PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The relation between walking speed and changes in cardiovascular risk factors during a 12–day walking tour to Santiago de Compostela: a cohort study
AUTHORS	Remy HH Bemelmans, Paulus P. Blommaert, Annemarie M.J. Wassink, Blai Coll, Wilko Spiering, Yolanda van der Graaf and Frank L.J. Visseren

VERSION 1 - REVIEW

REVIEWER	Peter W Grandjean, Ph.D., FACSM Director, Center for Healthy Living & Baylor Laboratories for Exercise Science & Technology Baylor University USA
REVIEW RETURNED	05/02/2012

GENERAL COMMENTS	The purpose of this prospective cohort study was to quantify the influence of walking speed, independent of walking volume, on clinical measures of CVD risk in middle-aged and older men and women. The authors should be commended for their experimental design, the clear and concise conveyance of experimental methods and important, unique results. The manuscript is well-written. The tables and figures support the text. There are a few minor revisions that should help the reader understand the context of the findings: 1. There appears to be a typographical error in the HDL-c finifings in the opening sentence of the Abstract. 2. The Abstract should express the idea that thes effects with 95% CI are adjusted for other covariats (Include age, gender, physical activity levels and walking speed) 3. It should be clearly conveyed that these participants already had a level of fitness that enabled them to walk almost 5.4 hrs/dy for 12 days. This separates the cohort from most general middle-aged and older indviduals. 4. The data does not include information on dietary or nutrient consumption over the 12-day period. If any generalized quantified data exist, this would be good to know...However, the authors should point out the effects of walking speed overcame potential differences in caloric and nutrient composition among walking speed groups to show distinct differences.

REVIEWER	Dr Ruth Hunter Research Fellow Centre for Public Health Queen's University Belfast Northern Ireland I confirm that I have no conflicts of interest.
REVIEW RETURNED	26/02/2012

THE STUDY	1) The authors acknowledge that little is known about the dose-response relationship of exercise. However, more research needs to be done in a controlled lab-based setting in order to fully inform a pragmatic study such as this; 2) Is it feasible to expect any medium to long term effects following a 12 day intervention. No post-intervention follow-up measures were taken; 3) The manuscript does not detail the specific instructions given to the participants. Were participants aware that the study was about walking speed?; 4) There is no rationale given for the size of cohort; 5) Important details regarding the placement and calibration of the pedometer are omitted. Was participant stride length used in order to obtain an accurate walking speed? Using a pedometer only gives average walking speed for the day. Accelerometry would have allowed more detailed analysis of bouts of walking at certain intensities within individuals; 6) There is no rationale given for the chosen parameters for 'high' and 'low' walking speeds; 7) A number of confounding factors have not been accounted for, for example, cardiorespiratory fitness.
RESULTS & CONCLUSIONS	1) No significance testing for baseline differences has been reported; 2) Given the small, heterogeneous sample (males/females, aged 40-70 years old), it is difficult to draw any firm conclusions from this study. In addition, to the small, heterogeneous population and the nature of the intervention, the generalisability or usefulness of the results are questionable.
REPORTING & ETHICS	1) Important details regarding the recruitment process are omitted including, the number of individuals expressing an interest, the number of exclusions and reasons for exclusion.
GENERAL COMMENTS	The authors should be commended on a unique, pragmatic study. However, I feel that there are a number of limitations in the study design that limit the generalisability of the findings.

REVIEWER	Ningqi Hou, PhD Postdoctoral Scholar University of Chicago Department of Health Studies The United States I have no conflict of competing interests with this study, as my current research focus on breast cancer epidemiology.
REVIEW RETURNED	28/02/2012

THE STUDY	The study design is unique and interesting. However, generalization of the findings may be limited, considering this small sample (15 men and 14 women) was from a non-randomized intervention group

	who participated a 281 km pilgrimage walk tour. The authors mentioned that they balanced gender when categorizing the sample into high vs. low walking speed. It is not clear what's the cutpoint they used, and how did it differ by gender. From table 1, ranges of the walking speed overlap between the high (4.2-5.0 km/h) and low (3.8-4.5 km/h) speed groups, though the means (4.6 vs. 4.1 km/h) were statistically significant (yet not indicated). It may be helpful to show some descriptions of speed by gender (e.g. men in high speed group, women in low speed group, etc.). The authors used continuous walking speed in the modeling. The walking speed was calculated as an overall average during the 12 days by self-report walking hours. It is possible that the walking speed would vary during the 12 days, esp. by contrasting the beginning and the end. A time-specific (e.g. per 2 days) walking speed would be very informative, if possible. The last, did the participants tend to have a similar diet during the tour?
GENERAL COMMENTS	In Page 7, lines 25-34, it was not clear to me why an interaction term would be necessary, since the outcome vars (mean of the per 2 day changes; page 21 line 27-28) and walking speed were all on average, without being time-specific.

VERSION 1 – AUTHOR RESPONSE

Response to reviewer's comments

From the managing editor:

The title uses the word 'influence' but the sort title uses 'relation'. Should both use 'relation' or 'association'?
- We agree, we changed 'influence' into 'relation' in the title.

Please delete the 'what this study adds' box.
-We have deleted the "what this study adds' box.

Reviewer: Peter W Grandjean, Ph.D., FACSM Director, Center for Healthy Living & Baylor Laboratories for Exercise Science & Technology Baylor University USA

The purpose of this prospective cohort study was to quantify the influence of walking speed, independent of walking volume, on clinical measures of CVD risk in middle-aged and older men and women.
The authors should be commended for their experimental design, the clear and concise conveyance of experimental methods and important, unique results. The manuscript is well-written. The tables and figures support the text.
There are a few minor revisions that should help the reader understand the context of the findings:

1. There appears to be a typographical error in the HDL-c findings in the opening sentence of the Abstract.
- We checked the manuscript, including the abstract, for typographical errors and made appropriate adjustments.

