STUDY PROTOCOL: SIT LESS OR EXERCISE MORE: IMPACT ON CARDIOMETABOLIC HEALTH IN MULTIPLE SCLEROSIS.

1. Background

To date, it is clear that sedentary behavior is strongly related to an increased risk of type II diabetes, cardiovascular disease and premature mortality[1]. People suffering from chronic disabilities appear to be particularly susceptible to a sedentary lifestyle and inactivity due to primary disease symptoms[2]. To date, this is an important new research topic in Multiple Sclerosis (MS, ~2.3 million people worldwide[3, 4], ~10-12.000 diagnosed in Belgium[5-7])[8] treatment. MS is a progressive, autoimmune, neurodegenerative disorder of the central nervous system (CNS) that predominantly affects young to middle-aged adults. It is characterized by a chronic inflammatory process that causes demyelination and axonal damage across the CNS[9]. Clinical manifestations include spasticity, tremor, paralysis, walking difficulties and cognitive abnormalities[10].

Persons with MS (PwMS) have a 40% lower daily step count compared to healthy inactive persons[11] and tend to accumulate their sedentary time in longer bouts[12]. As described above and similar to other chronic conditions, a sedentary lifestyle also makes PwMS more vulnerable to the accumulation of important cardiometabolic comorbidities that seem inactivity-related rather than a direct result of non-reversible tissue injury[13]. Such comorbidities include impaired whole body glycaemic control, an abnormal blood lipid profile, an unhealthy body composition and hypertension[14, 15]. In this respect, it is important to note that corticosteroids, which are often used to treat MS patients pharmacologically[16], elevate fasting glucose and insulin concentrations and induce insulin resistance in healthy controls (HC)[17] therefor probably also increase several cardiometabolic risk factors in MS.

An important first line treatment strategy to improve such cardiometabolic comorbidities involves exercise therapy and a more active lifestyle. So far however, more physical activity usually refers to moderate- to vigorous physical activity (MVPA), as evidenced in current activity guidelines. Here, adults aged 18 to 65 years are recommended to perform at least 30min of moderate-intensity aerobic physical activity on five days of the week or vigorous-intensity physical activity for a minimum of 20min on three days of the week[18]. These guidelines however do not describe the activity level of the remaining 1410min per day. In this respect, evidence is growing that sedentary time, independent of the (dis)practice of MVPA, is an important independent health risk factor[19, 20]. In MS, MVPA is a well-studied topic and frequently applied complementary treatment strategy leading to a wide range of important functional improvements in different areas of cardiorespiratory fitness, muscle strength, balance, fatigue, cognition, quality of life and respiratory function[21]. In this population effects on cardiometabolic risk markers however are less prominent and contradictory[22]. Consequently, any strategy that also improves cardiometabolic health may help to further optimize rehabilitation in MS. Breaking up and reducing sedentary time with easy, daily activities such as household activities and other activities which increase light-intensity walking and standing, known as non-exercise physical activity (NEPA) may be such a strategy.

NEPA has already been shown to significantly improve cardiometabolic risk markers in healthy, sedentary subjects[23], type II diabetes patients[24] and obese adults[25] and it involves lower intensity physical activities that are probably more feasible than MVPA for PwMS. Moreover, with comparable activity workloads, reducing sitting time by NEPA of longer duration decreases insulin levels and fasting lipid levels more than performing one MVPA bout[23, 24] that is usually described in current activity guidelines[18]. So far however, acute MVPA and NEPA effects on cardiometabolic health in this population have never been described. Therefore, the aim of this study is to investigate whether (1) cardiometabolic health (glycaemic control, blood lipids, inflammation markers and blood pressure) of persons with MS improves when sedentary time is reduced and (2) NEPA results in better cardiometabolic health parameters than (a shorter daily bout of) moderate-intensity exercise when workload of both activities is identical in this population.

