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Exploring mechanisms associated with the benefits of physical activity and the negatïve effects of sedentary behaviour

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Manuscripts based on the studies presented in this thesis

Chapter 2

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Chapter 3

Gubelmann C, Antiochos P, Vollenweider P, Marques-Vidal P. Association of activity behaviours and patterns with cardiovascular risk factors in Swiss middle-aged adults: The CoLaus study. *Preventive Medicine Reports.*

Chapter 4

Gubelmann C, Heinzer R, Haba-Rubio J, Vollenweider P, Marques-Vidal P. Physical activity is associated with higher sleep efficiency in the general population: The CoLaus study. *Sleep*. 2018.

Chapter 5

Gubelmann C, Kuehner C, Vollenweider P, Marques-Vidal P. Association of activity status and patterns with salivary cortisol: The population-based CoLaus study. *European Journal of Applied Physiology.* 2018

Chapter 6

Gubelmann C, Vollenweider P, Marques-Vidal P. Regularly actives have higher grip strength and lean mass but not Weekend warriors: The CoLaus study. *Submitted in Mayo Clinic Proceedings.*

Chapter 7

Gubelmann C, Vollenweider P, Marques-Vidal P. Association of grip strength with cardiovascular risk markers. *European Journal of Preventive Cardiology.* 2017;24(5):514-521.

Chapter 8

Gubelmann C, Vollenweider P, Marques-Vidal P. No association between grip strength and cardiovascular risk: The CoLaus population-based study. *International Journal of Cardiology.* 2017;236:478-482.

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List of Publications

Published

1. **Gubelmann C**, Antiochos P, Vollenweider P, Marques-Vidal P. Association of activity behaviours and patterns with cardiovascular risk factors in Swiss middle-aged adults: the CoLaus study. *Preventive Medicine Reports.* 2018.

2. **Gubelmann C**, Kuehner C, Vollenweider P, Marques-Vidal P. Association of activity status and patterns with salivary cortisol: the population-based CoLaus study. *European Journal of Applied Physiology*. 2018.

3. **Gubelmann C**, Heinzer R, Haba-Rubio J, Vollenweider P, Marques-Vidal P. Physical activity is associated with higher sleep efficiency in the general population: the CoLaus study. *Sleep*. 2018

4. **Gubelmann C**, Marques-Vidal P, Bringolf-Isler B, Suggs S, Vollenweider P, Kayser B. Correlates of weekday compliance to physical activity recommendations in Swiss youth non-compliant in weekend days. *Preventive Medicine Reports*. 2018;9:86-91.

5. **Gubelmann C**, Vollenweider P, Marques-Vidal P. Of weekend warriors and couch potatoes: Socio-economic determinants of physical activity in Swiss middle-aged adults. *Preventive Medicine*. 2017;105:350-355.

6. **Gubelmann C**, Vollenweider P, Marques-Vidal P. No association between grip strength and cardiovascular risk: the CoLaus population-based study. *International Journal of Cardiology.* 2017;236:478-482.

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8. **Gubelmann C**, Vollenweider P, Marques-Vidal P. Regularly actives have higher grip strength and lean mass but not weekend warriors: The CoLaus study. *Submitted in Mayo Clinic Proceedings.*

List of Communications during the thesis

1. D.Day, Lausanne University, Lausanne, Switzerland, 2016

   Gubelmann C, Vollenweider P, Marques-Vidal P. Association of grip strength with cardiovascular risk markers. *Oral*

2. D.Day, Lausanne University, Lausanne, Switzerland, 2017

   Gubelmann C, Vollenweider P, Marques-Vidal P. No association between grip strength and cardiovascular risk: The CoLaus population-based study. *Poster*

3. Congress of Swiss Society of General Internal Medicine, Lausanne, Switzerland, 2017

   Gubelmann C, Vollenweider P, Marques-Vidal P. No association between grip strength and cardiovascular risk: The CoLaus population-based study. *Poster*

4. MD-PhD retreat, MD-PhD Commision EPFL & Lausanne University, Lausanne, Switzerland, 2017

   Gubelmann C, Antiochos P, Vollenweider P, Marques-Vidal P. Association of activity behaviours and patterns with cardiovascular risk factors in Swiss middle-aged adults: The CoLaus study. *Poster*

5. D.Day, Lausanne University, Lausanne, Switzerland, 2018

   Gubelmann C, Heinzer R, Haba-Rubio J, Vollenweider P, Marques-Vidal P. Physical activity is associated with higher sleep efficiency in the general population: The CoLaus study. *Poster*

6. EuroPrevent, European Association of Preventive Cardiology, Ljubljana, Slovenia, 2018

   Gubelmann C, Antiochos P, Vollenweider P, Marques-Vidal P. Association of activity behaviours and patterns with cardiovascular risk factors in Swiss middle-aged adults: The CoLaus study. *Poster - finalist for best poster in epidemiology*

7. Congress of Swiss Society of General Internal Medicine, Basel, Switzerland, 2018

   Gubelmann C, Heinzer R, Haba-Rubio J, Vollenweider P, Marques-Vidal P. Physical activity is associated with higher sleep efficiency in the general population: The CoLaus study. *Oral – top 4 best student communication*

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   Thesis results presented as a short pitch for the general public. See the oral presentation on https://youtu.be/aGS9-JpOjc0.

Ancillary research not reported in this thesis

1. Research topic: Determinants of physical activity in youth

   Gubelmann C, Marques-Vidal P, Bringolf-Isler B, Suggs LS, Vollenweider P, Kayser B. Correlates of weekday compliance to physical activity recommendations in Swiss youth non-compliant in weekend days. *Preventive Medicine Reports.* 2018;9:86-91.

Summary

The benefits of physical activity on cardiovascular disease prevention are well established. Still, the impact of the distribution of physical activity over the week has been poorly explored, and the underlying mechanisms are incompletely understood. This work aimed to 1) characterize physical activity patterns during the week and 2) explore the associations between physical activity patterns and a series of established or potential cardiovascular risk factors. To achieve this, we conducted five studies in adults from the general population, where physical activity was objectively assessed using accelerometry and patterns defined according to its distribution over the week. The first study demonstrated that weekly physical activity patterns vary according to socio-economic status. The second study demonstrated that physically active adults have lower prevalence of established cardiovascular risk factors, such as obesity, hypertension and diabetes. In the latter studies, physically active adults had also a higher sleep efficiency, a lower cortisol secretion, and a higher muscle mass and strength. Mainly, both physical activity distributed evenly over the week or concentrated on weekends seemed to be beneficial for cardiovascular risk profile. However, physical activity concentrated on weekends was less beneficial on muscle mass and strength. Finally, the association of physical activity with cardiovascular risk was replicated by two other studies using grip strength, a correlate of physical activity. Overall, this work demonstrated that physical activity favorably influences a large number of cardiovascular risk factors, and that the amount of physical activity is more important than the timing of its practice during the week. These results could help update recommendations on the distribution of physical activity over week.

Résumé

Les bénéfices de l'activité physique sur l'incidence des maladies cardiovasculaires sont bien établis. Cependant, l'impact de la distribution de l’activité physique sur la semaine ainsi que les mécanismes sous-jacents ne sont que partiellement compris. Ce travail a cherché à 1) mieux caractériser les comportements d’activité physique sur la semaine, et 2) explorer leurs associations avec les facteurs de risque cardiovasculaire. Pour ce faire, cinq études ont été menées parmi des adultes de la population générale dont l’activité physique a été évaluée par accélérométrie et les comportements définis selon sa distribution sur la semaine. La première étude a montré que les comportements d’activité physique sur la semaine dépendent du niveau socio-économique. La deuxième étude a montré que les adultes actifs présentent une plus faible prévalence de facteurs de risque cardiovasculaire tels que l'obésité, l'hypertension et le diabète. Dans les dernières études, les adultes actifs ont également une meilleure efficacité du sommeil, une sécrétion de cortisol plus basse, et une masse et force musculaire plus grandes. Généralement, autant l'activité physique distribuée régulièrement sur la semaine que concentrée les week-ends est bénéfique sur le profil de risque cardiovasculaire. Cependant, pour la masse et force musculaire, l’activité physique concentrée le weekend semble moins bénéfique. Enfin, l’association de l’activité physique avec le risque cardiovasculaire a été répliquée par deux études en utilisant la force de préhension, un marqueur d’activité physique. Globalement, ce travail montre que l’activité physique influence un très grand nombre de paramètres de santé et de facteurs de risque cardiovasculaire, et que c’est le niveau d’activité physique plutôt que sa distribution sur la semaine qui est important. Nous espérons que ces résultats serviront pour la mise à jour des recommandations de la distribution de l’activité physique sur la semaine.

«Walking is man's best medicine»

Hippocrates (460 BC - 370 BC)

List of Abbreviations

ANOVA: One-Way Analysis of Variance

AUCg: Area Under Curve with respect to ground

BMI: Body Mass Index

BP: Blood Pressure

CAR: Cortisol Awakening Response

CBV: Cerebrovascular

CHD: Coronary Heart Disease

CI: Confidence Interval

CV: Cardiovascular

CVD: Cardiovascular Disease

CVRF: Cardiovascular Risk Factors

GS: Grip Strength

ISI: Insomnia Severity Index

LIPA: Light Intensity Physical Activity

LM: Lean Mass

LTPA: Leisure-Time Physical Activity

MET: Metabolic Equivalent of Task

MVPA: Moderate-to-Vigorous Intensity Physical Activity

OPA: Occupational Physical Activity

OR: Odds Ratio

PSQI: Pittsburgh Sleep Quality Index

RR: Relative-risk Ratio

SB: Sedentary Behaviour

SE: Sedentary

WHO: World Health Organization

Chapter 1

Introduction

Definitions

Physical activity (PA) refers as any bodily movement produced by skeletal muscles that requires energy expenditure (1). For the purpose of this thesis, PA was restricted to moderate-to-vigorous intensity activities (≥3 METs).

Sedentary behaviour (SB) is defined as any waking behaviour characterized by an energy expenditure ≤1.5 METs while in a sitting or reclining posture (2).

Metabolic Equivalent of Task (MET) is the ratio of the work metabolic rate to a standard resting metabolic rate. 1 MET is considered as resting metabolic rate obtained during quiet sitting. It can range from 0.9 METs (sleeping) to 18 METs (running at 16 km/h) (3).

Physical activity and cardiovascular disease

Cardiovascular diseases (CVD) such as myocardial infarction and stroke are the leading cause of death worldwide (4). Physical activity (PA) is protective against CVD and practice of PA reduces the risk of CVD death by 35% (5). In this context, recommendations regarding PA have been issued. The World Health Organization (WHO) recommends that adults spend at least 150 minutes of moderate-intensity PA (3-5.9 METs) or 75 minutes of vigorous-intensity PA (≥6 METs) per week (1). Still, over 60% of the world's population does not comply with these recommendations (6). Consequently, the economic impact of physical inactivity to health-systems worldwide is estimated at $53.8 billion (7). Switzerland is not an exception, as 27% of men and 26% of women never exercise (8). More recently, sedentary behaviour (SB) has emerged as an independent risk factor for CVD (9). SB is distinct from a lack of PA, as individuals compliant to WHO recommendations might spend the rest of the time sitting or lying. SB has been dramatically increasing in industrialized countries (10), currently averaging 7.7 hours per day in the USA (11). Finally, most of the knowledge on PA was based on self-reported PA or SB levels.

Measurement methods for physical activity

Several methods exist for assessing PA (12). Self-reported measures (e.g. questionnaires) were widely used in epidemiological studies because of their low burden to participants and low cost. Nevertheless, their validity remained limited by recall bias or social desirability (12, 13). Recently, devices such as accelerometers and heart rate monitors became more accessible and allowed researchers to measure objective PA in large samples of participants (12, 14). While the relationship between heart rate and PA is affected by factors such as physical fitness (12), accelerometers show a good ability to capture different PA intensities (15). Thus, accelerometers provide the opportunity to improve PA measurement; however, there is no consensus on the analytic method to process the data (16).

Activity behaviours and patterns

Accelerometers capture information that allows calculating new parameters related to CV health: 1) the distribution of PA over week, called weekly activity patterns; and 2) the combination between PA and SB levels, called activity behaviours. First, exercising only once or twice per week instead of being regularly active could decrease the benefits of PA on CVD, possibly due to the short-lived effects of PA (17). Further, the interaction between PA and SB levels have been also shown to impact CVD; indeed, high PA levels could attenuate the deleterious effect of SB (18). Physical activity patterns and behaviours have been recently defined in the literature. For instance, three weekly activity patterns are usually defined (19): 1) ‘Inactive’: low PA; 2) ‘Weekend warrior’: high PA concentrated in 1-2 sessions; or 3) ‘Regularly active’: high PA distributed in ≥ 3 sessions. Activity behaviours can be classified into (20): 1) ‘Couch potato’: low PA & high SB; 2) ‘Light mover’: low PA & low SB; 3) ‘Sedentary Exerciser’: high PA & high SB; or 4) ‘Busy bee’: high PA & low SB. Finally, these parameters might provide new insights regarding the relationship between PA, SB, and CV risk.

Determinants of activity

Many determinants of PA and SB have been studied (21, 22). In adults, socio-economic factors such as employment (23), high income and/or high educational level (22) have been associated with higher PA levels in adults. Paradoxically, high income and high education have also been related to higher SB levels, although this association is debated (21). These contradictory findings are likely because studies focused separately on PA or SB levels but not on their combination, i.e. on activity behaviours. Further, no study has explored the socio-economic determinants of weekly activity patterns.

Association of activity with cardiovascular risk

Most effects of PA on CVD are mediated through changes in traditional cardiovascular risk factors (CVRF) (24). High PA levels are associated with lower levels of body mass index (BMI), blood pressure (BP), lipids and glycaemia (25). Conversely, no association between levels of SB and CVRF has been reported (26, 27), although this finding is debated (28). These contradictory findings are likely because most studies focused separately on SB or on PA levels but not on their combinations, i.e. on activity behaviours. Further, which weekly activity pattern to adopt for optimal CV risk profile remains unknown.

Potentially novel CVRF such as sleep duration (29) and quality (30), cortisol secretion (i.e. a marker of stress) (31), or muscle mass (32) and strength (33) have been associated with incident CVD. As part of the effect of PA on CVD remains unknown (24), it can be speculated that PA and SB impact CVD by modulating these novel CVRF. Indeed, physically active individuals seem to have higher sleep duration (34) and quality (35), lower cortisol secretion (31), and higher muscle mass (36) and strength (37). Nevertheless, these findings were limited because they did not consider levels of SB or weekly activity patterns.

Aim of this thesis

In this thesis was aimed to explore the mechanisms associated with the benefits of PA and the negative effects of SB on CV health. This aim was further categorized into:

1. Characterize the determinants of PA, SB and their patterns in the general population.
2. Explore the associations between PA, SB and their patterns with traditional and novel CVRF.

Recruitment of participants and follow-up procedure

The analyses were based on participants of the CoLaus study, which is a population-based cohort exploring the biological, genetic and environmental determinants of CVD (38, 39). More information can be obtained from www.colaus-psycolaus.ch. The sampling procedure of the CoLaus study was as follows: the source population was defined as all subjects aged between 35 and 75 years registered in the population register of the city of Lausanne (Switzerland). A simple, non-stratified random sample of 19'830 subjects was drawn and the selected subjects were invited to participate by letter. If no response was obtained, a second letter was sent, and if no response was obtained several phone calls were made to contact the potential participant. The following inclusion criteria were applied: (a) written informed consent and (b) willingness to participate.

Recruitment was conducted between 2003 and 2006, enrolling 6733 total participants (34% of the initial random sample). Participants underwent a personal interview, a physical examination and laboratory testing. They also had to complete a questionnaire on family and personal history of cardiovascular disease and risk factors, lifestyle, medicines prescribed and bought over-the-counter. The first follow-up was performed between 2009 and 2012, 5.6 years on average after the collection of baseline data, and included 5064 participants. The second follow-up was performed between 2014 and 2017, 10.9 years on average after the collection of baseline data, and included 4881 participants. Both follow-ups collected the same information as the baseline examination, plus self-reported data on sleep, dietary intake and PA. In the second follow-up, further information regarding novel CVRF (i.e. cortisol secretion, and muscle mass and strength) was collected and an optional module on PA (using accelerometry) was proposed to all participants. Of the 4881 participants, 3060 (63%) accepted to participate in the optional module measuring their PA levels for 14 days.

During the 10.9 years of follow-up, 351 deaths and 437 incident CVD occurred. The vital status was systematically ascertained at the end of follow-up according to the population register. If the population register informed that a participant had died, the cause of death was medically documented by a trained investigator and further adjudicated by two internal medicine specialists. Incident CVD were elicited at follow-up using a standardized interview questionnaire and included coronary heart disease (i.e. myocardial infarction, angina pectoris, percutaneous revascularization or bypass grafting) and cerebrovascular disease (i.e. stroke or transient ischemic attack). Reported incident CVD were first checked and medically documented by a trained investigator, and further validated using pre-defined criteria by an adjudication committee composed of two cardiologists and one neurologist.

Accelerometry measurement

Participants had their PA assessed using a wrist-worn triaxial accelerometer (*GENEActiv*, Activinsights Ltd, United Kingdom). This device was validated against reference methods. The intra- and inter-instrument coefficients of variation were 1.4% and 2.1%; and high correlations with reference methods such as mechanical shaking (r=0.98) and indirect calorimetry (r=0.83) have been reported (15). 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. The resulting files included information for raw acceleration data for x, y and z axes. Using the *GENEActiv* software version 2.9

(*GENEActiv*, Activinsights Ltd, United Kingdom), data were downloaded and collapsed into 1-minute epoch signal vector magnitude ($\mathrm{SVM}\ [\mathrm{g.min}] = \sum\left|\sqrt{x^2+y^2+z^2}\ \text{- 1 g}\right|$).

A valid day of accelerometry measurement was defined as ≥10 h of diurnal wear-time. At least 5 weekdays and 2 weekend days of valid accelerometry data were required.

Data were analyzed using the *GENEActiv macro file* 'General physical activity' version 1.9 (40) based on validated intensity cutoffs (15): SB (<241 g.min), light intensity PA (LIPA) (241-338 g.min) and moderate-to-vigorous PA (MVPA) (>338 g.min). The *GENEActiv macro file* was validated among 60 middle-aged healthy adults performing activity tasks while wearing a portable metabolic gas analyzer. The algorithm showed a good ability to discriminate between SB, LIPA and MVPA (area under the receiver operating characteristic curve = 0.90) (15). Conversely, no information was available regarding the criteria used for non-wear time (proprietary). Sleep was analyzed using the R-package GGIR version 1.5-9 (https://cran.r-project.org) for which the sleep detection algorithm was validated by polysomnography (41). Sleep was defined as the time with no change in arm angle greater than 5° for a period ≥5 minutes during a predefined nocturnal sleep window (21:00-09:00). For each participant, the time spent in LIPA, MVPA and in SB was averaged for all valid days and separately for valid week and weekend days.

Activity behaviours were defined according to the combination of PA and SB status. For PA status, participants were split into tertiles of average MVPA time and classified as 'low PA' if they were in the first tertile and as 'high PA' otherwise. Previous studies have shown that LIPA could influence CV health (42). SB status was defined according to the ratio between the average SB time and the average LIPA time as performed by others (20, 43). Participants were classified as 'high SB' if they were in the highest tertile and as 'low SB' otherwise. This allowed creating four mutually exclusive activity behaviours (**Figure A**): 1) 'Couch potato': 'low PA' & 'high SB'; 2) 'Light mover': 'low PA' & 'low SB'; 3) 'Sedentary exerciser': 'high PA' & 'high SB'; and 4) 'Busy bee': 'high PA' & 'low SB'.

Activity patterns were defined according to PA status and its distribution throughout the week. For PA status, participants were classified as ‘low PA’ if they were in the first tertile of average MVPA time. For the distribution of PA, average MVPA time on weekend days was divided by average MVPA time 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 (**Figure B**): 1) ‘Inactive’: ‘low PA’; 2) ‘Weekend warrior’: ‘high PA’ & ‘PA mainly on weekends’; and 3) ‘Regularly active’: ‘high PA’ & ‘PA throughout the week’.

Figure A – Activity behaviours

	Low physical activity *1st tertile of MVPA*	High physical activity *2nd & 3rd tertile of MVPA*
High sedentary *3rd tertile of SB/LIPA*	Couch potato	Sedentary exerciser
Low sedentary 1st & 2nd tertile of SB/LIPA	Light mover	Busy bee

Figure B – Activity patterns

	Low physical activity *1st tertile of MVPA*	High physical activity *2nd & 3rd tertile of MVPA*
Physical activity mainly on week-ends *3rd tertile of MVPA weekend/week*	Inactive	Weekend warrior
Physical activity throughout the week *1st & 2nd tertile of MVPA weekend/week*		Regularly active

Outline of this thesis

Chapters 2 present the results of a cross-sectional study on the socio-economic determinants of PA, SB and their patterns (CoLaus study, Lausanne, Switzerland). The results show that PA determinants are different regarding 1) the distribution of PA over the week, or 2) the combinations between PA and SB levels.

Chapters 3 to 6 present the results of four cross-sectional studies investigating the association of PA, SB and their patterns with traditional and novel CVRF (CoLaus study, Lausanne, Switzerland). **Chapter 3** studies the relationship of activity patterns and behaviours with traditional CVRF such as obesity, hypertension, diabetes and dyslipidemia. **Chapters 4, 5** and **6** study the association of activity levels and patterns with novel CVRF such as sleep parameters, salivary cortisol, and muscle markers. The results show that sufficient PA improves CV risk profile regardless of PA distribution over the week. Further, they suggest that the effect of PA and SB on CVD is partly mediated by sleep efficiency, cortisol secretion, and muscle mass and strength.

Finally, chapters 7 and 8 study the association of GS, a correlate of PA (44), with CV risk (CoLaus study, Lausanne, Switzerland). **Chapter 7** studies, in a cross-sectional setting, the association of GS with both traditional and novel CVRF. **Chapter 8** studies the longitudinal relationship between GS and incident CVD events.

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Chapter 2

Determinants of activity behaviours and patterns

Based on **Gubelmann C**, Vollenweider P, Marques-Vidal P. Of weekend warriors and couch potatoes: Socio-economic determinants of physical activity in Swiss middle-aged adults. *Preventive Medicine.* 2017;105:350-355.

ABSTRACT

Determinants of the interplay between physical activity (PA) and sedentary (SE) status are poorly known. We assessed the socio-economic determinants of PA and SE behaviours and patterns in a population-based study (The CoLaus study, Lausanne, Switzerland, 2014-2017). 2229 adults (51.8% women, age range 45-86 years) had PA and SE levels measured for 14 days using a wrist-worn accelerometer. Four activity behaviours: (1) ‘Couch potato’: low PA & high SE; (2) ‘Light mover’: low PA & low SE; (3) ‘Sedentary exerciser’: high PA & high SE, and (4) ’Busy bee’: high PA & low SE; and three activity patterns: (1) ‘Inactive’, (2) ‘Weekend warrior’, and (3) ‘Regularly active’ were defined. Employment, household income and educational level were collected by questionnaire. For activity behaviours, relative to ‘Couch potatoes’, multivariate analysis showed that being employed and having a low educational level were positively associated with ‘Light movers’: relative risk ratios and (95% confidence interval): 1.54 (1.00-2.37) and 1.73 (1.11-2.69), respectively, and also with ‘Busy bees’: 1.49 (1.09-2.04) and 1.71 (1.26-2.32), respectively. High household income was negatively associated with ‘Light movers’: 0.58 (0.34-0.97) and positively with ‘Sedentary exercisers’: 1.85 (1.10-3.10). For activity patterns, relative to ‘Inactives’, being employed and having a high household income were positively associated with ‘Weekend warriors’: 1.78 (1.26-2.50) and 1.59 (1.07-2.36), respectively, while having a low educational level was positively associated with ‘Regularly actives’: 1.76 (1.32-2.34). Employment, educational level and household income are significantly but differently associated with activity behaviours and patterns.

INTRODUCTION

The beneficial effects of regular physical activity (PA) have been well established (1). According to the World Health Organization, adults should spend at least 150 minutes of moderate-intensity PA per week (2). Still, 60 percent of the world population does not adhere to these recommendations; further, interventions to increase PA levels are often ineffective (3). Beyond the dose-response effect, other components of PA have been shown to impact health: (i) its interplay with sedentary (SE) levels (i.e. ‘activity behaviour’) as described by Bakrania and al. (4); and (ii) its distribution over time (i.e. ‘activity pattern’) (5). Indeed, the benefits of PA could be altered either by being SE instead of performing light-intensity physical activity (LIPA) such as standing (4, 6), or by performing only 1-2 sessions per week (5). Hence, to promote optimal activity patterns and behaviours in the general population, a better understanding of their determinants is necessary.

Several socio-economic factors have been associated with PA and SE. Namely, employment (7), high income (8) and high educational level (8) are related to higher PA levels. Paradoxically, high income and high education have also been related to higher SE levels, although this association has been debated (9). This paradox is likely due to the fact that most studies focused either on PA (8) or on SE (9) but not on their combinations. For instance, high PA levels can be associated either with high or low SE levels, and reciprocally (10); hence, analysis of PA and SE combinations might provide more information than of PA or SE alone.

To date, little is known about the determinants of activity behaviours and patterns. The existent literature is limited as: (i) it took into account a single socio-economic factor (11, 12) or used socio-economic status instead of studying the different socio-economic factors (4), or (ii) the definition of behaviours and patterns relied on self-reported data (5, 10-12). Further, all previous findings were limited to simple descriptive analyses, and no adjustment for major confounders such as age, gender or lifestyle was performed (4, 10-12).

Therefore, this study aimed to assess the socio-economic determinants of activity behaviours and patterns in a population-based sample aged 45-86 years from the city of Lausanne, Switzerland (CoLaus study).

METHODS

Recruitment of participants

The detailed description of the recruitment of the CoLaus study and the follow-up procedures has been described previously (13, 14). Briefly, the CoLaus study is a population-based cohort exploring the biological, genetic and environment determinants of cardiovascular diseases. 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 levels for 14 days.

Physical activity measurement

Physical activity 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 1-minute epoch files. Data were analyzed using the *GENEActiv macro file* 'General physical activity' version 1.9 (15) which had been previously validated (16). A valid day was defined as ≥10 h (i.e. 600 min-epoch) and ≥8 h (i.e. 480 min-epoch) of diurnal wear-time on week days and weekend days, respectively. For each participant, the number of minutes spent in LIPA, moderate-to-vigorous intensity PA (MVPA) and in SE were averaged for all valid days and separately for valid week and weekend days. At least 5 week days and 2 weekend days of valid accelerometry data were required (see exclusion criteria) (17).

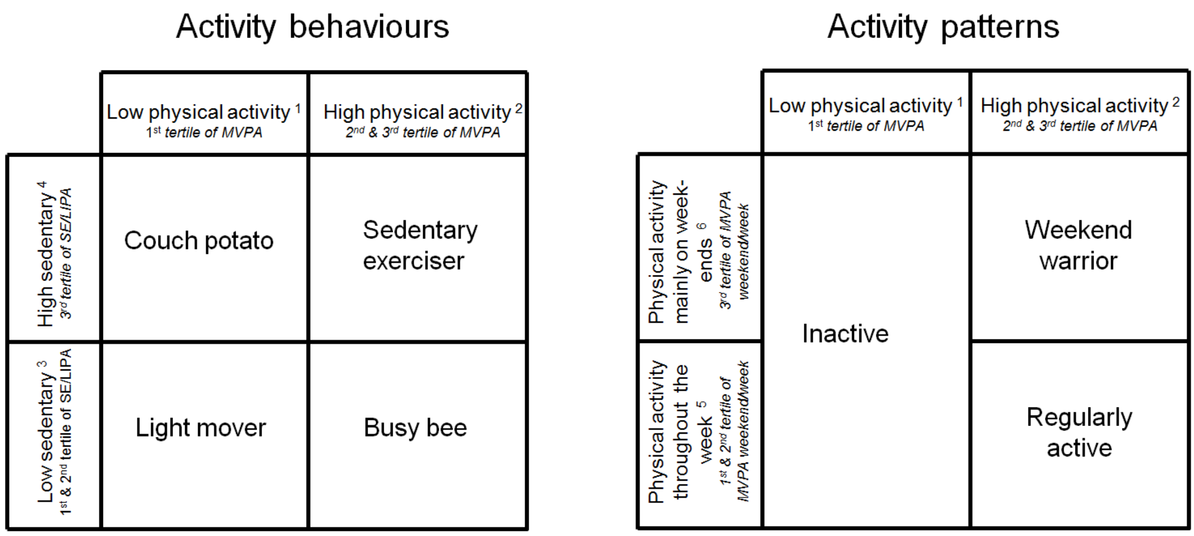
Activity behaviours

Activity behaviours were defined according to the interplay between MVPA and SE status. For MVPA status, participants were split into tertiles of average MVPA time and classified as ‘low PA’ if they were in the first tertile and as ‘high PA’ if they were in the second or third tertile. Based upon other studies (4, 18), SE status was defined according to the ratio between the average SE time and the average LIPA time. Participants were classified as ‘high SE’ if they were in the third tertile and as ‘low SE’ if they were in the first or second tertile. This classification allowed creating four mutually exclusive activity behaviours (**Figure 1**) as described by Bakrania and al. (4): 1) ‘Couch potato’: ‘low PA’ & ‘high SE’; 2) ‘Light mover’: ‘low PA’ & ‘low SE’; 3) ‘Sedentary Exerciser’: ‘high PA’ & ‘high SE’; and 4) ‘Busy bee’: ‘high PA’ & ‘low SE’.

Activity patterns

Activity patterns were defined according to MVPA status and its distribution throughout the week. For MVPA status, participants were classified as ‘low PA’ if they were in the first tertile of average MVPA time and as ‘high PA’ if they were in the second or third tertile. For the distribution of MVPA, average MVPA time on weekend days was divided by average MVPA time on week days and split into tertiles. Participants were categorized as ‘PA mainly on weekends’ if they were in the third tertile and as ‘PA throughout the week’ if they were in the first or second tertile. This classification allowed creating three mutually exclusive activity patterns (**Figure 1**) as described by O’Donovan and al. (12): 1) ‘Inactive’: ‘low PA’; 2) ‘Weekend warrior’: ‘high PA’ & ‘PA mainly on weekends’; and 3) ‘Regularly active’: ‘high PA’ & ‘PA throughout the week’.

Figure 1: Mutually exclusive activity behaviours and patterns. The CoLaus study, Switzerland, 2014-2017. [1] tertile 1 of average moderate-to-vigorous physical activity (MVPA) time; [2] tertile 2 or 3 of average MVPA time; [3] tertiles 1 or 2 of the ratio between average sedentary time (SE) and average light physical activity (LIPA) time; [4] tertiles 3 of the ratio between average SE and LIPA; [5] tertiles 1 or 2 of the ratio between average MVPA time on weekend days and average MVPA time on week days. [6] tertile 3 of the ratio between average MVPA time on weekend days and average MVPA time on week days.



Socio-economic and other data

Demographic, smoking status, employment and household income data were collected at second follow-up by questionnaire. Educational level was collected at baseline by questionnaire. Educational level was categorized as low (obligatory school or apprenticeship), medium (high school), or high (university degree). Participants were considered as employed if they were currently working. Conversely, no information regarding working patterns (i.e. which were the work and non-work days during the week) was collected. Monthly household income before social charges was collected and expressed in Swiss francs (1 CHF=1.007 US$ or 0.937 € as of 29 March 2017).

Exclusion criteria

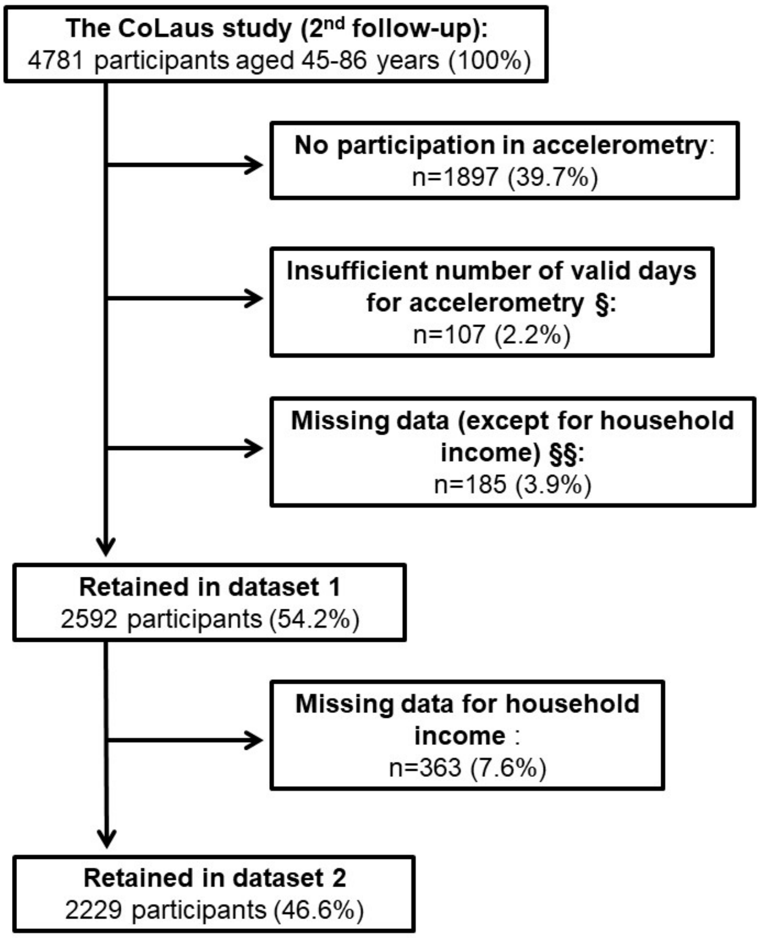
Participants were excluded if they: (i) did not participate in accelerometry; (ii) had less than 5 week days or 2 weekend days of valid accelerometry data, and (iii) had missing data for the other covariates. As a significant proportion of the participants refused to provide household income data, two datasets were used in the analysis: one with all included participants but without income data (dataset 1), and another including only participants who provided income data (dataset 2, **Figure 2**).

Statistical analysis

Statistical analyses were conducted using Stata version 14.0 for windows (Stata Corp, College Station, Texas, USA). Results were expressed as number of participants (percentage) for categorical variables or as average ± standard deviation for continuous variables. Between-group comparisons were performed using chi-square and one-way analysis of variance for categorical and continuous variables, respectively.