2. The Abstract should express the idea that these effects with 95% CI are adjusted for other covariates (Include age, gender, physical activity levels and walking speed)
- In the first sentence in the results section of the abstract, the differences in changes in HDL-c, LDL-c and total cholesterol between the high and low walking speed groups are presented. This is an observational description of absolute changes in the levels of cardiovascular risk factors, as shown in figure 1, and these results are not adjusted for confounding factors. The second sentence describes the relation between walking speed and changes in HDL-c, LDL-c and total cholesterol. We fully agree with the reviewer that for these analyses adjusting for potential confounding factors is important. We conducted these analyses as such. It is stated at the end of that sentence in the results section of the abstract (page 3, line 21-22): "….. adjusted for age, gender, smoking, BMI and heart rate, ….."
As we did not measure physical activity levels, we were not able to adjust for this, and we mentioned this as a limitation of our study as now stated in the discussion section (page 14, line 16): " Furthermore, we were not able to adjust for differences in the dietary pattern or cardiorespiratory fitness level of the participants, as these variables were not measured. However, by adjusting for the heart rate at baseline as a proxy for cardiorespiratory fitness and for other variables related to cardiorespiratory fitness or unhealthy dietary intake such as age, gender, BMI and smoking, residual confounding of cardiorespiratory fitness or dietary intake is unlikely."
We also added to the article summary (page 2, line 14)"……. adjusted for age, gender, smoking, BMI and heart rate".

3. It should be clearly conveyed that these participants already had a level of fitness that enabled them to walk almost 5.4 hrs/dy for 12 days. This separates the cohort from most general middle-aged and older indviduals.
- This is certainly an important point raised by the reviewer. The generalizability of the findings in this study is certainly somewhat restricted as our study participants were subjects with a baseline level of physical fitness enabling them to walk the pilgrimage to Santiago de Compostela. This is inherent to the study. In the original Santiago study (1), we addressed this issue, but we agree with the reviewer that this should be discussed as well in the current manuscript for better understanding. Therefore, we have added the following sentences, to the discussion section (page 13, line 18): "Walking a pilgrimage requires a considerable amount of time, a thorough preparation and a good physical and mental health. Our findings can be generalised to healthy middle-aged males and females who satisfy these conditions, and possibly to other types of exercise, consisting of prolonged daily periods of moderate intensity."

(1) Bemelmans RH, Coll B, Faber DR, Westerink J, Blommaert PP, Spiering W, et al. Vascular and metabolic effects of 12 days intensive walking to Santiago de Compostela. Atherosclerosis 2010 Oct;212(2):621-7.

4. The data does not include information on dietary or nutrient consumption over the 12-day period. If any generalized quantified data exist, this would be good to know...However, the authors should point out the effects of walking speed overcame potential differences in caloric and nutrient composition among walking speed groups to show distinct differences.
- This is another important aspect raised by the reviewer. We agree that this point should be discussed in the manuscript. We did not investigate the dietary pattern during the study and we have no information regarding this point. It could be argued that subjects walking at lower speed have a worse physical fitness level, which can be related to unhealthier dietary pattern. However, this unhealthier dietary pattern is not expected to arise during the 12-day walking tour, but is expected to be already present at baseline. An unhealthier dietary intake at baseline is associated with other covariates related to unhealthy behaviour, such as smoking, BMI, heart rate as a proxy for physical fitness etc. The relation between walking speed and changes in cardiovascular risk factors was

adjusted for the baseline variables age, gender, current smoking, BMI and heart rate, and in the exploratory analyses also for systolic and diastolic blood pressure, HDL-c, LDL-c and triglycerides, which did not change the results. By adjusting for these baseline covariates, we believe we will have adjusted to a very large part as well for an unhealthy baseline dietary pattern, whether present at baseline or possibly arising during the pilgrimage. We changed the sentences in the discussion section regarding this issue (page 14, line 16): “Furthermore, we were not able to adjust for differences in the dietary pattern or cardiorespiratory fitness level of the participants, as these variables were not measured. However, by adjusting for the heart rate at baseline as a proxy for cardiorespiratory fitness and for other variables related to cardiorespiratory fitness or unhealthy dietary intake such as age, gender, BMI and smoking, residual confounding of cardiorespiratory fitness or dietary intake is unlikely.”
Furthermore, we added the following sentence to the methods section (page 7, line 7): “No information about dietary intake at baseline or during the study was obtained. Participants were not instructed on their diet.”

Reviewer: Dr Ruth Hunter
Research Fellow
Centre for Public Health
Queen's University Belfast
Northern Ireland

1) The authors acknowledge that little is known about the dose-response relationship of exercise. However, more research needs to be done in a controlled lab-based setting in order to fully inform a pragmatic study such as this;
-We agree with the point made by the reviewer. We added the following sentence to the discussion section to emphasize this point (page 13, line 24): “The current study reports pragmatic research about exercise in real life, however, more research needs to be done in a controlled lab-based setting in order to fully explore and understand the results of this study.”

2) Is it feasible to expect any medium to long term effects following a 12 day intervention. No post-intervention follow-up measures were taken;
- We observed that two months after the study there was a significant difference in change in weight of -2.0 kg (95%CI -3.2 to -0.8) in the participants of the pilgrimage compared to age- and gender-matched controls who did not walk the pilgrimage (1). No other significant changes were seen between the pilgrims and the controls at two months post-intervention. To address this point of the reviewer, we have added the following sentence to the results section (page 9, line 24): “Most of these changes were short-lived; after two months, there was only a significant difference in change of weight (-2.0 kg; 95%CI -3.2 to -0.8) in the participants walking the pilgrimage compared to controls who did not walk the pilgrimage, while there were no differences in changes in the other cardiovascular risk factors between the groups (1).”