2. Methods

A flowchart of the study design is provided in Figure 1. The design of the study, the different activity regimes and the continuous activity monitoring are based on previous research in sedentary subjects[23] and type II diabetes patients[24]. For practical reasons (subjects are aware of their activity regimen), it is not possible to blind the study. The study is an intervention study with a cross-over design. Participants will follow four regimes of 4 days each including a control, a sit less, a sit and an exercise regime. The order of the sit and sit less regimes will be randomized. Each activity regime will be followed by a wash-out period of 10 days during which subjects will continue their normal lifestyle. Fasting glucose, insulin, inflammation parameters, lipids and blood pressure will be determined at the end of each regime (on day 5).

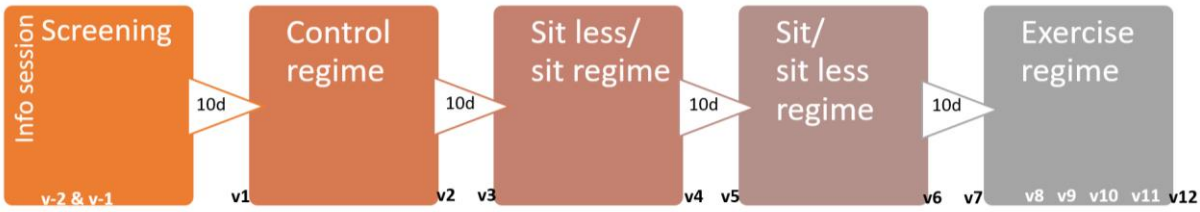

Fig. 1: Flowchart study design. The order of the sit less and sit regime will be randomised. Between each activity regime a wash-out period of 10 days takes place. Abbreviations: d: days, v: visit (v-1,-2: screening visits, v1,3,5,7: attachment of ActivPAL – v2,4,6,12: test days + removing of ActivPAL – v8,9,10,11: exercise sessions)

2.1 Visits

An overview of study visits is provided in Table 1, they include screening visits before start of the study, activPAL (activity monitor) installation/attachments before the start of each activity regimen, training sessions during the training regime and test days and removal of the ActivPAL following each intervention period. The visit structure is described in Table 2.

Table 1. Overview of visits

Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1					v1: activPAL	Control regime	Control regime
2	Control regime	Control regime	v2: blood samples				
3					v3: activPAL	Sit less/sit regime	Sit less/sit regime
4	Sit less/sit regime	Sit less/sit regime	v4: blood samples				
5					v5: activPAL	Sit/sit less regime	Sit/sit less regime
6	Sit/sit less regime	Sit/sit less regime	v6: blood samples				
7					v7: activPAL	v8: training session 1	v9: training session 2
8	v10: training session 3	v11: training session 4	v12: blood samples				

Table 2: specific visit information		
Info session	Day -...	Informed consent
Screening	Visit -2 Visit -1	- Resting ECG - Graded exercise test + explanation polar M200
Visit 1	Day 0	End of wash-out period (= day before the start of the control regime; day 1) - attachment of ActivPAL
Visit 2	Day 5	Start of wash-out period (= day after the end of the control regime) - Blood sampling + OGTT + blood pressure - Removing ActivPAL
Visit 3	Day 14	Last day of wash-out period (= day before the start of the sit less/sit regime) - Attachment of ActivPAL + instructions for activity pattern
Visit 4	Day 19	Start of wash-out period - Blood sampling + OGTT + blood pressure - Removing ActivPAL
Visit 5	Day 28	Last day of wash-out period (= day before the start of the sit/sit less regime) - Attachment of ActivPAL + instructions for activity pattern
Visit 6	Day 33	Start of wash-out period - Blood sampling + OGTT + blood pressure - Removing ActivPAL + feedback on accelerometer data
Visit 7	Day 42	Last day of wash-out period (= day before the start of the exercise regime) - Attachment of ActivPAL + instructions for activity pattern
Visit 8	Day 43	Start of exercise regime - Cycling
Visit 9	Day 44	Day 2 of exercise regime - Cycling + feedback on accelerometer data
Visit 10	Day 45	Cycling
Visit 11	Day 46	Day 4 of exercise regime - Cycling + feedback on accelerometer data
Visit 12	Day 47	Day after the end of the exercise regime - Blood sampling + OGTT + blood pressure - Removing ActivPAL