Multivariate analyses using the activity behaviours or patterns as the dependent variables were conducted using multinomial logistic regression. For activity behaviours, the ‘Couch potato’ group was considered as base outcome and the variables associated with ‘Light mover’, ‘Sedentary exerciser’ and ‘Busy bee’ behaviours were assessed. For activity patterns, the ‘Inactive’ pattern was considered as base outcome and the variables associated with the ‘Weekend warrior’ and ‘Regularly active’ patterns were assessed. The variables included in the model were: age (continuous), gender (male/female), marital status (yes/no), smoking status (current/former/never), employment (no/yes), educational level (high/medium/ low), and household income (<5000/5000-9499/>9500 CHF). Results were expressed as relative-risk ratio and 95% confidence interval. Trends were assessed using the **test** function of Stata.

Figure 2: Selection procedure. The CoLaus study, Switzerland, 2014-2017. §: less than 5 week days with minimum 10 h of diurnal wear-time or less than 2 weekend days with minimum 8 h of diurnal wear-time. §§: missing data in marital status, smoking status, employment or educational level. Percentages were calculated using the total sample size as denominator.



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

Selection procedure and characteristics of excluded and included participants

The selection procedure is indicated in **Figure 2.** Of the initial 4781 participants, 2592 (54.2%) and 2229 (46.6%) were retained in datasets 1 and 2, respectively.

Included and excluded participants' characteristics are presented in **Supplementary Table 1**. For both datasets, included participants were younger, more likely a male and to be married, and more prone to be employed and to have a higher household income or educational level than excluded ones. No significant difference was found for smoking status.

The characteristics of the participants included and excluded due to insufficient number of valid days for accelerometry are presented in **Supplementary Table 2** (for dataset 2). Noncompliers were more frequently women, while no differences were found for the other variables. Among included participants, the number of days with valid accelerometry data was 9.4±1.2 on weekdays and 3.7±0.6 on weekends (mean ± standard deviation). Average time (± standard deviation) of accelerometer wear during the day was 14.2±1.5 hours.

Socio-economic determinants of activity behaviours

The bivariate associations between the socio-economic factors and the activity patterns are described in **Supplementary tables 3** (dataset 1) and **4** (dataset 2). The multivariate analyses are presented in **Table 1**. Significant differences were found for all demographical variables assessed; being younger, less frequently married, and more frequently former or never smokers were associated with the 'Sedentary exerciser' behaviour.

The associations with the socio-economic factors were similar within both datasets. On bivariate analysis, being employed was positively associated with the 'Busy bee' and 'Sedentary exerciser' patterns in comparison to the 'Couch potato' one. Low educational levels were related to higher prevalence rates of 'Light movers' and 'Busy bees'. High household income was negatively associated with the 'Light movers' and positively with the 'Sedentary exercisers'. After multivariate adjustment, all the associations persisted. Finally, being employed was significantly associated with the 'Light movers' (**Table 1**).

Socio-economic determinants of activity patterns

The bivariate associations between the socio-economic factors and the activity patterns are described in **Supplementary table 5**. The multivariate analyses are presented in **Table 2**. Significant differences were found for all demographical variables assessed; being younger, a female, less frequently married, and more frequently never smokers were associated with the 'Weekend warrior' pattern.

The associations with the socio-economic factors were similar within both datasets. On bivariate analysis, being employed was positively associated with the 'Weekend warrior' and 'Regularly active' patterns in comparison to the 'Inactive' one. Low educational levels were related to higher prevalences of 'Regularly actives' and lower prevalences of 'Weekend warriors'. Finally, having a high income was associated with the 'Weekend warrior' pattern. After multivariate adjustment, most of the associations persisted, except for employment that was no longer associated with the 'Regularly actives'.

Table 1: Multivariate analysis of the socio-economic factors associated with activity behaviours. The CoLaus study, Switzerland, 2014-2017.

	Dataset 1 §			Dataset 2 §§		
	Light mover (N=305)	***Sedentary exerciser*** (N=322)	***Busy bee*** (N=1415)	***Light mover*** (N=255)	***Sedentary exerciser*** (N=289)	***Busy bee*** (N=1212)
Employment						
No	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Yes	1.60 (1.09 - 2.36)	1.79 (1.20 - 2.66)	1.68 (1.27 - 2.23)	1.54 (1.00 - 2.37)	1.55 (0.99 - 2.43)	1.49 (1.09 - 2.04)
Educational level						
High	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Medium	1.26 (0.82 - 1.93)	0.94 (0.64 - 1.39)	1.49 (1.11 - 2.00)	1.29 (0.81 - 2.06)	0.99 (0.66 - 1.50)	1.32 (0.96 - 1.81)
Low	1.74 (1.19 – 2.55)	1.06 (0.75 - 1.51)	1.88 (1.44 - 2.46)	1.73 (1.11 - 2.69)	1.34 (0.90 – 2.00)	1.71 (1.26 - 2.32)
P for trend	0.01	0.79	<0.01	0.04	0.21	<0.01
Household income [1]						
<5000 CHF	.	.	.	1 (ref)	1 (ref)	1 (ref)
5000-9499 CHF	.	.	.	0.71 (0.48 - 1.06)	1.31 (0.83 - 2.06)	0.99 (0.73 - 1.34)
≥9500 CHF	.	.	.	0.58 (0.34 - 0.97)	1.85 (1.10 - 3.10)	0.78 (0.54 - 1.14)
P for trend	.	.	.	0.10	0.05	0.27

§: all included participants but without household income data; §§: only participants with household income data. [1] 1 CHF=1.007 US$ or 0.937 € as of 29 March 2017. Results are expressed as multivariate-adjusted relative-risk ratio and (95% confidence interval). Statistical analyses performed by multinomial logistic regression comparing the 'Light mover', 'Sedentary exerciser' and 'Busy bee' behaviours to the 'Couch potato' one. Variable included in the model: age (continuous), gender (2 categories), marital status (2 categories), smoking status (3 categories), and listed covariates (except household income for dataset 1).

Table 2: Multivariate analysis of the socio-economic factors associated with activity patterns. The CoLaus study, Switzerland, 2014-2017.

	Dataset 1 §		**Dataset 2 §§**	
	Weekend warrior (N=605)	***Regularly active*** (N=1132)	***Weekend warrior*** (N=527)	***Regularly active*** (N=974)
Employment				
No	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Yes	1.94 (1.43 - 2.62)	1.23 (0.96 - 1.58)	1.78 (1.26 - 2.50)	1.10 (0.83 - 1.45)
Educational level				
High	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Medium	0.94 (0.70 - 1.28)	1.54 (1.16 - 2.04)	0.91 (0.66 - 1.26)	1.36 (1.01 - 1.84)
Low	0.75 (0.57 - 0.99)	1.95 (1.51 - 2.50)	0.85 (0.62 - 1.17)	1.76 (1.32 - 2.34)
P for trend	0.08	<0.01	0.60	<0.01
Household income [1]				
<5000 CHF	.	.	1 (ref)	1 (ref)
5000-9499 CHF	.	.	1.23 (0.88 - 1.71)	1.17 (0.90 - 1.53)
≥9500 CHF	.	.	1.59 (1.07 - 2.36)	0.96 (0.68 - 1.34)
P for trend	.	.	0.07	0.21

§: all included participants but without household income data; §§: only participants with household income data. [1] 1 CHF=1.007 US$ or 0.937 € as of 29 March 2017. Results are expressed as multivariate-adjusted relative-risk ratio and (95% confidence interval). Statistical analyses performed by multinomial logistic regression comparing the 'Weekend warrior' and 'Sedentary exerciser' patterns to the 'Inactive' one. Variable included in the model: age (continuous), gender (2 categories), marital status (2 categories), smoking status (3 categories), and listed covariates (except household income for dataset 1).

DISCUSSION

This study assessed the socio-economic determinants of PA and SE behaviours and patterns using a 14-day accelerometry measurement in a population-based setting. Our results suggest that employment, educational level and household income are differently associated with PA and SE behaviours and patterns.

Employment

Employment was positively associated with the ‘Light mover’, the ‘Sedentary exerciser’ and ‘Busy bee’ behaviours. Positive associations were also found with the ‘Weekend warrior’, whereas no association was found with the ‘Regularly active’ pattern. These findings are partly in agreement with previous studies showing that workers are more physically-active (7, 10, 19) and less sedentary (10, 20, 21) than nonworkers. However, a longitudinal study showed that nonworking is protective against any decrease in PA, but these results were restricted to leisure-time PA (22). Thus, our findings suggest that employed individuals are more prone to adopt high PA or low SE levels than others. Further, they are more likely to concentrate their PA on weekends, probably due to a lack of time during the week. Finally, the absence of a significant association with the ‘Regularly active’ pattern is possibly explained by the high proportion of retired participants (age≥65 years, 36%), which may have blurred the association.

Educational level

Low educational level was positively associated with the ‘Light mover’ and the ‘Busy bee’ patterns, a finding in agreement with Bakrania and al (4). Low educational level was also related to a lower prevalence of ‘Weekend warriors’ and a higher prevalence of ‘Regularly actives’, a finding partly in agreement with another study that reported higher educational levels among the ‘Weekend warriors’ (11). However, education has been positively related to sufficient PA levels in cross-sectional (10, 23) and longitudinal studies (22, 24, 25). Still, these conflicting findings were found for leisure-time PA, not occupational

PA. Thus, our results suggest that poorly educated individuals are more prone to adopt low SE levels than others. Further, they are more likely regularly active whereas highly educated individuals tend to concentrate their PA on weekends. A possible explanation is that the higher the educational level the less likely the employment is active. Still, these findings need to be further confirmed in other studies.

Household income

High household income was associated with a lower prevalence of ‘Light movers’ and with a higher prevalence of ‘Sedentary exercisers’. Sugiyama and al. confirmed these findings for the ‘Sedentary exercisers’, but also found an association between high income and a higher prevalence of ‘Light mover’ and ‘Busy bee’ behaviours (10). These discrepancies are possibly due to the fact that Sugiyama and al. restricted their analysis to leisure-time PA and SE, therefore misclassifying active workers as ‘Couch potatoes’ (10). High household income was also associated with a higher prevalence of the ‘Weekend warrior’ pattern, whereas no association was found for the ‘Regularly actives’. This latter finding disagrees with other studies which have shown a positive association between household income and PA (8, 26, 27). Several explanations can be put forward to explain the absence of association between household income and the ‘Regularly active’ pattern. First, we used objectively measured PA, which has been recently shown to be differently associated with income than self-reported PA (28). Second, we studied the relationship between household income and PA distribution, which has not been addressed so far. Overall, our results suggest that individuals with a high household income are more prone to adopt high PA and high SE levels, and to concentrate their PA on weekends. This is possibly explained by a more SE employment but needs to be further explored.

Study strengths and limitations

As far as we know, this is the first study exploring socio-economic determinants for both activity behaviours and patterns. Importantly, and contrary to other studies (10-12), PA and SE were objectively assessed using a 14-day accelerometry measurement and the analyses were adjusted for major confounders.

This study also has several limitations. Firstly, the cross-sectional design of our study precludes the assessment of any causal effect of socio-economic factors on activity behaviours and patterns; the next follow-up of the CoLaus participants will enable assessing causal effects. Secondly, the accelerometer was worn on the right wrist. Although it might be more prone to noisy movements, previous findings found no impact on PA assessment (16, 29). Thirdly, because the GENEActiv accelerometers considerably over-report MVPA levels (30), PA was categorized into tertiles of MVPA but not according to recommendations (2). Fourthly, PA patterns were defined according to a Monday-Friday week. Therefore, weekend workers could be misclassified as 'Weekend warriors'. However, it is most likely that the majority of participants adopt a traditional Monday-Friday pattern. Finally, educational level was collected at baseline and not updated during follow-up; however, it is unlikely that a sizable fraction of middle-aged adults would change their educational levels, so the impact of this non-update might be limited.

Conclusion

In a population-based sample aged 45 to 86 years, socio-economic determinants were differently associated with activity behaviours and patterns. Thus, taking into account PA and SE combinations might explain the contradictory findings when only PA or SE is assessed.

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SUPPLEMENTARY MATERIAL

Supplementary table 1: Characteristics of excluded and included participants. The CoLaus study, Switzerland, 2014-2017.

	Dataset 1 §			Dataset 2 §§		
	Included (N=2592)	**Excluded** (N=2189)	**P-value**	**Included** (N=2229)	**Excluded** (N=2552)	**P-value**
Age (years)	62.0 ± 10.0	64.2 ± 10.8	<0.01	61.5 ± 9.9	64.2 ± 10.7	<0.01
Age group			<0.01			<0.01
[45-65[	61.5	54.2		63.6	53.4	
65+	38.5	45.8		36.4	46.6	
Female	53.6	56.7	0.03	51.8	57.8	<0.01
Married	56.6	52.1	<0.01	56.5	52.9	0.01
Smoking status			0.10			0.38
Current	17.9	20.5		18.1	19.8	
Former	39.7	38.3		39.6	38.7	
Never	42.4	41.3		42.3	41.6	
Employment			<0.01			<0.01
No	43.5	52.0		40.7	53.4	
Yes	56.5	48.1		59.4	46.6	
Educational level			<0.01			<0.01
High	22.0	20.1		23.2	19.3	
Medium	27.1	24.4		27.7	24.2	
Low	50.9	55.6		49.0	56.6	
Household income [1]			.			<0.01
<5000 CHF	.	.		25.3	30.9	
5000-9499 CHF	.	.		43.3	44.2	
>9499 CHF	.	.		31.4	25.0	

§: all included participants but without household income data; §§: only participants with household income data; [1] 1 CHF=1.007 US$ or 0.937 € as of 29 March 2017. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and Student t-test.

Supplementary table 2: Characteristics of included participants and excluded participants due to insufficient number of valid day for accelerometry, in dataset 2. The CoLaus study, Switzerland, 2014-2017.

	Included (N=2229)	**Excluded** (N=106)	**P-value**
Age (years)	61.5 ± 9.9	61.2 ± 9.5	0.70
Age group			0.76
[45-65[	63.6	65.1	
65+	36.4	34.9	
Female	51.8	62.6	0.03
Married	56.5	51.0	0.28
Smoking status			0.12
Current	18.1	25.0	
Former	39.6	41.7	
Never	42.3	33.3	
Employment			0.15
No	40.7	48.0	
Yes	59.4	52.0	
Educational level			0.16
High	23.2	16.8	
Medium	27.7	25.2	
Low	49.0	57.9	
Household income [1]			0.11
<5000 CHF	25.3	31.7	
5000-9499 CHF	43.3	47.6	
>9499 CHF	31.4	20.7	

[1] 1 CHF=1.007 US$ or 0.937 € as of 29 March 2017. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and Student t-test.

Supplementary table 3: Participants' characteristics of dataset 1, by activity behaviours. The CoLaus study, Switzerland, 2014-2017.

	Dataset 1 §				
	Couch potato	***Light mover***	***Sedentary exerciser***	***Busy bee***	**P-value**
	N=550 (21.2%)	N=305 (11.8%)	N=322 (12.4%)	N=1415 (54.6%)	
Age (years)	65.7 ± 10.7	66.8 ± 10.2	57.9 ± 8.8	60.4 ± 9.1	<0.01
Age group					
[45-65[	45.8	40.0	77.0	68.6	<0.01
65+	54.2	60.0	23.0	31.2	
Female	36.0	61.6	42.2	61.2	<0.01
Married	57.8	51.8	50.6	58.5	0.02
Smoking status					0.02
Current	21.5	21.3	12.7	16.9	
Former	38.9	40.0	42.2	39.4	
Never	39.6	38.7	45.0	43.8	
Employment					<0.01
No	59.1	59.0	27.0	37.9	
Yes	40.9	41.0	73.0	62.1	
Educational level					<0.01
High	26.7	17.4	31.4	19.1	
Medium	27.3	24.6	25.8	27.8	
Low	46.0	58.0	42.9	53.1	

§: all included participants but without household income data. Couch potato: low physical activity (PA) & high sedentary (SE); Light mover: low PA & low SE; Sedentary exerciser: high PA & high SE; Busy bee: high PA & low SE. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and one-way analysis of variance, comparing activity behaviours.

Supplementary table 4: Participants' characteristics of Dataset 2, by activity behaviours. The CoLaus study, Switzerland, 2014-2017.

	Dataset 2 §				
	Couch potato	***Light mover***	***Sedentary exerciser***	***Busy bee***	**P-value**
	N=473 (21.2%)	N=255 (11.4%)	N=289 (13.0%)	N=1212 (54.4%)	
Age (years)	65.3 ± 10.8	66.1 ± 10.3	57.7 ± 8.6	60.0 ± 8.9	<0.01
Age group					
[45-65[	47.6	43.5	78.2	70.6	<0.01
65+	52.4	56.5	21.8	29.4	
Female	34.9	58.8	39.5	59.8	<0.01
Married	60.3	51.8	50.2	57.6	0.02
Smoking status					0.02
Current	21.8	22.4	12.1	17.2	
Former	38.7	39.2	42.2	39.4	
Never	39.5	38.4	45.7	43.4	
Employment					<0.01
No	55.4	56.1	24.2	35.6	
Yes	44.6	43.9	75.8	64.4	
Educational level					<0.01
High	28.1	16.9	32.2	20.5	
Medium	28.8	25.5	26.0	28.2	
Low	43.1	57.7	41.9	51.2	
Household income [1]					<0.01
<5000 CHF	25.2	38.8	15.9	24.8	
5000-9499 CHF	43.3	40.8	36.7	45.5	
>9499 CHF	31.5	20.4	47.4	29.8	

§: only participants with household income data; [1] 1 CHF=1.007 US$ or 0.937 € as of 29 March 2017. Couch potato: low physical activity (PA) & high sedentary (SE); Light mover: low PA & low SE; Sedentary exerciser: high PA & high SE; Busy bee: high PA & low SE. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and one-way analysis of variance, comparing activity behaviours.

Supplementary table 5: Characteristics of participants, by activity patterns. The CoLaus study, Switzerland, 2014-2017.

	Dataset 1 §				**Dataset 2 §§**			
	Inactive	***Weekend warrior***	***Regularly active***	**P-value**	***Inactive***	***Weekend warrior***	***Regularly active***	**P-value**
	N=855 (33.0%)	N=605 (23.3%)	N=1132 (43.7%)		N=728 (32.7%)	N=527 (23.6%)	N=974 (43.7%)	
Age (years)	66.1 ± 10.6	58.7 ± 8.8	60.6 ± 9.1	<0.01	65.6 ± 10.6	58.2 ± 8.4	60.3 ± 9.1	<0.01
Age group								
[45-65[	43.7	76.4	67.1	<0.01	46.2	78.4	68.7	<0.01
65+	56.3	23.6	33.0		53.9	21.6	31.3	
Female	45.2	60.5	56.2	<0.01	43.3	58.4	54.5	<0.01
Married	55.7	52.7	59.4	0.02	57.3	52.8	58.0	0.13
Smoking status				<0.01				<0.01
Current	21.4	16.9	15.7		22.0	16.7	16.0	
Former	39.3	37.4	41.3		38.9	37.0	41.5	
Never	39.3	45.8	43.0		39.2	46.3	42.5	
Employment				<0.01				<0.01
No	59.1	27.4	40.4		55.6	24.3	38.3	
Yes	40.9	72.6	59.6		44.4	75.7	61.7	
Educational level				<0.01				<0.01
High	23.4	30.7	16.3		24.2	32.5	17.6	
Medium	26.3	29.8	26.2		27.6	29.2	27.0	
Low	50.3	39.5	57.4		48.2	38.3	55.4	
Household income [1]				.				<0.01
<5000 CHF	.	.	.		30.0	18.6	25.5	
5000-9499 CHF	.	.	.		42.5	38.3	46.7	
>9499 CHF	.	.	.		27.6	43.1	27.8	

§: all included participants but without household income data; §§: only participants with household income data; [1] 1 CHF=1.007 US$ or 0.937 € as of 29 March 2017. Inactive: low physical activity (PA); Weekend warrior: high PA & PA mainly on weekends; Regularly active: high PA & PA throughout the week. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and one-way analysis of variance, comparing activity patterns.

Chapter 3

Association of activity behaviours and patterns with traditional cardiovascular risk factors

Based on **Gubelmann C**, Antiochos P, Vollenweider P, Marques-Vidal P. Association of activity behaviours and patterns with cardiovascular risk factors in Swiss middle-aged adults: The CoLaus study. *Preventive Medicine Reports.* 2018.

ABSTRACT

The impact of the combination between physical activity (PA) and sedentary (SE) levels on cardiovascular health is poorly known. We assessed the association of activity behaviours and patterns with cardiovascular risk factors in the general population (The CoLaus study, Switzerland, 2014-2017). 2605 adults (54.4% women, age range 45-86 years) had PA and SE levels measured for 14 days using wrist-worn accelerometry. Four activity behaviours: ‘Couch potato’: low PA & high SE; ‘Light mover’: low PA & low SE; ‘Sedentary exerciser’: high PA & high SE, and ’Busy bee’: high PA & low SE; and three activity patterns: ‘Inactive’, ‘Weekend warrior’, and ‘Regularly active’ were defined. Smoking, obesity, hypertension, dyslipidemia and diabetes were assessed. Relative to ‘Couch potatoes’, ‘Sedentary exercisers’ and ‘Busy bees’ had a lower likelihood of smoking: Odds Ratio (95% confidence interval): 0.40 (0.27-0.61) and 0.62 (0.47-0.81), obesity: 0.43 (0.29-0.63) and 0.41 (0.31-0.54), and diabetes: 0.53 (0.30-0.95) and 0.62 (0.42-0.89), respectively. Relative to ‘Inactives’, ‘Weekend warriors’ and ‘Regularly actives’ had a lower likelihood of smoking: 0.58 (0.43-0.78) and 0.56 (0.44-0.72), obesity: 0.41 (0.30-0.56) and 0.41 (0.32-0.53), hypertension: 0.66 (0.51-0.85) and 0.72 (0.59-0.89), and diabetes: 0.61 (0.38-0.98) and 0.60 (0.42-0.86), respectively. High PA is associated with a favourable cardiovascular risk profile, even when concomitant with high SE or when PA is concentrated on weekends. These findings suggest that being ‘Sedentary exerciser’ or ‘Weekend warrior’ might be sufficient to prevent cardiovascular disease.

INTRODUCTION

The beneficial effect of regular physical activity (PA) on cardiovascular disease (CVD) is well established (1, 2). Beyond the dose-response effect, other components of PA have been shown to impact cardiovascular (CV) health: (i) its combination with sedentary (SE) levels (i.e. 'activity behaviour') as described by Bakrania and al. (3); and (ii) its distribution over time (i.e. 'activity pattern') as described by Lee and al. (4) and O'Donovan and al. (5). Indeed, the benefits of PA could be altered either by a high SE level (6, 7), or by exercising only 1-2 times per week (4).

Part of the effect of PA on CVD is mediated through changes in cardiovascular risk factors (CVRF) (8). High PA levels are associated with lower levels of body mass index (BMI), blood pressure (BP), lipids and glycaemia (2, 9). Paradoxically, several studies reported no association between SE levels and CVRF (9, 10) but those findings have been questioned (11-13). These contradictory findings are likely due to the fact that most studies focused separately on SE or on PA but not on their combinations. Indeed, a recent meta-analysis (14) described an interaction between PA and SE, showing that high PA levels could attenuate the deleterious effect of SE. Hence, analysis of PA and SE combinations seems necessary to provide more valuable information with regards to their association with CVRF, and thus with CVD risk.

Nevertheless, to date, little is known on the association of activity behaviours and patterns with CVRF. The existent literature is limited as: (i) it did not take into account all traditional CVRF (3, 15); (ii) the definition of behaviours (7, 16) or patterns (4, 5) relied on self-reported data, or (iii) it did not adjust for major confounders such as age, gender or socio-economic factors (4, 5).

Therefore, this study aimed to assess the association of activity behaviours and patterns with traditional CVRF in a population-based sample aged 45-86 years from the city of Lausanne, Switzerland (CoLaus study).

METHODS

Recruitment of participants

The detailed description of the recruitment of the CoLaus study and the follow-up procedures has been described previously (17, 18). 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: 2605 subjects participated in an optional module assessing their PA levels for 14 days and were sufficiently studied to be included in the analysis (see exclusion criteria). For this study, we performed a cross-sectional analysis using data of the second follow-up only.

Physical activity measurement

PA was assessed using a wrist-worn triaxial accelerometer (*GENEActiv*, Activinsights Ltd, United Kingdom). This device has been validated against reference methods (19). The intra- and inter-instrument coefficients of variation were 1.4% and 2.1%; and the correlations with methods such as mechanical shaking and indirect calorimetry were strong (r=0.98 and r=0.83)(19). The accelerometers were pre-programmed with a 50 Hz sampling frequency, and subsequently attached to the participants' right wrist irrespective of their dominant wrist (20). To optimally capture PA gradient between week and weekend days, participants were requested to wear the device continuously, day and night, 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 (21) which is based on validated intensity cutoffs (19): SE (<241 g.min), light intensity PA

(241-338 g.min) and moderate-to-vigorous PA (>338 g.min). Conversely, no information was available regarding the criteria used for sleep and non-wear time (proprietary). A valid day was defined as ≥10 h (i.e. 600 min) of diurnal wear-time. For each participant, the time (in minutes) spent in light intensity PA, moderate-to-vigorous intensity PA (MVPA) and in SE was averaged for all valid days and separately for valid week and weekend days. At least 5 week days and 2 weekend days of valid accelerometry data were required (see exclusion criteria).

Activity behaviours

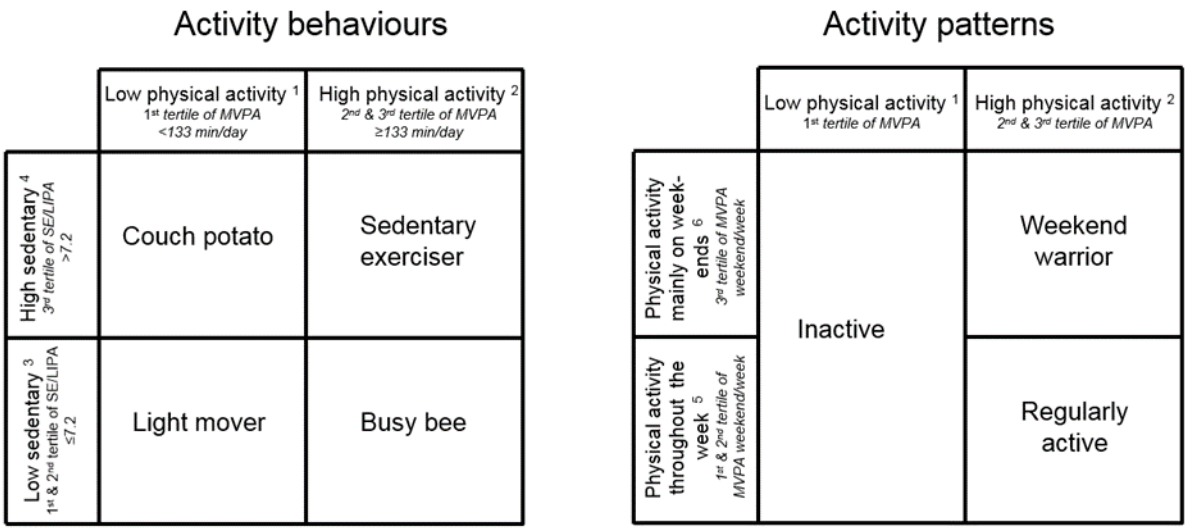
Activity behaviours were defined according to the combination between PA and SE status. For PA status, participants were split into tertiles of average MVPA time and classified as 'low PA' if they were in the first tertile (<133 min/day) and as 'high PA' otherwise. Previous studies have shown that light intensity PA could influence CV health (6). SE status was defined according to the ratio between the average SE time and the average light intensity PA time as performed by others (3, 15). Participants were classified as 'high SE' if they were in the highest tertile (>7.2) and as 'low SE' otherwise. This allowed creating four mutually exclusive activity behaviours (**Figure 1**): 1) 'Couch potato': 'low PA' & 'high SE'; 2) 'Light mover': 'low PA' & 'low SE'; 3) 'Sedentary Exerciser': 'high PA' & 'high SE'; and 4) 'Busy bee': 'high PA' & 'low SE'.

Activity patterns

Activity patterns were defined according to PA status and its distribution throughout the week. For PA status, participants were classified as 'low PA' if they were in the first tertile of average MVPA time (<133 min/day). For the distribution of PA, average MVPA time on weekend days was divided by average MVPA time 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 (**Figure 1**): 1) 'Inactive': 'low PA'; 2) 'Weekend warrior':

‘high PA’ & ‘PA mainly on weekends’; and 3) ‘Regularly active’: ‘high PA’ & ‘PA throughout the week’.

Figure 1: Mutually exclusive activity behaviours and patterns. The CoLaus study, Switzerland, 2014-2017. [1] tertile 1 of average moderate-to-vigorous physical activity time; [2] tertile 2 or 3 of average moderate-to-vigorous physical activity time; [3] tertiles 1 or 2 of the ratio between average sedentary time and average light physical activity time; [4] tertiles 3 of the ratio between average sedentary time and light physical activity time; [5] tertiles 1 or 2 of the ratio between average moderate-to-vigorous physical activity time on weekend days and average moderate-to-vigorous physical activity time on week days. [6] tertile 3 of the ratio between average moderate-to-vigorous physical activity time on weekend days and average moderate-to-vigorous physical activity time on week days.



Cardiovascular risk factors

CVRF were assessed at second follow-up, when PA was measured.

Smoking status was collected by questionnaire. Participants were considered as smokers if they reported current smoking (any type of tobacco combustion) and non-smokers otherwise.

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 by a BMI ≥30 kg/m^2.

In accordance with US recommendations (22), blood pressure (BP) was measured three times using an Omron® HEM-907 automated oscillometric sphygmomanometer after at least 10 minutes' rest in a seated position and the average of the last two measurements was used. Hypertension was defined as a systolic BP ≥140 mmHg and/or a diastolic BP ≥90 mmHg and/or if the participant reported having an anti-hypertensive treatment.

A fasting venous blood sample was drawn and measurements performed by the clinical laboratory of the Lausanne university hospital. CVRF included glucose, and LDL-cholesterol that was calculated using the Friedewald formula if triglycerides were <4.6 mmol/L. Diabetes was defined by a fasting glucose ≥7.0 mmol/l and/or if the participant reported having an anti-diabetic treatment. Dyslipidemia was defined either by using the LDL-cholesterol thresholds according to the PROspective CArdiovascular Münster (PROCAM) risk score (23) adapted for Switzerland (24), or if the participant reported having a lipid lowering treatment. Although HDL-cholesterol and triglycerides can also be influenced by PA, they were not considered as only LDL-cholesterol is used in the Swiss definition of dyslipidemia (24).

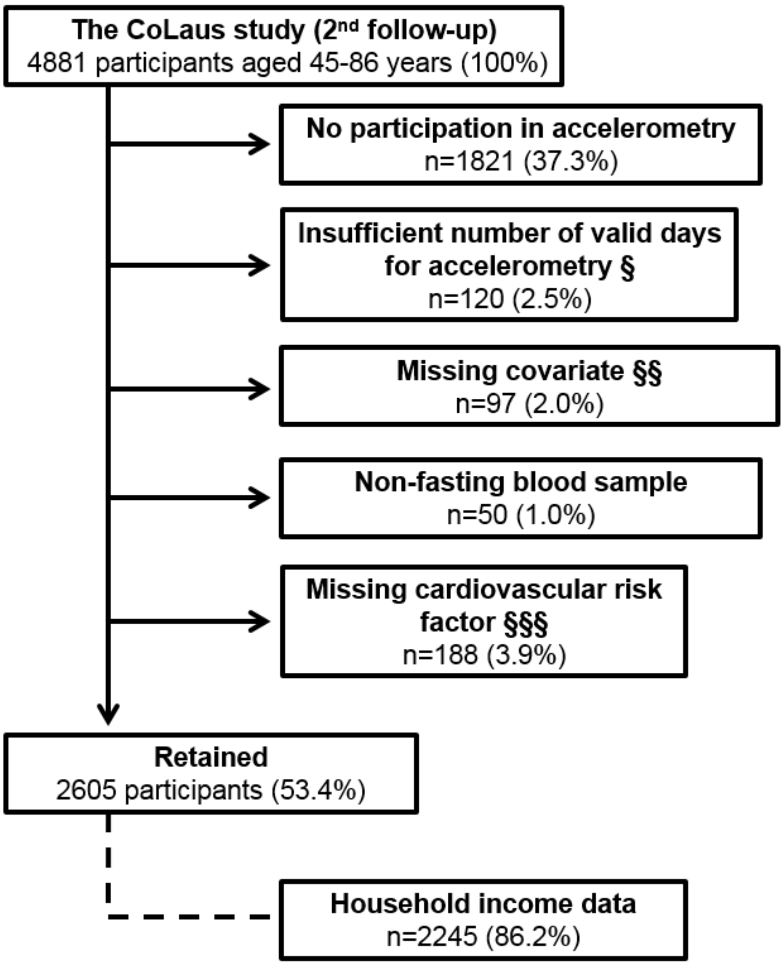
Socio-economic data

Demographic, professional occupation and household income data were collected by questionnaire. Monthly household income before social charges was expressed in Swiss francs (1 CHF=1.012 US$ or 0.913 € as of 16 May 2017). Educational level was collected at baseline by questionnaire and categorized as low (obligatory school or apprenticeship), medium (high school), or high (university degree).

Exclusion criteria

Participants were excluded if they: (i) did not participate in accelerometry; (ii) had less than 5 weekdays or 2 weekend days of valid accelerometry data, (iii) had missing data for covariates (professional occupation, educational level, or body mass index), (iv) were non-fasting, or (v) had missing data in CVRF (**Figure 2**).

Figure 2: Selection procedure. The CoLaus study, Switzerland, 2014-2017. §: less than 5 week days or less than 2 weekend days with minimum 10 h of diurnal wear-time. §§: any missing data in professional occupation, educational level, or body mass index. §§§: any missing data in smoking, obesity, hypertension, dyslipidemia or diabetes. Percentages were calculated using the total sample size as denominator.