3) The manuscript does not detail the specific instructions given to the participants. Were participants aware that the study was about walking speed?;
- The present study is a sub-study of the Santiago study, a study about the metabolic and vascular effects of walking a pilgrimage to Santiago de Compostela (1). Participants were aware that the effect of walking the pilgrimage was measured on vascular and metabolic parameters. Participants recorded daily walking distance and time, and carried a pedometer, however they were free to walk at their individually preferred speed and were unaware that the effects of walking speed would be evaluated later. We agree with the reviewer that it is important to state that the participants were unaware that the effects of walking speed would be investigated; therefore we changed the following sentence in the methods section (page 6, line 12): “Participants walked at their individually preferred speed and were unaware that the effects of their walking speed would become subject of evaluation.”

4) There is no rationale given for the size of cohort;
- The reviewer is right that no sample size calculation is reported for the present analyses of the original Santiago study (1). A sample size calculation was given in the original study to detect a difference in endothelial function between the participants walking the pilgrimage and the matched control subjects who stayed at home, as this was the primary end point of the Santiago study (1). To make this point clear we have added the following sentence to the methods section (page 5, line 11): "The cohort size of 30 participants was based on a sample size calculation to detect a difference in endothelial function in the original Santiago study (1)."

5) Important details regarding the placement and calibration of the pedometer are omitted. Was participant stride length used in order to obtain an accurate walking speed? Using a pedometer only gives average walking speed for the day. Accelerometry would have allowed more detailed analysis of bouts of walking at certain intensities within individuals;
- In the current study, the main results are based on walking speed expressed in km/h based on the recorded walking distance and time by the participants. For sensitivity purposes, the analyses were repeated with walking speed expressed in number of steps/hour, recorded with a pedometer. The pedometer only counts the number of steps. The participants were instructed to wear the pedometer at their belt or waistband at the left or right side of their body. We did not measure the participants stride length neither did we calibrate the pedometer. However, as the results of this sensitivity analyses with walking speed expressed in steps/hour were comparable to the results of the main analyses with walking speed expressed in km/hour, we believe the results are valid. We added the following sentence to the methods section (page 6, line 15): "The participants were instructed to wear the pedometer at their belt or waistband at the left or right side of the body."

6) There is no rationale given for the chosen parameters for 'high' and 'low' walking speeds;
- We agree with the reviewer that it should be clear what the rationale is for the creation of the high and low speed groups. As there is no clinically significant definition or cut-off point of 'high' or 'low' walking speed, we decided to use an objective criterium, namely the median walking speed, which also has the advantage of creating groups of equal size. To further clarify this point we added the following sentence to the methods section (page 7, line 16): "As there is no generally accepted cut-off point for high or low walking speed, the study population was divided based on median walking speed, which also has the advantage of creating groups of equal size."

7) A number of confounding factors have not been accounted for, for example, cardiorespiratory fitness.
- The reviewer is right, we did not measure cardiorespiratory fitness, which could be a potential confounding variable in the relation between walking speed and changes in cardiovascular risk factors. We address this point at the end of the discussion section under study limitations (page 14, line 16), with the following sentence: "Furthermore, we were not able to adjust for differences in the dietary pattern or cardiorespiratory fitness level of the participants, as these variables were not measured. However, by adjusting for the heart rate at baseline as a proxy for cardiorespiratory fitness and for other variables related to cardiorespiratory fitness or unhealthy dietary intake such as age, gender, BMI and smoking, residual confounding of cardiorespiratory fitness or dietary intake is unlikely."
1) No significance testing for baseline differences has been reported;
- This is correct, we did not test the baseline variables for statistical differences as we believed the groups were too small for meaningful statistical evaluation. When groups are small (15 resp 14 subjects), it is possible that changes in baseline variables are not statistically significant, but can very well be clinically significant, as is also illustrated in the modified baseline table below, with the requested p-values added:

high speed low speed group group p-values All subjects
Mean walking speed (km/h) 4.6 ± 0.2 4.1 ± 0.2 < 0.01 4.4 ± 0.3
Walking speed range (km/h) 4.2-5.0 3.8-4.5 3.8-5.0
Number of steps/hour 6309 ± 582 5547±437 <0.01 5941 ± 639
Total walking time (hours) 62 ± 3 68 ± 3 0.01 65 ± 4
Total walking distance (km) 284 ± 7 278 ± 11 0.13 281 ± 10
Male subjects 8 (53%) 7 (50%) 0.86 15 (52%)
Age (years) 60.9 ± 3.5 58.1±6.6 0.17 59.5 ± 5.3
Current smoking 3 (20%) 2 (14%) 0.68 5 (17%)
Systolic blood pressure (mmHg) 148 ± 18 138 ± 18 0.16 143 ± 19
Diastolic blood pressure (mmHg) 87 ± 10 81 ± 9 0.11 84 ± 10
Heart rate (beats/minute) 69 ± 10 63 ± 10 0.14 66 ± 11
BMI (kg/m²) 24.2 ± 2.2 27.0 ± 2.7 <0.01 25.5 ± 2.8
Waist circumference (cm) 88 ± 10 92 ± 11 0.32 90 ± 10
Glucose (mmol/L) 5.2 ± 0.6 5.2 ± 0.4 0.90 5.2 ± 0.5
Total cholesterol (mmol/L) 5.3 ± 0.7 5.6 ± 0.8 0.29 5.5 ± 0.8
LDL-cholesterol (mmol/L) 3.4 ± 0.5 3.7 ± 0.8 0.22 3.5 ± 0.7
HDL-cholesterol (mmol/L) 1.45±0.39 1.24±0.36 0.14 1.35±0.38
Triglycerides (mmol/L) 1.1 ± 0.5 1.5 ± 0.9 0.12 1.3 ± 0.8
Total cholesterol/HDL-c ratio 3.8 ± 1.0 5.0 ± 2.1 0.07 4.4 ± 1.7
LDL-c/HDL-c ratio 2.5 ± 0.7 3.3 ± 1.5 0.06 2.9 ± 1.2

Differences between the high and low speed group were tested with independent samples t-test for continuous variables, and with Chi-square test for categorical variables.