2.2 Subjects

Sample size

The study population will involve 31 PwMS. The number of subjects needed for this study has been calculated based on triglyceride level changes in a comparable study by Duvivier and colleagues[24] with 3 activity regimes (paired t-test: effect size 0.71, correlation 0.50, 2-sided α of 0.05/6, power 80%, drop-out 10%). Serum triglycerides, which are significantly elevated in PwMS[15], are an important cardiometabolic risk marker[26] and improve consistently in 2 comparable studies in sedentary subjects and type II diabetes patients[23, 24].

Recruitment

PwMS will be recruited using online and paper advertisements (local physiotherapists, MS-Liga, hospitals). All subjects will be informed in detail and will be asked to provide written informed consent. The study will be approved by the medical ethical committee of Hasselt University and will be performed at Hasselt University (Diepenbeek, Belgium) between February 2019 and September 2020 in accordance with the principles of the Declaration of Helsinki. The present study is registered at clinicaltrials.gov.

Screening

The following subject characteristics will be assessed: age, sex, medications, smoking history and family history of coronary artery disease, type of MS (relapsing remitting, secondary progressive, primary progressive), duration of MS and Expanded disability status scale (EDSS) score.

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Signed informed consent
- Men and women ≥18 years old
- Clinical diagnosis of MS (McDonald criteria)
- EDSS ≤ 5
- Daily internet access

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Reported participation in another biomedical trial which may have an effect on blood parameters one month before the pre-study examination or during the study
- Blood donation in the past three months
- Pregnancy or intention of becoming pregnant
- Reported dietary habits: medically prescribed diet, slimming diet
- Reported weight loss (>2kg) in the last three months prior to the screening
- Alcohol use > 20 units per week (during the last 3 months)
- Experimental drug use (during the last 3 months)
- Medication changes (during the last month)
- Medical conditions which make participation in the study not responsible:
  - Heart failure NYHA 3 or higher
  - Angina pectoris or signs of cardiac ischemia during exercise testing
  - Mental or physical disability
- Based on historical information not able to walk for 3h per day and stand for 4h per day (e.g. intermittent claudication)

There will be two screening visits, organized at least 10 days before start of the study (Saturday of week 1, see table 1). During the first screening visit, a resting electrocardiogram (ECG) will be assessed, which will be evaluated by a trained professional for medical safety of maximal exercise testing (until volitional exhaustion in combination with a respiratory exchange ratio ≥1.1 or failure to maintain 45 RPM). This will be done with an incremental exercise test on a bicycle ergometer during the second screening visit, to assess maximal workload capacity (W_{max}). The W_{max} is necessary to calculate intensity of training sessions in the exercise regime (see 3.6 exercise intensity). Subsequently, participants will receive a Polar M200 activity tracker and explanation about its use (see 3.5 activity tracking).

2.3. Randomization

At the end of visit -1 subjects will be randomised, if no contra-indications have been observed during the screening. The two below-mentioned schemes (Fig. 2) will be taken into randomisation 15 times. For the last subject the scheme will be chosen randomly