Statistical analysis

Statistical analyses were conducted using Stata version 14.0 for windows (Stata Corp, College Station, Texas, USA). Results were expressed as number of participants (percentage) for categorical variables or as average ± standard deviation for continuous variables. Between-group comparisons were performed using chi-square and one-way analysis of variance for categorical and continuous variables, respectively.

Multivariate analyses were conducted using logistic regression with CVRF as the dependent variable. All multivariate models were adjusted for age (continuous), gender (male/female), professional occupation (no/yes), educational level (high/medium/low), and accelerometer diurnal wear-time (continuous); with an additional adjustment on BMI for the associations with hypertension, dyslipidemia and diabetes. Analyses were further adjusted for household income. Finally, several sensitivity analyses were performed: (i) using medians instead of tertiles for the definition of activity behaviours and patterns; (ii) by excluding participants with history of CVD; (iii) by including all participants irrespective of missing data in CVRF; or (iv) without adjustment for BMI. Results were expressed as odds ratio and 95% confidence interval. Statistical significance was assessed for a two-sided test with $p<0.05$. As this was mainly an exploratory analysis, we decided not to adjust for multiple comparisons in order to capture any potential interesting association.

Ethical statement and consent

The Ethics Commission of Canton Vaud approved the second follow-up of the CoLaus study (reference 26/14, decision of 11th March 2014). 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

Selection procedure and characteristics of excluded and included participants

The selection procedure is indicated in **Figure 2.** Of the initial 4881 participants, 2605 (53.4%) were retained for analysis. Included and excluded participants' characteristics are presented in **Supplementary table 1**. Included participants were younger, less likely smoking, more prone to have a professional occupation, a higher educational level or household income, and had lower accelerometer diurnal wear-time than excluded ones; they had also a lower CV risk (PROCAM), and lower prevalences of obesity, hypertension, diabetes and dyslipidemia. Among included participants, average time (±standard deviation) of accelerometer diurnal wear on valid days was 15.4±1.1 hours. The number of valid accelerometry days was 9.3±1.2 on weekdays and 3.7±0.7 on weekends (mean±standard deviation).

Association of activity behaviours with cardiovascular risk factors

Of the final 2605 participants, 545 (20.9%) were categorized as 'Couch potatoes', 306 (11.8%) as 'Light movers', 321 (12.3%) as 'Sedentary Exercisers', and finally 1433 (55.0%) as 'Busy bees'. The 'Light movers' and 'Busy bees' were more frequently female (**Supplementary table 2**).

The bivariate associations between activity behaviours and CVRF are described in **Supplementary tables 2** while the multivariate analyses are presented in **Table 1** and **2**. On bivariate analysis, the 'Sedentary exerciser' and 'Busy bee' behaviours were related to lower rates to smoke, obesity, hypertension, dyslipidemia and diabetes, compared to the 'Couch potatoes'. The 'Light movers' presented higher rates of dyslipidemia. After multivariate adjustment, all associations remained excepted that the 'Sedentary exerciser' and 'Busy bee' behaviours were no longer associated with dyslipidemia, and only non-significant trends persisted between the 'Light movers' and higher rates of hypertension (p=0.10) and between the 'Sedentary exercisers' and lower rates of hypertension (p=0.11) (**Table 1**). Additional adjustment for household income lead mostly to similar findings (**Table 2**). The ‘Busy bees’ were negatively associated with smoking, obesity, hypertension and diabetes. It was similar for the ‘Sedentary exercisers’ but only a non-significant trend was found with lower rates of diabetes (p=0.08). Furthermore, a non-significant trend persisted between the ‘Light movers’ and higher rates of dyslipidemia (p=0.28). Most associations remained in sensitivity analyses (**Supplementary table 4-7**).

Table 1: Multivariate analysis of the cardiovascular risk factors associated with activity behaviours and patterns. The CoLaus study, Switzerland, 2014-2017.

	Smoking	Obesity	Hypertension [1]	Dyslipidemia [1]	Diabetes [1]
Activity behaviours					
Couch potato	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Light mover	1.03 (0.72 - 1.46)	1.00 (0.72 - 1.39)	1.31 (0.95 - 1.80)	**1.44 (1.05 - 1.97)**	0.97 (0.63 - 1.50)
Sedentary exerciser	**0.40 (0.27 - 0.61)**	**0.43 (0.29 - 0.63)**	0.77 (0.56 - 1.06)	1.09 (0.79 - 1.52)	**0.53 (0.30 - 0.95)**
Busy bee	**0.62 (0.47 - 0.81)**	**0.41 (0.31 - 0.54)**	**0.77 (0.61 - 0.98)**	1.07 (0.84 - 1.36)	**0.62 (0.42 - 0.89)**
Activity patterns					
Inactive	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Weekend warrior	**0.58 (0.43 - 0.78)**	**0.41 (0.30 - 0.56)**	**0.66 (0.51 - 0.85)**	0.90 (0.69 - 1.18)	**0.61 (0.38 - 0.98)**
Regularly active	**0.56 (0.44 - 0.72)**	**0.41 (0.32 - 0.53)**	**0.72 (0.59 - 0.89)**	0.95 (0.77 - 1.18)	**0.60 (0.42 - 0.86)**

Results are expressed as odds ratio (OR) and (95% confidence interval). Statistical analyses performed by logistic regressions adjusted for age, gender, professional occupation, educational level and accelerometer diurnal wear-time; with a further adjustment on body mass index [1]. Significant ($p<0.05$) odds ratio are indicated in bold.

Table 2: Multivariate analysis of the cardiovascular risk factors associated with activity behaviours and patterns, with adjustment on household income. The CoLaus study, Switzerland, 2014-2017.

	Smoking	Obesity	Hypertension [1]	Dyslipidemia [1]	Diabetes [1]
Activity behaviours					
Couch potato	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Light mover	1.03 (0.70 - 1.51)	0.92 (0.64 - 1.32)	1.22 (0.86 - 1.72)	1.24 (0.87 - 1.75)	1.06 (0.66 - 1.71)
Sedentary exerciser	**0.37 (0.24 - 0.58)**	**0.48 (0.31 - 0.73)**	**0.69 (0.49 - 0.97)**	0.95 (0.67 - 1.35)	0.58 (0.32 - 1.06)
Busy bee	**0.62 (0.46 - 0.82)**	**0.45 (0.33 - 0.60)**	**0.73 (0.57 - 0.94)**	1.05 (0.81 - 1.37)	**0.63 (0.42 - 0.95)**
Activity patterns					
Inactive	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Weekend warrior	**0.54 (0.40 - 0.75)**	**0.49 (0.36 - 0.69)**	**0.62 (0.47 - 0.81)**	0.85 (0.64 - 1.13)	0.64 (0.39 - 1.06)
Regularly active	**0.57 (0.43 - 0.74)**	**0.45 (0.35 - 0.59)**	**0.70 (0.56 - 0.88)**	1.00 (0.80 - 1.26)	**0.59 (0.40 - 0.87)**

Results are expressed as odds ratio (OR) and (95% confidence interval). Statistical analyses performed by logistic regressions adjusted for age, gender, professional occupation, educational level, household income, and accelerometer diurnal wear-time; with a further adjustment on body mass index [1]. Significant ($p<0.05$) odds ratio are indicated in bold.

Association of activity patterns with cardiovascular risk factors

Of the final 2605 participants, 851 (32.7%) were categorized as ‘Inactives’, 592 (22.7%) as ‘Weekend warriors’, and finally 1162 (44.6%) as ‘Regularly actives’. The ‘Weekend warriors’ and ‘Regularly actives’ were more frequently female (**Supplementary table 3**).

The bivariate associations between activity patterns and CVRF are described in **Supplementary table 3** and the multivariate analyses are presented in **Table 1 and 2**. On bivariate analysis, the ‘Weekend warrior’ and ‘Regularly active’ patterns were related to lower rates of smoking, obesity, hypertension, dyslipidemia and diabetes, compared to the ‘Inactives’. After multivariate adjustment, all associations remained excepted that the ‘Weekend warrior’ and ‘Busy bee’ patterns were no longer related to dyslipidemia (**Table 1**). Results did not change after additional adjustment for household income excepted that only a non-significant trend persisted between the ‘Weekend warrior’ and lower rates of diabetes (p=0.09) (**Table 2**). Most associations remained in sensitivity analyses (**Supplementary table 4-7**). It is to note that without adjustment for BMI the ‘Weekend warrior’ and ‘Regularly active’ patterns were negatively associated with dyslipidemia (**Supplementary table 7**).

DISCUSSION

This study assessed the association of PA and SE behaviours and patterns with traditional CVRF using a 14-day accelerometry measurement in a population-based setting. Our results indicate that, among activity behaviours, the ‘Busy bees’ and ‘Sedentary exercisers’ are associated to a lower prevalence of CVRF whereas no association was found for the ‘Light movers’. Similarly, among activity patterns, the ‘Regularly actives’ and ‘Weekend warriors’ were related to lower prevalence of CVRF. Thus, adopting sufficient PA despite high SE levels or concentrating PA on weekends might be enough to prevent CVD.

Activity behaviours

The ‘Sedentary exerciser’ and ‘Busy bee’ behaviours were negatively associated with smoking whereas no association was found for the ‘Light movers’. These findings are partly in agreement with Bakrania and al. (3) that demonstrated lower prevalence rates of smoking among the ‘Sedentary exercisers’ but higher ones for the ‘Busy bees’ and the ‘Light movers’; but these results were not adjusted for potential confounders. Overall, PA has been negatively associated with smoking (25). The ‘Sedentary exercisers’ and ‘Busy bees’ were also negatively associated with obesity whereas no association was found for the ‘Light movers’, a finding in agreement with other studies (3, 15, 16) but not with another one (7) showing also lower prevalence rates of obesity among the ‘Light movers’. This discrepancy is possibly due to the fact that they restricted their analysis to leisure-time PA, therefore misclassifying active workers as ‘Light movers’. Finally, both ‘Sedentary exerciser’ and ‘Busy bee’ behaviours were negatively associated with hypertension whereas a non-significant positive trend was found for the ‘Light movers’, a finding in agreement with another study (16). Finally, our results suggest that individuals adopting high PA levels are less prone to smoke and less likely obese or hypertensive, independently of their SE levels.

The ‘Sedentary exerciser’ and ‘Busy bee’ behaviours showed no association with dyslipidemia. ‘Light movers’ had higher prevalence rates of dyslipidemia relative to ‘Couch potatoes’, but this association was no longer significant after full adjustment. These findings are in agreement with previous studies (15, 16), and with the fact that PA (2) and SE (12) do not significantly alter LDL-cholesterol levels.

The ‘Busy bees’ and ‘Sedentary exercisers’ were negatively associated with diabetes whereas no association was found for the ‘Light movers’. Whether activity behaviours are associated with diabetes is still debated. A recent study showed lower likelihoods of diabetes among the ‘Busy bees’, ‘Sedentary exercisers’ and ‘Light movers’ (16) while Bakrania and al. (3) showed lower glycated haemoglobin levels only among the ‘Busy bees’ and ‘Sedentary exercisers’. Another study reported no association with glycaemia (15). Discrepancies with our results are possibly due to the fact that: 1) they used self-reported PA and SE (16); or 2) they took continuous markers of diabetes with no threshold allowing the distinction between diabetic and non-diabetic participants (3, 15) . Finally, our results suggest that adopting low SE levels might be necessary for PA to be beneficial on glucometabolism but it should be further explored.

Activity patterns

The 'Weekend warrior' and 'Regularly active' patterns were related to lower prevalence rates of smoking, a finding in agreement with other studies (4, 5). They were also related to lower prevalence rates of obesity but it remains a matter of debate in literature: a study reported slightly higher BMI levels among the 'Weekend warriors' (4) while another reported no difference (5); however, none of these contradictive findings adjusted for potential confounders. The 'Weekend warriors' and 'Regularly actives' were related to lower prevalence rates of hypertension, which is in agreement with a previous study (4). Finally, our results suggest that individuals with high PA levels are less likely to smoke, and less prone to be obese or hypertensive, independently of PA distribution.

In our study, no association remained between activity patterns and dyslipidemia after adjustment for BMI. This observation was contradicted by a previous study showing a slightly lower prevalence of self-reported dyslipidemia among the 'Weekend warriors' (4); however this contradictory study did not adjust for potential confounders. Finally, our results suggest that the effect of PA on dyslipidemia is mediated by changes in BMI.

The 'Weekend warriors' and 'Regularly actives' were related to lower prevalence rates of diabetes whereas no association was found for the 'Light movers'. High PA levels protect against diabetes, mainly due to an increase in glucose transporters (GLUT4) (26). Interventional studies also indicated that regular PA (≥3 days per week) is associated with improved insulin sensitivity and glycaemic control (27). Our results confirm these findings at a population level, and further suggest that concentrating PA on weekends also exert a beneficial effect on glucometabolism. These findings should be confirmed in longitudinal studies exploring the effect of activity patterns on incident impaired fasting glucose or diabetes.

Study strengths and limitations

As far as we know, this is the first study exploring the association of both activity behaviours and patterns with CVRF. Importantly, and contrary to recent findings (3, 5, 16), PA and SE were objectively assessed and the analyses included all traditional CVRF.

This study also has several limitations. Firstly, the cross-sectional design of our study precludes the assessment of any causal effect of activity behaviours and patterns on CVRF; the next follow-up of the CoLaus participants will enable assessing causal effects. Secondly, the accelerometer was worn on the right wrist. Although it might be more prone to noisy movements, previous findings found no impact on PA assessment (19, 20). Thirdly, *GENEActiv* accelerometers have been suggested to over-report MVPA (28); still, as MVPA levels were categorized into tertiles and not absolute values this should not impact the validity of our results. Fourthly, it was not possible to know how accelerometer non-wear time was computed, as the algorithm was proprietary and the *GENEActiv* company did not provide it. Fifthly, the definition of dyslipidemia has been developed for the Swiss population; therefore, our findings might not be generalizable to other countries. Sixthly, as the Swiss definition for dyslipidemia (24) is limited to ages <75 years, participants older than 75 had their risk calculated using 75 years instead of their real age. This could underestimate the prevalence of dyslipidemia in this age group. Finally, included participants had lower CV risks and higher socio-economic levels than excluded ones. This is a common selection bias also observed in other large epidemiological studies using accelerometry (29, 30), and it would be interesting that our findings be replicated in other cohorts with a different socioeconomic background.

Conclusion

In a population-based sample aged 45 to 86 years, high PA levels are associated with a favourable CV risk profile, even in presence of high SE levels or when PA is concentrated on weekends. Thus, being a ‘Sedentary exerciser’ or a ‘Weekend warrior’ might be enough to prevent CVD.

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SUPPLEMENTARY MATERIAL

Supplementary table 1: Characteristics of excluded and included participants. The CoLaus study, Switzerland, 2014-2017.

	Included	Excluded	P-value
Sample size	2605	2276	
Age (years)	61.8 ± 9.9	64.2 ± 10.9	<0.01
Female	54.4	55.9	0.27
Professional occupation	57.2	47.4	<0.01
Educational level			0.02
High	22.3	19.8	
Medium	26.4	25.1	
Low	51.3	55.1	
Household income [1]			<0.01
<5000 CHF	25.2	31.1	
5000-9499 CHF	43.4	43.8	
>9499 CHF	31.4	25.1	
Smoking	17.2	21.5	<0.01
Cardiovascular risk (PROCAM)			<0.01
Very low	63.8	57.9	
Low	20.5	24.5	
Intermediate	11.1	12.6	
High	4.6	5.1	
High physical activity	67.3	62.9	0.06
Average MVPA time (min/day)	178.3 ± 85.8	171.1 ± 95.3	0.11
Low sedentary	72.1	67.8	0.09
Average sedentary time (min/day)	636.6 ± 105.2	622.1 ± 116.6	0.01
Average LIPA time (min/day)	109.1 ± 33.7	107.5 ± 36.6	0.36
Accelerometer diurnal wear-time (hour/day)	15.4 ± 1.1	15.0 ± 1.4	<0.01
Obesity	17.5	20.9	<0.01
Body mass index (kg/m^2)	26.3 ± 4.6	26.6 ± 4.8	0.02
Hypertension	43.1	54.8	<0.01
Systolic blood pressure (mmHg)	125.7 ± 17.4	128.5 ± 18.5	<0.01
Diastolic blood pressure (mmHg)	77.2 ± 10.5	77.7 ± 10.8	0.11
Diabetes	9.0	13.5	<0.01
Fasting glucose (mmol/l)	5.4 ± 1.0	5.5 ± 1.2	<0.01
Dyslipidemia	36.2	43.6	<0.01
LDL-cholesterol (mmol/l)	3.2 ± 0.9	3.1 ± 0.9	0.10

[1] 1 CHF=1.012 US$ or 0.913 € as of 16 May 2017. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and Student t-test.

Supplementary table 2: Characteristics of participants, by activity behaviours. The CoLaus study, Switzerland, 2014-2017.

	Couch potato	***Light mover***	***Sedentary exerciser***	***Busy bee***	**P-value**
Sample size	545 (20.9%)	306 (11.8%)	321 (12.3%)	1433 (55.0%)	
Age (years)	65.6 ± 10.6	66.4 ± 10.1	58.2 ± 8.7	60.2 ± 9.0	<0.01
Female	37.4	62.1	42.4	61.8	<0.01
Professional occupation	41.1	43.5	71.0	63.2	<0.01
Educational level					<0.01
High	26.8	17.7	30.8	19.7	
Medium	26.8	24.8	24.9	26.9	
Low	46.4	57.5	44.2	53.4	
Household income [1]					<0.01
<5000 CHF	25.8	39.5	16.4	24.1	
5000-9499 CHF	43.1	39.8	36.9	45.8	
>9499 CHF	31.1	20.7	46.7	30.1	
Smoking	20.6	21.2	11.8	16.3	<0.01
Obesity	25.9	26.8	13.1	13.4	<0.01
Hypertension	57.3	61.1	35.8	35.5	<0.01
Dyslipidemia	46.1	51.3	30.8	30.4	<0.01
Diabetes	17.4	14.7	5.6	5.4	<0.01

[1] 1 CHF=1.012 US$ or 0.913 € as of 16 May 2017. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and one-way analysis of variance, comparing activity behaviours.

Supplementary table 3: Characteristics of participants, by activity patterns. The CoLaus study, Switzerland, 2014-2017

	Inactive	*Weekend warrior*	*Regularly active*	**P-value**
Sample size	851 (32.7%)	592 (22.7%)	1162 (44.6%)	
Age (years)	65.8 ± 10.4	58.5 ± 8.7	60.5 ± 9.1	<0.01
Female	53.7	40.5	42.3	<0.01
Professional occupation	42.0	74.2	59.8	<0.01
Educational level				<0.01
High	23.5	30.9	17.0	
Medium	26.1	29.4	25.1	
Low	50.4	39.7	57.8	
Household income [1]				<0.01
<5000 CHF	30.6	18.0	25.1	
5000-9499 CHF	42.0	39.1	46.8	
>9499 CHF	27.4	42.9	28.2	
Smoking status	20.8	16.1	15.2	<0.01
Obesity	26.2	12.2	13.9	<0.01
Hypertension	58.6	31.1	37.9	<0.01
Dyslipidemia	47.9	26.2	32.7	<0.01
Diabetes	16.5	4.6	5.9	<0.01

[1] 1 CHF=1.012 US$ or 0.913 € as of 16 May 2017. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square and one-way analysis of variance, comparing activity patterns.

Supplementary table 4: Multivariate analysis of the cardiovascular risk factors associated with activity behaviours and patterns defined using medians. The CoLaus study, Switzerland, 2014-2017.

	Smoking	**Obesity**	**Hypertension** [1]	**Dyslipidemia** [1]	**Diabetes** [1]
Activity behaviours					
Couch potato	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Light mover	1.14 (0.80 - 1.63)	0.89 (0.63 - 1.26)	**1.38 (1.01 - 1.90)**	1.06 (0.77 - 1.45)	1.26 (0.79 - 2.01)
Sedentary exerciser	**0.52 (0.35 - 0.77)**	**0.51 (0.35 - 0.76)**	**0.69 (0.51 - 0.94)**	0.90 (0.66 - 1.24)	0.65 (0.36 - 1.20)
Busy bee	**0.73 (0.56 - 0.96)**	**0.45 (0.34 - 0.60)**	0.81 (0.65 - 1.03)	1.01 (0.79 - 1.28)	0.77 (0.50 - 1.18)
Activity patterns					
Inactive	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Weekend warrior	**0.63 (0.48 - 0.84)**	**0.45 (0.33 - 0.61)**	**0.65 (0.51 - 0.82)**	0.88 (0.68 - 1.13)	0.83 (0.53 - 1.31)
Regularly active	**0.64 (0.48 - 0.86)**	**0.51 (0.38 - 0.68)**	**0.78 (0.62 - 0.99)**	1.03 (0.81 - 1.32)	**0.55 (0.34 - 0.90)**

Results are expressed as odds ratio (OR) and (95% confidence interval). Statistical analyses performed by logistic regressions adjusted for age, gender, professional occupation, educational level and accelerometer diurnal wear-time; with a further adjustment on body mass index [1]. Significant ($p<0.05$) odds ratio are indicated in bold.

Supplementary table 5: Multivariate analysis of the cardiovascular risk factors associated with activity behaviours and patterns, excluding participants with history of cardiovascular disease. The CoLaus study, Switzerland, 2014-2017.

	Smoking	Obesity	Hypertension [1]	Dyslipidemia [1]	Diabetes [1]
Activity behaviours					
Couch potato	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Light mover	1.00 (0.68 - 1.46)	1.01 (0.71 - 1.44)	1.27 (0.90 - 1.78)	**1.49 (1.05 - 2.10)**	0.97 (0.60 - 1.57)
Sedentary exerciser	**0.44 (0.29 - 0.67)**	**0.41 (0.27 - 0.62)**	0.83 (0.60 - 1.15)	1.23 (0.86 - 1.75)	0.55 (0.30 - 1.01)
Busy bee	**0.66 (0.49 - 0.88)**	**0.42 (0.32 - 0.56)**	**0.78 (0.61 - 1.00)**	1.09 (0.84 - 1.42)	**0.57 (0.38 - 0.86)**
Activity patterns					
Inactive	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Weekend warrior	**0.63 (0.46 - 0.86)**	**0.40 (0.29 - 0.56)**	**0.68 (0.52 - 0.89)**	0.91 (0.68 - 1.21)	**0.50 (0.29 - 0.87)**
Regularly active	**0.60 (0.47 - 0.78)**	**0.42 (0.33 - 0.55)**	**0.74 (0.59 - 0.92)**	0.99 (0.79 - 1.25)	**0.60 (0.41 - 0.87)**

Results are expressed as odds ratio (OR) and (95% confidence interval). Statistical analyses performed by logistic regressions adjusted for age, gender, professional occupation, educational level and accelerometer diurnal wear-time; with a further adjustment on body mass index [1]. Significant ($p<0.05$) odds ratio are indicated in bold.

Supplementary table 6: Multivariate analysis of the cardiovascular risk factors associated with activity behaviours and patterns, including all participants irrespective of missing data in cardiovascular risk factors. The CoLaus study, Switzerland, 2014-2017.

	Smoking	Obesity	Hypertension [1]	Dyslipidemia [1]	Diabetes [1]
Activity behaviours					
Couch potato	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Light mover	1.01 (0.72 - 1.44)	0.98 (0.72 - 1.34)	1.30 (0.95 - 1.77)	**1.52 (1.12 - 2.08)**	0.94 (0.62 - 1.42)
Sedentary exerciser	**0.38 (0.25 - 0.57)**	**0.44 (0.31 - 0.64)**	0.79 (0.58 - 1.08)	1.11 (0.80 - 1.53)	**0.46 (0.26 - 0.81)**
Busy bee	**0.61 (0.47 - 0.80)**	**0.40 (0.31 - 0.52)**	**0.73 (0.58 - 0.92)**	1.08 (0.85 - 1.37)	**0.61 (0.43 - 0.86)**
Activity patterns					
Inactive	1 (ref)	1 (ref)	1 (ref)	1 (ref)	1 (ref)
Weekend warrior	**0.57 (0.43 - 0.76)**	**0.40 (0.30 - 0.53)**	**0.66 (0.51 - 0.84)**	0.89 (0.69 - 1.16)	**0.58 (0.37 - 0.92)**
Regularly active	**0.56 (0.44 - 0.71)**	**0.42 (0.33 - 0.53)**	**0.68 (0.56 - 0.83)**	0.94 (0.76 - 1.15)	**0.59 (0.43 - 0.82)**

Results are expressed as odds ratio (OR) and (95% confidence interval). Statistical analyses performed by logistic regressions adjusted for age, gender, professional occupation, educational level and accelerometer diurnal wear-time; with a further adjustment on body mass index [1]. Significant ($p<0.05$) odds ratio are indicated in bold.

Supplementary table 7: Multivariate analysis of the cardiovascular risk factors associated with activity behaviours and patterns, without adjustment on body mass index. The CoLaus study, Switzerland, 2014-2017.

	Hypertension	**Dyslipidemia**	**Diabetes**
Activity behaviours			
Couch potato	1 (ref)	1 (ref)	1 (ref)
Light mover	1.29 (0.95 - 1.76)	**1.43 (1.05 - 1.96)**	0.96 (0.63 - 1.45)
Sedentary exerciser	**0.65 (0.48 - 0.88)**	0.96 (0.70 - 1.33)	**0.40 (0.23 - 0.70)**
Busy bee	**0.61 (0.49 - 0.77)**	0.90 (0.71 - 1.14)	**0.41 (0.29 - 0.58)**
Activity patterns			
Inactive	1 (ref)	1 (ref)	1 (ref)
Weekend warrior	**0.52 (0.41 - 0.66)**	**0.77 (0.59 - 0.99)**	**0.42 (0.26 - 0.66)**
Regularly active	**0.58 (0.48 - 0.71)**	**0.81 (0.66 - 1.00)**	**0.41 (0.30 - 0.58)**

Results are expressed as odds ratio (OR) and (95% confidence interval). Statistical analyses performed by logistic regressions adjusted for age, gender, professional occupation, educational level and accelerometer diurnal wear-time. Significant ($p<0.05$) odds ratio are indicated in bold.

Chapter 4

Association of activity levels and patterns with sleep parameters

Based on **Gubelmann C**, Heinzer R, Haba-Rubio J, Vollenweider P, Marques-Vidal P. Physical activity is associated with higher sleep efficiency in the general population: The CoLaus study. *Sleep*. 2018

ABSTRACT

Study objectives: To evaluate the association of objective physical activity (PA) and sedentary behaviour (SB) with sleep duration and quality.

Methods: Cross-sectional study including 2649 adults (53.5% women, 45-86 years) from the general population. Proportions of time spent in PA and SB were measured using 14-day accelerometry. Low PA and high SB status were defined as the lowest and highest tertile of each behaviour. ‘Inactive’, ‘Weekend warrior’ and ‘Regularly active’ weekly patterns were also defined. Sleep parameters were derived from the accelerometer and validated questionnaires.

Results: High PA, relative to low PA, was associated with higher sleep efficiency [76.6 *vs.* 73.8%, p<0.01] and lower likelihood of evening chronotype [relative-risk ratio (RR) and 95%CI: 0.71 (0.52; 0.97)]. Similar associations were found for low SB relative to high SB. ‘Weekend warriors’, relative to ‘Inactives’, had higher sleep efficiency [76.4 *vs.* 73.9%, p<0.01] and lower likelihood of evening chronotype [RR: 0.63 (0.43; 0.93)]. ‘Regularly actives’, relative to ‘Inactives’, had higher sleep efficiency [76.7 *vs.* 73.9%, p<0.01] and tended to have less frequently an evening chronotype [RR: 0.75 (0.54; 1.04), p=0.09]. No associations were found for PA and SB with sleep duration, daytime sleepiness, insomnia, and risk of sleep apnea (after adjustment for body mass index).

Conclusions: High PA and low SB individuals, even if they do not sleep longer, have higher sleep efficiency and have less frequently an evening chronotype.

INTRODUCTION

The impact of physical activity (PA) (1) and sedentary behaviour (SB) (2) on cardiovascular disease (CVD) is well established, but the underlying mechanisms are incompletely understood. Mora et al. (3) suggested that only half of PA-mediated reduction in CVD incidence was explained by known cardiovascular risk factors.

Sleep duration and sleep disorders are associated with incident CVD (4, 5). Therefore, it can be speculated that PA and SB might impact CVD by modulating sleep. In small clinical trials, PA was related to better subjective and objective sleep (6). These findings have also been replicated in epidemiological studies, where physically active individuals had higher sleep duration (7, 8), quality and efficiency (9), and lower risks of insomnia (10, 11), excessive daytime sleepiness (7, 12) and sleep apnoea (13, 14). However, all these findings were limited by the fact that they were based on: (i) self-reported PA (8-12, 14, 15), that is prone to recall bias, or (ii) non-validated sleep questionnaire (7, 10-12). Interestingly, a recent study found that objective PA shows little associations with sleep when exploring a large panel of parameters (16). Finally, previous studies only considered PA levels; however it has been shown that PA distribution over week (i.e. weekly activity pattern) also exerts an effect on CVD. Indeed, exercising 1-2 times per week, called the 'Weekend warrior' pattern, could decrease the benefits of PA possibly due to the short-lived effects of PA (17).

Today, light and wearable accelerometers allow an easy and objective assessment of PA and SB (18), as well as sleep estimation (19). Also, well validated sleep questionnaires such as the Pittsburgh Sleep Quality Index (PSQI) (20), the Epworth sleepiness scale (21), the Berlin questionnaire for risk of sleep apnoea (22), and the Insomnia Severity Index (ISI) (23) are currently available.

Therefore, this study aimed to assess sleep parameters according to PA and SB status and patterns in a large population-based sample aged 45-86 years from the city of Lausanne, Switzerland. Our hypothesis was that sleep characteristics would differ between activity status and weekly patterns.

METHODS

Recruitment of participants

The detailed description of the recruitment of the CoLaus study and the follow-up procedures has been described previously (24, 25). 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 for 14 days.

Physical activity

PA was assessed using a wrist-worn triaxial accelerometer (GENEActiv, Activinsights Ltd, United Kingdom). This device has been validated against reference methods (26). 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 collapsed into 60-second epoch files. Data were analyzed using the GENEActiv macro file 'General physical activity' version 1.9 (27) based on intensity cutoffs validated among middle-aged adults (26): SB (<241 g.min), light intensity PA (241-338 g.min) and moderate-to-vigorous PA (MVPA) (>338 g.min). Conversely, no information was available regarding the criteria used for non-wear time (proprietary). Based upon a previous study (28), a valid day was defined as ≥10 h (i.e. 600 min) and ≥8 h (i.e. 480 min) of diurnal wear-time on week days and weekend days, respectively. For each participant, the proportion of time (in percentage) spent in MVPA and in SB was averaged for all valid days and separately for valid week and weekend days. At least 5 week days and 2 weekend days of valid accelerometry data were required (see exclusion criteria).

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.

Weekly activity patterns were defined according to PA status and its distribution throughout the week (**Supplementary 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 weekdays, 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 previously described (28): 1) ‘Inactive’: low PA; 2) ‘Weekend warrior’: high PA & PA mainly on weekends; and 3) ‘Regularly active’: high PA & PA throughout the week.

Sleep measurement

Objective sleep duration and efficiency were derived from accelerometry and analyzed with R-package GGIR version 1.5-9 (http://cran.r-project.org) (19). Sleep duration was defined as time with no change in arm angle greater than 5° for 5 min or more during a predefined nocturnal sleep window (21:00-09:00). Data cleaning was performed by replacing sleep duration or efficiency as missing values if they were lower than 3h or 40%, respectively.

Subjective sleep quality was derived from the PSQI (20), a 19-item questionnaire evaluating sleep over the previous month. Seven items scaling 0-3 are derived: sleep quality, latency, efficiency, duration, disturbances, daytime dysfunction, and use of sleep medications; and then summed to obtain the global PSQI score (range: 0-21). Poor sleep quality was defined as a PSQI score >5 (20).

Self-reported sleep duration was derived from one item of the PSQI. Participants indicated the average number of hours of actual sleep per night in the previous month. A sleep duration ≤6 hours per night was considered as ‘short sleep’ (29).

Daytime sleepiness was derived from the Epworth Sleepiness Scale (21). Participants rated how likely they were to doze off in eight daily situations scaling 0-3. Items were then summed to obtain the total daytime sleepiness score (range: 0-24). Daytime sleepiness was defined as an Epworth score >10 (21).

Risk of sleep apnoea was derived from the Berlin questionnaire (22), asking participants about the presence of snoring behaviour and waketime sleepiness or fatigue, and the history of obesity or hypertension. Participants with persistent and frequent symptoms in any two of these three domains were considered to be at high risk for sleep apnoea (22).

Participants reporting no sleep problems and not taking any sleep medication were considered as having no insomnia. For the other participants, insomnia severity was derived from the Insomnia Severity Index (ISI) (23), a 7-item questionnaire evaluating the nature, severity, and impact of insomnia over the last month; namely difficulties falling sleep, sleep maintenance problems, and early morning awakening, sleep dissatisfaction, interference of sleep disturbances with daytime functioning, noticeability of sleep problems by others, and distress caused by the sleep difficulties. Items were scaled 0-4 and then summed to obtain the global ISI score (range: 0-28). Clinically significant insomnia was defined as an ISI score ≥15 (moderate to severe intensity) (23).

Chronotype assessment was derived from the classification of the Morningness-Eveningness Questionnaire of Horne and Ostberg (30), i.e. participants were asked to characterize themselves as ‘definite evening’, ‘moderate evening’, ‘intermediate’, ‘moderate morning’, or ‘definite morning’. The chronotype was then summarized into three categories (intermediate/morning/evening).

Other data

Socio-demographic factors included age, gender and professional occupation. Participants were considered as having a professional occupation if they were currently working. Self-rated health (very good/good/average or bad) was collected during an interview. Behavioural factors such as smoking and alcohol consumption were assessed by self-reported questionnaire. Alcohol consumption was considered as low if the participant reported to drink 0-13 units per week and high otherwise. Depression risk was assessed by the Center for Epidemiological Studies-Depression Scale (CES-D), and increased depression risk was defined by a CES-D score ≥17 for men and ≥23 for women (31). Participants indicated their current medication which was then coded according to the Anatomical Therapeutics Chemical (ATC) Classification System of the World Health Organization. Psycholeptic or psychoanaleptic medications were defined by an ATC code beginning with ‘N05’ and ‘N06’, respectively.