The results of the study were based on model III (adjusted for age, gender, BMI, current smoking and baseline heart rate), which also adjusts for the only covariate (BMI) which was statistically significantly different between the groups at baseline (walking speed, number of steps per hour and total walking time are (part of) the determinant).
In our sensitivity analyses, we additionally adjusted for baseline values of systolic and diastolic blood pressure, HDL-c, LDL-c and triglycerides, which are not statistically significant at baseline between the groups, but the absolute differences between the groups may be clinically relevant. However, the results were not essentially different in this sensitivity analyses. In the results section where we describe the baseline characteristics of the participants and differences between the high and low speed group (page 9, lines 3-18), we added the p-values on the appropriate places (for differences in age, walking speed, BMI, total walking distance, systolic and diastolic blood pressure, heart rate, LDL, HDL, and triglycerides between the high and low speed group).

2) Given the small, heterogeneous sample (males/females, aged 40-70 years old), it is difficult to draw any firm conclusions from this study. In addition, to the small, heterogeneous population and the nature of the intervention, the generalizability or usefulness of the results are questionable.
- In general, one could argue that a heterogeneous study population makes the results of a study more generalisable than a study population with for example only males with a narrow age-range. This is especially true if there is no significant interaction. The sample size in the present study is small and therefore no analyses on interaction or subgroup analyses can be performed. We agree with the reviewer that this is a limitation. Therefore, we have changed the following sentences in the discussion section (page 13, line 21) to: “However, the results of the present study are based on a relatively small group of subjects walking 281 km in 12 days. Therefore no statistical interaction tests and no subgroup analyses could be performed.”

1) Important details regarding the recruitment process are omitted including, the number of individuals expressing an interest, the number of exclusions and reasons for exclusion.
- We agree with the reviewer that this is important information, partly reported in the original publication of the Santiago study. In the revised manuscript we have added the following sentence to the methods section (page 5, line 14): “There were 49 subjects responding to the advertisement and applied for participation in the intervention group of the Santiago study. One subject was not eligible because of a history of diabetes mellitus, and 1 subject was not eligible because of uncontrolled hypertension (systolic blood pressure >170 mmHg). From the remaining 47 eligible subjects, the first 15 males and 15 females were recruited for participation. After signing the informed consent form but before start of the intervention period, 1 female subject ended participation for personal reasons.”

The authors should be commended on a unique, pragmatic study. However, I feel that there are a number of limitations in the study design that limit the generalizability of the findings.

Reviewer: Ningqi Hou, PhD
Postdoctoral Scholar
University of Chicago
Department of Health Studies
The United States

The study design is unique and interesting. However, generalization of the findings may be limited, considering this small sample (15 men and 14 women) was from a non-randomized intervention group who participated a 281 km pilgrimage walk tour.
- The reviewer is right that the small sample size limits the possibility to statistically test for interaction or to perform subgroup analyses. We have added the following sentence to the discussion section (page 13, line 21):“ However, the results of the present study are based on a relatively small group of subjects walking 281 km in 12 days. Therefore no statistical interaction tests and no subgroup analyses could be performed.”

The authors mentioned that they balanced gender when categorizing the sample into high vs. low walking speed. It is not clear what's the cut point they used, and how did it differ by gender. From table 1, ranges of the walking speed overlap between the high (4.2-5.0 km/h) and low (3.8-4.5 km/h) speed groups, though the means (4.6 vs. 4.1 km/h) were statistically significant (yet not indicated). It may be helpful to show some descriptions of speed by gender (e.g. men in high speed group, women in low speed group, etc.).
- The male and female subjects were categorized into ‘high’ vs ‘low’ walking speed according to the median speed of their sex, as indicated in the methods section. However, the reviewer is right that we did not report these median values, which can be of interest to the reader. Please see below:

Speed group Gender n Walking speed median (IQR)
High speed group Male 8 4.62 (4.57 - 4.92)
Female 7 4.52 (4.24 - 4.62)
Low speed group Male 7 4.23 (4.01 - 4.33)
Female 7 4.08 (3.94 - 4.10)

We added the requested p-value for the mean walking speed in the high and low speed group in the results section (page 9, line 3) in the following sentence:“The high speed group consisted of 8 men and 7 women, 60.9±3.5 years old, who walked with an average speed of 4.6±0.2 km/h, while the low speed group comprised 7 men and 7 women, 58.1±6.6 years old, with a mean walking speed of 4.1±0.2 km/h (p-value between groups <0.01) (Table 1).”

Furthermore, to clarify this issue we added the following sentence in the results section (page 9, line 6): " The median speed of the men (n=8) in the high speed group was 4.62 (IQR 4.57-4.92) km/h, of the women in the high speed group (n=7) 4.52 (IQR 4.24-4.62), of the men in the low speed group (n=7) this was 4.23 (IQR 4.01-4.33) km/h and of the women in the low speed group (n=7) this was 4.08 (IQR 3.94-4.10) km/h".