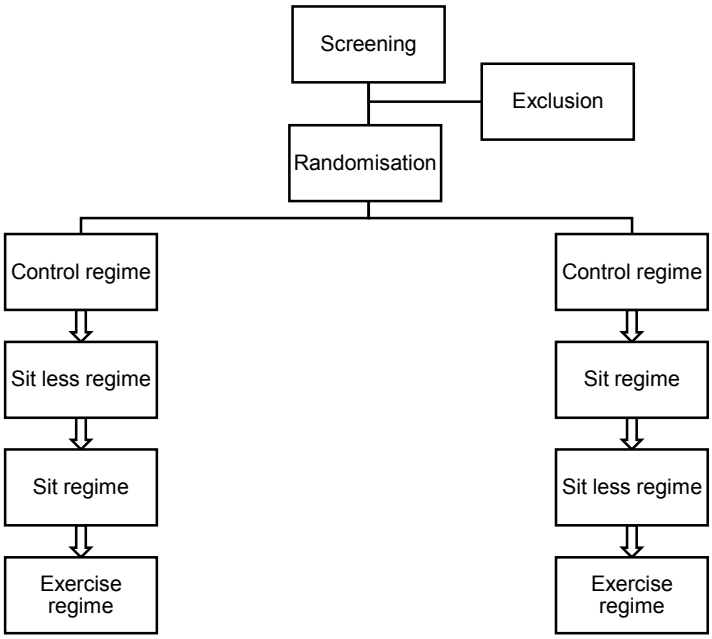

Fig. 2: allocation to activity regime schemes.

The randomisation will be done by a member of the research team by drawing a sealed, non-translucent envelop with herein written the scheme. After randomisation, the first activity regime will start within ±10 days. The regimes will always start on the same day (Saturday) to minimize bias.

2.4 Regimes

During each activity regime (4 days), participants will be asked to spend 8h sleeping/lying. Subjects will be allowed to sleep ± 2 hours less or more and replace this sleeping time by sitting (more or less) as long as sleeping time is the same during all regimes. Each activity regime is followed by a wash-out period of 10 days during which subjects can continue their normal lifestyle, but are not allowed to perform more than 60min of MVPA.

Control regime

To provide feedback on how to change activity patterns in order to comply with the subsequent activity regimes, subjects will start with the control regime. This is a baseline measurement of physical activity during which subjects will be instructed not to change activity patterns during these four days and to note all activities they perform.

Sit regime

Here, participants spend 14h of their day sitting, 1h walking and 1h standing. According to the compendium of Ainsworth et al. (2011)[27], this corresponds with a daily workload of activities (DWA) of 27 metabolic equivalents (MET's) per day.

Sit less regime

Each day will consist of 3h walking, 4h standing and 9h sitting. These time frames are chosen to result in a comparable DWA increase as the exercise regime compared to the sit regime (+7 MET's)[27]. The additional 2h of walking and 3h of standing, compared to the sitting regime, will be done in a minimum of four bouts with a time interval of > 1h. The subjects will be instructed to walk on a slow pace. i.e. 2-3 km/h (e.g. walking during shopping and work related walking in an office).

Exercise regime

One hour of sitting in the sit regime will be replaced with 1 training session (1h) on a cycle ergometer in the research center. The remaining hours of each day have to be spent as follows: 13h sitting, 1h walking and 1h standing for daily care. The intensity of the training session (50-60% of W_{max}) results in a DWA of 34.5 MET's according to the compendium of physical activities[27]. Duration of training sessions will be adapted individually with ActivPAL data of the sit less and sit regime to identically match
DWA increase between the sit less and exercise regime, compared to the sitting regime. Details can be found in '3.6 exercise intensity'.

2.5 Activity tracking

The activPAL will be placed on the upper leg with a waterproof adhesive film by a researcher one working day before each activity regime (Friday). The subjects will be asked to wear the activPAL 24 h per day, showering is allowed but swimming is not. The ActivPAL objectively determines the sort and intensity of physical activity (how much time has been spent walking, standing or sitting/lying: Fig. 3) and is already validated in PwMS[28]. Physical (in)activity can therefore be accurately determined and

adherence with the activity regimes is measured correctly. Participants are asked to note down the exact time spent sleeping and lying, since the ActivPAL is not able to distinguish between sitting and sleeping.

To facilitate adherence with the activity regimes, participants receive a Polar M200. This is an activity tracker which monitors the number of steps, time spent lying, sitting, walking and running per day (Fig. 4). These data can be controlled online by members of the research team to provide feedback by phone when necessary.