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. A fasting venous blood sample was drawn and glucose measurement was performed by the clinical laboratory of the Lausanne university hospital. Diabetes was defined by a fasting glucose ≥7.0 mmol/l and/or if the participant reported having an anti-diabetic treatment.

Exclusion criteria

Participants were excluded if they: (i) did not participate in accelerometry; (ii) had less than 5 weekdays or 2 weekend days of valid accelerometry data or (iii) had any missing data in professional occupation, self-rated health, alcohol consumption or psychotropic medication (**Figure 1**).

Statistical analysis

Statistical analyses were conducted using Stata version 14.1 for windows (Stata

Corp, College Station, Texas, USA). In bivariate analyses, 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 (32). Categorical variables were expressed as percentage and between-group comparisons were performed using chi-square test of independence.

For continuous parameters of sleep, multivariable analysis comparing sleep parameters between activity status and weekly patterns groups were conducted using ANOVA and results were expressed as multivariable-adjusted average ± standard error. Post-hoc pairwise comparisons were performed using the method of Scheffe (32).

For dichotomous parameters of sleep, multivariable analyses were conducted using logistic regression and results were expressed as multivariable-adjusted odds-ratio and 95% confidence interval (CI).

For chronotype, multivariable analyses were conducted using multinomial logistic regression, with the 'Intermediate' group as base outcome and results were expressed as multivariable-adjusted relative-risk ratio (RR) and 95% CI.

Further analyses were performed including all participants irrespective of objective sleep duration and efficiency, and of missing items in daytime sleepiness questionnaire.

Additional analyses for PA and SB status were conducted to evaluate the effect of (i) a 10%- increment of the proportion of time spent in each activity and (ii) a 10h-increment of weekly PA. Additional analyses for weekly activity patterns were conducted to evaluate the effect of one standard deviation increase in daily PA while controlling for PA level. For continuous parameters of sleep, statistical analyses were conducted using linear regression and results were expressed as multivariable-adjusted coefficient and 95% CI. For dichotomous and categorical variables, multivariable analyses were conducted using simple and multinomial logistic regression, respectively.

All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes). Further adjustments for BMI (continuous), diabetes (no/yes), or increased depression risk (no/yes) were performed. 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

Selection procedure and characteristics of participants

Of the initial 4881 participants, 2649 (54.3%) were retained for analysis. The selection procedure is indicated in **Figure 1.** The response rates for sleep questionnaires varied from 63.9% (PSQI) to 82.2% (ISI), mainly due to missing items. Included and excluded participants' characteristics are presented in **Table 1**. Included participants were younger, less likely female, had a better self-rated health and lower prevalence of diabetes, and were more prone to have a professional occupation than excluded ones.

Figure 1: 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], alcohol consumption, neurotropic medication or professional occupation.

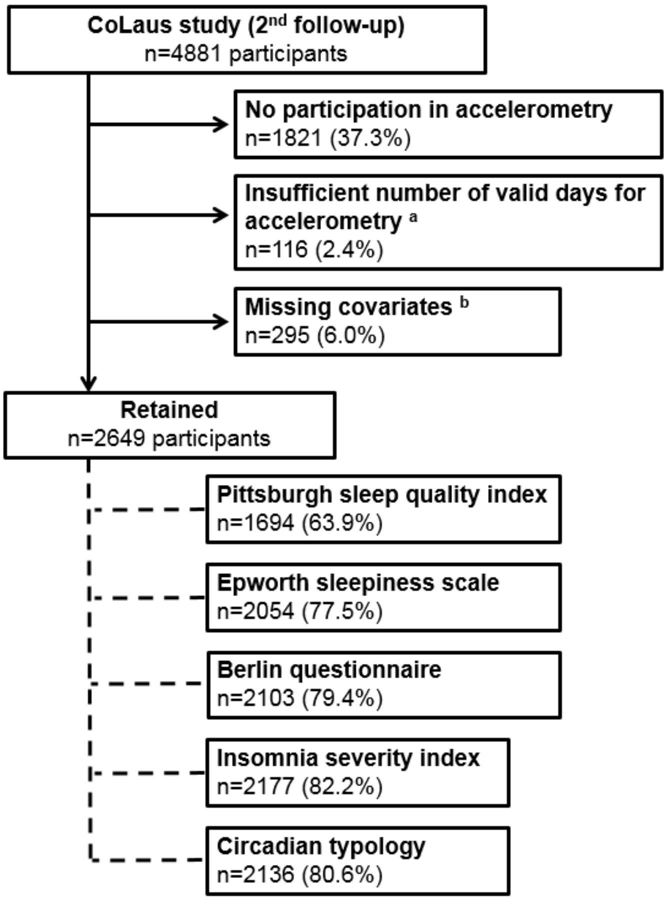

Table 1: Characteristics of excluded and included participants. The CoLaus study, Switzerland, 2014-2017.

	Included	Excluded	P-value
Sample size	2649	2232	
Age (years)	61.6 ± 9.8	64.5 ± 10.9	<0.01
Body mass index (kg/m^2)	26.4 ± 4.6	26.5 ± 4.8	0.27
Female	53.5	56.9	0.02
Self-rated health			<0.01
Very good	22.8	19.6	
Good	56.9	55.1	
Average or bad	20.3	25.3	
Smoking status			0.08
Never	42.6	41.0	
Former	39.5	38.4	
Current	17.9	20.6	
High alcohol consumption	14.0	13.0	0.38
Work	57.5	46.8	<0.01
High PA status	66.7	66.4	0.91
Diabetes	9.2	13.4	<0.01
Increased depression risk	11.9	11.9	0.99

PA, physical activity. Results expressed as mean ± standard deviation for continuous variables and as percentage for categorical variables. Between-group comparisons performed using student t-test for continuous variables and using chi-square test of independence for categorical variables.

Participants' characteristics per activity status are presented in **Table 2**. Younger age, lower BMI, female gender, lower prevalence of diabetes, reporting a better health, and being professionally active were associated with high PA and low SB status, non-smoking status with high PA only. Participants' characteristics per weekly activity patterns are presented in **Table 3**. Younger age, lower BMI, female gender, non-smoking status, lower prevalence of diabetes, reporting a better health, and being professionally active were associated with the 'Weekend warrior' pattern.

Table 2: Characteristics of participants, stratified by activity status. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			Sedentary behaviour		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Sample size	882	1767		893	1756	
Age (years)	65.6 ± 10.5	59.6 ± 8.8	<0.01	64.6 ± 10.6	60.1 ± 9.1	<0.01
Body mass index (kg/m^2)	27.7 ± 5.0	25.7 ± 4.3	<0.01	27.7 ± 5.0	25.7 ± 4.3	<0.01
Female	44.7	58.0	<0.01	40.3	60.3	<0.01
Self-rated health			<0.01			<0.01
Very good	16.4	26.0		17.4	25.6	
Good	57.3	56.8		57.8	56.5	
Average or bad	26.3	17.3		24.9	17.9	
Smoking status			<0.01			0.12
Never	39.5	44.2		40.5	43.7	
Former	39.5	39.5		39.7	39.4	
Current	21.1	16.3		19.8	16.9	
High alcohol consumption	15.0	13.5	0.30	14.7	13.6	0.46
Work	43.5	64.5	<0.01	47.3	62.7	<0.01
Diabetes	16.1	5.7	<0.01	14.9	6.3	<0.01
Increased depression risk	13.6	11.1	0.07	13.7	11.0	0.06

Results expressed as mean ± standard deviation for continuous variables and as percentage for categorical variables. Between-group comparisons performed using student t-test for continuous variables and using chi-square test of independence for categorical variables.

Table 3: Characteristics of participants, stratified by weekly activity patterns. The CoLaus study, Switzerland, 2014-2017.

	Inactive	**Weekend warrior**	**Regularly active**	**P-value**
Sample size	882	617	1150	
Age (years)	65.6 ± 10.5	57.4 ± 8.1	60.8 ± 9.0	<0.01
Body mass index (kg/m^2)	27.7 ± 5.0	25.1 ± 4.0	26.0 ± 4.4	<0.01
Female	44.7	58.8	57.5	<0.01
Smoking status				0.03
Never	39.5	45.0	43.8	
Former	39.5	38.9	39.8	
Current	21.1	16.1	16.4	
Self-rated health				<0.01
Very good	16.4	28.0	24.9	
Good	57.3	56.6	56.9	
Average or bad	26.3	15.4	18.3	
High alcohol consumption	15.0	14.4	13.0	0.40
Work	43.5	79.6	56.4	<0.01
Diabetes	16.1	4.6	6.4	<0.01
Increased depression risk	13.6	10.5	11.3	0.18

Results expressed as mean ± standard deviation for continuous variables and as percentage for categorical variables. Between-group comparisons performed using one-way analysis of variance for continuous variables and using chi-square test of independence for categorical variables.

Association of activity status with sleep

The associations between PA and SB status and sleep parameters are described in **Table 4**. In bivariate analysis, high PA and low SB status were associated with higher objective sleep efficiency, lower risk of sleep apnoea, and lower likelihood of evening chronotype. These associations persisted after multivariable adjustment (**Table 4**). No associations were found for the other sleep parameters (objective and self-reported sleep durations, subjective sleep quality, daytime sleepiness, and insomnia) (**Table 4**). Results did not change after including all participants irrespective of objective sleep duration and efficiency, and of missing items in daytime sleepiness questionnaire (**Supplementary table 1**). Most associations persisted after additional adjustments for BMI (**Supplementary table 2**), diabetes (**Supplementary table 3**), or depression risk (**Supplementary table 4**). Nevertheless, no association remained for PA and SB with sleep apnoea risk when adjusted for BMI (**Supplementary table 2**), and only a non-significant trend (p=0.06) persisted for PA with lower likelihood of evening chronotype when adjusted for depression risk (**Supplementary table 4**).

Additional analyses that evaluated 10%-increment of the proportion of time spent in PA and SB and 10h-increment of weekly PA are presented in **Supplementary table 5** and **6**. Similar associations were found: increases in proportion of time spent in PA and increases in weekly PA were associated with higher objective sleep efficiency, lower risk of sleep apnoea and lower likelihood of evening chronotype.

Table 4: Association of physical activity and sedentary behaviour status with sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			Sedentary behaviour		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Sample size	882	1767		893	1756	
Objective sleep duration (h) §						
Bivariate	7.1 ± 1.0	7.1 ± 1.0	0.48	7.1 ± 1.0	7.1 ± 1.0	0.76
Multivariable-adjusted	7.1 ± 0.03	7.1 ± 0.02	0.88	7.1 ± 0.03	7.1 ± 0.02	0.56
Objective sleep efficiency (%) §						
Bivariate	73.5 ± 8.4	76.8 ± 8.0	<0.01	73.1 ± 8.4	77.0 ± 7.9	<0.01
Multivariable-adjusted	73.8 ± 0.29	76.6 ± 0.20	<0.01	73.6 ± 0.28	76.7 ± 0.20	<0.01
Self-reported sleep duration (h) §						
Bivariate	7.0 ± 1.1	7.0 ± 1.0	0.95	6.9 ± 1.1	7.0 ± 1.0	0.46
Multivariable-adjusted	6.9 ± 0.05	7.0 ± 0.03	0.49	6.9 ± 0.05	7.0 ± 0.03	0.33
Short sleep †						
Bivariate	27.6	25.1	0.27	27.9	24.9	0.18
Multivariable-adjusted	1 (ref)	0.89 (0.69; 1.14)	0.34	1 (ref)	0.87 (0.68; 1.10)	0.25
Poor sleep quality †						
Bivariate	34.6	31.8	0.25	33.8	32.2	0.52
Multivariable-adjusted	1 (ref)	1.08 (0.85; 1.39)	0.52	1 (ref)	1.05 (0.82; 1.33)	0.72
Excessive daytime sleepiness †						
Bivariate	10.3	11.0	0.63	9.5	11.4	0.21
Multivariable-adjusted	1 (ref)	0.94 (0.68; 1.30)	0.70	1 (ref)	1.15 (0.84; 1.59)	0.38
Increased risk of sleep apnoea †						
Bivariate	28.2	18.8	<0.01	27.9	18.8	<0.01
Multivariable-adjusted	1 (ref)	0.72 (0.57; 0.91)	<0.01	1 (ref)	0.73 (0.58; 0.92)	<0.01
Insomnia †						
Bivariate	4.4	5.9	0.17	4.5	5.8	0.21
Multivariable-adjusted	1 (ref)	1.56 (0.98; 2.48)	0.06	1 (ref)	1.47 (0.93; 2.32)	0.10
Chronotype						
Bivariate			<0.01			<0.01
Intermediate	11.6	13.7		11.6	13.7	
Morning	38.4	45.1		35.7	46.5	
Evening	50.0	41.2		52.7	39.8	
Multivariable-adjusted						
Morning	1 (ref)	1.07 (0.78; 1.47)	0.66	1 (ref)	1.18 (0.87; 1.62)	0.29
Evening	1 (ref)	0.71 (0.52; 0.97)	0.03	1 (ref)	0.64 (0.47; 0.86)	<0.01

For continuous variables (§), statistical analyses were performed using student t-test (bivariate) and ANOVA (multivariable); results were expressed as average ± standard deviation (bivariate) and as multivariable-adjusted average ± standard error. For dichotomous categorical variables (†), statistical analyses were performed using chi-square test of independence (bivariate) and logistic regression (multivariable); results were expressed as percentage (bivariate) and as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the 'Morning' and 'Evening' groups to the 'Intermediate' one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes).

Association of weekly activity patterns with sleep

The associations between weekly activity patterns and sleep parameters are presented in **Table 5**. In bivariate analysis, the 'Weekend warriors' had a higher prevalence of daytime sleepiness in comparison to the other patterns, and a lower risk of sleep apnoea with respect to the 'Inactives' while the 'Regularly actives' stood in between (**Table 5**). Both 'Weekend warrior' and 'Regularly active' patterns had higher objective sleep efficiency and lower likelihood of evening chronotype relative to the 'Inactives' (**Table 5**). After multivariable adjustment, the 'Weekend warriors' had higher objective sleep efficiency, lower risk of sleep apnoea, and lower likelihood of evening chronotype than the 'Inactives'. Similarly, 'Regularly actives' had higher objective sleep efficiency and lower risk of sleep apnoea while a non-significant trend remained for lower likelihood of evening chronotype (p=0.09) than the 'Inactives'. There was no persisting association between activity patterns and daytime sleepiness (**Table 5**). Finally, no associations were found between patterns and the other sleep parameters (objective and self-reported sleep durations, subjective sleep quality, and insomnia). Results did not change after including all participants irrespective of objective sleep duration and efficiency, and of missing items in daytime sleepiness questionnaire (**Supplementary table 7**). Adjusting for BMI led to similar results except that activity patterns were no longer associated with risk of sleep apnoea (**Supplementary table 8**). Additional analyses that evaluated 10%-increment in standard deviation of daily proportion of time spent in PA showed no association (**Supplementary table 9**).

Table 5: Association of weekly activity patterns with sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Inactive	Weekend warrior	Regularly active	P-value
Sample size	882	617	1150	
Objective sleep duration (h) §				
Bivariate	7.1 ± 1.0	7.0 ± 0.9	7.1 ± 1.0	0.72
Multivariable-adjusted	7.1 ± 0.03	7.1 ± 0.04	7.1 ± 0.03	0.87
Objective sleep efficiency (%) §				
Bivariate	73.5 ± 8.4 [a]	76.7 ± 7.6 [b]	76.8 ± 8.1 [b]	<0.01
Multivariable-adjusted	73.9 ± 0.29 [a]	76.4 ± 0.34 [b]	76.7 ± 0.24 [b]	<0.01
Self-reported sleep duration (h) §				
Bivariate	7.0 ± 1.1	6.9 ± 1.0	7.0 ± 1.0	0.38
Multivariable-adjusted	6.9 ± 0.05	7.0 ± 0.05	7.0 ± 0.04	0.77
Short sleep †				
Bivariate	27.6	25.0	25.2	0.54
Multivariable-adjusted	1 (ref)	0.84 (0.62; 1.15)	0.91 (0.70; 1.19)	
Poor sleep quality †				
Bivariate	34.6	30.5	32.6	0.39
Multivariable-adjusted	1 (ref)	1.07 (0.78; 1.46)	1.09 (0.84; 1.42)	
Excessive daytime sleepiness †				
Bivariate	10.3	14.1	9.2	0.02
Multivariable-adjusted	1 (ref)	1.15 (0.79; 1.69)	0.83 (0.58; 1.18)	
Increased risk of sleep apnoea †				
Bivariate	28.2	16.6	20.1	<0.01
Multivariable-adjusted	1 (ref)	0.61 (0.44; 0.83) *	0.77 (0.60; 1.00) *	
Insomnia †				
Bivariate	4.4	5.4	6.1	0.32
Multivariable-adjusted	1 (ref)	1.50 (0.84; 2.68)	1.59 (0.97; 2.59)	
Chronotype				
Bivariate				<0.01
Intermediate	11.6	15.1	12.9	
Morning	38.4	44.2	45.5	
Evening	50.0	40.7	41.5	
Multivariable-adjusted				
Morning	1 (ref)	0.98 (0.67; 1.45)	1.12 (0.80; 1.56)	
Evening	1 (ref)	0.63 (0.43; 0.93) *	0.75 (0.54; 1.04)	

For continuous variables (§), statistical analyses were performed using student t-test (bivariate) and ANOVA (multivariable); results were expressed as average ± standard deviation (bivariate) and as multivariable-adjusted average ± standard error. For dichotomous categorical variables (†), statistical analyses were performed using chi-square test of independence (bivariate) and logistic regression (multivariable); results were expressed as percentage (bivariate) and as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the 'Morning' and 'Evening' groups to the 'Intermediate' one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes). Post-hoc pairwise comparisons of averages were performed using the method of Scheffe; values with differing superscripts differ at $p<0.05$. Significant ($p<0.05$) odds ratios or relative-risk ratios are indicated with *.

DISCUSSION

This study showed that high PA and low SB are related to higher objective sleep efficiency, and lower likelihood of evening chronotype. Further, both PA evenly distributed over the week or concentrated on weekends are associated with improved sleep efficiency.

Association of activity status with sleep

High PA and low SB status were related to higher objective sleep efficiency, which is consistent with a previous study that used polysomnography (9). Even if changes in sleep efficiency seem moderate (i.e. 2.8% and 3.1% within PA and SB status), they might be clinically relevant (33) as they are in the same magnitude order as decrement in sleep efficiency due to obstructive sleep apnoea (34) or periodic limb movement disorder (35). Since lower sleep efficiency has been related to mortality (33), and conditions disturbing sleep structure such as obstructive sleep apnoea have been shown to be associated with increased CVD and mortality (36), it is possible that the lower sleep efficiency might be one of the mechanisms mediating low PA and high SB association with CVD.

Participants adopting high PA or low SB had lower risk of sleep apnoea, but this difference was no longer significant after controlling for BMI. This finding is in agreement with a prior epidemiological study (14), but it has been contradicted by others showing an independent association (13, 15). Overall, exercise interventions have been shown to improve sleep apnoea without decreasing BMI (37). Finally, our results suggest that the effect of PA on sleep apnoea is mediated by changes in BMI, or that the association is too small to be detected using our sample size.

High PA and low SB status were negatively associated with evening chronotype, which is in agreement with another study showing lower PA levels among evening type adolescents (38). Interestingly, a study indicated that participants with evening chronotype had a higher likelihood of type 2 diabetes and hypertension as compared with morning types

(39). Still, any influence of PA on chronotype needs to be further tested in longitudinal studies.

No associations were found for PA and SB status with objective and self-reported sleep durations, subjective sleep quality, daytime sleepiness and insomnia. This is in agreement with some previous studies (16, 40, 41) but not with others showing longer sleep duration (7, 8), increased subjective sleep quality (8, 9), lower rate of insomnia (10, 11), and lower daytime sleepiness (7, 12) among active individuals. For sleep duration, the lack of association may be due to the older age range of our sample (45-86 years old) since it was previously shown that the influence of PA on sleep decreases with age (7). Other contradictory findings could be due to the use of self-reported PA (9, 10), since it has been shown to be differently associated with sleep than objective PA (8).

Association of weekly activity patterns with sleep

In comparison to the 'Inactive' pattern, the 'Weekend warriors' had higher objective sleep efficiency, lower risk of sleep apnoea, and lower likelihood of evening chronotype. Relative to the 'Inactives', the 'Regularly actives' had also higher objective sleep efficiency and lower risk of sleep apnoea while only a tendency remained for lower likelihood of evening chronotype. After adjustment for BMI, the associations with sleep apnoea risk were no longer significant. We failed to find any study to which we could compare our results. Our findings suggest that either distributing PA throughout the week or concentrating it on weekends improves sleep efficiency and is associated with lower likelihood of evening chronotype. Therefore, PA distribution does not seem to significantly impact the beneficial effect of PA on sleep.

Strengths and limitations

To the best of our knowledge, this is the first study exploring the association of both objectively-measured activity and sleep among adults. Importantly, and contrary to other studies (7, 16), self-reported sleep characteristics were collected using validated questionnaires. Finally, both PA and SB were assessed, as high PA levels can be associated either with high or low SB levels, and reciprocally.

This study also has several limitations. First, due to its cross-sectional setting, reverse causation (i.e. sleep disturbances leading to changes in PA and SB levels and weekly activity patterns) cannot be ruled out. It would thus be important to confirm prospectively the results of this study, so that directional causality can be established. The next follow-up of the CoLaus cohort will hopefully solve this issue. Second, the accelerometer was worn on the right wrist, which is the dominant side for most people; hence, overall PA might have been overestimated. Still, previous findings found no impact of device location on PA assessment (26). Third, *GENEActiv* accelerometers have been suggested to over-report MVPA (42); still, as MVPA levels were categorized into tertiles and not absolute values this should not impact the validity of our results. Fourth, although sleep detection algorithm has been validated by polysomnography and predicted sleep duration with an accuracy of 83% (19), the validation procedure was conducted among 28 sleep clinic patients wearing the accelerometer on their non-dominant wrist. Further, the algorithm overestimated sleep duration by an average of 31 minutes. Hence, the validation data might not be applicable to our sample, as most participants had no sleep complaints and the accelerometer was worn on the dominant wrist. Still, it has been shown that wear side does not influence PA assessment (26), and in the absence of other validation procedures, this is the best methodology that could be applied in our study. For future studies, it would be important that the algorithm be also validated in a larger sample of subjects without sleep complains. Finally, due to an important exclusion rate (i.e. 45.7%), the retained sample might be no longer representative of the general population. Still, included participants showed demographic characteristics relatively similar to the Lausanne population (**Supplementary table 10**).

Conclusion

High PA and low SB individuals, even if they do not sleep longer, have higher sleep efficiency and less evening chronotype.

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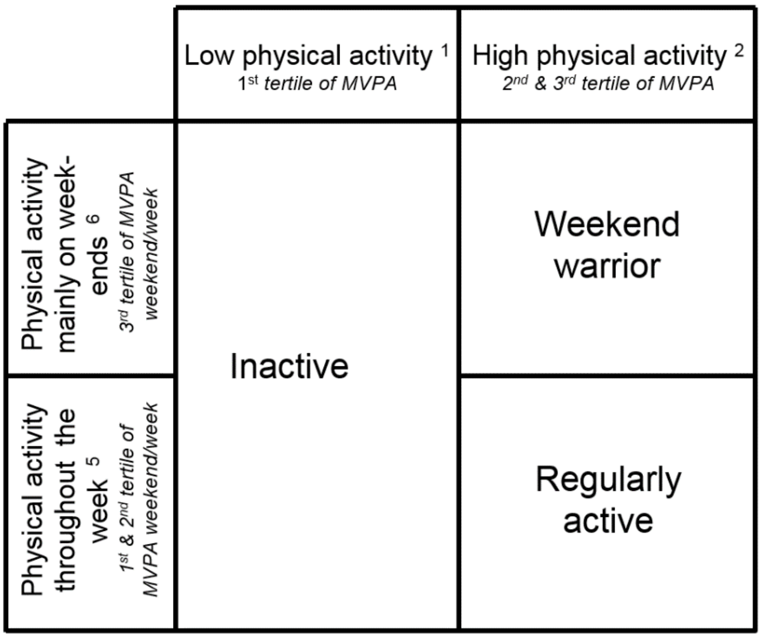
SUPPLEMENTARY MATERIAL

Supplementary figure 1: Mutually exclusive weekly 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.



Supplementary table 1: Additional analysis for the association between physical activity and sedentary behaviour status with sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			Sedentary behaviour		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Objective sleep duration (h) §	6.8 ± 0.06	6.7 ± 0.04	0.73	6.8 ± 0.06	6.7 ± 0.04	0.79
Objective sleep efficiency (%) §	70.4 ± 0.60	73.0 ± 0.42	<0.01	70.1 ± 0.59	73.2 ± 0.42	<0.01
Excessive daytime sleepiness †	1 (ref)	0.91 (0.67; 1.25)	0.57	1 (ref)	1.07 (0.78; 1.46)	0.68

Multivariable analysis including all participants irrespective of objective sleep duration and efficiency, and of missing items in daytime sleepiness questionnaire. For continuous variables (§), statistical analyses were performed using ANOVA and results were expressed as multivariable-adjusted average ± standard error. For categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes).

Supplementary table 2: Multivariable association of physical activity and sedentary behaviour status with sleep parameters, with a further adjustment for body mass index. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			**Sedentary behaviour**		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Objective sleep duration (h) §	7.1 ± 0.03	7.1 ± 0.02	0.43	7.1 ± 0.03	7.0 ± 0.02	0.20
Objective sleep efficiency (%) §	74.0 ± 0.29	76.5 ± 0.20	<0.01	73.8 ± 0.29	76.6 ± 0.20	<0.01
Self-reported sleep duration (h) §	6.9 ± 0.05	7.0 ± 0.03	0.50	6.9 ± 0.05	7.0 ± 0.03	0.34
Short sleep †	1 (ref)	0.92 (0.71; 1.18)	0.49	1 (ref)	0.89 (0.70; 1.14)	0.37
Poor sleep quality †	1 (ref)	1.06 (0.83; 1.36)	0.64	1 (ref)	1.02 (0.80; 1.31)	0.86
Excessive daytime sleepiness †	1 (ref)	0.99 (0.71; 1.37)	0.95	1 (ref)	1.23 (0.88; 1.70)	0.22
Increased risk of sleep apnoea †	1 (ref)	0.93 (0.72; 1.20)	0.60	1 (ref)	0.99 (0.77; 1.27)	0.91
Insomnia †	1 (ref)	1.55 (0.97; 2.48)	0.07	1 (ref)	1.46 (0.92; 2.31)	0.11
Chronotype						
Morning	1 (ref)	1.06 (0.77; 1.46)	0.72	1 (ref)	1.17 (0.86; 1.61)	0.32
Evening	1 (ref)	0.71 (0.52; 0.98)	0.04	1 (ref)	0.64 (0.47; 0.87)	<0.01

For continuous variables (§), statistical analyses were performed using ANOVA and results were expressed as multivariable-adjusted average ± standard error. For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the ‘Morning’ and ‘Evening’ groups to the ‘Intermediate’ one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), body mass index (continuous), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes).

Supplementary table 3: Association of physical activity and sedentary behaviour status with sleep parameters, with a further adjustment for diabetes. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			Sedentary behaviour		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Objective sleep duration (h) §	7.1 ± 0.03	7.1 ± 0.02	0.68	7.1 ± 0.03	7.1 ± 0.02	0.45
Objective sleep efficiency (%) §	73.9 ± 0.29	76.5 ± 0.20	<0.01	73.7 ± 0.28	76.7 ± 0.20	<0.01
Self-reported sleep duration (h)	6.9 ± 0.05	7.0 ± 0.03	0.48	6.9 ± 0.05	7.0 ± 0.03	0.35
Short sleep †	1 (ref)	0.89 (0.70; 1.14)	0.37	1 (ref)	0.88 (0.69; 1.12)	0.29
Poor sleep quality †	1 (ref)	1.07 (0.83; 1.37)	0.60	1 (ref)	1.04 (0.82; 1.33)	0.74
Excessive daytime sleepiness †	1 (ref)	0.97 (0.70; 1.34)	0.84	1 (ref)	1.17 (0.85; 1.62)	0.34
Increased risk of sleep apnoea	1 (ref)	0.75 (0.59; 0.95)	0.02	1 (ref)	0.74 (0.59; 0.94)	0.01
Insomnia †	1 (ref)	1.61 (1.00; 2.58)	0.05	1 (ref)	1.51 (0.95; 2.39)	0.08
Chronotype						
Morning	1 (ref)	1.08 (0.78; 1.47)	0.65	1 (ref)	1.18 (0.86; 1.62)	0.29
Evening	1 (ref)	0.71 (0.52; 0.97)	0.03	1 (ref)	0.64 (0.47; 0.87)	<0.01

For continuous variables (§), statistical analyses were performed using ANOVA and results were expressed as multivariable-adjusted average ± standard error. For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the ‘Morning’ and ‘Evening’ groups to the ‘Intermediate’ one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), diabetes (no/yes), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes).

Supplementary table 4: Multivariable association of physical activity and sedentary behaviour status with sleep parameters, with a further adjustment for depression risk. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			Sedentary behaviour		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Objective sleep duration (h) §	7.1 ± 0.04	7.1 ± 0.03	0.78	7.1 ± 0.04	7.1 ± 0.03	0.83
Objective sleep efficiency (%) §	73.8 ± 0.31	76.6 ± 0.21	<0.01	73.6 ± 0.30	76.7 ± 0.21	<0.01
Self-reported sleep duration (h) §	6.9 ± 0.05	7.0 ± 0.03	0.48	6.9 ± 0.05	7.0 ± 0.03	0.30
Short sleep †	1 (ref)	0.89 (0.69; 1.14)	0.36	1 (ref)	0.86 (0.68; 1.10)	0.24
Poor sleep quality †	1 (ref)	1.06 (0.82; 1.37)	0.67	1 (ref)	1.00 (0.78; 1.29)	0.99
Excessive daytime sleepiness †	1 (ref)	0.93 (0.67; 1.29)	0.65	1 (ref)	1.15 (0.82; 1.60)	0.42
Increased risk of sleep apnoea †	1 (ref)	0.74 (0.58; 0.95)	0.02	1 (ref)	0.74 (0.59; 0.94)	0.02
Insomnia †	1 (ref)	1.62 (0.99; 2.65)	0.06	1 (ref)	1.61 (0.99; 2.62)	0.06
Chronotype						
Morning	1 (ref)	1.07 (0.77; 1.48)	0.68	1 (ref)	1.20 (0.88; 1.65)	0.25
Evening	1 (ref)	0.74 (0.54; 1.01)	0.06	1 (ref)	0.66 (0.49; 0.91)	0.01

For continuous variables (§), statistical analyses were performed using ANOVA and results were expressed as multivariable-adjusted average ± standard error. For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the 'Morning' and 'Evening' groups to the 'Intermediate' one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), increased depression risk (no/yes), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes).

Supplementary table 5: Multivariate analysis of the effect of a 10%-increment in the proportion of time spent in physical activity and sedentary behaviour on sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Physical activity	**P-value**	**Sedentary behaviour**	**P-value**
Objective sleep duration (h) §	-0.01 (-0.05; 0.04)	0.81	0.03 (-0.01; 0.07)	0.14
Objective sleep efficiency (%) §	1.95 (1.58; 2.33)	<0.01	-1.75 (-2.06; -1.44)	<0.01
Self-reported sleep duration (h) §	0.01 (-0.05; 0.08)	0.70	-0.00 (-0.06; 0.05)	0.86
Short sleep †	0.93 (0.80; 1.07)	0.30	1.05 (0.93; 1.18)	0.40
Poor sleep quality †	0.93 (0.81; 1.08)	0.33	1.07 (0.95; 1.20)	0.27
Excessive daytime sleepiness †	1.14 (0.96; 1.36)	0.14	0.87 (0.76; 1.01)	0.07
Increased risk of sleep apnoea †	0.77 (0.67; 0.89)	<0.01	1.20 (1.07; 1.35)	<0.01
Insomnia †	1.07 (0.84; 1.35)	0.60	0.94 (0.77; 1.14)	0.51
Chronotype				
Morning	1.08 (0.92; 1.27)	0.36	0.90 (0.78; 1.03)	0.13
Evening	0.78 (0.66; 0.92)	<0.01	1.19 (1.04; 1.37)	0.01

For continuous variables (§), statistical analyses were performed using linear regression and results were expressed as multivariable-adjusted coefficient and (95% confidence interval). For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the 'Morning' and 'Evening' groups to the 'Intermediate' one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes).

Supplementary table 6: Multivariate analysis of the effect of a 10h-increment in weekly hours of physical activity on sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Weekly physical activity	**P-value**
Objective sleep duration (h) §	-0.04 (-0.08; -0.00)	0.05
Objective sleep efficiency (%) §	1.62 (1.28; 1.95)	<0.01
Self-reported sleep duration (h) §	-0.00 (-0.06; 0.06)	0.98
Short sleep †	0.95 (0.83; 1.08)	0.41
Poor sleep quality †	0.94 (0.82; 1.07)	0.36
Excessive daytime sleepiness †	1.15 (0.98; 1.34)	0.08
Increased risk of sleep apnoea †	0.81 (0.71; 0.93)	<0.01
Insomnia †	1.05 (0.84; 1.30)	0.69
Chronotype		
Morning	1.08 (0.93; 1.25)	0.34
Evening	0.80 (0.68; 0.93)	<0.01

For continuous variables (§), statistical analyses were performed using linear regression and results were expressed as multivariable-adjusted coefficient and (95% confidence interval). For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the 'Morning' and 'Evening' groups to the 'Intermediate' one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes), professional occupation (no/yes), and diurnal wearing time (continuous).

Supplementary table 7: Additional analysis for the association between weekly activity patterns and sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Inactive	**Weekend warrior**	**Regularly active**	**P-value**
Objective sleep duration (h)	6.8 ± 0.06	6.8 ± 0.07	6.7 ± 0.05	0.66
Objective sleep efficiency	70.4 ± 0.60 [a]	73.2 ± 0.71 [b]	72.9 ± 0.51 [b]	<0.01
Excessive daytime	1 (ref)	1.13 (0.78; 1.63)	0.80 (0.57; 1.13)	

Multivariable analysis including all participants irrespective of objective sleep duration and efficiency, and of missing items in daytime sleepiness questionnaire. For continuous variables (§), statistical analyses were performed using ANOVA and results were expressed as multivariable-adjusted average ± standard error. For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes). Post-hoc pairwise comparisons of averages were performed using the method of Scheffe; values with differing superscripts differ at $p<0.05$.