The authors used continuous walking speed in the modeling. The walking speed was calculated as an overall average during the 12 days by self-report walking hours. It is possible that the walking speed would vary during the 12 days, esp. by contrasting the beginning and the end. A time-specific (e.g. per 2 days) walking speed would be very informative, if possible.
- The analysis of the results in this study was conducted with a mixed linear effects model. An advantage of this way of analyses is that multiple measurements of each outcome variable during time can be used to increase precision. Analysing walking speed per 2 days on the per 2 days measured outcomes, is not possible in this way, as there are no longitudinal sets. Therefore, we had to restrict to overall mean walking speed during the 12 days when analysing the results. However, the reviewer raises the point that walking speed could vary during the 12 days. In the table below, we provide data on walking speed per 2 days during the 12 days. It appears that speed within the groups is about the same during the 12 days:

	High speed (n=15)	Low speed (n=14)	All participants (n=29)
Day 1-2	4.70 (4.40-4.87)	4.30 (4.29-4.51)	4.41 (4.30-4.73)
Day 3-4	4.52 (4.35-4.67)	4.16 (4.07-4.34)	4.35 (4.10-4.57)
Day 5-6	4.39 (4.12-4.90)	3.90 (3.78-4.02)	4.03 (3.89-4.43)
Day 7-8	4.37 (4.21-4.80)	3.77 (3.50-4.07)	4.19 (3.77-4.48)
Day 9-10	4.71 (4.42-4.87)	4.09 (3.93-4.42)	4.42 (4.09-4.74)
Day 11-12	5.01 (4.78-5.16)	4.23 (3.99-4.54)	4.58 (4.23-5.03)

We address this point in the results section (page 9, line 9) by adding: "Walking speed varied during the 12-day pilgrimage from 4.37 (IQR 4.21-4.80) to 5.01 (IQR 4.78-5.16) in the high speed group, and from 3.77 (IQR 3.50-4.07) to 4.30 (IQR 4.29-4.51) in the low speed group".

The last, did the participants tend to have a similar diet during the tour?
- This is an important point raised by the reviewer. We did not record dietary habits during the walking tour and cannot formally report on this. However, participants all walked the same stages each day and ended their daily stage together in the same, mostly very small villages. Generally, they enjoyed dinner together in the mostly very limited amount of restaurants. In this respect, they had comparable, Northern Spanish diets during the walking tour, however, as stated, no recordings were made, so no formal statement can be made. We added the following sentence to the methods section (page 7, line 7):
"No information about dietary intake at baseline or during the study was obtained. Participants were not instructed on their diet."
In Page 7, lines 25-34, it was not clear to me why an interaction term would be necessary, since the outcome vars (mean of the per 2 day changes; page 21 line 27-28) and walking speed were all on average, without being time-specific.
- This is inherent to the method of analyses with a mixed linear effect model. The determinant is an interaction variable of the variable of interest (in this study, walking speed) and time, while the outcome measures are time-specific measurements of the outcome variables (LDL-c, HDL-c etc). The results are expressed as the change in outcome variables related to a change in 1 unit of the determinant (1 km/h) per time point, which is 2 days in our analysis.
Reference List

(1) Bemelmans RH, Coll B, Faber DR, Westerink J, Blommaert PP, Spiering W, et al. Vascular and metabolic effects of 12 days intensive walking to Santiago de Compostela. Atherosclerosis 2010 Oct;212(2):621-7.

VERSION 2 – REVIEW

REVIEWER	Peter W Grandjean, Ph.D., FACSM Director, Center for Healthy Living & Baylor Laboratories for Exercise Science & Technology Baylor University USA
REVIEW RETURNED	28/03/2012

THE STUDY	First, the participants cannot be classified as patients. Second, the participants are significantly healthier and more fit than the average population to which these results might be extrapolated.
RESULTS & CONCLUSIONS	Upon a second review of this manuscript, it is apparent that the authors have not considered at least two - very plausible - possibilities for their findings. The blood lipid differences between walking intensity groups may result from differences in plasma volume decrements that would be expected during the 12-day pilgrimage. (NOTE: The authors fleetingly refer to plasma volume expansion in the discussion; however, given the dehydration-rehydration literature, expansion would not be expected to occur with prolonged exercise and heat exposure.) Second, the authors adjust the blood lipid responses for BMI. The BMI was calculated from baseline data. As such, adjusting for BMI would not account for the blood lipid changes that are likely to result from a modest reduction in body weight with the 12-days of exercise. The changes in blood lipids should be adjusted for the change in body weight (or BMI) that occurred with the 12-day pilgrimage.
GENERAL COMMENTS	The authors have improved the manuscript considerably. I have few remaining concerns and some additional comments. GENERAL It seems that the walking group intensities would be better described as "Faster" and "Slower" walking pace groups. The current group descriptions "High-speed" and "Low-Speed" is misleading. The high-speed group averaged 4.6 km/hr or just under 3 mph; whereas, the low-speed group 4.1 km/h or 2.5 mph. First, the faster group could be classified as walking briskly. Second, there is not very much difference to distinguish these walking speeds - especially when the number and length of breaks during the daily walking sessions were included in these average velocity calculations. Third, the authors merely used the median as a cutpoint to distinguish groups not a random assignment. Upon a second review of this manuscript, it is apparent that the authors have not considered at least two - very plausible - possibilities for their findings. The blood lipid differences between walking intensity groups may result from differences in plasma volume decrements that would be expected during the 12-day pilgrimage. (NOTE: The authors fleetingly refer to plasma volume expansion in the discussion; however, given the dehydration-rehydration literature, expansion would not be expected to occur with prolonged exercise and heat exposure.) Plasma volume shifts of just a small percentage can influence blood concentrations. If plasma volume decreased moreso in the faster walking group, the