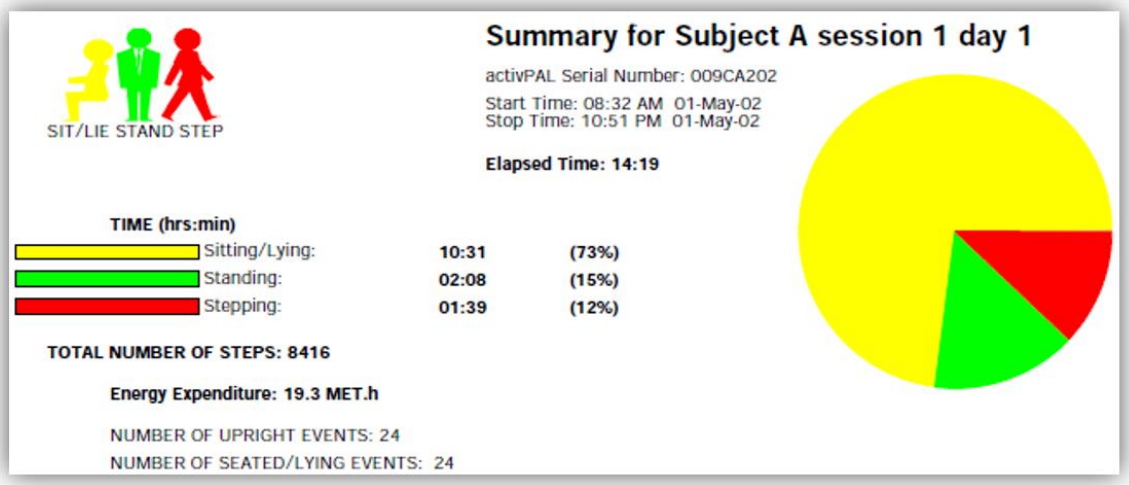

Figure 3: example of summary ActivPAL data

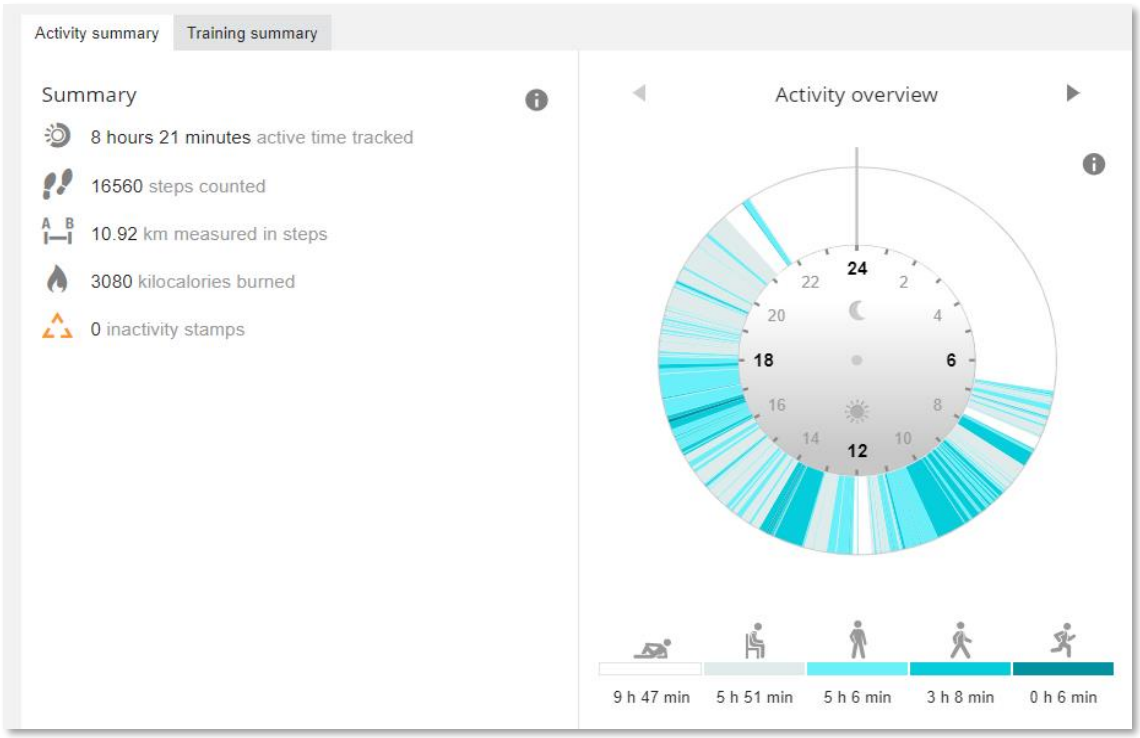

Figure 4: example of summary Polar data

2.6. Exercise intensity

To ensure that DWA will be the same during the sit less and exercise regime, ActivPAL data of the sit less and sit regime will be used to individualize training sessions during the exercise regime. ActivPAL is able to estimate workload of activities in MET's. One MET is the energy expenditure of an individual in resting condition (3.5 $mL*kg^{-1}*min^{-1}$). The amount of MET's as provided by the activPAL is based on how many steps are taken, acceleration and amount of hours walking, standing and sitting/lying. The ActivPAL will be taken off during cycling in the exercise regime. The measurement error of the activPAL in estimating this energy expenditure has been reported to be low for sedentary and light-intensity activities but high for moderate- and vigorous-intensity activities[29].

It will be calculated how long subjects need to cycle at an intensity of 50-60% W_{max} to match the workload of the training session with the DWA increase of the sit less regime, compared to the sit regime. The workload of cycling at 50-60% of W_{max} will be calculated using the following equation[24]: MET = 0.0545 * Watt + 1.4561 (R^2 = 0.946), which describes the relationship between MET's and Watts, based on literature included in the compendium of physical activities[27] that actually measured energy expenditure over a range of workloads. The DWA in the sit less and sit regime will be measured with the ActivPAL as described above.

2.7 Diet

Subjects will be asked not to consume any alcohol during the study and to only consume one serving of alcohol per day during the wash-out period. The use of dietary supplements (e.g. fish oil capsules, polyphenols and vitamins) will be prohibited after screening and during the study. All medication needs to be continued, no changes in the dose or type of medication are allowed during the study and timing of intake will be standardized.