Supplementary table 8: Association of weekly activity patterns with sleep parameters, with a further adjustment for body mass index. The CoLaus study, Switzerland, 2014-2017.

	Inactive	**Weekend warrior**	**Regularly active**
Objective sleep duration (h) §	6.9 ± 0.05	7.0 ± 0.05	7.0 ± 0.04
Objective sleep efficiency (%) §	7.1 ± 0.03	7.1 ± 0.04	7.1 ± 0.03
Self-reported sleep duration (h) §	74.1 ± 0.29 [a]	76.2 ± 0.34 [b]	76.6 ± 0.24 [b]
Short sleep †	1 (ref)	0.87 (0.64; 1.20)	0.94 (0.72; 1.22)
Poor sleep quality †	1 (ref)	1.04 (0.76; 1.43)	1.07 (0.82; 1.40)
Excessive daytime sleepiness †	1 (ref)	1.26 (0.85; 1.86)	0.86 (0.60; 1.23)
Increased risk of sleep apnoea †	1 (ref)	0.87 (0.62; 1.22)	0.96 (0.74; 1.26)
Insomnia †	1 (ref)	1.46 (0.81; 2.64)	1.58 (0.97; 2.59)
Chronotype			
Morning	1 (ref)	0.97 (0.66; 1.44)	1.11 (0.79; 1.55)
Evening	1 (ref)	0.64 (0.43; 0.94) *	0.75 (0.54; 1.05)

For continuous variables (§), statistical analyses were performed using linear regression and results were expressed as multivariable-adjusted coefficient and (95% confidence interval). For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the ‘Morning’ and ‘Evening’ groups to the ‘Intermediate’ one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), body mass index (continuous), alcohol consumption (low/high), psychotropic medication (no/yes) and professional occupation (no/yes). Post-hoc pairwise comparisons of averages were performed using the method of Scheffe; values with differing superscripts differ at $p<0.05$. Significant ($p<0.05$) odds ratios or relative-risk ratios are indicated with *.

Supplementary table 9: Multivariate analysis of the effect of a 10%-increment in standard deviation of daily proportion of time spent in physical activity on sleep parameters. The CoLaus study, Switzerland, 2014-2017.

	Standard deviation of daily PA	**P-value**
Objective sleep duration (h) §	0.14 (-0.04; 0.32)	0.13
Objective sleep efficiency (%)	0.61 (-0.87; 2.09)	0.42
Self-reported sleep duration (h)	-0.07 (-0.31; 0.17)	0.57
Short sleep †	1.00 (0.58; 1.72)	0.99
Poor sleep quality †	1.02 (0.59; 1.77)	0.93
Excessive daytime sleepiness	1.22 (0.66; 2.26)	0.52
Increased risk of sleep apnoea	0.94 (0.55; 1.60)	0.82
Insomnia †	2.15 (0.92; 5.04)	0.08
Chronotype		
Morning	0.87 (0.47; 1.63)	0.67
Evening	1.25 (0.67; 2.35)	0.48

PA, physical activity. For continuous variables (§), statistical analyses were performed using linear regression and results were expressed as multivariable-adjusted coefficient and (95% confidence interval). For dichotomous categorical variables (†), statistical analyses were performed using logistic regression and results were expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For chronotype, statistical analyses were performed using multinomial logistic regression comparing the ‘Morning’ and ‘Evening’ groups to the ‘Intermediate’ one and results were expressed as multivariable-adjusted relative-risk ratio and (95% confidence interval). All multivariable models were adjusted for age (continuous), gender (male/female), self-rated health (very good/good/average or bad), alcohol consumption (low/high), psychotropic medication (no/yes) professional occupation (no/yes), and average proportion of time spent in PA (continuous).

Supplementary table 10: Distribution of age groups in included participants and in the Lausanne population, stratified by gender.

	Included participants		Lausanne population [1]	
Age (years)	**Male**	**Female**	**Male**	**Female**
45-54	28.2	33.3	40.0	35.1
55-64	32.9	33.0	29.2	26.9
65-74	28.0	23.3	19.3	22.1
78-84	11.0	10.4	11.5	15.9

Proportions expressed as percentage. [1] Data from Statistical Office of Canton Vaud (http://www.scris.vd.ch/).

Chapter 5

Association of activity levels and patterns with salivary cortisol

Based on **Gubelmann C**, Kuehner C, Vollenweider P, Marques-Vidal P. Association of activity status and patterns with salivary cortisol: The population-based CoLaus study. *European Journal of Applied Physiology.* 2018.

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.

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.

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

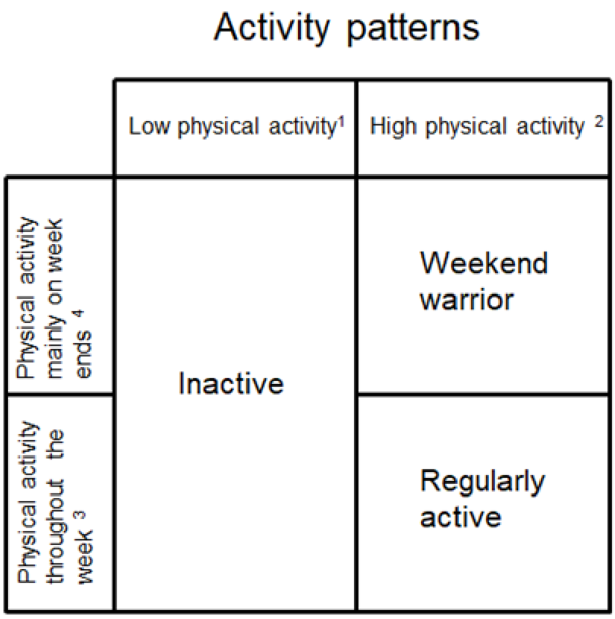
PA was assessed using a wrist-worn triaxial accelerometer (*GENEActiv*, Activinsights Ltd, United Kingdom). The accelerometers were pre-programmed with a 50 Hz sampling frequency and subsequently attached to the participants' right wrist. Participants were requested to wear the device continuously for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

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.



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

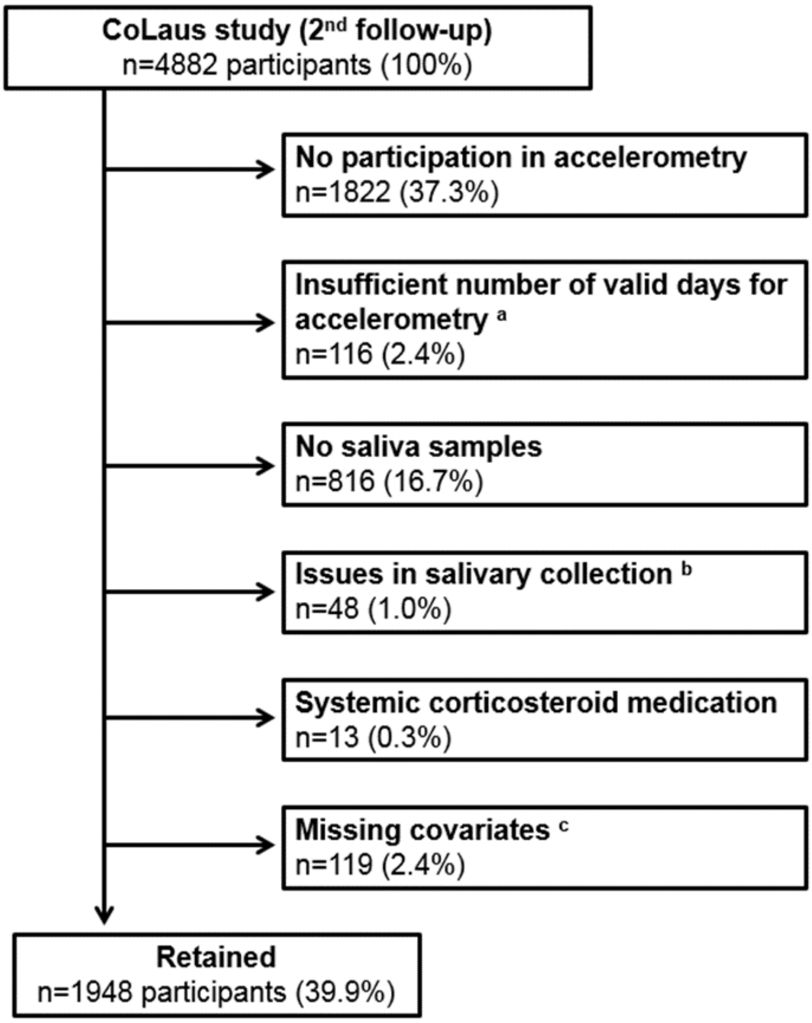
Low SB status was associated to steeper diurnal cortisol slopes. Non-significant trends were observed for high PA status with lower values in AUCg and steeper slopes. For PA patterns, the ‘Regularly actives’ and ‘Weekend warriors’ had respectively lower values in AUCg and steeper slopes in comparison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

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.



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**).

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				
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 $p<0.05$.

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 SB 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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SUPPLEMENTARY MATERIAL

Supplementary table 1: Characteristics of excluded and included participants. The CoLaus study, Switzerland, 2014-2017.

	Included	Excluded	P-value
Age (years)	62.2 ± 9.8	63.4 ± 10.8	<0.01
Female (%)	54.9	55.2	0.86
Professional occupation (%)	55.3	51.4	<0.01
Educational level (%)			0.09
High	21.7	20.8	
Medium	27.2	24.9	
Low	51.2	54.3	
Current smoker (%)	15.3	21.9	<0.01
Body mass index (kg/m^2)	26.2 ± 4.5	26.6 ± 4.9	<0.01
Obesity (%)	17.0	20.5	<0.01

Results are expressed as percentage for categorical variables or as mean ± standard deviation for continuous variables. Between-group comparisons performed by chi-square for categorical variables and by student t-test for continuous variables.

Supplementary table 2: Characteristics of participants, stratified by activity status. The CoLaus study, Switzerland, 2014-2017.

	Physical activity			**Sedentary behaviour**		
	Low	**High**	**P-value**	**High**	**Low**	**P-value**
Sample size	625	1323		617	1331	
Age (years)	66.3 ± 10.2	60.3 ± 9.0	<0.01	65.5 ± 10.2	60.7 ± 9.2	<0.01
Female (%)	45.4	59.4	<0.01	39.6	62.1	<0.01
Professional occupation (%)	40.6	62.2	<0.01	43.6	60.7	<0.01
Educational level (%)			0.88			0.10
High	21.9	21.5		24.2	20.5	
Medium	26.4	27.5		27.9	26.8	
Low	51.7	50.9		48.0	52.7	
Current smoker (%)	18.6	13.8	<0.01	17.0	14.5	0.15
Body mass index (kg/m^2)	27.5 ± 4.8	25.6 ± 4.2	<0.01	27.6 ± 4.8	25.6 ± 4.2	<0.01
Obesity (%)	24.5	13.5	<0.01	24.5	13.5	<0.01

Results are expressed as percentage for categorical variables or as mean ± standard deviation for continuous variables. Between-group comparisons performed by chi-square for categorical variables and by student t-test for continuous variables.

Supplementary table 3: Characteristics of participants, stratified by activity patterns. The CoLaus study, Switzerland, 2014-2017.

	Inactive	**Weekend warrior**	**Regularly active**	**P-value**
Sample size	625	442	881	
Age (years)	66.3 ± 10.2	57.8 ± 8.3	61.5 ± 9.1	<0.01
Female (%)	45.4	61.5	58.3	<0.01
Professional occupation (%)	40.6	77.4	54.6	<0.01
Educational level (%)				<0.01
High	21.9	31.7	16.5	
Medium	26.4	29.2	26.7	
Low	51.7	39.1	56.9	
Current smoker (%)	18.6	14.5	13.4	0.02
Body mass index (kg/m^2)	27.5 ± 4.8	24.9 ± 4.0	25.9 ± 4.2	<0.01
Obesity (%)	24.5	11.8	14.3	<0.01

Results are expressed as percentage for categorical variables or as mean ± standard deviation for continuous variables. Between-group comparisons performed by chi-square for categorical variables and by one-way analysis of variance for continuous variables.

Supplementary table 4: Multivariate association of physical activity status and patterns with salivary cortisol parameters, adjusting for physical activity during the day of saliva sampling. The CoLaus study, Switzerland, 2014-2017.

	Physical activity status			Physical activity patterns			
	Low	**High**	**P-value**	**Inactive**	**Weekend warrior**	**Regularly active**	**P-value**
AUCg [nmol.hour/l]	216.6 ± 3.8	206.8 ± 2.4	0.04	217.1 ± 3.8 [a]	215.3 ± 4.1 [a]	202.7 ± 2.9 [b]	<0.01
Awakening response [nmol/l]	14.4 ± 0.8	13.4 ± 0.5	0.37	14.4 ± 0.8	14.0 ± 0.9	13.1 ± 0.6	0.47
Slope [nmol/l per hour]	-1.40 ± 0.05	-1.54 ± 0.03	0.03	-1.40 ± 0.05 [a]	-1.60 ± 0.05 [b]	-1.51 ± 0.04 [a, b]	0.03

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

Supplementary table 5: Multivariate association of activity patterns with salivary cortisol parameters, by adjusting on week day. The CoLaus study, Switzerland, 2014-2017.

	Inactive	Weekend warrior	Regularly active	P-value
AUCg [nmol.hour/l]	215.4 ± 2.9 ^{a}	216.0 ± 3.5 ^{a}	205.4 ± 2.4 ^{b}	<0.01
Awakening response [nmol/l]	13.4 ± 0.7	14.1 ± 0.8	13.8 ± 0.5	0.79
Slope [nmol/l per hour]	-1.44 ± 0.04 ^{a}	-1.61 ± 0.05 ^{b}	-1.50 ± 0.03 ^{a,b}	0.02

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

Chapter 6

Association of activity levels and patterns with muscle markers

Based on **Gubelmann C**, Vollenweider P, Marques-Vidal P. Regularly actives have higher grip strength and lean mass but not Weekend warriors: The CoLaus study. *Submitted in Mayo Clinic Proceedings.*

ABSTRACT

Background: Physical activity (PA) levels has been associated with muscle mass and strength, but the impact of PA distribution has never been assessed.

Methods: Cross-sectional study including 2338 adults (53.4% women, 45-86 years). PA was measured by 14-day accelerometry. Low PA status was defined as the lowest tertile. 'Inactive', 'Weekend warrior' and 'Regularly active' PA patterns were also defined. Grip strength was measured by hand dynamometer and percentage of lean mass by bioimpedance. Low grip strength was defined according to US criteria.

Results: High PA men had lower likelihood of low grip strength [odds ratio (OR) and 95% confidence interval (95%IC): 0.46 (0.24; 0.87)], and a tendency for lower likelihood of low lean mass [OR: 0.67 (0.45; 1.01)]. In men, relative to 'Inactives', 'Regularly actives' had lower likelihood of low grip strength [OR: 0.41 (0.20; 0.84)] and low lean mass [OR: 0.61 (0.40; 0.95)]; no differences were found for 'Weekend warriors' with low grip strength [OR: 0.60 (0.23; 1.55)] and low lean mass [OR: 0.85 (0.47; 1.54)]. In women, no associations were found for PA status and patterns.

Conclusion: In men, high PA is related to higher grip strength and lean mass. This relationship is valid for regularly active individuals but not when PA is concentrated on weekends. No such associations were found in women.

INTRODUCTION

The impact of physical activity (PA) (1) and sedentary behaviour (SB) (2) on cardiovascular disease (CVD) is well established, but the underlying mechanisms are incompletely understood (3). Muscular strength, commonly measured using grip strength (GS), and lean mass (LM) have been shown to predict CVD mortality (4, 5). Therefore, it can be speculated that PA and SB might impact CVD by modulating muscle strength and mass. Several epidemiological studies showed that physically active individuals have higher GS (6-8) and LM (7, 9), but they were limited by self-reported PA (7, 8), or restricted to the elderly (6, 9). Finally, it has been suggested that the benefits of PA could be altered by exercising only 1-2 times per week (10). Still, no previous study took into account the distribution of PA (i.e. PA patterns). Therefore, this study aimed to evaluate the association of objective PA and SB status and patterns with GS and LM in a population-based sample aged 45-86 years from the city of Lausanne, Switzerland.

METHODS

Participants were recruited during the second follow-up of the CoLaus study (11), which included a module on PA. As previously described (12), PA was measured by wrist-worn accelerometry (*GENEActiv*, Activinsights Ltd, United Kingdom) during 14 days. For PA status, participants were classified as 'low PA' if they were in the first tertile of average proportion of time spent in moderate-to-vigorous PA (MVPA), and as 'high PA' otherwise. For SB status, participants were classified as 'high SB' if they were in the highest tertile of average proportion of time spent in SB, and as 'low SB' otherwise. Finally, participants were categorized as 'PA mainly on weekends' if they were in the highest tertile of the ratio between the average proportion of time spent in MVPA on weekend days and the average proportion of time spent in MVPA on week days, and as 'PA throughout the week' otherwise. This classification allowed creating three mutually exclusive PA patterns (**Supplementary figure 1**): 1) 'Inactive': low PA; 2) 'Weekend warrior': high PA & PA mainly on weekends; and 3) 'Regularly active': high PA & PA throughout the week.

Grip strength (GS) was assessed using the Baseline® Hydraulic Hand Dynamometer (Fabrication Enterprises Inc, Elmsford, NY, USA) according to the American Society of Hand Therapists' guidelines (13). Three measurements were performed consecutively with the right hand and only the highest value (expressed in kg) was included in the analyses. Grip strength was further categorized as low or normal according to Fried criterion (14). Lean mass (in percent of total body weight) was assessed by electrical bioimpedance in the lying position after a 5-min rest using the Bodystat® 1500 body mass analyzer (Bodystat Ltd, Isle of Man, England). The results obtained using this device have been shown to correlate well with measurements from dual energy X-ray absorptiometry (r=0.968) (15). Participants in the lowest sex-specific quartile were considered as presenting low LM.

Demographic data and smoking status were collected by questionnaire. Educational level was collected at baseline by questionnaire and categorized as low (obligatory school or apprenticeship), medium (high school), or high (university degree). Perceived health (very good/good/average or bad) was collected during an interview. 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.

Participants were excluded if they: (i) did not participate in accelerometry, or had less than 5 weekdays or 2 weekend days of valid accelerometry data; (ii) were not assessed for GS, or presented any condition precluding adequate measurement (i.e. pain or arthrosis); (iii) were not assessed for LM; or (iv) had any missing data in smoking status, educational level, perceived health, weight or height.

Statistical analyses were conducted using Stata version 14.1 for windows (Stata Corp, College Station, Texas, USA). In bivariate analysis, 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). Multivariable analyses were conducted using logistic regression for categorical variables and ANOVA for continuous variables. Results were expressed as odds ratio and 95% confidence interval for logistic regression and as multivariable-adjusted average ± standard error for ANOVA. For ANOVA, post-hoc pairwise comparisons were performed using the method of Scheffe. All multivariable models were adjusted for age, height, weight, smoking status, perceived health and educational level. Statistical significance was assessed for a two-sided test with $p<0.05$. Sensitivity analyses were conducted among post-menopausal women, or using 10%-increment of proportion of time spent in PA and SB.

The CoLaus study was approved by the Ethics Committee of the University of Lausanne. 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

Of the initial 4881 participants, 2338 (47.9%) were retained for analysis; the selection procedure is indicated in **Supplementary figure 2**. Participants' characteristics are presented in **Supplementary table 1** and **2**.

The associations between activity status and muscle markers are described in **Table 1** (PA status) and **Supplementary table 3** (SB status). After multivariate adjustment, high PA men had lower likelihood of low GS and had higher LM values relative to low PA. Non-significant trends were also found for GS values ($p=0.13$) and low LM ($p=0.06$). No associations were found for women, even after restricting to postmenopausal ones (**Supplementary table 4**). Low SB participants had higher GS values relative to high SB, while low SB men had also lower likelihood of low GS. No associations were found between SB status and LM. Most associations remained identical with 10%-increment of proportion of time spent in PA and SB (**Supplementary table 5**).

Table 1: Association of physical activity status with muscle markers, stratified by gender. The CoLaus study, Switzerland, 2014-2017.

	Women			**Men**		
	Low PA	**High PA**	**P-value**	**Low PA**	**High PA**	**P-value**
Sample size	329	919		429	661	
Grip strength (kg)						
Bivariate	25.0 ± 5.7	26.7 ± 6.0	**<0.01**	43.0 ± 9.6	45.5 ± 8.8	**<0.01**
Multivariable-adjusted	26.0 ± 0.3	26.4 ± 0.2	0.28	44.0 ± 0.4	44.8 ± 0.3	0.13
Low grip strength						
Bivariate	13.1	7.4	**<0.01**	10.0	3.0	**<0.01**
Multivariable-adjusted	1 (ref)	1.14 (0.70; 1.85)	0.59	1 (ref)	0.46 (0.24; 0.87)	**0.02**
Lean mass (%)						
Bivariate	60.0 ± 8.4	63.4 ± 7.8	**<0.01**	71.6 ± 5.7	74.9 ± 5.5	**<0.01**
Multivariable-adjusted	62.4 ± 0.3	62.5 ± 0.2	0.65	73.1 ± 0.2	73.9 ± 0.2	**<0.01**
Low lean mass						
Bivariate	35.9	17.9	**<0.01**	36.8	15.7	**<0.01**
Multivariable-adjusted	1 (ref)	0.88 (0.55; 1.39)	0.58	1 (ref)	0.67 (0.45; 1.01)	0.06

PA, physical activity. For categorical variables, statistical analyses performed by chi-square (bivariate) and logistic regression (multivariable); results expressed as percentage (bivariate) and as multivariable-adjusted odds-ratio and (95% confidence interval). For continuous variables, statistical analyses performed by student t-test (bivariate) and ANOVA (multivariable); results expressed as average ± standard deviation (bivariate) or as multivariable-adjusted average ± standard error. Multivariable models were adjusted for age (continuous), height (continuous), weight (continuous), smoking status (no/yes), perceived health (very good/good/average or bad) and educational level (high/medium/low).

Table 2: Association of activity patterns with muscle markers, stratified by gender. The CoLaus study, Switzerland, 2014-2017.

	Women				**Men**			
	Inactive	**Weekend warrior**	**Regularly active**	**P-value**	**Inactive**	**Weekend warrior**	**Regularly active**	**P-value**
Sample size	329	325	594		429	232	429	
Grip strength (kg)								
Bivariate	25.0 ± 5.7 [a]	27.7 ± 6.0 [b]	26.2 ± 5.9 [c]	**<0.01**	43.0 ± 9.6 [a]	46.8 ± 8.8 [b]	44.8 ± 8.7 [c]	**<0.01**
Multivariable-adjusted	26.0 ± 0.3	26.4 ± 0.3	26.3 ± 0.2	0.55	44.0 ± 0.4	44.7 ± 0.5	44.9 ± 0.4	0.30
Low grip strength								
Bivariate	13.1	4.0	9.3	**<0.01**	10.0	2.6	3.3	**<0.01**
Multivariable-adjusted	1 (ref)	0.80 (0.39; 1.63)	1.26 (0.77; 2.07)		1 (ref)	0.60 (0.23; 1.55)	**0.41 (0.20; 0.84)**	
Lean mass (%)								
Bivariate	60.0 ± 8.4 [a]	64.9 ± 7.4 [b]	62.6 ± 7.9 [c]	**<0.01**	71.6 ± 5.7 [a]	75.8 ± 5.5 [b]	74.4 ± 5.5 [c]	**<0.01**
Multivariable-adjusted	62.4 ± 0.3	62.6 ± 0.3	62.5 ± 0.2	0.88	73.1 ± 0.2 [a]	73.8 ± 0.3 [a, b]	74.0 ± 0.2 [b]	**0.01**
Low lean mass								
Bivariate	35.9	13.2	20.4	**<0.01**	36.8	12.9	17.3	**<0.01**
Multivariable-adjusted	1 (ref)	0.80 (0.43; 1.48)	0.91 (0.56; 1.48)		1 (ref)	0.85 (0.47; 1.54)	**0.61 (0.40; 0.95)**	

For continuous variables, statistical analyses performed by student t-test (bivariate) and ANOVA (multivariable); results expressed as average ± standard deviation (bivariate) and as multivariable-adjusted average ± standard error. For categorical variables, statistical analyses performed by chi-square (bivariate) and logistic regression (multivariate); results expressed as percentage (bivariate) and as multivariable-adjusted odds-ratio and (95% confidence interval). Multivariable models were adjusted for age (continuous), height (continuous), weight (continuous), smoking status (no/yes), perceived health (very good/good/average or bad) and educational level (high/medium/low). Post-hoc pairwise comparisons of averages were performed using the method of Scheffe; values with differing subscripts differ at p<0.05. Significant (p<0.05) odds ratio are indicated in bold.

The associations between activity patterns and muscle markers are described in **Table 2**. After multivariate adjustment, relative to the 'Inactives', the 'Regularly actives' men had a lower likelihood of low GS and of low LM, and had higher LM values, whereas no association persisted for the 'Weekend warriors'. No associations were found for women.

DISCUSSION

Our results suggest that individuals who concentrate their PA on weekends benefit less from PA than subjects who exercise regularly regarding muscle mass and strength.

High PA men had higher GS and LM whereas no association was found for women, which is in agreement with a recent study (7). On the other hand, other studies showed that women also benefit of PA (8, 9) but they were restricted to the elderly. Our results suggest that high PA is beneficial on both muscle mass and strength in men, but not in women. This gender discrepancy is possibly explained by lower PA intensities performed by women.

Low SB participants had higher GS whereas no association was found for LM. Whether SB is deleterious on muscle has been debated. Some studies reported negative associations with GS (6) and LM (9) while others reported no association (16). However, these different findings were focusing on the elderly (9, 16), and are therefore not representative of the general population. Therefore, our findings suggest that low SB is beneficial on muscle strength, but not on muscle mass.

In comparison to the 'Inactive' pattern, the 'Regularly actives' men had higher GS and LM whereas no significant difference was found for the 'Weekend warriors'. No associations were found for women. We failed to find any study to which we could compare our results. Our findings suggest that PA should be distributed throughout the week to be beneficial on muscle mass and strength, but it needs to be confirmed in longitudinal studies.

As far as we know, this is the largest study investigating the relationship of objective PA with GS or LM, and the first one to focus on PA distribution. Further, and contrary to other studies (7, 8), it was conducted among a large sample of middle-aged adults, and considered SB. However, the study has also some limitations. First, the cross-sectional design precludes the assessment of any causal effect of activity on muscle markers; the next follow-up of the CoLaus cohort will solve this issue. Second, *GENEActiv* accelerometers have been suggested to over-report MVPA (17); still, MVPA levels categorized into tertiles should not impact the validity of our results.

In conclusion, high PA is related to higher GS and LM in men. This beneficial association only applies when PA is evenly distributed over the week.

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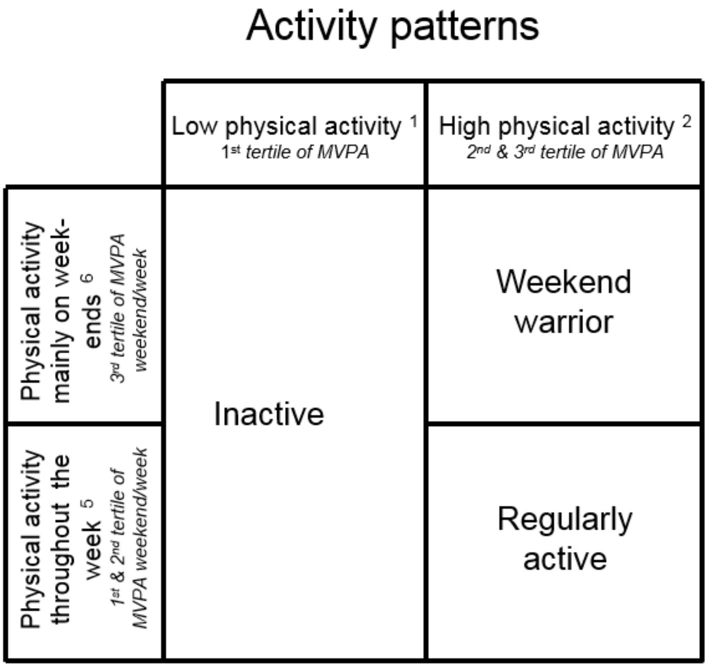
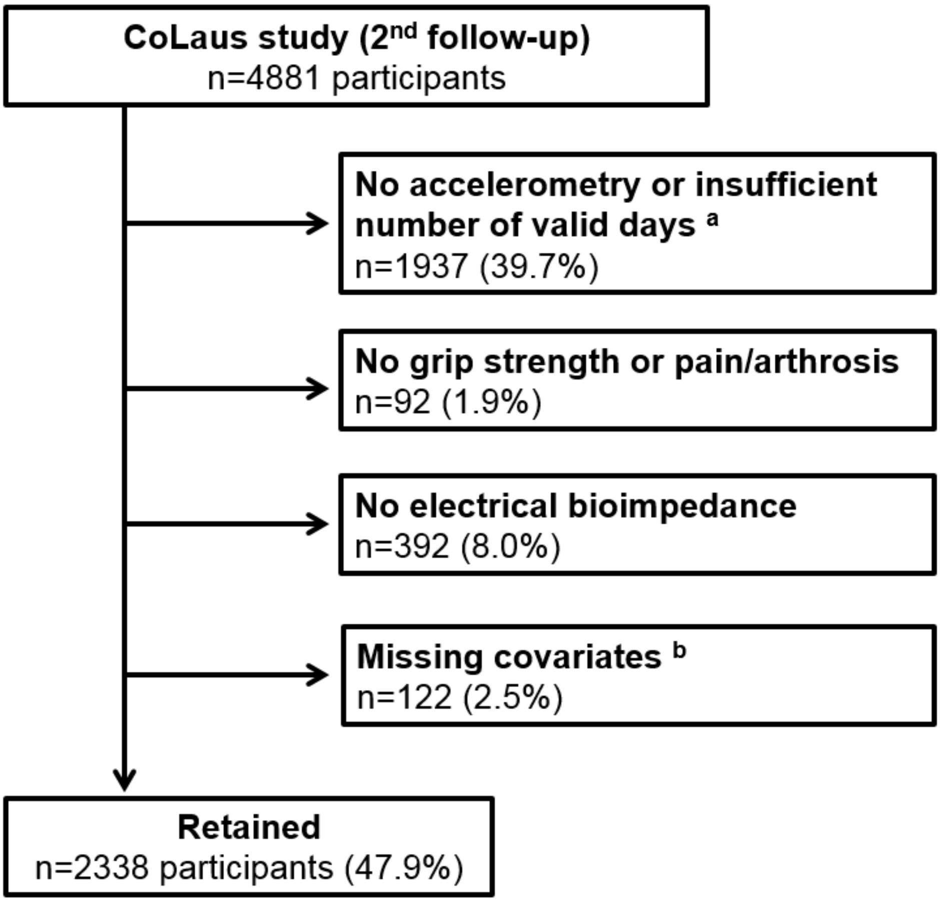
SUPPLEMENTARY MATERIAL

Supplementary 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.



Supplementary 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], perceived health, smoking status, educational level, height and weight.



Supplementary table 1: Characteristics of excluded and included participants. The CoLaus study, Switzerland, 2014-2017.

	Included	**Excluded**	**P-value**
Sample size	2338	2543	
Age (years)	61.5 ± 9.7	64.3 ± 10.9	<0.01
Female	53.4	56.7	0.02
Educational level			<0.01
High	22.8	19.7	
Medium	26.2	25.4	
Low	51.0	54.9	
Smoke	17.6	20.6	0.01
Perceived health			<0.01
Very good	22.2	20.6	
Good	58.6	53.8	
Average or bad	19.3	25.6	
High PA status	67.6	63.7	0.05
Low grip strength	7.4	14.4	<0.01
Low lean mass	23.3	28.1	<0.01

PA, physical activity. Results expressed as mean ± standard deviation for continuous variables and as percentage for categorical variables. Between-group comparisons performed by student t-test for continuous variables and by chi-square for categorical variables.

Supplementary table 2: Characteristics of participants, stratified by gender. The CoLaus study, Switzerland, 2014-2017.

	Women	**Men**	**P-value**
Sample size	1248	1090	
Age (years)	61.9 ± 9.7	60.9 ± 9.7	<0.01
Educational level			<0.01
High	18.8	27.3	
Medium	26.6	25.8	
Low	54.7	46.9	
Smoke	16.9	18.4	0.36
Perceived health			<0.01
Very good	20.4	24.2	
Good	57.7	59.5	
Average or bad	21.9	16.2	
Height (cm)	162.0 ± 6.9	174.8 ± 7.3	<0.01
Weight (kg)	67.4 ± 13.3	82.3 ± 13.6	<0.01
High PA status	73.6	60.6	<0.01
Grip strength (kg)	26.3 ± 6.0	44.5 ± 9.2	<0.01
Low grip strength	8.9	5.8	<0.01
Lean mass (%)	62.5 ± 8.1	73.6 ± 5.8	<0.01
Low lean mass	22.6	24.0	0.41

Results expressed as mean ± standard deviation for continuous variables and as percentage for categorical variables. Between-group comparisons performed by student t-test for continuous variables and by chi-square for categorical variables.

Supplementary table 3: Association of sedentary behaviour status with muscle markers, among men. The CoLaus study, Switzerland, 2014-2017.