	shift would influence all blood lipid values similarly. This is exactly what we observe; greater HDLC and attenuated decreases in TC, LDLC and TG when compared to the slower walking group. It is possible that the slower group took more frequent or longer breaks and consumed more fluids during their daily treks versus their faster counterparts. The authors should speak to these possibilities. To be sure, there are manuscripts reporting on blood lipid responses to exercise that show plasma volume decreases - not the expansion that the authors refer to in Ferguson et al. Second, the authors adjust the blood lipid responses for BMI. The BMI was calculated from baseline data. As such, adjusting for BMI would not account for the blood lipid changes that are likely to result from a modest reduction in body weight with the 12-days of exercise. The changes in blood lipids should also be adjusted for the change in body weight (or BMI) that occurred with the 12-day pilgrimage. SPECIFIC COMMENTS The opening sentence in the "Changes in cardiovascular risk factors..." in the results section describes decreases in TC, LDLC and TG that occur across groups with daily walking. However, in the discussion, the authors write, "A higher walking speed was related to an increase in HDLc, LDLc and total cholesterol." This does not seem to be consistent. In the discussion (pg 10, lines 29 - 46): The issue of whether increasing HDLc or lowering LDLc is of greater health significance is not very controversial. Scores of empirical evidence supports the primary target for dyslipidemia is to lower LDLc first. Increasing HDLc is of secondary or tertiary significance. This issue is completely different from the issue of determining what walking speed is best for improving blood lipid profiles. The authors discussion in this section is confusing. The authors use the term "pathophysiological" to introduce a discussion of mechanisms (pg 10, lines 48-49). The proper term would be "physiological"...because the descriptions that follow describe normal physiological events that might explain exercise-induced blood lipid changes. (pg 14, lines 19-20) "We also acknowledge study limitations."...is this a stand-alone sentence? What limitations do the authors acknowledge? The weight loss that occurred with the pilgrimage reflects an energy imbalance favoring expenditure over consumption. This concept should be recognized and discussed by the authors along with their brief recognition that dietary/nutrient intake data was not obtained. Energy deficits with or without exercise can induce blood lipid changes similar to what is reported in this manuscript.

VERSION 2 – AUTHOR RESPONSE

Response to reviewer(s)

Reviewer: Peter W Grandjean, Ph.D., FACSM
Director, Center for Healthy Living & Baylor Laboratories for Exercise Science & Technology Baylor University USA

First, the participants cannot be classified as patients. Second, the participants are significantly healthier and more fit than the average population to which these results might be extrapolated.
- The reviewer is right, this study is conducted in healthy volunteers which should be referred to as participants in stead of patients. This is the reason we did not use the word patients in our manuscript, but referred to the study population as “participants” or “subjects”. Furthermore, the participants are certainly healthier and more fit than the average population. We addressed this point in the following passage in the discussion section (page, line): “Walking a pilgrimage requires a considerable amount of time, a thorough preparation and a good physical and mental health. Our findings can be generalised to healthy middle-aged males and females who satisfy these conditions, and possibly to other types of exercise, consisting of prolonged daily periods of moderate intensity”.

The authors have improved the manuscript considerably. I have few remaining concerns and some additional comments.

GENERAL
It seems that the walking group intensities would be better described as "Faster" and "Slower" walking pace groups. The current group descriptions "High-speed" and "Low-Speed" is misleading. The high-speed group averaged 4.6 km/hr or just under 3 mph; whereas, the low-speed group 4.1 km/h or 2.5 mph. First, the faster group could be classified as walking briskly. Second, there is not very much difference to distinguish these walking speeds - especially when the number and length of breaks during the daily walking sessions were included in these average velocity calculations. Third, the authors merely used the median as a cutpoint to distinguish groups not a random assignment.
- This is a valid point raised by the reviewer. We report the results of a real life study, were participants walked a part of a pilgrimage at their individually preferred walking speed. As this was an observational intervention study, participants were not randomly assigned a certain walking speed. Participants were not instructed to walk at a certain speed and were even unaware that their walking speed would become subject of evaluation. The walking speed was calculated from recorded walking distance and time; the number and length of breaks during the daily walking stages were excluded when the walking speed was calculated. As there is no generally accepted cut-off point for high or low walking speed, we used the median speed to divide the study population in two groups, which also has the advantage of creating two groups of equal size. We agree with the reviewer that the difference in mean walking speed between both groups is not very large, 0.5 km/h, but the range in walking speeds of all participants was not very large as well, from 3.8 to 5.0 km/h. By using the median speed as cut off point for the high and low speed group we created the maximum contrast between the two groups. This difference in walking speed between the groups is statistically significant ($p<0.01$). We agree with the reviewer that the terms “faster” and “slower” walking pace groups are better descriptions than “high-speed” and “low-speed”, as both groups were walking at a moderate pace, and high- and low-speed suggest a larger contrast.
We made the following adjustments to the manuscript: In the methods section (page 7, line 137): “From these data, the walking speed was calculated in km/h by dividing the total distance covered during the study by the total walking time, without including the resting time”. Furthermore, the terms “high-speed” and “low-speed” group were substituted throughout the manuscript by “faster” and “slower” walking speed group.

Upon a second review of this manuscript, it is apparent that the authors have not considered at least two - very plausible - possibilities for their findings. The blood lipid differences between walking intensity groups may result from differences in plasma volume decrements that would be expected during the 12-day pilgrimage. (NOTE: The authors fleetingly refer to plasma volume expansion in the discussion; however, given the dehydration-rehydration literature, expansion would not be expected to occur with prolonged exercise and heat exposure.) Plasma volume shifts of just a small percentage can influence blood concentrations. If plasma volume decreased moreso in the faster walking group,