Subjects will be asked to continue their normal diet and to write down in a dairy each day what they eat and drink. The diet that is consumed during the first regime needs to be exactly the same during the following regimes. This ensures that energy intake and proportionality of macronutrients will be similar during all four regimes. The meal on the evening before the test day needs to be consumed at similar time points, no food is allowed after this last meal and coffee and tea only up till 20h.

3. Endpoints

On the fifth day of each activity regime the subjects will come in the morning to the laboratory to undergo fasting blood sampling, an oral glucose tolerance test and to measure body weight and blood pressure. Subjects will be asked to use public transportation or car, in order to ensure that subjects are not physically high active on the test day. The subjects need to refrain from smoking, food and drinks except water starting at 20h the day before. Water is allowed until 6h of the test day.

Primary endpoints:

Physical activity

Physical activity and sedentary behaviour will be measured with the ActivPAL3^{TM} activity monitor (PAL Technologies Ltd, Glasgow, UK).

Venous blood samples will be collected to assess glycaemic control and fasting blood parameters:

- Glycaemic control

A standardized two-hour oral glucose tolerance test (OGTT) will be performed to explore effects on insulin sensitivity. The OGTT is chosen as a measure for insulin sensitivity because of its relative simplicity and its acceptable correlation with the gold standard (hyperinsulinemic euglycemic clamp)[30]. Subjects ingest a solution (250ml) containing 75g dextrose, and venous blood samples are obtained at t=-5, 15, 30, 45, 60, 90 and 120min for assessment of venous glucose and insulin concentration. From glucose and insulin concentrations, homeostatic model assessment for insulin resistance (HOMA-IR) is calculated by: fasting glucose (mg/dl) * fasting insulin (µU/ml)/405[31] and whole-body insulin sensitivity index (ISI): 10000/√[fasting glucose (mg/dl) * fasting insulin (µU/ml)) * (mean glucose during OGTT (mg/dl) * mean insulin during OGTT (µU/ml)][30] will be calculated. The area under the curve (AUC) for glucose and insulin for the 2-hour period is calculated using the trapezoidal method[32].