	Women			Men		
	High SB	**Low SB**	**P-value**	**High SB**	**Low SB**	**P-value**
Sample size	306	942		462	628	
Grip strength (kg)						
Bivariate	25.1 ± 5.9	26.6 ± 5.9	<0.01	43.3 ± 9.6	45.4 ± 8.8	<0.01
Multivariable-adjusted	25.8 ± 0.3	26.4 ± 0.2	0.05	43.9 ± 0.4	44.9 ± 0.3	0.04
Low grip strength						
Bivariate	13.7	7.3	<0.01	9.1	3.3	<0.01
Multivariable-adjusted	1 (ref)	0.89 (0.55; 1.43)	0.62	1 (ref)	0.48 (0.26; 0.89)	0.02
Lean mass (%)						
Bivariate	60.1 ± 8.4	63.2 ± 7.8	<0.01	72.3 ± 5.9	74.6 ± 5.6	<0.01
Multivariable-adjusted	62.4 ± 0.3	62.5 ± 0.2	0.73	73.5 ± 0.2	73.7 ± 0.2	0.38
Low lean mass						
Bivariate	35.0	18.6	<0.01	32.9	17.5	<0.01
Multivariable-adjusted	1 (ref)	0.89 (0.56; 1.42)	0.62	1 (ref)	0.82 (0.55; 1.22)	0.33

SB, sedentary behaviour. For categorical variables, statistical analyses performed by chi-square (bivariate) and logistic regression (multivariable); results expressed as percentage (bivariate) and as multivariable-adjusted odds-ratio and (95% confidence interval). For continuous variables, statistical analyses performed by student t-test (bivariate) and ANOVA (multivariable); results expressed as average ± standard deviation (bivariate) or as multivariable-adjusted average ± standard error. Multivariable models were adjusted for age (continuous), height (continuous), weight (continuous), smoking status (no/yes), perceived health (very good/good/average or bad) and educational level (high/medium/low).

Supplementary table 4: Multivariate association of physical activity and sedentary behaviour status with muscle markers, among postmenopausal women. The CoLaus study, Switzerland, 2014-2017.

	Low PA	**High PA**	**P-value**	**High SB**	**Low SB**	**P-value**
Sample size	205	565		184	586	
Grip strength (kg)	25.3 ± 0.4	25.7 ± 0.2	0.36	25.0 ± 0.4	25.8 ± 0.2	0.07
Low grip strength	1 (ref)	1.07 (0.58; 1.97)	0.84	1 (ref)	0.70 (0.39; 1.26)	0.23
Lean mass (%)	62.3 ± 0.4	61.9 ± 0.2	0.42	62.2 ± 0.4	62.0 ± 0.2	0.62
Low lean mass	1 (ref)	0.99 (0.52; 1.85)	0.96	1 (ref)	1.12 (0.59; 2.12)	0.74

PA, physical activity; SB, sedentary behaviour. For categorical variables, statistical analyses performed by logistic regression; results expressed as multivariable-adjusted odds-ratio and (95% confidence interval). For continuous variables, statistical analyses performed by ANOVA (multivariable); results expressed as multivariable-adjusted average ± standard error. Multivariable models were adjusted for age (continuous), height (continuous), weight (continuous), smoking status (no/yes), perceived health (very good/good/average or bad), educational level (high/medium/low) and hormonal replacement therapy (no/yes).

Supplementary table 5: Multivariable association of 10%-increment of proportion of time spent in physical activity and sedentary behaviour with muscle markers, stratified by gender. The CoLaus study, Switzerland, 2014-2017.

	Women		**Men**	
	Physical activity	**Sedentary behaviour**	**Physical activity**	**Sedentary behaviour**
Grip strength (kg)	0.27 (-0.07; 0.60)	-0.15 (-0.44; 0.13)	0.57 (-0.03; 1.16)	**-0.48 (-0.95; -0.01)**
Low grip strength	0.96 (0.75; 1.24)	0.97 (0.78; 1.19)	**0.39 (0.23; 0.64)**	**1.82 (1.29; 2.58)**
Lean mass (%)	0.07 (-0.28; 0.43)	-0.08 (-0.37; 0.22)	**0.40 (0.09; 0.70)**	**-0.28 (-0.52; -0.04)**
Low lean mass	0.83 (0.64; 1.07)	1.23 (0.99; 1.52)	**0.75 (0.58; 0.98)**	1.16 (0.95; 1.42)

For continuous variables, statistical analyses performed by linear regression; results expressed as multivariable-adjusted coefficient and (95% confidence interval). For categorical variables, statistical analyses performed by logistic regression; results expressed as multivariable-adjusted odds-ratio and (95% confidence interval). Multivariable models were adjusted for age (continuous), height (continuous), weight (continuous), smoking status (no/yes), perceived health (very good/good/average or bad) and educational level (high/medium/low). Significant ($p<0.05$) coefficient and odds ratio are indicated in bold.

Chapter 7

Association of grip strength with cardiovascular risk factors

Based on **Gubelmann C**, Vollenweider P, Marques-Vidal P. Association of grip strength with cardiovascular risk markers. *European Journal of Preventive Cardiology.* 2017;24(5):514-521.

ABSTRACT

Background: Mechanisms underlying the association between grip strength (GS) and cardiovascular mortality are poorly understood. We aimed to assess the association of GS with a panel of cardiovascular risk markers.

Design: Cross-sectional analysis of 3468 adults aged 50 to 75 years (1891 women) from a population-based sample in Lausanne, Switzerland.

Methods: GS was measured using a hydraulic hand dynamometer. Cardiovascular risk markers included anthropometry, blood pressure (BP), lipids, glucose, adiposity, inflammatory and other metabolic markers.

Results: In both genders, GS was negatively associated with fat mass (Pearson correlation coefficient: women: -0.170, men: -0.198), systolic blood pressure (women: -0.096, men: -0.074), fasting glucose (women: -0.048, men: -0.071), log-transformed leptin (women: -0.074, men: -0.065), log-transformed hs-CRP (women: -0.101, men: -0.079) and log-transformed homocysteine (women: -0.109, men: -0.060). In men, GS was also positively associated with diastolic BP (0.068), total (0.106) and LDL-cholesterol (0.082), and negatively associated with interleukin-6 (-0.071); in women, GS was negatively associated with triglycerides (-0.064) and uric acid (-0.059). After multivariate adjustment, GS was negatively associated with waist circumference (change per 5 kg increase in GS: -0.82 cm in women and -0.77 cm in men), fat mass (-0.56% in women; -0.27% in men) and hs-CRP (-6.8% in women; -3.2% in men) in both genders, and with body mass index (0.22 kg/m^2) and leptin (-2.7%) in men.

Conclusion: GS shows only moderate associations with cardiovascular risk markers. The effect of muscle strength as measured by GS on CVD does not seem to be mediated by cardiovascular risk markers.

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INTRODUCTION

Muscle strength is an important predictor of health (1), partly explained by the beneficial effect of muscle resistance activities on physical fitness (2). Compared to other muscular tests such as trunk and knee extension or flexion, grip strength is the most appropriate marker of muscle strength (3) and has also been related to fitness (4). Therefore, it remains the simplest and most largely recommended technique to assess muscle strength in clinical practice (5). Grip strength has been shown to be inversely associated with overall and cardiovascular mortality in all age groups (6, 7), but the mechanisms involved have been less well established. Several cross-sectional studies assessed the associations between grip strength and cardiovascular (CV) risk factors, metabolic syndrome or inflammatory markers, but have been limited by the fact that they assessed a small set of variables (8, 9), relied on a small sample size (10) or were based only on elderly participants (9, 10). Further, several studies have suggested that fitness can exert its effects independently of physical activity levels (11), and that not all types of physical activity are beneficial for health (12). For instance, leisure-time physical activity (LTPA) has been shown to be beneficial while occupational physical activity (OPA) has been shown to be deleterious regarding all-cause mortality (13). Still, no previous study took into account this finding.

Thus, the aim of this study was to assess the associations between grip strength and nineteen CV risk markers using a large population-based sample aged 50-75 years from the city of Lausanne, Switzerland (CoLaus study), taking into account the effects of LTPA and OPA.

METHODS

Recruitment

A detailed description of the recruitment of the CoLaus study has been published previously (14). Briefly, the CoLaus study assesses the prevalence and determinants of CV disease in the city of Lausanne, Switzerland. A non-stratified, representative sample of the Lausanne population aged 35-75 years was drawn from the population register of the city. A letter was sent to these individuals, and subjects who volunteered to participate were then contacted by phone to set up an appointment. The baseline Colaus study was conducted between 2003 and 2006 and included 6733 participants.

Grip strength

Participants of the CoLaus study aged over 50 were invited to participate in a sub-study on frailty, which included grip strength. Grip strength was assessed using the Baseline® Hydraulic Hand Dynamometer and positioning of the participants was done according to the American Society of Hand Therapists's guidelines (5): subject seated, shoulders adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral and wrist between 0 and 30° of dorsiflexion. Three measurements were performed consecutively at the right hand and the highest value (expressed in kg) was included in the analyses. Participants were also asked about their handedness.

Exclusion criteria

Participants were excluded if they presented any condition precluding adequate measurement of grip strength, i.e. pain, injury, recent surgery, osteoarthritis and rheumatoid arthritis, among others.

Other data

A self administered questionnaire collected demographic data. Information on education level, job and on several lifestyle factors, including tobacco and LTPA (weekly number of ≥20min bouts of exercise) were also collected. OPA was categorized as non-physical (when sitting or standing) and physical (carrying light or heavy load). History of CVD and CV risk factor was elicited with a standardized interview-based questionnaire filled in by a trained recruiter. Participants indicated if they have been diagnosed with hypertension, dyslipidemia, diabetes, and if they were treated for these conditions.

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 weigth/height^2. Waist circumference (WC) was measured at mid-way between the lowest rib and the iliac crest as recommended (15). Body composition was assessed by bioimpedance (Bodystat® 1500 analyzer, Isle of Man, UK) and expressed as percentage of fat. Blood pressure (BP) was measured using an Omron® HEM-907 automated oscillometric sphygmomanometer after at least 10 minutes' rest in a seated position and the average of the last two measurements was used. Hypertension was defined as a systolic BP ≥140 mmHg and/or a diastolic BP ≥90 mmHg and/or presence of an anti-hypertensive treatment.

A fasting venous blood sample was drawn and most measurements performed by the clinical laboratory of the Lausanne university hospital. Lipid markers included total and HDL-cholesterol, triglycerides and apolipoprotein B; LDL-cholesterol was calculated using the Friedewald formula if triglycerides were <4.6 mmol/L. Dyslipidemia was defined either by the presence of a lipid lowering drug or using the LDL-cholesterol thresholds according to the PROCAM cardiovascular score adapted for Switzerland (16). Glucometabolic markers included glucose and insulin; diabetes was defined by a fasting glucose ≥7.0 and/or presence of antidiabetic drug treatment. Inflammatory markers included high sensitivity C-reactive protein (hs-CRP), interleukin 6 (IL-6) and tumour necrosis factor alpha (TNF-α). Other markers included leptin, adiponectin, homocysteine and uric acid.

CV absolute risk was calculated using the European Society of Cardiology SCORE recalibrated and validated for the Swiss population (17). This risk equation uses age, gender, smoking, systolic BP and total cholesterol to compute the 10-year absolute risk of fatal CV disease. No CV absolute risk was calculated for participants with history of CV disease.

Statistical analysis

Statistical analyses were stratified by gender and conducted using Stata version 14.0 for windows (Stata Corp, College Station, Texas, USA). Descriptive results were expressed as number of participants (percentage) or as average ± standard deviation. Between-group comparisons were performed using chi-square or Student t-test for categorical and continuous variables, respectively. Natural log transformation was applied to variables with a skewed distribution: triglycerides, insulin, leptin, adiponectin, hs-CRP, IL-6, TNF-α and homocysteine. Bivariate associations were assessed by Pearson correlation. Multivariate associations were assessed using linear regression and the results were expressed as multivariate-adjusted standardized coefficients, which can be interpreted as multivariate-adjusted correlation coefficients.

The effect of a 5 kg increase in grip strength on the different CV risk markers was assessed by linear regression, and the results were expressed as coefficient and (95% confidence interval). For log-transformed dependent variables, results were expressed as percentage change of the untransformed dependent variable and (95% confidence interval), as recommended (18). Multivariate analyses were conducted using linear or quadratic regression models and the adequacy of the linear model relative to the quadratic one was tested by likelihood ratio test. Multicollinearity of the dependent variables was assessed by computing the variance inflation factor; values ranged from 1.02 to 1.21, suggesting lack of collinearity.

All multivariate models were adjusted for age (continuous), smoking status (current/other), LTPA (3 categories), OPA (physical/non-physical) and BMI (except for anthropometry). Further adjustments were performed on: weight (continuous) for WC; hypertensive drug treatment (yes/no) for BP; lipid lowering drug treatment (yes/no) for lipid markers and antidiabetic drug treatment (yes/no) for glucometabolic markers. Sensitivity analyses were performed by further stratifying on tertiles of age. Statistical significance was assessed for a two-sided test with $p<0.05$.

Ethical statement

The CoLaus study was approved by the Ethics Committee of the University of Lausanne and all participants gave their signed informed consent before entering the study.

RESULTS

Characteristics of excluded participants

Of the initial 3704 participants invited to the sub-study on frailty, 3550 (95.8%) accepted. A further 82 (2.3%) participants were excluded because of issues related to grip strength measurement. Included and excluded participants' characteristics are presented in **Supplementary Table 1**. Included participants were more likely right-handed than the excluded ones, while no significant differences were found for all other variables analysed.

The final sample consisted of 3468 participants; their characteristics overall and according to gender are summarized in **Supplementary Table 2**. Men had higher grip strength, were more likely to be current or former smoker, to have a university level of education, to be full-time worker, to perform a physical job, and to have a higher 10-year CV absolute risk than women.

Association of grip strength with cardiovascular risk markers

The bivariate and multivariate-adjusted associations using linear regression between grip strength and CV risk markers are described in **Table 1**; the corresponding changes in CV risk markers due to a 5 kg-increase in grip strength are described in **Table 2**. Bivariate analysis showed that grip strength was negatively associated with fat mass, systolic BP, fasting glucose, leptin, hs-CRP and homocysteine in both genders. In men, grip strength was positively associated with diastolic BP, total and LDL-cholesterol, and negatively associated with IL-6; in women, grip strength was negatively associated with triglycerides and uric acid. Finally, grip strength was negatively associated with 10-year CV absolute risk as assessed by the SCORE equation in both genders (Pearson correlation coefficient: women: -0.245, $p<0.001$, men: -0.264, $p<0.001$). Most of the previous associations were no longer significant after multivariate adjustment. In both genders, grip strength was negatively associated with WC, fat mass and hs-CRP; in men, grip strength was positively associated with BMI and negatively associated with leptin (**Table 1 and 2**).

Comparison between linear and quadratic models for homocysteine, total and LDL-cholesterol are expressed in **Supplementary Table 3**. For log-transformed homocysteine, total and LDL-cholesterol, the quadratic regression model showed a better fit than the linear one. An inverse U-shaped association between grip strength and total and LDL-cholesterol was found in women. A U-shaped association between grip strength and homocysteine was found in men.

The linear associations between grip strength and CV risk markers stratified by tertiles of age are represented in **Supplementary Tables 4** (women) **and 5** (men), and the quadratic associations for homocysteine, total and LDL-cholesterol in **Supplementary Table 6**. Most associations remained identical through tertiles of age.

Table 1: Bi- and multivariate associations between grip strength and cardiovascular risk markers.

	Pearson correlation		Multivariate-adjusted	
	Women	**Men**	**Women**	**Men**
Anthropometry				
Body mass index (kg/m^2)	-0.034	0.022	-0.000	0.092 **
Waist circumference (cm)	-0.005	0.039	-0.069 [1] **	-0.114 [1] **
Fat mass (%)	-0.170**	-0.198**	-0.078 *	-0.084 *
Blood pressure (mmHg)				
Systolic	-0.096**	-0.074*	0.038 [2]	0.003 [2]
Diastolic	0.007	0.068*	0.015 [2]	0.045 [2]
Lipid markers (mmol/L)				
Total cholesterol	-0.028	0.106**	0.004 [3]	0.082 [3] *
HDL-cholesterol	0.015	0.002	-0.001 [3]	0.029 [3]
LDL-cholesterol	-0.025	0.082*	0.001 [3]	0.055 [3] *
Triglycerides §	-0.064*	0.048	-0.003 [3]	0.026 [3]
Apolipoprotein B (mg/dL)	-0.007	0.010	0.003 [3]	-0.006 [3]
Glucometabolic markers				
Fasting glucose (mmol/L)	-0.048*	-0.071*	-0.006 [4]	-0.036 [4]
Insulin (µU/mL) §	-0.031	-0.049	0.007 [4]	-0.032 [4]
Adipokines (µU/mL)				
Leptin §	-0.074*	-0.065*	-0.026 [5]	-0.059 [5] *
Adiponectin §	-0.036	-0.014	-0.024 [5]	0.012 [5]
Inflammatory markers				
hs-CRP (mg/L) §	-0.101**	-0.079*	-0.071 [5] *	-0.052 [5] *
IL-6 (pg/mL) §	-0.009	-0.071*	-0.009 [5]	-0.054 [5]
TNF-α (pg/mL) §	-0.005	-0.043	0.016 [5]	-0.024 [5]
Homocysteine (µmol/L) §	-0.109**	-0.060*	-0.022 [5]	0.032 [5]
Uric acid (µmol/L)	-0.059*	0.012	0.017 [5]	0.018 [5]

§, log-transformed. hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha. Bivariate associations assessed using Pearson correlation or multivariable linear regression; results are expressed as Pearson correlation coefficient or as multivariate-adjusted standardized coefficient. Multivariable linear model was adjusted for age, current smoking, leisure-time physical activity and occupational physical activity, with a further adjustment on [1] weight; [2] body mass index and antihypertensive drug treatment; [3] body mass index and lipid lowering drug treatment; [4] body mass index and antidiabetic drug treatment; [5] body mass index. *, $p<0.05$; **, $p<0.001$.

Table 2: Unadjusted and multivariate-adjusted changes in cardiovascular risk marker levels per 5 kg increase in grip strength, stratified by gender.

	Women				Men			
	Unadjusted	**P-value**	**Multivariate-adjusted**	**P-value**	**Unadjusted**	**P-value**	**Multivariate-adjusted**	**P-value**
Anthropometry								
Body mass index (kg/m^2)	-0.16 (-0.37 ; 0.05)	0.143	0.00 (-0.22 ; 0.22)	0.985	0.05 (-0.07 ; 0.17)	0.384	0.22 (0.10 ; 0.35)	<0.001
Waist circumference (cm)	-0.06 (-0.60 ; 0.49)	0.839	-0.82 (-1.13 ; -0.52) [1]	<0.001	0.26 (-0.07 ; 0.59)	0.121	-0.77 (-0.93 ; -0.61) [1]	<0.001
Fat mass (%)	-1.23 (-1.55 ; -0.90)	<0.001	-0.56 (-0.89 ; -0.22)	0.001	-0.63 (-0.78 ; -0.47)	<0.001	-0.27 (-0.43 ; -0.11)	0.001
Blood pressure (mmHg)								
Systolic	-1.67 (-2.46 ; -0.89)	<0.001	0.66 (-0.12 ; 1.43) [2]	0.098	-0.77 (-1.28 ; -0.26)	0.003	0.04 (-0.48 ; 0.56) [2]	0.892
Diastolic	0.07 (-0.38 ; 0.52)	0.762	0.15 (-0.31 ; 0.61) [2]	0.522	0.44 (0.12 ; 0.76)	0.007	0.29 (-0.05 ; 0.63) [2]	0.090
Lipid markers (mmol/L)								
Total cholesterol	-0.03 (-0.07 ; 0.02)	0.230	0.00 (-0.04 ; 0.05) [3]	0.863	0.06 (0.03 ; 0.09)	<0.001	0.05 (0.02 ; 0.08) [3]	0.002
HDL-cholesterol	0.01 (-0.01 ; 0.03)	0.505	0.00 (-0.02 ; 0.02) [3]	0.969	0.00 (-0.01 ; 0.01)	0.939	0.01 (0.00 ; 0.02) [3]	0.257
LDL-cholesterol	-0.02 (-0.06 ; 0.02)	0.273	0.00 (-0.04 ; 0.04) [3]	0.961	0.04 (0.02 ; 0.07)	0.001	0.03 (0.00 ; 0.06) [3]	0.036
Triglycerides §	-2.7 (-4.5 ; -0.8)	0.006	-0.1 (-2.0 ; 1.7) [3]	0.884	1.6 (-0.1 ; 3.2)	0.058	0.8 (-0.8 ; 2.5) [3]	0.312
Apolipoprotein B (mg/dL)	-0.91 (-7.02 ; 5.20)	0.770	0.35 (-6.21 ; 6.90) [3]	0.918	0.83 (-3.34 ; 5.01)	0.695	-0.52 (-5.01 ; 3.97) [3]	0.820
Glucometabolic markers								
Fasting glucose (mmol/L)	-0.05 (-0.10 ; 0.00)	0.036	-0.01 (-0.05 ; 0.04) [4]	0.777	-0.06 (-0.10 ; -0.02)	0.005	-0.03 (-0.07 ; 0.01) [4]	0.116
Insulin (µU/mL) §	-1.6 (-4.0 ; 0.9)	0.215	0.4 (-2.0 ; 2.7) [4]	0.764	-1.7 (-3.4 ; 0.1)	0.069	-1.1 (-2.7 ; 0.6) [4]	0.201
Adipokines (µU/mL)								
Leptin §	-4.9 (-8.0 ; -1.7)	0.003	-1.8 (-4.4 ; 0.9) [5]	0.198	-2.9 (-5.2 ; -0.5)	0.016	-2.7 (-4.6 ; -0.6) [5]	0.010
Adiponectin §	-2.1 (-4.8 ; 0.6)	0.129	-1.4 (-4.2 ; 1.5) [5]	0.337	-0.5 (-2.4 ; 1.4)	0.598	0.4 (-1.6 ; 2.5) [5]	0.671
Inflammatory markers								
hs-CRP (mg/L) §	-9.6 (-13.5 ; -5.5)	<0.001	-6.8 (-10.7 ; -2.8) [5]	0.001	-4.7 (-7.6 ; -1.8)	0.002	-3.2 (-6.1 ; -0.2) [5]	0.039
IL-6 (pg/mL) §	-1.1 (-6.6 ; 4.8)	0.713	-1.1 (-7.0 ; 5.2) [5]	0.730	-5.4 (-9.1 ; -1.5)	0.007	-4.1 (-8.2 ; 0.1) [5]	0.055
TNF-α (pg/mL) §	-0.4 (-4.0 ; 3.3)	0.828	1.2 (-2.6 ; 5.3) [5]	0.534	-2.1 (-4.5 ; 0.4)	0.094	-1.2 (-3.8 ; 1.5) [5]	0.392
Homocysteine (µmol/L) §	-2.9 (-4.0 ; -1.7)	<0.001	-0.6 (-1.8 ; 0.7) [5]	0.359	-1.1 (-2.0 ; -0.2)	0.019	0.6 (-0.4 ; 1.6) [5]	0.212
Uric acid (µmol/L)	-3.93 (-6.92 ; -0.94)	0.010	1.12 (-1.79 ; 4.04) [5]	0.449	0.53 (-1.75 ; 2.80)	0.650	0.84 (-1.52 ; 3.21) [5]	0.485

hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha. Statistical analyses performed using linear regression. Results are expressed as effect of a 5 kg increase in grip strength and (95% confidence interval). §, on log-transformed data; results are expressed as % change of the risk marker related to a 5 kg increase in grip strength. Multivariate adjustment for age, current smoking, leisure-time physical activity and occupational physical activity, with a further adjustment on [1] weight; [2] body mass index and antihypertensive drug treatment; [3] body mass index and lipid lowering drug treatment; [4] body mass index and antidiabetic drug treatment; [5] body mass index.

DISCUSSION

This study assessed the associations between grip strength and a large panel of CV risk markers in a population-based setting. Our results suggest that grip strength is only moderately associated with CV risk markers and CV absolute risk. Thus, the reported associations between grip strength and CV disease might not be mediated via those CV risk markers.

Grip strength, anthropometric and adiposity-related markers

Grip strength was negatively associated with WC and fat mass in both genders, and positively with BMI in men. The negative association with WC is consistent with a large cross-sectional population-based study (8) but not with another including older participants (10). Fitness and regular exercise have been shown to improve body composition by reducing fat mass (19, 20), but the effect of grip strength on CV mortality has also been suggested to be independent of body composition (21). According to a large 8.3-year follow-up study (22), muscle strength (measured using bench and leg press tests) showed a strong inverse prediction of excessive WC and fat mass after adjusting for fitness. The results suggest that grip strength is negatively related to body fat and positively to BMI, possibly due to the larger muscle mass of overweight and obese subjects. Still, the changes in WC, fat mass and BMI induced by 5 kg change in grip strength were modest (1.2 cm, 1.2% and 0.30 kg/m^2, respectively) at the individual level.

A negative association between grip strength and leptin was found in men but not in women, and no association was observed for adiponectin. These findings are partly in agreement with a cross-sectional study (10) where no association was found between grip strength and adiposity-related hormones. Exercise has been shown to decrease leptin levels (23) but not adiponectin levels (23). Overall, our results suggest that grip strength is moderately associated with leptin levels in men, but further studies should be conducted to confirm this association.

Grip strength, blood pressure, lipids and glucometabolic markers

On multivariate analysis, no significant association was found between grip strength and BP levels. These findings are in agreement with a recent cross-sectional study (10) but not with another (8). Fitness and regular exercise have been shown to decrease BP levels (24), while muscle strength (measured using bench and leg press tests) showed no effect on 19-year incidence of hypertension after adjustment for fitness (25). Overall, our results suggest that grip strength is not associated with BP levels, or that the association is too small to be detected using our sample size.

In both genders, an inverse U-shaped association between grip strength and total and LDL-cholesterol was found, this association being more prominent in women. Conversely, no association was found between grip strength and HDL-cholesterol, triglycerides and apolipoprotein B. These findings are partly in agreement with a cross-sectional study (10) which found no association between grip strength and triglycerides, total and HDL-cholesterol. The inverse U-shaped association between grip strength and total and LDL cholesterol might be explained by two differing phenomena: first, increased fitness is associated with an improved lipids profile (19), which would explain the negative association between high grip strength values and lipid levels on the right hand side of the curve. Second, low lipid levels have been associated with mortality in an elderly cohort (26); as low grip strength is also associated with increased mortality, this would explain the positive association between grip strength and lipid levels on the left hand side of the curve. Thus, our results suggest that grip strength has a complex association with the lipid profile, high values of grip strength being associated with a “beneficial” low lipid profile, while low values of grip strength are associated with a “deleterious” low lipid profile. Nevertheless, these findings should be further confirmed in other studies.

No association was found between grip strength and fasting glucose and insulin, a finding in agreement with two cross-sectional studies (8, 10). Fitness and regular exercise have been shown to improve glucose profile (19, 27) while muscle strength showed no beneficial effects on glucose levels after adjustment for fitness (28). The results suggest that grip strength is not associated with glucose metabolism or that the association is too small to be detected using the current sample size.

Grip strength and inflammation

Grip strength was negatively associated with hs-CRP levels, a finding in agreement with the literature (9, 10). Fitness and regular exercise decrease CRP levels (29), probably by a decrease in adiposity levels and adiposity-related inflammation. Indeed, a previous study (30) showed an association between poor muscle quantity and quality (i.e. fat deposition in skeletal muscle) and adiposity-related inflammation. Conversely, the association between grip strength and IL-6 or TNF-α is still a matter of debate : some studies reported a negative association (9, 31) while others reported no association (10). Thus, our findings confirm that grip strength is negatively associated with hs-CRP levels, but not with IL-6 or TNF-α. Still, the change in CRP levels were moderate (8.5% decrease per 5 kg increase in grip strength) compared for example to the reduction induced by statin treatment (32). Thus, whether decrease in CRP levels due to grip strength is clinically significant remains to be assessed.

Grip strength, homocysteine and uric acid

A U-shaped association between grip strength and homocysteine was found in men. Low grip strength was associated with high homocysteine levels, a finding also reported in a recent review (33), while the high homocysteine levels found among subjects with high grip strength deserve further clarification. Finally, no clear association was found between grip strength and uric acid levels, a finding in agreement with the literature (34).

Grip strength and cardiovascular absolute risk

Grip strength was negatively associated with CV absolute risk in both genders, a finding in agreement with the beneficial effects of fitness (11) and muscle strength (7) on CV mortality.

Study strengths and limitations

This is one of the largest studies assessing the associations between grip strength and a wide panel of cardiovascular risk markers. Importantly, the specific effects of grip strength were separated from those of LTPA and OPA.

This study also has several limitations worth acknowledging. Firstly, grip strength was assessed on the right hand whereas approximately 8% of our participants were left-handed. However, it has been shown that grip strength does not differ between dominant and non-dominant hands in left-handed people (5). Secondly, the cross-sectional design of our study precludes the assessment of any causal effect of grip strength on CV risk markers; the ongoing follow-up of the CoLaus participants will enable assessing the prospective effects of grip strength on CV risk markers. Thirdly, only participants aged between 50 and 75 were included, so our findings cannot be extrapolated to younger or older ages. Finally, most of the associations between grip strength and CV risk markers were weak, suggesting that grip strength might exert its effect on CV disease via other pathways, such as changes in endothelial function or autonomic nervous system.

Conclusion

In a population-based sample aged between 50 and 75 years, grip strength was only moderately associated with some CV risk markers. Thus, the reported associations between grip strength and CV disease might not be mediated via CV risk markers.

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SUPPLEMENTARY MATERIAL

Supplementary table 1: socio-demographic and clinical characteristics of excluded and included participants.

	Included	**Excluded**	**P-value**
N	3468	82	
Right-handedness (%)	91.6	79.0	<0.001
Grip strength (kg)	33.5 ± 10.8	28.2 ± 12.9	<0.001
Age (years)	60.8 ± 6.8	61.3 ± 7.5	0.46
Smoking			0.86
Former (%)	36.6	34.6	
Never (%)	40.2	43.2	
Current (%)	23.2	22.2	
University level (%)	16.3	12.4	0.34
Working			0.88
Full time (%)	46.9	48.2	
Part time (%)	46.8	46.9	
None (%)	6.3	4.9	
Physical job (%)	15.7	21.3	0.18
10-year CV absolute risk (%)	3.3 ± 3.9	3.6 ± 4.5	0.51
Body mass index (kg/m^2)	26.4 ± 4.7	26.4 ± 4.8	0.93
Fat mass (%)	32.1 ± 8.7	33.2 ± 7.0	0.24
Hypertension (%)	50.1	53.7	0.53
Dyslipidemia (%)	41.1	45.1	0.47
Diabetes (%)	9.8	13.4	0.27

CV, cardiovascular. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square for categorical variables or Student's t-test for quantitative variables.

Supplementary table 2: Characteristics of participants, overall and by gender.

	All	Women	Men	P-value
N	3468	1891	1577	
Right-handedness (%)	91.6	92.1	91.1	0.49
Grip strength (kg)	33.5 ± 10.8	26.0 ± 5.4	42.6 ± 8.4	<0.001
Age (years)	60.8 ± 6.8	60.8 ± 6.8	60.7 ± 6.8	0.80
Smoking				<0.001
Former (%)	36.6	29.2	45.5	
Never (%)	40.2	49.4	29.1	
Current (%)	23.2	21.4	25.4	
University level (%)	16.3	11.8	21.7	<0.001
Working				<0.001
Full time (%)	46.9	39.4	55.8	
Part time (%)	46.8	54.4	37.7	
None (%)	6.3	6.2	6.5	
Physical job (%)	15.7	13.0	18.9	<0.001
10-year CV absolute risk (%)	3.3 ± 3.9	2.3 ± 3.1	4.6 ± 4.5	<0.001
Anthropometry				
Body mass index (kg/m^2)	26.4 ± 4.7	25.8 ± 5.0	27.1 ± 4.1	<0.001
Waist circumference (cm)	91.5 ± 13.6	85.9 ± 12.8	98.2 ± 11.3	<0.001
Fat mass (%)	32.1 ± 8.7	37.0 ± 7.7	26.1 ± 5.4	<0.001
Lean mass (%)	67.9 ± 8.7	63.0 ± 7.7	73.9 ± 5.4	<0.001
Blood pressure				
Systolic BP (mmHg)	133.7 ± 18.5	130.8 ± 18.7	137.1 ± 17.6	<0.001
Diastolic BP (mmHg)	80.7 ± 10.9	79.1 ± 10.6	82.8 ± 11.0	<0.001
Hypertension (%)	50.1	43.3	58.3	<0.001
Lipid markers				
Total cholesterol (mmol/L)	5.8 ± 1.0	5.9 ± 1.0	5.6 ± 1.0	<0.001
HDL-cholesterol (mmol/L)	1.7 ± 0.5	1.8 ± 0.5	1.5 ± 0.4	<0.001
LDL-cholesterol (mmol/L)	3.5 ± 0.9	3.5 ± 0.9	3.4 ± 0.9	<0.001
Triglycerides §	1.4 ± 1.0	1.3 ± 0.7	1.7 ± 1.3	<0.001
Apolipoprotein B (mg/dL)	182.1 ± 140.0	182.4 ± 141.7	181.7 ± 137.9	0.90
Dyslipidemia (%)	88.9	89.3	88.3	0.36
Glucometabolic markers				
Fasting glucose (mmol/L)	5.7 ± 1.3	5.5 ± 1.1	6.0 ± 1.4	<0.001
Insulin (µU/mL) §	9.2 ± 6.4	8.3 ± 5.4	10.2 ± 7.3	<0.001
Diabetes (%)	9.8	5.7	14.6	<0.001
Adipokines				
Leptin (µU/mL) §	14.2 ± 11.1	18.0 ± 12.0	9.5 ± 7.7	<0.001
Adiponectin (µU/mL) §	10767 ± 8610	13213 ± 9754	7860 ± 5801	<0.001
Inflammatory markers				
hs-CRP (mg/L) §	2.7 ± 3.6	2.8 ± 3.8	2.6 ± 3.4	0.20
IL-6 (pg/mL) §	9.0 ± 105.4	9.1 ± 128.1	8.8 ± 69.2	<0.001
TNF-α (pg/mL) §	5.3 ± 18.0	5.6 ± 23.2	4.9 ± 8.2	0.25
Homocysteine (µmol/L) §	11.0 ± 4.7	10.0 ± 3.3	12.2 ± 5.7	<0.001
Uric acid (µmol/L)	324.3 ± 85.0	286.3 ± 71.0	369.8 ± 77.6	<0.001

CV, cardiovascular. §, on log-transformed data. Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square or Student's t-test.

Supplementary table 3: Comparison between the linear and the quadratic model for the associations between grip strength and selected cardiovascular risk markers.