the shift would influence all blood lipid values similarly. This is exactly what we observe; greater HDLC and attenuated decreases in TC, LDLC and TG when compared to the slower walking group. It is possible that the slower group took more frequent or longer breaks and consumed more fluids during their daily treks versus their faster counterparts. The authors should speak to these possibilities. To be sure, there are manuscripts reporting on blood lipid responses to exercise that show plasma volume decreases - not the expansion that the authors refer to in Ferguson et al.
- We did not measure possible changes in plasma volume or markers of it, such as haematocrit. Therefore, we can only speculate about this point raised by the reviewer. All measurements were conducted in early morning, while the walking stages ended the day before in the early afternoon (the participants started walking the dialy stage between 6 and 8 am, and generally finished between noon and 2 pm). There was at least more than 12 hours before ending the daily walking stage and the next measurement, enough time to replenish possible fluid losses. Furthermore, the pilgrimage was conducted in Galicia, the North-West of Spain, were the climate is very mild, temperatures during the pilgrimage were between 10 and 20 degrees Celcius, although not formally measured in our study, so there was only very moderate heat exposure. The reviewer raises the possibility that the slower walking group took longer breaks and consumed more fluid than the faster walking group. As explained above, the walking speed was calculated without the breaks, so the length of the breaks does not determine whether participants were classified in the slow of fast walking group. Furthermore, one could also argue that the participants in the faster walking group finish earlier and have more time to replenish their possible fluid losses before the measurements the next morning. Other arguments against more pronounced decreases in plasma volume of the high speed walking group versus the low speed group is that walking speed was not related to blood pressure and heart rate, nor in plasma glucose level. Finally, the reported relation between walking speed and changes in lipids is per 2 days, if plasma volume changes would explain this relation, this means that plasma volume should decrease every 2 days during the whole pilgrimage. This seems rather unlikely, you would expect possible plasma volume decreases during the first days, but not continuing decreases in plasma volume during 12 days. To conclude, on this point we do not agree with the reviewer that it is likely that our results can be explained for a large part by changes in plasma volume. However, we agree with the reviewer that we should specifically mention this topic in the discussion section. As there are arguments before and against the possible influence of changes in plasma volume on the results of the study, and we did not measure it, we made the following adjustment to the text in the discussion section (page 15, line 311-314): “We did not measure (markers of) plasma volume changes, which could possibly be of influence on the results. However, as the reported results are linear during 12 days, and the measurements were conducted early in the morning, more than 12 hours after the ending of the previous walking stage, we believe the influence of changes in plasma volume on the results to be small”.

Second, the authors adjust the blood lipid responses for BMI. The BMI was calculated from baseline data. As such, adjusting for BMI would not account for the blood lipid changes that are likely to result from a modest reduction in body weight with the 12-days of exercise. The changes in blood lipids should also be adjusted for the change in body weight (or BMI) that occurred with the 12-day pilgrimage
- We did not adjust the relation between walking speed and cardiovascular risk factors for changes in body weight or BMI during the walking stages, because body weight was one of our outcome variables. As we show in table 2 and 3 of the manuscript, there was no relation between walking speed and changes in body weight (an increase in walking speed of 1 km/h was related with an increase in body weight of 0.06 (95%CI -0.06-0.19) kg per 2 days, while an increase in walking speed of 1000 steps/h was related to an increase in body weight of 0.01 (95% CI -0.05-0.08) kg per 2 days (Model III). For the specific relation between walking speed and changes in lipids, we did not correct for changes in BMI as these could be in the causal pathway. This is the exact question of the reviewer: whether walking speed influences blood lipids via changes in body weight. We agree with the reviewer that this is a very interesting point, and we did an exploratory analysis, adjusting our

model III for changes in body weight during the walking program. Please see the results in the table below:
For walking speed expressed in km/h (as in table 2 in the manuscript):

	Total Cholesterol β (95% CI)	LDL-cholesterol β (95% CI)	HDL-cholesterol β (95% CI)	TG β (95% CI)
model I	0.05(0.01-0.09)	0.02(-0.02-0.06)	0.03(0.02-0.05)	-0.02(-0.06-0.03)
model II	0.05(0.01-0.10)	0.02(-0.02-0.06)	0.04(0.02-0.05)	-0.01(-0.06-0.03)
model III	0.06(0.02-0.10)	0.03(-0.01-0.07)	0.04(0.02-0.05)	0.00(-0.05-0.04)
model III +Δweight	0.06(0.02-0.10)	0.02(-0.02-0.06)	0.04(0.02-0.05)	-0.01(-0.05-0.04)

For walking speed expressed in steps/h (as in table 3 in the manuscript):

	Total Cholesterol β (95% CI)	LDL-cholesterol β (95% CI)	HDL-cholesterol β (95% CI)	TG β (95% CI)
model I	0.02(0.00-0.05)	0.02(0.00-0.04)	0.01(0.00-0.02)	-0.01(-0.03-0.01)
model II	0.02(0.00-0.05)	0.02(0.00-0.04)	0.01(0.00-0.02)	-0.01(-0.03-0.01)
model III	0.03(0.00-0.05)	0.02(0.00-0.04)	0.01(0.00-0.02)	0.00(-0.03-0.02)
model III +Δweight	0.02(0.00-0.05)	0.02(0.00-0.04)	0.01(0.00-0.02)	-0.01(-0.03-0.02)

Mode I: crude
Model II: age and gender
Model III: BMI at baseline, current smoking, pulse frequency at baseline.
Model III + Δweight: Model III additionally adjusted for changes in body weight.

Additional adjusting the relation between walking speed and changes in blood lipids for changes in BMI does not alter the results. We made the following adjustments to the manuscript: in the methods section (page 9, line 179-181): “We conducted an exploratory analysis with additional adjustment for changes in body weight, to see if changes in body weight during the walking tour were in the causal pathway of the relation between walking speed and changes in blood lipids”. In the results section (page 12, line 241-243): “Exploratory adjustment of the relation between walking speed and changes in total cholesterol, LDL-c, HDL-c and triglycerides for changes in body weight did not change the results”. And in the discussion section (page 12, line 251-254) “A higher walking speed was related to a higher increase in HDL-c and attenuated decrease in LDL-c and total cholesterol, a relation that was not explained by changes in body weight.” And (page 15, line 315-317): “Furthermore, we showed in an exploratory analysis that the relation between walking speed and changes in blood lipids were not explained by changes in body weight”. And we added to our conclusion (discussion section page 16, line 351 and abstract page 4, line 73): “independent of changes in body weight.”