- Fasting blood parameters

After antecubital catheter placement, fasting blood samples are obtained for the measurement of glucose, insulin, lipid spectrum parameters (total cholesterol, high density lipoprotein cholesterol (HDL-chol), low density lipoprotein cholesterol (LDL-chol), non HDL cholesterol, triglycerides (TG), apolipoprotein B (apo B), apolipoprotein A1 (apo A1), free fatty acids) and systemic inflammatory markers (C-reactive protein (CRP), interleukin 1 (IL-1) and interleukin 6 (IL-6)).

For blood glucose assessment, blood will be collected in NAF/KO tubes (6ml) and stored in a refrigerator at 4°C for a maximum of 30min. For the assessment of insulin, inflammation parameters and lipid spectrum parameters, blood will be collected in 3 SST tubes (6ml), which will be allowed to clot for 30 min at room temperature. After 30min all tubes will be centrifuged during 12min at 1020 x g at a temperature of 20°C. Afterwards, plasma and serum samples are immediately portioned into aliquots and stored at -80°C until analysis at the end of the trial.

Secondary endpoints:

Blood pressure

After an initial resting period of 10min with participants in a supine position in a quiet room with constant temperature (19-21°C), blood pressure (BP) is measured 3 times at 5-min intervals using an electronic sphygmomanometer (Omron®, Omron Healthcare, IL, USA) from the dominant arm and documented as the mean value of the final 2 measurements.

Body weight

Body weight (in underwear) is determined using a digital-balanced weighting scale to the nearest 0.1kg.

4. Statistical analysis

Statistical analyses are performed by IBM SPSS® version 25.0 (IBM SPSS Statistics for Windows, Chicago, IL, USA). Data are expressed as mean ± SD.

The differences in outcome between regimens are analysed using linear mixed model analyses with activity regimen and period (order of activity regimen) as fixed factors, and an unstructured covariance structure for the four repeated measurements for each person. Shapiro-Wilk test will be used to test normality of the data ($p<0.05$) and natural log transformations are performed if the outcome is not normally distributed. Likelihood based methods are used for missing values. Numerical variables are presented as mean ± SD or as medians (first quartile, third quartile) for baseline characteristics (measured during screening) and estimated mean (SEM) for the other values. Regimens are compared pairwise, whereby P-values ≤0.05/6 (Bonferroni correction) are considered statistically significant to account for multiple testing.

The activPAL will be read out and analysed with the activPAL software. The software is able to determine how much time the subjects spent walking, standing and sitting/lying and is also able to estimate energy expenditure (Fig. 1). This makes it possible to objectively determine the proportion of activity/inactivity. We will determine sleeping time based on reported data since the activPAL is not able to distinguish between sitting and sleeping.

5. Data anonymity

All data will be handled confidentially and coded (RST-subject number 0→31). A subject identification list will be used to link data to the subjects. The key to the code will be safeguarded by an independent investigator that is not involved in the study. All collected data will be stored in Case Report Forms at the Google drive file stream and only members of the research team will have access to the source data. Source data will be stored for 25 years in accordance with GCP standards to re-use them or to validate results. Future research in line with the current study (re-use of the data) will only be conducted with subject's approval on the informed consent form for re-use of the data and approval of the new study by the ethical committee.

Abbreviations:

Apo: apolipoprotein
AUC: area under the curve
BP: blood pressure
CRP: C-reactive protein
DWA: daily workload of activities
ECG: electrocardiogram
EDSS: Expanded disability status scale
HC: healthy controls
HDL chol: high-density lipoprotein cholesterol
HOMA-IR: homeostatic model assessment for insulin resistance
IL-1: interleukin 1
IL-6: interleukin 6
LDL chol: low-density lipoprotein cholesterol
MET: metabolic equivalent of task
MVPA: moderate to vigorous physical activity
NAF/KO tube: sodium fluoride / potassium oxalate tube
NEPA: non-exercise physical activity
OGTT: oral glucose test
PwMS: persons with multiple sclerosis
RPM: rounds per minute
SD: standard deviation
SST: serum seperator tube
TG: triglycerides
WBISI: whole-body insulin sensitivity index
W_{max}: maximal workload capacity

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