusted	-1.45 ± 0.04	-1.54 ± 0.03	0.06	-1.44 ± 0.04	-1.54 ± 0.03	0.03

Results are expressed as average ± standard deviation (bivariate) or as multivariable-adjusted average ± standard error. Statistical analyses performed by student t-test (bivariate) or ANOVA (multivariable). Multivariable models were adjusted for age (continuous), gender (male/female), smoking status (no/yes), BMI (continuous), awakening time (continuous), professional occupation (no/yes) and educational level (high/medium/low).

Table 2: Association of activity patterns with salivary cortisol parameters. The CoLaus study, Switzerland, 2014-2017.

	Inactive	Weekend warrior	Regularly active	P-value
Sample size	625	442	881	
AUCg [nmol.hour/l]				
Bivariate	215.8 ± 71.9 [a]	217.2 ± 67.6 [a]	204.5 ± 67.1 [b]	<0.01
Multivariable-adjusted	215.5 ± 2.9 [a]	215.9 ± 3.5 [a]	205.4 ± 2.4 [b]	<0.01
Awakening response [nmol/l]				
Bivariate	12.8 ± 14.1	14.8 ± 14.9	14.0 ± 15.4	0.09
Multivariable-adjusted	13.4 ± 0.7	14.1 ± 0.8	13.9 ± 0.5	0.80
Slope [nmol/l per hour]				
Bivariate	-1.44 ± 0.87 [a]	-1.61 ± 0.88 [b]	-1.50 ± 0.92 [a, b]	0.02
Multivariable-adjusted	-1.44 ± 0.04 [a]	-1.61 ± 0.05 [b]	-1.50 ± 0.03 [a, b]	0.02

Results are expressed as average ± standard deviation (bivariate) or as multivariable-adjusted average ± standard error. Statistical analyses performed by one-way (bivariate) or multivariable ANOVA. Multivariable models were adjusted for age (continuous), gender (male/female), smoking status (no/yes), BMI (continuous), awakening time (continuous), professional occupation (no/yes) and educational level (high/medium/low). Post-hoc pairwise comparisons of multivariable-adjusted averages were performed using the method of Scheffe; values with differing subscripts differ at p<0.05.

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