	Grip strength	Grip strength^2	Likelihood ratio §§	P-value
Men				
Total cholesterol, linear model	0.082 [1]	-	3.88	0.049
Total cholesterol, quadratic	0.367 [1] *	-0.288 [1]		
LDL cholesterol, linear model	0.055 [1]	-	3.54	0.060
LDL cholesterol, quadratic	0.345 [1] *	-0.293 [1]		
Homocysteine §, linear model	0.032	-	7.11	0.008
Homocysteine §, quadratic	-0.488 *	0.526 *		
Women				
Total cholesterol, linear model	0.004 [1]	-	9.23	0.002
Total cholesterol, quadratic	0.432 [1] *	-0.434 [1] *		
LDL cholesterol, linear model	0.001 [1]	-	8.22	0.004
LDL cholesterol, quadratic	0.427 [1] *	-0.432 [1] *		
Homocysteine §, linear model	-0.022	-	1.23	0.268
Homocysteine §, quadratic	-0.271	0.252		

§ log-transformed, §§ likelihood ratio test comparing the quadratic to the linear model. Results are expressed as standardized coefficients. Adjustments for age, current smoking, leisure-time physical activity, occupational physical activity and body mass index with a further adjustment on [1] lipid lowering drug treatment; *, $p<0.05$; **, $p<0.001$.

Supplementary table 4: Multivariate-adjusted changes in cardiovascular risk marker levels per 5 kg increase in grip strength, women, stratified by tertile of age.

	All	P-value	First tertile	P-value	Second tertile	P-value	Third tertile	P-value
Anthropometry								
Body mass index (kg/m^2)	0.00 (-0.22 ; 0.22)	0.985	-0.28 (-0.67 ; 0.10)	0.151	-0.01 (-0.39 ; 0.37)	0.946	0.33 (-0.04 ; 0.71)	0.079
Waist circumference (cm)	-0.82 (-1.13 ; -0.52) [1]	<0.001	-1.00 (-1.49 ; -0.51) [1]	<0.001	-0.68 (-1.17 ; -0.18) [1]	0.008	-1.18 (-1.77 ; -0.59) [1]	<0.001
Fat mass (%)	-0.56 (-0.89 ; -0.22)	0.001	-1.21 (-1.78 ; -0.64)	<0.001	-0.27 (-0.86 ; 0.32)	0.362	-0.43 (-0.99 ; 0.13)	0.132
Blood pressure (mmHg)								
Systolic	0.66 (-0.12 ; 1.43) [2]	0.098	-0.60 (-1.71 ; 0.50) [2]	0.284	1.73 (0.33 ; 3.12) [2]	0.015	0.02 (-1.51 ; 1.56) [2]	0.979
Diastolic	0.15 (-0.31 ; 0.61) [2]	0.522	-0.51 (-1.25 ; 0.22) [2]	0.170	0.91 (0.12 ; 1.71) [2]	0.025	0.52 (-0.32 ; 1.37) [2]	0.222
Lipid markers								
Total cholesterol (mmol/L)	0.00 (-0.04 ; 0.05) [3]	0.863	-0.08 (-0.15 ; -0.01) [3]	0.035	0.07 (-0.01 ; 0.15) [3]	0.067	-0.01 (-0.08 ; 0.07) [3]	0.893
HDL-cholesterol (mmol/L)	0.00 (-0.02 ; 0.02) [3]	0.969	-0.02 (-0.05 ; 0.01) [3]	0.277	0.00 (-0.03 ; 0.04) [3]	0.752	0.01 (-0.02 ; 0.05) [3]	0.475
LDL-cholesterol (mmol/L)	0.00 (-0.04 ; 0.04) [3]	0.961	-0.06 (-0.13 ; 0.01) [3]	0.071	0.05 (-0.02 ; 0.12) [3]	0.147	-0.01 (-0.08 ; 0.07) [3]	0.877
Triglycerides (mmol/L) §	-0.1 (-2.0 ; 1.7) [3]	0.884	-0.6 (-3.6 ; 2.6) [3]	0.719	1.7 (-1.5 ; 5.0) [3]	0.306	-2.4 (-5.6 ; 0.9) [3]	0.154
Apolipoprotein B (mg/dL)	0.35 (-6.21 ; 6.90) [3]	0.918	-4.67 (-15.60 ; 6.27) [3]	0.402	4.23 (-7.94 ; 16.40) [3]	0.495	0.49 (-10.10 ; 11.08) [3]	0.927
Glucometabolic markers								
Fasting glucose (mmol/L)	-0.01 (-0.05 ; 0.04) [4]	0.777	-0.01 (-0.07 ; 0.06) [4]	0.860	0.02 (-0.06 ; 0.11) [4]	0.615	-0.04 (-0.11 ; 0.03) [4]	0.288
Insulin (µU/mL) §	0.4 (-2.0 ; 2.7) [4]	0.764	0.7 (-3.1 ; 4.6) [4]	0.725	0.5 (-3.6 ; 4.7) [4]	0.828	-0.9 (-4.9 ; 3.3) [4]	0.664
Adipokines (µU/mL)								
Leptin §	-1.8 (-4.4 ; 0.9) [5]	0.198	-3.9 (-7.9 ; 0.4) [5]	0.072	-2.4 (-7.0 ; 2.4) [5]	0.314	1.2 (-3.7 ; 6.4) [5]	0.639
Adiponectin §	-1.4 (-4.2 ; 1.5) [5]	0.337	0.1 (-4.4 ; 4.9) [5]	0.963	-3.6 (-8.4 ; 1.5) [5]	0.161	-0.5 (-5.3 ; 4.5) [5]	0.838
Inflammatory markers								
hs-CRP (mg/L) §	-6.8 (-10.7 ; -2.8) [5]	0.001	-7.7 (-13.9 ; -1.0) [5]	0.024	-3.8 (-10.3 ; 3.3) [5]	0.289	-8.5 (-15.3 ; -1.2) [5]	0.024
IL-6 (pg/mL) §	-1.1 (-7.0 ; 5.2) [5]	0.730	3.4 (-7.3 ; 15.3) [5]	0.552	-5.8 (-15.3 ; 4.7) [5]	0.265	-0.6 (-10.7 ; 10.6) [5]	0.909
TNF-α (pg/mL) §	1.2 (-2.6 ; 5.3) [5]	0.534	3.8 (-3.1 ; 11.1) [5]	0.288	1.9 (-4.5 ; 8.7) [5]	0.574	-4.1 (-10.4 ; 2.6) [5]	0.223
Homocysteine (µmol/L) §	-0.6 (-1.8 ; 0.7) [5]	0.359	0.4 (-1.6; 2.5) [5]	0.682	0.3 (-1.7; 2.3) [5]	0.790	-4.4 (-6.6; -2.1) [5]	<0.001
Uric acid (µmol/L)	1.12 (-1.79 ; 4.04) [5]	0.449	-1.28 (-5.87 ; 3.32) [5]	0.586	4.68 (-0.19 ; 9.56) [5]	0.060	-2.6 (-8.13; 2.99) [5]	0.365

hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha. Statistical analyses performed using linear regression. Results are expressed as effect of a 5 kg increase in grip strength and (95% confidence interval). §, on log-transformed data; results are expressed as % change of the risk marker related to a 5 kg increase in grip strength. Multivariate adjustment for age (not for tertiles of age) current smoking, leisure-time physical activity and occupational physical activity, with a further adjustment on [1] weight; [2] body mass index and antihypertensive drug treatment; [3] body mass index and lipid lowering drug treatment; [4] body mass index and antidiabetic drug treatment; [5] body mass index.

Supplementary table 5: Multivariate-adjusted changes in cardiovascular risk marker levels per 5 kg increase in grip strength, men, stratified by tertile of age.

	All	P-value	First tertile	P-value	Second tertile	P-value	Third tertile	P-value
Anthropometry								
Body mass index	0.22 (0.10 ; 0.35)	<0.001	0.22 (0.01 ; 0.43)	0.038	0.30 (0.10 ; 0.51)	0.004	0.12 (-0.12 ; 0.35)	0.328
Waist circumference	-0.77 (-0.93 ; -0.61) [1]	<0.001	-0.71 (-0.96 ; -0.46) [1]	<0.001	-0.78 (-1.06 ; -0.50) [1]	<0.001	-0.94 (-1.24 ;-0.63) [1]	<0.001
Fat mass (%)	-0.27 (-0.43 ; -0.11)	0.001	-0.17 (-0.45 ; 0.10)	0.215	-0.26 (-0.53 ; 0.02)	0.065	-0.54 (-0.81 ; -0.27)	<0.001
Blood pressure (mmHg)								
Systolic	0.04 (-0.48 ; 0.56) [2]	0.892	0.34 (-0.43 ; 1.11) [2]	0.386	0.21 (-0.67 ; 1.09) [2]	0.642	-1.01 (-2.05 ; 0.03) [2]	0.056
Diastolic	0.29 (-0.05 ; 0.63) [2]	0.090	0.21 (-0.35 ; 0.77) [2]	0.458	0.74 (0.19 ; 1.30) [2]	0.008	0.03 (-0.61 ; 0.66) [2]	0.935
Lipid markers								
Total cholesterol	0.05 (0.02 ; 0.08) [3]	0.002	0.06 (0.00 ; 0.11) [3]	0.033	0.00 (-0.05 ; 0.05) [3]	0.946	0.11 (0.05 ; 0.17) [3]	<0.001
HDL-cholesterol	0.01 (0.00 ; 0.02) [3]	0.257	0.00 (-0.01 ; 0.02) [3]	0.630	0.01 (-0.01 ; 0.02) [3]	0.531	0.01 (-0.01 ; 0.03) [3]	0.527
LDL-cholesterol	0.03 (0.00 ; 0.06) [3]	0.036	0.04 (-0.01 ; 0.08) [3]	0.091	-0.03 (-0.08 ; 0.02) [3]	0.237	0.09 (0.04 ; 0.14) [3]	<0.001
Triglycerides	0.8 (-0.8 ; 2.5) [3]	0.312	0.5 (-2.3 ; 3.5) [3]	0.712	1.4 (-1.3 ; 4.2) [3]	0.311	0.6 (-2.1 ; 3.4) [3]	0.656
Apolipoprotein B	-0.52 (-5.01 ; 3.97) [3]	0.820	-4.33 (-12.63 ; 3.96) [3]	0.305	-2.09 (-8.60 ; 4.37) [3]	0.525	4.67 (-3.72 ; 13.06) [3]	0.275
Glucometabolic markers								
Fasting glucose	-0.03 (-0.07 ; 0.01) [4]	0.116	0.00 (-0.06 ; 0.05) [4]	0.944	-0.07 (-0.14 ; -0.01) [4]	0.029	-0.02 (-0.10 ; 0.06) [4]	0.621
Insulin (µU/mL) §	-1.1 (-2.7 ; 0.6) [4]	0.201	-3.2 (-6.0 ; -0.3) [4]	0.029	-0.1 (-2.9 ; 2.7) [4]	0.934	-0.3 (-3.2 ; 2.6) [4]	0.814
Adipokines (µU/mL)								
Leptin §	-2.7 (-4.6 ; -0.6) [5]	0.010	-3.9 (-7.3 ; -0.4) [5]	0.031	-0.3 (-3.5 ; 3.0) [5]	0.859	-4.9 (-8.4 ; -1.4) [5]	0.007
Adiponectin §	0.4 (-1.6 ; 2.5) [5]	0.671	-0.2 (-3.5 ; 3.2) [5]	0.904	-1.3 (-4.6 ; 2.1) [5]	0.437	2.1 (-1.5 ; 5.9) [5]	0.252
Inflammatory markers								
hs-CRP (mg/L) §	-3.2 (-6.1 ; -0.2) [5]	0.039	-1.1 (-5.9 ; 3.9) [5]	0.657	-2.1 (-7.0 ; 3.10) [5]	0.416	-8.1 (-13.3 ; -2.6) [5]	0.004
IL-6 (pg/mL) §	-4.1 (-8.2 ; 0.1) [5]	0.055	-2.3 (-9.2 ; 5.1) [5]	0.534	-1.9 (-9.0 ; 5.7) [5]	0.611	-9.0 (-15.5 ; -1.9) [5]	0.013
TNF-α (pg/mL) §	-1.2 (-3.8 ; 1.5) [5]	0.392	-2.2 (-6.2 ; 2.1) [5]	0.308	-0.5 (-4.9 ; 4.0) [5]	0.811	-0.4 (-5.5 ; 4.9) [5]	0.869
Homocysteine (µmol/L) §	0.6 (-0.4 ; 1.6) [5]	0.212	2.1 (0.5 ; 3.7) [5]	0.009	0.3 (-1.4 ; 2.0) [5]	0.741	-1.9 (-3.7 ; 0.0) [5]	0.050
Uric acid (µmol/L)	0.84 (-1.52 ; 3.21) [5]	0.485	1.80 (-1.92 ; 5.52) [5]	0.342	0.34 (-3.84 ; 4.52) [5]	0.873	-0.40 (-4.78 ; 3.98) [5]	0.858

hs-CRP, high sensitivity C-reactive protein; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha. Statistical analyses performed using linear regression. Results are expressed as effect of a 5 kg increase in grip strength and (95% confidence interval). §, on log-transformed data; results are expressed as % change of the risk marker related to a 5 kg increase in grip strength. Multivariate adjustment for age (not for tertiles of age), current smoking, leisure-time physical activity and occupational physical activity, with a further adjustment on [1] weight; [2] body mass index and antihypertensive drug treatment; [3] body mass index and lipid lowering drug treatment; [4] body mass index and antidiabetic drug treatment; [5] body mass index.

Supplementary table 6: Multivariate associations between grip strength and selected cardiovascular risk markers using quadratic model, stratified by tertile of age.

	All		**First tertile**		**Second tertile**		**Third tertile**	
	Grip strength	Grip strength^2	Grip strength	Grip strength^2	Grip strength	Grip strength^2	Grip strength	Grip strength^2
Men								
Total cholesterol	0.367 [1] *	-0.288 [1]	0.090 [1]	0.010 [1]	0.483 [1]	-0.497 [1]	0.456 [1]	-0.315 [1]
LDL cholesterol	0.345 [1] *	-0.293 [1]	0.046 [1]	0.034 [1]	0.551 [1]	-0.622 [1] *	0.304 [1]	-0.160 [1]
Homocysteine §	-0.488 *	0.526 *	-0.228	0.350	-0.305	0.341	-0.745 *	0.712 *
Women								
Total cholesterol	0.432 [1] *	-0.434 [1] *	0.694 [1] *	-0.773 [1] *	0.023 [1]	0.059 [1]	0.302 [1]	-0.314 [1]
LDL cholesterol	0.427 [1] *	-0.432 [1] *	0.662 [1] *	-0.729 [1] *	0.065 [1]	-0.004 [1]	0.294 [1]	-0.304 [1]
Homocysteine §	-0.271	0.252	0.050	-0.016	-0.033	0.056	-0.619 *	0.501

§ log-transformed. Statistical analyses performed using quadratic regression model. Results are expressed as standardized coefficients. Adjustments for age, current smoking, leisure-time physical activity, occupational physical activity and body mass index with a further adjustment on [1] lipid lowering drug treatment; *, $p<0.05$; **, $p<0.001$.

Chapter 8

Association of grip strength with incident cardiovascular events

Based on **Gubelmann C**, Vollenweider P, Marques-Vidal P. No association between grip strength and cardiovascular risk: The CoLaus population-based study. *International Journal of Cardiology.* 2017;236:478-482.

ABSTRACT

Background: Decreased grip strength (GS) is predictive of cardiovascular (CV) disease but whether it improves CV risk prediction has not been evaluated. We assessed the predictive value of low GS on incident CV events and overall mortality taking into account CV risk equations in a population-based study from Switzerland.

Methods: 2707 adults (54.8% women, age range 50-75 years) were followed for a median time of 5.4 years. GS was assessed using a hydraulic hand dynamometer. CV absolute risk at baseline was assessed using recalibrated SCORE, Framingham and PROCAM risk equations. Incident CV events were adjudicated by an independent committee.

Results: 160 deaths and 188 incident CV events occurred during follow-up. On bivariate analysis, low GS was associated with increased incident CV events: Hazard Ratio (HR) and (95% confidence interval) 1.76 (1.13-2.76), p<0.01 but not with overall mortality: HR=1.51 (0.94-2.45), p=0.09. The association between low GS and incident CV events disappeared after adjusting for baseline CV risk: HR=1.23 (0.79-1.94), p=0.36; 1.34 (0.86-2.10), p=0.20 and 1.47 (0.94-2.31), p=0.09 after adjusting for SCORE, Framingham and PROCAM scores, respectively.

Conclusion: Low GS is not predictive of incident CV events when taking into account CV absolute risk.

INTRODUCTION

Grip strength (GS) has been shown to be inversely associated with risk of incident cardiovascular (CV) events (1, 2) and overall mortality (1, 3). The effect of low GS on CV events might be partly mediated by changes in CV risk factors (4); thus, the analysis of the effect of low GS on CV events and overall mortality should take into account basal CV risk. Basal CV risk can be estimated using equations such as SCORE (5), Framingham (6) and PROCAM (7). Although the associations of GS with incident CV events (1, 2) and overall mortality (1, 3, 8) have been assessed in several longitudinal studies, they were only partially adjusted on CV risk factors. Finally, whether low GS improves the predictive value of the existing CV risk equations remains to be assessed.

Thus, the aim of this study was to assess the predictive value of low GS on CV events incidence and overall mortality, taking into account absolute CV risk at baseline as assessed by SCORE, Framingham or PROCAM equations, in a well-characterised population-based sample from the city of Lausanne, Switzerland (CoLaus study).

METHODS

Recruitment

The detailed description of the recruitment of the CoLaus study has been published previously (9). Briefly, the CoLaus study is a population-based cohort exploring the biological, genetic and environment determinants of CV diseases. A non-stratified, representative sample of the population of Lausanne (Switzerland) was recruited between 2003 and 2006 based on the following inclusion criteria: a) age 35-75 years and b) willingness to participate. Participants aged over 50 years (3704 of the 6733 initially recruited, 55%) were invited to participate in a sub-study on frailty, which included GS assessment.

Grip strength

GS was assessed using the Baseline® Hydraulic Hand Dynamometer and positioning of the participants was done according to the American Society of Hand Therapists's guidelines (10): subject seated, shoulders adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral position and wrist between 0 and 30° of dorsiflexion. Three measurements were performed consecutively with the right hand. Coefficient of variation between measurements was 5.3%. The highest value (expressed in kg) was included in the analyses. Participants were also asked about their handedness. Grip strength was categorized as low or normal according to Fried criterion (11) that takes into account gender and body mass index.

Clinical data

Socio-demographic data such as education level, job position and social help, together with tobacco, leisure-time and occupational physical activity data were collected by questionnaire. Leisure-time physical activity was categorized as <2 or ≥2 periods of ≥20 minutes per week. Occupational activity was categorized as non-physical (when sitting or standing) and physical (carrying light or heavy load). Personal and family history of CV disease was elicited with a standardized interview questionnaire filled in by a trained recruiter. Participants also indicated if they were treated for hypertension, dyslipidemia or diabetes.

Body weight and height were measured to the nearest 0.1 kg and 5 mm, respectively, using a Seca® scale and height gauge (Hamburg, Germany), with participants in light indoor clothes standing without shoes. Waist and hip circumferences were measured as recommended (12) at mid-way between the lowest rib and the iliac crest, and at the greater trochanters, respectively. Blood pressure (BP) was measured using an Omron® HEM-907 automated oscillometric sphygmomanometer (13) after at least 10 minutes' rest in a seated position and the average of the last two measurements was used. Hypertension was defined as a systolic BP ≥140 mmHg and/or a diastolic BP ≥90 mmHg and/or presence of an anti-hypertensive treatment.

Biological data

A fasting venous blood sample was drawn and measurements performed by the clinical laboratory of the Lausanne university hospital. CV risk factors included glucose, total and HDL-cholesterol, triglycerides; LDL-cholesterol was calculated using the Friedewald formula if triglycerides were <4.6 mmol/L. Diabetes was defined by a fasting glucose ≥7.0 and/or presence of antidiabetic drug treatment. Dyslipidemia was defined either by the presence of a hypolipidemic drug or using the LDL-cholesterol thresholds according to the PROCAM CV score (7) adapted for Switzerland (14).

Cardiovascular risk assessment

CV risk was calculated using internationally used risk equations. As there is no consensus regarding which risk equation to use in Switzerland (15), we opted for the three most used equations: the European Society of Cardiology SCORE (5), Framingham-2001 (6) and PROCAM-2007 (7). Framingham-2001 and SCORE have been recalibrated (16, 17) and validated on the Swiss population (17, 18). The SCORE, Framingham 2001 and PROCAM 2007 risk equations use age, gender, parental history, smoking, blood pressure, lipids and diabetes data to compute the 10-year absolute risk of CV death, coronary heart disease (CHD) and CV events, respectively. Participants were categorized as low, medium, high or very high CV risk according to cutoffs shown in **Supplementary Table 1**. Participants with previous history of CV disease were considered at very high CV risk.

Outcomes

Outcomes of interest were CV events and overall deaths. CV events included cerebrovascular events (CBV) and CHD. CBV events were defined as transient ischemic attack, ischemic or hemorrhagic stroke, *amaurosis fugax* and transient global amnesia. CHD events were defined as myocardial infarction, stable or unstable angina, coronary revascularization or bypass grafting. Outcomes were first verified and medically documented by a trained investigator, and further validated using pre-defined criteria by an independent adjudication committee composed of internists, cardiologists and a neurologist.

Exclusion criteria

Participants were excluded if they presented a questionable GS or if no follow-up data were available. Questionable GS values were considered if the participant reported any condition precluding adequate measurement (i.e. pain, injury, recent surgery, osteoarthritis and rheumatoid arthritis, among others), irrespectively of the observed value.

Statistical analysis

Statistical analyses were conducted using Stata version 14.0 for windows (Stata Corp, College Station, Texas, USA). Descriptive analyses were expressed as number of participants (percentage) for categorical variables or as average ± standard deviation for continuous variables. Between-group comparisons were performed using chi-square and Student t-test for categorical and continuous variables, respectively.

The effect of low GS on incident CV events and overall mortality was assessed using Cox proportional hazards models and results were expressed as hazard ratio (HR) and 95% confidence interval (95%CI). Bivariate and multivariate analyses were performed, and the following multivariate models were used: 1) adjusted on age and gender; ; 2) age, gender, education level, job position and social help; 3) age, gender, education level, job position, social help, waist-to-hip ratio and height; 4) adjusted on absolute CV risk according to SCORE; 5) adjusted on absolute CV risk according to Framingham 2001, and 6) adjusted on absolute CV risk according to PROCAM 2007. Adjustments on CV risk factors' treatment were also performed. To take into account the decline in muscular performance occurring with age, sensitivity analyses were performed by further stratifying on tertiles of age. Statistical significance was assessed for a two-sided test with $p<0.05$.

Power analysis was conducted using the **power cox** function of Stata. The following parameters were calculated: 1) power to consider the observed HR as statistically significant at p=0.05; 2) the minimum sample size to consider the observed HR as statistically significant at a power of 0.80 and p=0.05, and 3) the minimum detectable HR taking into account a sample size of 2707, 160 deaths and 188 incident CV events, a power of 0.80 and p=0.05. Power analyses were not performed if the observed HR was less than 1.

Ethical statement

The institutional Ethics Committee of the University of Lausanne (19) approved the baseline CoLaus study (protocol reference 16/03, decisions of 13th January and 10th February 2003) and the approval was renewed for its follow-up (protocol reference 33/09, decision of 23rd February 2009). All participants gave their signed informed consent before entering the study.

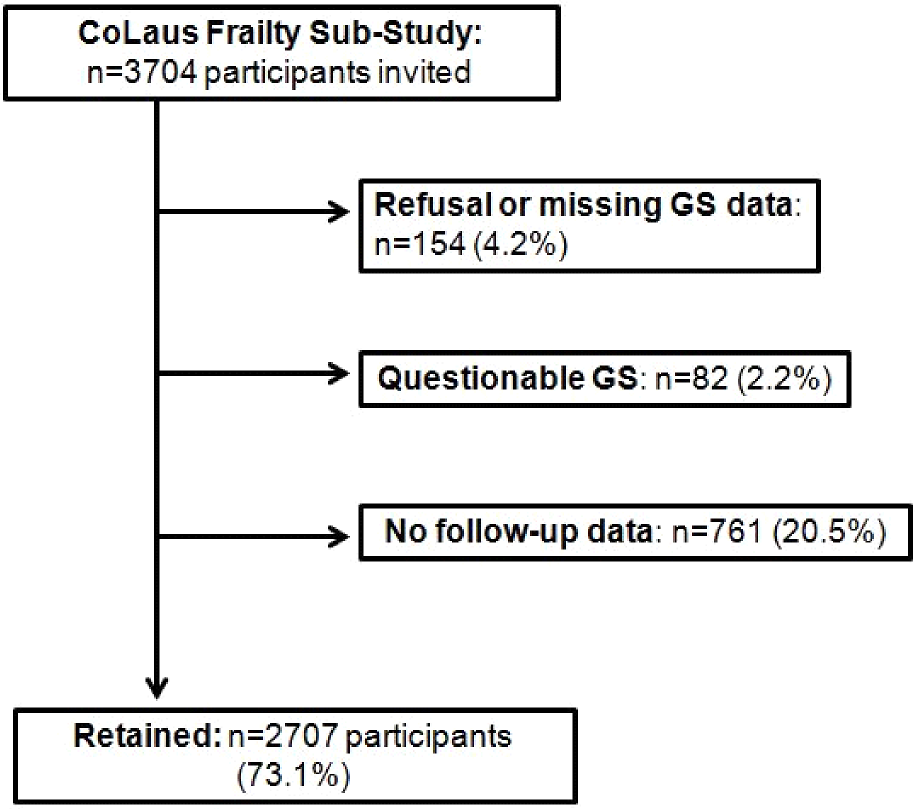
RESULTS

Characteristics of included and excluded participants

The selection procedure is indicated in **Figure 1.** Of the initial 3704 participants aged 50 and over, 2707 (73.1%) were retained for analysis. The characteristics of the included and excluded participants are summarized in **Supplementary Table 2**. Included participants were more likely right-handed and to perform leisure-time physical activity, more educated, had a higher job position and were less prone to smoke, to receive social help, to present with hypertension or dyslipidemia than excluded ones. No association was found in absolute CV risk using SCORE and Framingham risk equations, whereas excluded participants had slightly higher CV risk according to the PROCAM risk equation.

Participants' characteristics overall and according to GS category are summarized in **Table 1**. Participants with a low GS were older, less likely to have a high education level, working or performing leisure-time physical activity. Participants with a low GS were also more likely to receive social help and had a higher baseline absolute CV risk. GS values according to gender are represented in **Supplementary Figure 1**. Mean±standard deviation GS were 26.1±5.3 kg for women and 42.7±8.4 kg for men.

Figure 1: Selection procedure. CoLaus Study, Lausanne, Switzerland, 2003-2012.



GS: grip strength. Percentages were calculated using the baseline sample size as denominator.

Table 1: Characteristics of participants, overall and by grip strength categories. CoLaus Study, Lausanne, Switzerland, 2003-2012.

	All	Normal	Low	P value
N	2707	2521	186	
Right-handedness (%)	92.0	91.9	93.2	0.52
Grip strength (kg)	33.6 ± 10.7	34.5 ± 10.5	21.7 ± 6.5	<0.01
Age (years)	60.7 ± 6.8	60.4 ± 6.7	64.5 ± 7.0	<0.01
Female (%)	54.8	55.0	51.6	0.37
Smoking (%)				0.42
Current	22.9	23.2	19.4	
Never	39.1	38.8	42.5	
Former	38.0	38.0	38.2	
Physical job (%)	15.2	15.2	14.1	0.67
Weekly leisure-time physical activity				<0.01
<2 periods of 20+ minutes	42.2	41.4	53.2	
≥2 periods of 20+ minutes	57.8	58.6	46.8	
Living alone (%)	35.1	34.9	38.2	0.37
Education level (%)				<0.01
Low	58.5	57.7	69.4	
Middle	24.5	24.9	19.4	
High	17.0	17.4	11.3	
Job position (%)				<0.01
Low	12.7	12.4	16.7	
Middle	33.8	35.1	15.1	
High	10.7	11.2	4.8	
Not working	42.9	41.3	63.4	
Receiving social help (%)	30.0	28.1	55.4	<0.01
Risk categories (SCORE) (%)				<0.01
Low	41.3	42.6	24.3	
Medium	14.3	14.4	12.4	
High	16.7	17.1	11.9	
Very high	27.7	25.9	51.4	
Risk categories (Framingham) (%)				<0.01
Low	75.8	76.8	61.8	
Medium	10.1	10.0	11.3	
High	3.7	3.6	5.9	
Very high	10.4	9.6	21.0	
Risk categories (PROCAM) (%)				<0.01
Low	55.7	56.7	43.3	
Medium	20.4	20.1	23.3	
High	10.5	10.7	7.8	
Very high	13.5	12.6	25.6	

Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square or Student's t-tests comparing normal and low grip strength categories.

Association of grip strength with outcomes

During a median follow-up time of 5.4 years, there were 160 deaths and 188 incident CV events. Survival curves for all causes and CV events according to GS category are shown in **Supplementary Figure 2**. Five-year overall survival was 96.9% (95% confidence interval: 96.1-97.5) and 93.5% (88.9-96.3) for normal and low GS (P value: 0.09), respectively. Five-year CV events-free survival was 95.5% (94.6-96.3) and 89.0% (83.4-92.7) for normal and low GS (P value: 0.01), respectively.

The unadjusted and multivariate-adjusted associations between low GS and overall mortality or incident CV events are described in **Table 2**. Unadjusted analyses showed that low GS was associated with a higher incidence of CV events, while no association was found with overall mortality. The association between low GS and incident CV events was no longer significant after multivariate adjustment (**Table 2**). Results did not change after adjustment on CV risk factors' treatment (**Supplementary Table 3**) or after stratification by tertiles of age (**Supplementary Tables 4** and **5**).

Table 2: Association between low grip strength, overall mortality and incident cardiovascular events, unadjusted and multivariate-adjusted. CoLaus Study, Lausanne, Switzerland, 2003-2012.

	Overall mortality			Incident cardiovascular events		
	HR	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**
Unadjusted	1.51	0.94-2.45	0.09	1.76	1.13-2.76	0.01
Model 1	1.15	0.71-1.88	0.57	1.22	0.78-1.93	0.39
Model 2	1.08	0.66-1.77	0.75	1.07	0.68-1.70	0.76
Model 3	0.98	0.59-1.63	0.95	0.96	0.60-1.55	0.87
Model 4	1.13	0.69-1.85	0.62	1.23	0.79-1.94	0.36
Model 5	1.40	0.86-2.27	0.17	1.34	0.86-2.10	0.20
Model 6	1.40	0.86-2.27	0.18	1.47	0.94-2.31	0.09

Results are expressed as Hazard Ratio (HR) and 95% confidence interval (CI) for low grip strength using normal grip strength as the reference. Statistical analyses performed by Cox proportional hazard model, unadjusted and adjusted for: 1) age and gender; 2) age, gender, education level, job position and social help; 3) age, gender, education level, job position, social help, waist-to-hip ratio and height; 4) absolute CV risk according to SCORE risk equation; 5) absolute CV risk according to Framingham 2001 risk equation, and 6) absolute CV risk according to PROCAM 2007 risk equation.

DISCUSSION

This study assessed the impact of low GS on overall mortality and incident CV events in a prospective, population-based sample with a median 5.4-year follow-up time. Our results suggest that the association between low GS and incident CV events is no longer significant after adjusting for baseline absolute CV risk. Thus, GS measurement does not seem to be useful in assessing CV risk beyond traditional CV risk estimation equations.

Grip strength and incident cardiovascular events

Low GS was significantly associated with an increase in incident CV events on bivariate analysis, but this association disappeared after multivariate adjustment. These findings are in agreement with the study by Fujita et al. from Japan (20). However our results differ from those of the PURE study (1). It has to be mentioned that in the latter study, GS was reported as 5-kg decrease and not dichotomized in low and normal, and furthermore CV risk factors were self-reported. Discrepancies could therefore possibility result from those methodological aspects. Other longer follow-up studies (2, 3, 21, 22) also showed an inverse association between different markers of GS (i.e. standard deviation, deciles or tertiles) and incident CV events, after adjustment on a small number of CV risk factors. Thus, several studies have shown an inverse association between GS and incident CV events, but the results are difficult to apply in a clinical setting as different metrics for GS have been used and no threshold below which the CV risk can be considered as increased was suggested. Similarly, although several studies (1, 22) adjusted the results for gender, this adjustment might not have cancelled out the considerable difference in GS levels between genders. In this study, we assessed whether a common definition of low GS was associated with incident CV events. Our results suggest that the effect of low GS on incident CV events is mediated by CV risk factors, as the association disappears after adjusting for absolute CV risk. Still, it would be of interest to replicate our study in other population-based samples, in order to confirm or infirm if a low GS is associated with incident CV events independently of the other CV risk factors.

Grip strength and overall mortality

Low GS was associated with overall mortality neither on bivariate, nor on multivariate analysis. These findings are partially in agreement with two studies (20, 22) showing similar results for women though not for men but it has been contradicted by other studies (1, 3, 8, 21) showing that different markers of GS were negatively associated with overall mortality. A possible explanation might be the relatively short follow-up time in our sample, or the fact that we adjusted for absolute CV risk while the other studies only adjusted on self-reported (1) or on a limited number of CV risk factors (3, 8, 21). Overall, our results suggest that low GS has no impact on overall mortality when absolute CV risk is taken into account.