SPECIFIC COMMENTS
The opening sentence in the "Changes in cardiovascular risk factors..." in the results section describes decreases in TC, LDLC and TG that occur across groups with daily walking. However, in the discussion, the authors write, "A higher walking speed was related to an increase in HDLc, LDLc and total cholesterol." This does not seem to be consistent.
- The reviewer is right, this formulation could be improved. Overall there are marked decreases in total cholesterol, LDL-c and triglycerides, and an increase in HDL-c in the total study population, as described in the original publication of the Santiago study (1) and also in the results section (page 10-11, line 208-216) of the present manuscript. In the present manuscript, we investigated if there was a relation between walking speed and the changes in cardiovascular risk factors. A higher walking speed was related to a higher increase in HDL-c and a smaller decrease in LDL-c and total cholesterol. The statement in the discussion section: "A higher walking speed was related to an

increase in HDLc, LDLc and total cholesterol", means that on top of the earlier described decreases in LDL-c and total cholesterol, and increase in HDL-c, a higher walking speed was related to a higher increase in HDL-c and an attenuated decrease in LDL-c and total cholesterol. Therefore, as suggested by the reviewer, we rephrased the sentence in the discussion section (page 12, line 251-254) to: “A higher walking speed was related to a higher increase in HDL-c and attenuated decrease in LDL-c and total cholesterol, a relation that was not explained by changes in body weight”.
(1) Bemelmans RH, Coll B, Faber DR, Westerink J, Blommaert PP, Spiering W, et al. Vascular and metabolic effects of 12 days intensive walking to Santiago de Compostela. Atherosclerosis 2010 Oct;212(2):621-7.

In the discussion (pg 10, lines 29 - 46): The issue of whether increasing HDLc or lowering LDLc is of greater health significance is not very controversial. Scores of empirical evidence supports the primary target for dyslipidemia is to lower LDLc first. Increasing HDLc is of secondary or tertiary significance. This issue is completely different from the issue of determining what walking speed is best for improving blood lipid profiles. The authors discussion in this section is confusing.
- This is correct. LDL-c is the primary target in the treatment and prevention of cardiovascular disease. The intended message of the paragraph in the discussion section was to speculate about what walking speed is preferred for improving blood lipid profiles. We rephrased this paragraph (discussion section page 13-14, line 271-296) into: “There is no doubt that physical exercise should be advised to everyone who is capable to exercise, as physical exercise has multiple beneficial health effects.[1-3] Furthermore, more exercise is better, as there is a clear inverse dose-response relation between exercise and all-cause mortality.[2] However, what walking speed is optimal for improving the lipid profile is not sure. Should we advise people to walk with high speed or with low speed when the goal is improvement of the lipid profile? In the present study, walking with higher speed increases HDL-c more, but at the expense of less LDL-c decrease, and walking with lower speed leads to less HDL-c increase but a more profound LDL-c decrease. Does the extra increase in HDL-c related to a higher walking speed outweighs the less decrease in LDL-c? This question cannot be answered with the results of the current study. In general, the primary lipid target in the prevention and treatment of cardiovascular disease is LDL-c, which is best reached with lower walking speed, according to the results of the present study. However, in large prospective cohort studies in the healthy population, an increased walking speed assessed by a questionnaire has been related to a lower risk for coronary heart disease and diabetes, independent of walking volume.[17-20] This finding can lead to the speculation that the extra increase in HDL-c related to a higher walking speed could be more important than the less decrease in LDL-c. However, drawing conclusions from the combined findings of these two completely different types of studies is a step to far.”

The authors use the term "pathophysiological" to introduce a discussion of mechanisms (pg 10, lines 48-49). The proper term would be "physiological"...because the descriptions that follow describe normal physiological events that might explain exercise-induced blood lipid changes.
- This is correct, the present study in conducted with healthy volunteers and the results are exercise-induced physiological changes, possibly explained by the physiological mechanisms as stated in the discussion section. We changed “pathophysiological” into “physiological” in the indicated sentence in the discussion section (page 14, line 297).
(pg 14, lines 19-20) "We also acknowledge study limitations."...is this a stand-alone sentence? What limitations do the authors acknowledge?
- This is a result of the first revision round, the first sentence following the sentence “We also acknowledge study limitations” was removed then. The next sentence starts with “secondly”, which is not correct anymore, as it denoted the first limitation. We should have rephrased the next sentence for better understanding, we now removed the word “secondly” (discussion section page 16, line 338), so the paragraph after the sentence “We also acknowledge study limitations” mentions the limitations of the study.

The weight loss that occurred with the pilgrimage reflects an energy imbalance favoring expenditure over consumption. This concept should be recognized and discussed by the authors along with their brief recognition that dietary/nutrient intake data was not obtained. Energy deficits with or without exercise can induce blood lipid changes similar to what is reported in this manuscript.
- The reviewer is right that weight loss is a reflection of an energy imbalance favoring expenditure over consumption. However, we showed before that there was no relation between walking speed and changes in body weight. Furthermore, we now added the exploratory analysis showing that the relation between walking speed and changes in blood lipids are not caused by changes in body weight (see above). Although the reviewer is right that weight loss, whether caused by more expenditure (exercise) or less consumption (dietary intake), can induce blood lipid changes, we believe we have shown that this is not the case in the present study, with the adaptations made before (see above).