Study limitations

This study has several limitations worth acknowledging. Firstly, GS was assessed on the right hand whereas approximately 7% of our participants were left-handed. Although the use of the non-dominant hand might lead to lower GS values, most studies reported no difference (23-25), while some reported slightly higher values for the dominant compared to the non-dominant hand (26, 27). Thus, GS measurement at the right hand irrespective of handedness will have a limited impact on the observed values. Secondly, the exclusion of questionable GS was based on self-reported information given by the participant (i.e. condition that may preclude adequate measurement), and did not rely on objective criteria. However, including all GS measurements led to similar conclusions for overall mortality and partially for incident CV events, for which small significant positive associations ($p<0.05$) were found after adjustment for Framingham or PROCAM risk equations (see **Supplementary Table 6**). Still, the p-values would not resist Bonferroni correction, and the PROCAM risk equation hasn't been validated for the Swiss population. Thirdly, some events such as *amaurosis fugax* (AF) and transient global amnesia (TGA) might be wrongly reported as CV. Still, in this study, AF (N=1) and TGA (N=4) represented only 2.7% of CV events, so that the impact of a possible ascertainment bias is low. Further, excluding AF and TGA events led to similar conclusions (see **Supplementary Table 7**). Fourthly, our sample size and follow-up time period are relatively small for our low-risk population. However, on the whole sample, power calculations showed that the overall power to consider the bivariate and multivariate-adjusted HR as significant was higher than 70% in most cases (**Table 3**). The ongoing follow-up of the CoLaus study will enable assessing the 10-year outcomes of the participants. Fifthly, one-fifth of the participants did not participate to follow-up, but this participation rate is comparable to the literature (5), and loss to follow-up has only limited impact on relative risks for exposure-risk associations (28). Sixthly, our data have been collected between 2003 and 2012, whereas some previous findings' data were collected before 2000 (2, 22, 29). At this time, the incidence of fatal CV events was higher (30), which might have allowed to demonstrate the association between GS and incident CV events.

Finally, only participants aged between 50 and 75 were included, so our findings cannot be extrapolated to other ages.

Table 3: Power analyses for the results indicated in table 2. CoLaus Study, Lausanne, Switzerland, 2003-2012.

	Overall mortality			Incident cardiovascular events		
	Power	**MSS**	**MDHR**	**Power**	**MSS**	**MDHR**
Unadjusted	0.899	5,722	1.82	0.966	2,225	1.67
Model 1	0.719	80,981	2.15	0.756	36,694	2.08
Model 2	0.657	308,097	2.27	0.659	397,587	2.27
Model 4	0.689	113,599	2.21	0.756	33,857	2.08
Model 5	0.866	9,593	1.88	0.836	13,820	1.94
Model 6	0.866	9,593	1.88	0.896	6,630	1.83

Results are expressed as power to consider the observed HR>1 as statistically significant at p=0.05; the minimum sample size (MSS) to consider the observed HR>1 as statistically significant at a power of 0.80 and p=0.05, and the minimum detectable HR (MDHR) taking into account a sample size of 2707, 160 deaths and 188 incident CV events, a power of 0.80 and p=0.05. Calculations using the **power cox** function of Stata. Power analyses were not performed for model 3 as the observed HR were less than 1.

Conclusion

In a prospective, population-based sample aged 50 to 75 years, low GS was associated neither with overall mortality nor with incident CV events when adjusting for absolute CV risk.

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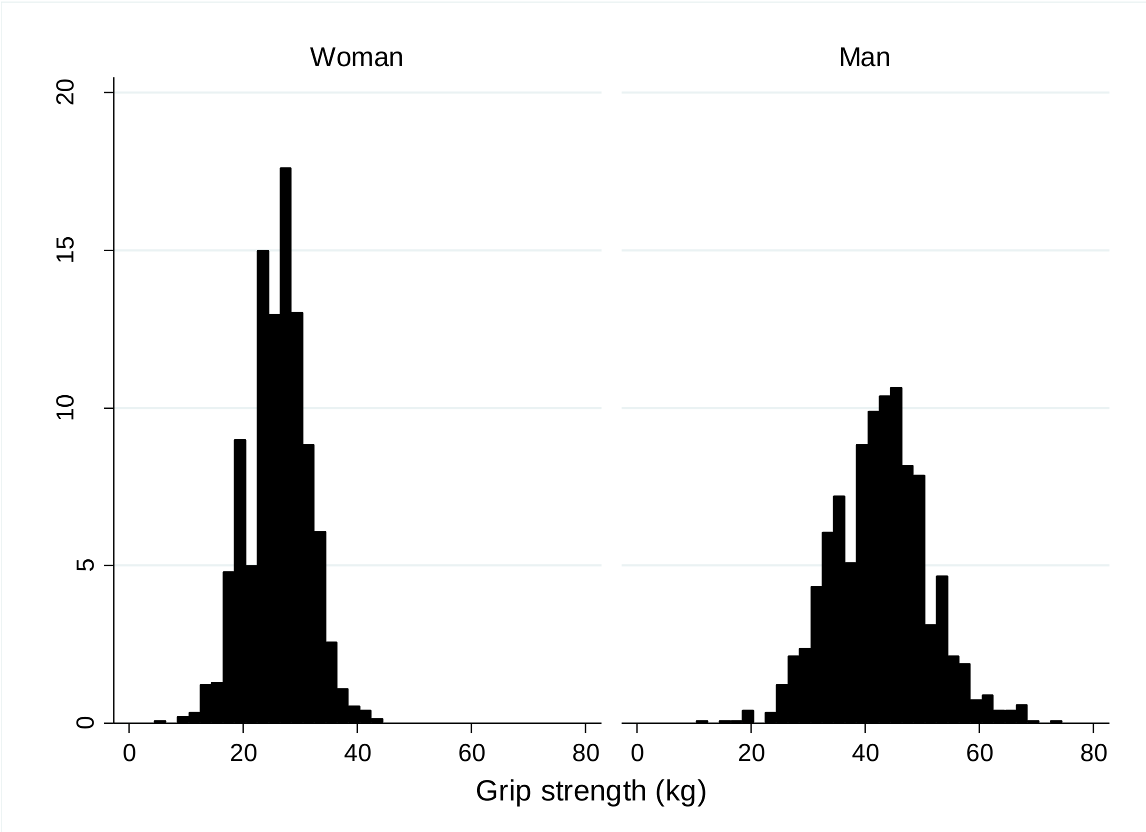
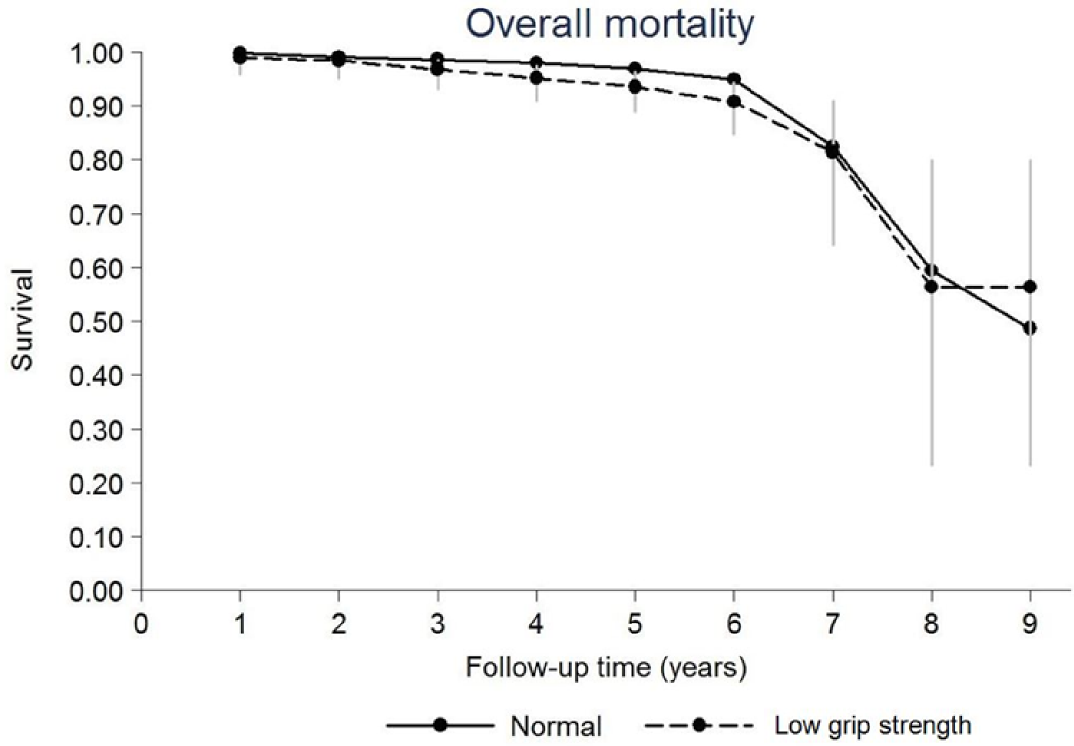
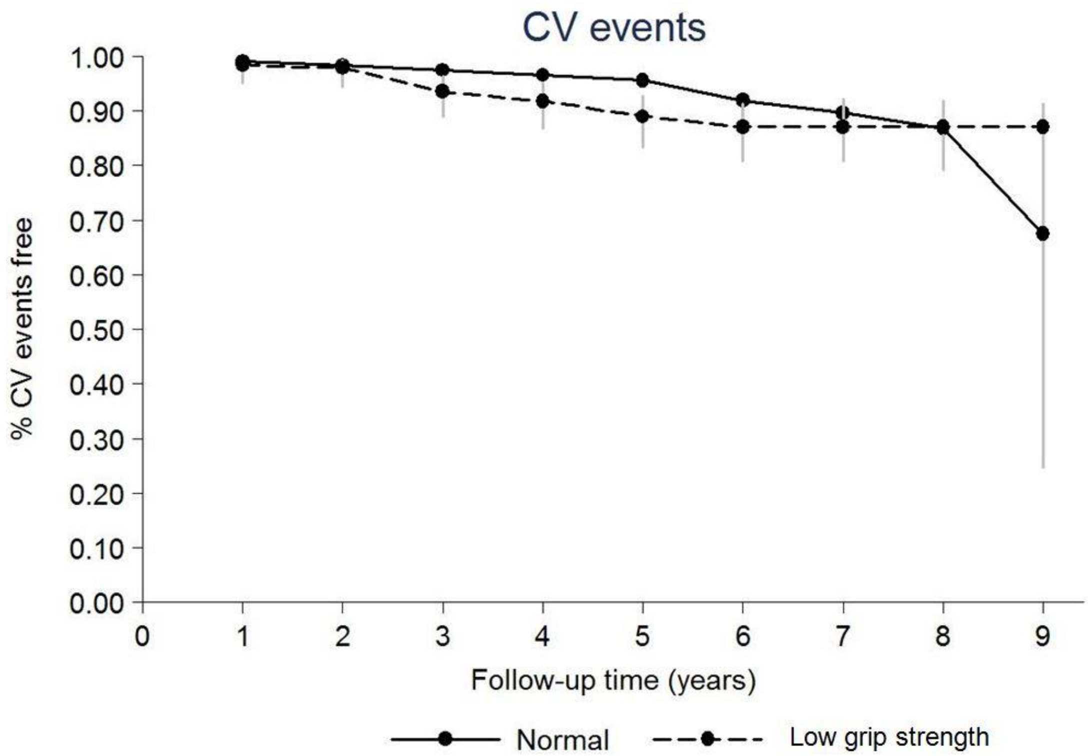
SUPPLEMENTARY MATERIAL

Supplementary figure 1: Distribution of grip strength according to gender. CoLaus study, Lausanne, Switzerland, 2003-2012.



Supplementary figure 2: Survival and incidence graphs for overall mortality and cardiovascular events. CoLaus Study, Lausanne, Switzerland, 2003-2012.





Supplementary table 1: 10-year absolute CV risk categorization for SCORE, Framingham and PROCAM cardiovascular risk equations. CoLaus study, Lausanne, Switzerland, 2003-2012.

Risk categories	**SCORE**	**Framingham**	**PROCAM**
Low (%)	[0, 1.5[	[0, 5[	[0, 5[
Medium (%)	[1.5, 2.5[	[5, 10[	[5, 10[
High (%)	[2.5, 5.0[	[10, 20[	[10, 20[
Very high (%)	[5.0 +	[20 +	[20 +

Supplementary table 2: Socio-demographic and clinical characteristics of included and excluded participants. CoLaus study, Lausanne, Switzerland, 2003-2012.

	Included	**Excluded**	**P value**
N	2707	843	
Right-handedness (%)	92.0	89.3	0.02
Grip strength (kg)	33.6 ± 10.7	32.7 ± 11.2	0.03
Age (years)	60.7 ± 6.8	61.0 ± 6.9	0.30
Female (%)	54.8	54.6	0.91
Smoking status (%)			<0.01
Current	22.9	24.0	
Never	39.1	44.1	
Former	38.0	31.9	
Physical job (%)	15.2	17.9	0.06
Weekly leisure-time physical activity			<0.01
<2 periods of 20+ minutes	42.2	48.8	
≥2 periods of 20+ minutes	57.8	51.3	
Living alone (%)	35.1	35.9	0.69
Education level (%)			<0.01
Low	58.5	68.1	
Middle	24.5	18.3	
High	17.0	13.6	
Job position (%)			<0.01
Low	12.7	19.9	
Middle	33.8	27.9	
High	10.7	6.8	
Not working	42.9	45.4	
Receive social help (%)	30.0	36.3	<0.01
Hypertension (%)	47.9	57.4	<0.01
Dyslipidemia (%)	38.7	45.2	<0.01
Diabetes (%)	9.6	10.6	0.42
Risk categories (SCORE)			0.19
Low	41.3	37.3	
Medium	14.3	14.4	
High	16.7	17.9	
Very high	27.7	30.4	
Risk categories (Framingham)			0.27
Low	75.8	73.4	
Medium	10.1	12.5	
High	3.7	3.6	
Very high	10.4	10.6	
Risk categories (PROCAM)			0.01
Low	55.7	49.6	
Medium	20.4	21.8	
High	10.5	13.4	
Very high	13.5	15.2	

Results are expressed as mean ± standard deviation or as percentage. Statistical analyses by chi-square or Student t-test.

Supplementary table 3: Association between low grip strength, overall mortality and incident cardiovascular events, unadjusted and adjusted for cardiovascular absolute risk and cardiovascular risk factors' treatment. CoLaus study, Lausanne, Switzerland, 2003-2012.

	Overall mortality			Incident cardiovascular events		
	HR	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**
Unadjusted	1.51	0.94-2.45	0.09	1.76	1.13-2.76	0.01
Model A	0.99	0.60-1.64	0.97	1.12	0.71-1.77	0.62
Model B	1.13	0.68-1.87	0.65	1.21	0.76-1.91	0.42
Model C	1.12	0.67-1.87	0.66	1.37	0.86-2.17	0.18

Results are expressed as Hazard Ratio (HR) and 95% confidence interval (CI) for low grip strength using normal grip strength as the reference. Statistical analyses performed by Cox proportional hazard model, unadjusted and adjusted for treatment for hypertension, dyslipidemia and diabetes, with a further adjustment on: A) absolute CV risk according to SCORE risk equation; B) absolute CV risk according to Framingham 2001 risk equation, and C) absolute CV risk according to PROCAM 2007 risk equation.

Supplementary table 4: Association between low grip strength and overall mortality, unadjusted and multivariate-adjusted, stratified by tertiles of age. CoLaus study, Lausanne, Switzerland, 2003-2012.

	1st tertile			2nd tertile			3rd tertile		
	HR	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**
Unadjusted	1.14	0.15-8.43	0.897	0.80	0.19-3.34	0.762	1.32	0.77-2.28	0.316
Model 1	1.18	0.16-8.77	0.870	0.85	0.20-3.57	0.826	1.20	0.70-2.07	0.508
Model 2	0.81	0.10-6.32	0.842	0.42	0.09-1.88	0.256	1.24	0.72-2.16	0.442
Model 3	0.63	0.08-5.01	0.661	0.43	0.09-2.06	0.289	1.05	0.59-1.89	0.866
Model 4	1.16	0.16-8.59	0.883	0.57	0.13-2.48	0.455	1.23	0.71-2.13	0.458
Model 5	1.07	0.14-7.98	0.946	0.61	0.14-2.66	0.508	1.34	0.77-2.30	0.293
Model 6	1.48	0.20-11.1	0.703	0.57	0.13-2.49	0.458	1.42	0.82-2.46	0.207

Results are expressed as Hazard Ratio (HR) and 95% confidence interval (CI) for low grip strength using normal grip strength as the reference. Statistical analyses performed by Cox proportional hazard model, unadjusted and adjusted for: 1) age and gender; 2) age, gender, education level, job position and social help; 3) age, gender, education level, job position, social help, waist-to-hip ratio and height; 4) absolute CV risk according to SCORE risk equation; 5) absolute CV risk according to Framingham 2001 risk equation, and 6) absolute CV risk according to PROCAM 2007 risk equation.

Supplementary table 5: Association between low grip strength and cardiovascular event incidence, unadjusted and multivariate-adjusted, stratified by tertiles of age. CoLaus study, Lausanne, Switzerland, 2003-2012.

	1st tertile			2nd tertile			3rd tertile		
	HR	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**
Unadjusted	1.00	0.14-7.41	0.993	1.35	0.49-3.75	0.562	1.41	0.84-2.38	0.195
Model 1	1.09	0.15-8.03	0.934	1.49	0.54-4.15	0.444	1.21	0.71-2.04	0.483
Model 2	0.65	0.09-5.02	0.683	0.95	0.34-2.70	0.927	1.14	0.67-1.94	0.628
Model 3	0.51	0.06-4.02	0.523	0.95	0.33-2.77	0.924	0.99	0.57-1.73	0.971
Model 4	1.05	0.14-7.74	0.964	1.06	0.38-2.98	0.906	1.30	0.77-2.19	0.332
Model 5	0.94	0.13-7.02	0.950	1.15	0.41-3.23	0.795	1.21	0.72-2.04	0.473
Model 6	1.09	0.15-8.10	0.930	1.23	0.44-3.45	0.695	1.40	0.83-2.37	0.208

Results are expressed as Hazard Ratio (HR) and 95% confidence interval (CI) for low grip strength using normal grip strength as the reference. Statistical analyses performed by Cox proportional hazard model, unadjusted and adjusted for: 1) age and gender; 2) age, gender, education level, job position and social help; 3) age, gender, education level, job position, social help, waist-to-hip ratio and height; 4) absolute CV risk according to SCORE risk equation; 5) absolute CV risk according to Framingham 2001 risk equation, and 6) absolute CV risk according to PROCAM 2007 risk equation.

Supplementary table 6: Association between low grip strength, overall mortality and incident cardiovascular events, unadjusted and multivariate-adjusted, including questionable grip strength measurements. CoLaus Study, Lausanne, Switzerland, 2003-2012.

	Overall mortality			Incident cardiovascular events		
	HR	**[95% CI]**	**P value**	**HR**	**[95% CI]**	**P value**
Unadjusted	1.46	0.92-2.32	0.11	1.95	1.30-2.93	<0.01
Model 1	1.13	0.70-1.80	0.62	1.37	0.90-2.07	0.14
Model 2	1.00	0.62-1.62	1.00	1.20	0.79-1.83	0.39
Model 3	0.91	0.55-1.49	0.70	1.11	0.72-1.71	0.65
Model 4	1.08	0.67-1.73	0.76	1.35	0.90-2.04	0.15
Model 5	1.37	0.86-2.18	0.18	1.53	1.01-2.30	0.04
Model 6	1.32	0.82-2.11	0.25	1.65	1.09-2.48	0.02

Results are expressed as Hazard Ratio (HR) and 95% confidence interval (CI) for low grip strength using normal grip strength as the reference. Statistical analyses performed by Cox proportional hazard model, unadjusted and adjusted for: 1) age and gender; 2) age, gender, education level, job position and social help; 3) age, gender, education level, job position, social help, waist-to-hip ratio and height; 4) absolute CV risk according to SCORE risk equation; 5) absolute CV risk according to Framingham 2001 risk equation, and 6) absolute CV risk according to PROCAM 2007 risk equation.

Supplementary table 7: Association between low grip strength and cardiovascular event incidence, unadjusted and multivariate-adjusted, after exclusion of a*maurosis fugax* and transient global amnesia events. CoLaus study, Lausanne, Switzerland, 2003-2012.

	Incident cardiovascular events		
	HR	**[95% CI]**	**P value**
Unadjusted	1.72	1.09-2.72	0.02
Model 1	1.19	0.75-1.89	0.46
Model 2	1.03	0.65-1.65	0.90
Model 3	0.93	0.57-1.51	0.77
Model 4	1.21	0.76-1.91	0.43
Model 5	1.30	0.82-2.06	0.26
Model 6	1.43	0.90-2.26	0.13

Results are expressed as Hazard Ratio (HR) and 95% confidence interval (CI) for low grip strength using normal grip strength as the reference. Statistical analyses performed by Cox proportional hazard model, unadjusted and adjusted for: 1) age and gender; 2) age, gender, education level, job position and social help; 3) age, gender, education level, job position, social help, waist-to-hip ratio and height; 4) absolute CV risk according to SCORE risk equation; 5) absolute CV risk according to Framingham 2001 risk equation, and 6) absolute CV risk according to PROCAM 2007 risk equation.

Chapter 9

General Discussion

SUMMARY OF RESULTS AND COMPARISON WITH THE LITERATURE

Determinants of physical activity and sedentary behaviour

Chapter 2 showed that socio-economic factors are differently associated with PA regarding 1) its distribution over the week (i.e. activity patterns) or 2) its combination with SB levels (i.e. activity behaviours). For activity behaviours, relative to the ‘Couch potatoes’, having a low educational level was positively associated with the ‘Light movers’ and ‘Busy bees’. High household income was negatively associated with the ‘Light movers’ and positively with the ‘Sedentary exercisers’. For activity patterns, relative to the ‘Inactives’, being employed and having a high household income were positively associated with the ‘Weekend warriors’. Low educational level was negatively associated with the ‘Weekend warriors’ and positively with the ‘Regularly actives’. These results are in agreement with prior studies showing different socio-economic levels within activity behaviours (1, 2) and patterns (3) although they did not adjust for major confounders. Overall, the findings suggest that low socio-economic subjects are more likely distributing PA over the week while high socio-economic ones are more prone to concentrate their PA on weekends and adopt high SB levels the rest of the week. This is likely explained by the fact that high socio-economic subjects have a more sedentary employment. Finally, the association between activity and socio-economic factors is more complicated than initially expected, and taking into account PA distribution over the week (i.e. weekly activity patterns) and the combination between PA and SB levels (i.e. activity behaviours) seem necessary to bring more valuable information.

Table A – Activity levels within behaviours and patterns

	SB (min/day)	LIPA (min/day)	MVPA (min/day)
Activity behaviours			
Couch potato	744 ± 71	75 ± 18	85 ± 32
Light mover	677 ± 66	122 ± 24	106 ± 21
Sedentary exerciser	681 ± 60	79 ± 12	173 ± 35
Busy bee	577 ± 88	126 ± 29	230 ± 74
Activity patterns			
Inactive	720 ± 76	92 ± 30	93 ± 30
Weekend warrior	622 ± 84	114 ± 31	204 ± 60
Regularly active	583 ± 94	119 ± 32	228 ± 77

SB, sedentary behaviour; LIPA, light intensity physical activity; MVPA, moderate-to-vigorous intensity physical activity. Results are expressed as mean ± standard deviation

Association of activity with cardiovascular risk

Chapter 3 studied the association of activity behaviours and patterns with traditional CVRF such as smoking, obesity, hypertension, diabetes. For activity behaviours, relative to the ‘Couch potatoes’, the ‘Sedentary exercisers’ and ‘Busy bees’ had a lower likelihood of smoking, obesity, and diabetes. No association was found for the ‘Light movers’. For activity patterns, relative to the ‘Inactives’, the ‘Weekend warriors’ and ‘Regularly actives’ had a lower likelihood of smoking, obesity, hypertension, and diabetes. Overall, the results show that high PA levels are associated with a favourable CV risk profile, even when concomitant with high SB levels or when PA is concentrated on weekends (**table B**). Conversely, adopting low SB levels without PA practice seems not enough to improve CV risk profile. These findings are in agreement with prior studies (1, 2) despite a lack of information regarding weekly activity patterns. Finally, our findings suggest that being ‘Sedentary exerciser’ or ‘Weekend warrior’ might be sufficient to prevent CVD. This was recently confirmed by studies showing that the 'Weekend warriors' and 'Sedentary exercisers' have similar CVD mortality rates than 'Regularly active' (4) and 'Busy bees' (5), respectively.

Chapters 4 studied the association of PA, SB and their patterns with sleep parameters. High PA and low SB statuses were associated with higher sleep efficiency (of around 3%) and lower likelihood of evening chronotype. However, no association were found for PA and SB with parameters such as sleep duration, daytime sleepiness, insomnia and risk of sleep apnea. For activity patterns, relative to the ‘Inactives’, both the ‘Weekend warriors’ and ‘Regularly actives’ were related to higher sleep efficiency and less frequent evening chronotype. Overall, our results show that PA levels, either evenly distributed over the week or concentrated on weekends, are associated with higher sleep efficiency (**table B**) and less frequent evening chronotype. These results are in agreement with prior studies showing higher sleep efficiency (6) and less evening chronotype (7) in active individuals. Several findings showed improvements in additional sleep characteristics but were limited by self-reported PA (8-10). We found no study to which we could compare our results on weekly activity patterns. Finally, since lower sleep efficiency has been related to mortality (11), our findings suggest that the effect of PA and SB on CVD might be partly mediated by sleep efficiency.

Chapters 5 studied the association of PA, SB and their patterns with salivary cortisol. Low SB status was associated to steeper diurnal cortisol slopes. Trends were also observed for high PA status with lower values in cortisol AUCg (area under the curve to ground) and steeper slopes. For activity patterns, the ‘Regularly actives’ and ‘Weekend warriors’ had respectively lower values in cortisol AUCg and steeper slopes in comparison to the ‘Inactives’. No associations were found with cortisol awakening response. Overall, our results show that PA levels, either evenly distributed over the week or concentrated on weekends, are associated with a lower cortisol secretion; however, the effects are small (**table B**). These findings are in agreement with two other community-dwelling studies (12, 13). Nevertheless, PA has been shown to acutely increase salivary cortisol secretion in athletes after high intensity activities (14), but these contradictive results might not be applicable to our setting. Finally, since lower cortisol secretion has been related to CVD (15), our findings suggest that the effect of PA and SB on CVD might be partly mediated by cortisol secretion.

Chapters 6 studied the association of PA, SB and their patterns with muscle markers. High PA men were associated with higher grip strength and lean mass, while low SB men were only related to higher grip strength. For activity patterns, relative to the 'Inactives', the 'Regularly actives' men had higher grip strength and lean mass; however, no differences were found for the 'Weekend warriors' with grip strength and lean mass. No such associations were found in women. Overall, our results show that physically active individuals have higher muscle mass and strength; however, the effects are small. These findings are in agreement with previous studies (16, 17). The lack of association for women is possibly because they adopt lower PA intensities. Further, our results show that individuals who concentrate their PA on weekends benefit less from PA than subjects who exercise regularly regarding muscle mass and strength (**table B**). We found no study to which we could compare these latter results. Finally, since muscle mass (18) and strength (19) have been related to CVD, these findings suggest that effect of PA and SB on CVD might be partly mediated by muscle markers.

Table B – Associations of the 'Weekend warrior' and 'Regularly active' patterns with cardiovascular risk factors, relative to the 'Inactives'.

	Weekend warrior	Regularly active
Traditional cardiovascular risk factors		
Lower likelihood of obesity	++	++
Lower likelihood of hypertension	++	++
Lower likelihood of diabetes	++	++
Novel cardiovascular risk factors		
Higher sleep efficiency	+	+
Lower cortisol secretion	+	+
Higher muscle strength	ø	+
Higher muscle mass	ø	+

+(+): Positive association; ø: No association

Chapters 7 and **8** assessed the association of GS, a correlate of PA, with CVRF (**chapter 7**) and with incidence of CVD (**chapter 8**). The importance of PA in predicting incident CVD independently of the traditional CVRFs was also assessed in **chapter 8**. High GS was related to more favourable traditional and novel CVRFs (**chapter 7**). These findings are in agreement with a prior study showing lower prevalence rates of CVRF among high GS individuals (20). High GS was also associated with a lower incidence of CVD events but this association was no longer significant after controlling for baseline CV risk (**chapter 8**). Although several large-sampled studies found an independent association between GS and CVD incidence, most only partially adjusted for CV risk (19, 21). Hence, despite a recent meta-analysis concluding that PA remains independently associated with incident CVD (22), GS did not seem to be useful in assessing CVD risk beyond established CVRF. Interestingly, most CV risk equations such as SCORE (23), Framingham (24) and PROCAM (25) do not include PA (**table C**), the most likely reason being the lack of standardisation in PA measurements. If PA is to be included in future risk equations, simple metrics such as being physical active (dichotomous yes/no) could be used, provided adequate definitions are made available. Future research should be conducted on how to define PA and which types of measurements (i.e. accelerometers and/or questionnaires) should be used. The situation is encouraging because raw accelerometry data (in gravitational unit) can now be collected (26) and processed using open-access algorithms (27). Finally, as PA patterns are associated with many health conditions (28, 29), they should continue to be explored.

Table C – Risk factors included in cardiovascular risk equations

	SCORE	**Framingham**	**PROCAM**
Age	x	x	x
Gender	x	x	x
Family history			x
Smoking	x	x	x
Hypertension	x	x	x
Dyslipidemia	x	x	x
Diabetes		x	x

STRENGTHS AND LIMITATIONS

Strengths

Activity patterns and behaviours have been understudied, and little information existed regarding their determinants and their relationship with CV risk. To our knowledge, this project was the first epidemiological study to consider both 1) the distribution of PA over the week (i.e. weekly activity patterns), and 2) the combinations between PA and SB levels (i.e. activity behaviours). We believe it brought important knowledge that will be used to update recommendations regarding PA distribution and its combination with SB levels. This project was also one of the few studies on activity patterns or behaviours using objectively-measured instead of self-reported activity. The extended accelerometry measurement time (up to 14 days) allowed a precise estimation of PA and SB levels, and to assess PA levels during the week and the weekend. Moreover, as participants were extensively assessed for their CV phenotype, this work was able to explore a large palette of potential CVRF. Finally, due to the sampling strategy, we expect that our results can be generalized to all Swiss citizens.

Limitations

This project has also several limitations. First, due to its cross-sectional design, we cannot exclude reverse causality (i.e. high CV risk leading to inactive behaviours and patterns). Thus, it would be important to confirm prospectively the results, so that directional causality can be established. Second, the *GENEActiv* accelerometer has been shown to over-report PA levels (30). However, this should not impact the validity of our results as activity patterns and behaviours were defined according to tertiles of PA levels and not to absolute values. Third, the *GENEActiv* accelerometer was worn on the right wrist, which is the dominant side for most people and thus more prone to noisy movements; however, previous studies found no impact of device location on PA assessment (31). Fourth, participants included in the analyses had higher socio-economic levels and lower CV risks than excluded ones. This is a common selection bias also observed in other large epidemiological studies using accelerometry (32, 33). Hence, it would be interesting that our results be replicated in other cohorts with a different socioeconomic background. Fifth, the independence between PA and SB in their relationship with CVRF was not assessed. In our setting, PA and SB levels were strongly correlated (r=-0.96), raising the issue of multicollinearity in the multivariable models. Future studies using more sophisticated statistical models accounting for multicollinearity will assess the independent effect of PA and SB on novel CVRF. Sixth, the association of PA with CVRF was not adjusted for physical fitness, as no data regarding fitness was available, a limitation also encountered in other studies (2, 34). Indeed, adequate assessment of fitness levels requires methods (e.g. ergometry) which are difficult to implement in large epidemiological studies. Still, a population-based study demonstrated that PA relates to CVRF independently of fitness level (35). Seventh, PA patterns were defined according to a traditional, "western-type" week, i.e. considering the Monday to Friday period as working and the Saturday-Sunday period as weekend. Therefore, subjects concentrating their PA on 1 or 2 days during the "weekday" period were not considered as 'Weekend warriors'. Future studies should explore alternative definitions focusing on PA frequency during the entire 7-day period rather than splitting weekdays and weekends. Eighth, body composition was measured using single-frequency bioimpedance, a method less precise than underwater weighting or dual energy X-ray absorptiometry (DEXA). Still, in a subsample of 794 women of the CoLaus study who were screened for osteoporosis using DEXA, the correlation between bioimpedance and DEXA was high (r=0.852). Hence, we consider that body composition from bioimpedance relates to body composition obtained using more precise and sophisticated methods.

RELEVANCE AND PROPOSALS FOR FUTURE RESEARCH

Our results raise important information on PA and SB. First, our findings show that activity determinants differ regarding 1) PA distribution over the week, and 2) the combinations between PA and SB levels. Low socio-economic individuals are more likely distributing PA evenly over week while high socio-economic ones are more prone to concentrate PA on weekends and adopt high SB levels the rest of the week. This discrepancy is likely explained by the fact that low socio-economic individuals adopt occupational (i.e. work-related) PA rather than leisure-time PA. Second, our results demonstrate that physically active individuals have an optimal profile of traditional CVRF (obesity, hypertension and diabetes), even in presence of high SB levels or when PA is concentrated on weekends. Interestingly, adopting low SB levels without PA practice is not enough to be beneficial on CV risk profile. Finally, our findings indicate that PA and SB are also associated with novel CVRF such as sleep efficiency, cortisol secretion, muscle mass and strength. Nevertheless, the effects are small suggesting that they might only partly explain the effect of PA and SB on CVD.

From a public health standpoint, our results suggest that PA patterns and behaviours are unevenly distributed according to socio-economic status. Those differences could be partly attenuated by simple interventions such as changes in the built environment to promote active commuting (i.e. cycling and walking) (36). Our results also suggest that concentrating PA on short periods (i.e. the weekend) has benefits regarding CVRF, although the effect is smaller than distributing PA throughout the week. Hence, people who cannot achieve adequate levels of PA during the week might have some benefit from exercising during short periods such as in the weekend.

Therefore, we make these three proposals for future research:

1. Promotion of activity should be adapted for: 1) low socio-economic subjects by increasing their levels of leisure-time PA; and 2) high socio-economic subjects by decreasing their weekday SB levels (i.e. decreasing sitting time at their workplace). Further exploring the determinants of activity behaviours and patterns will allow a better tailoring promotion of activity in the general population.

2. Test prospectively the association of activity behaviours and patterns with CVD incidence. This will allow updating activity recommendations on 1) the distribution of PA over the week, and 2) the combinations between PA and SB levels that should be adopted.

3. Test prospectively whether sleep efficiency, cortisol secretion, muscle mass and strength mediate the association of PA and SB on CVD incidence. If not, other candidates such as inflammation or adiposity markers should be explored.

CONCLUSION

Physical activity favorably influences a large number of traditional and novel cardiovascular risk factors. The amount of physical activity is more important than the timing of its practice during the week or the level of sedentary behaviour.

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