Clin Physiol Funct Imaging (2017) 37, pp8–16 doi: 10.1111/cpf.12260

Increased ventilatory response to exercise in symptomatic and asymptomatic *LMNA* mutation carriers: a follow-up study

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Accepted for publication
Received 1 December 2014; accepted 19 April 2015

Key words
cardiomyopathy; clinical exercise testing; $FetCO_2$; spiroergometry; $\dot{V}E/\dot{V}CO_2$ slope

Summary

Background *LMNA* mutations are an important cause of cardiomyopathy often leading to cardiac arrhythmias, heart failure and even heart transplantation. An increasing number of asymptomatic mutation carriers are identified, as family members of the index patients are screened. Our aim was to study the disease progression in asymptomatic *LMNA* mutation carriers and in patients with symptomatic cardiolaminopathy by repeated spiroergometric testing in a prospective clinical follow-up study.

Methods and Results We studied 26 *LMNA* mutation carriers once a year during 5 years up to 6 times by spiroergometry, clinical assessment, laboratory tests and echocardiography. The 23 control subjects underwent clinical assessment and spiroergometry once. Twelve of the mutation carriers were asymptomatic, and 14 had some clinical manifestations of the mutation ranging from clinically relevant rhythm disturbances to DCM and heart failure. Compared to controls, the symptomatic carriers showed a higher slope of the ventilatory equivalent for CO_2 ($\dot{V}E/\dot{V}CO_2$ slope) and a lower fraction of end-tidal CO_2 ($FetCO_2$). The asymptomatic mutation carriers also showed an increased ventilatory response to exercise during the follow-up as indicated by increased $\dot{V}E/\dot{V}CO_2$ slope and decreased $FetCO_2$.

Conclusions The study suggests that an increased ventilatory response during exercise might reveal a preclinical manifestation of DCM in *LMNA* mutation carriers.

Introduction

LMNA mutations cause a variety of clinical phenotypes including cardiomyopathy, lipodystrophy, muscular dystrophies, neuropathy and progeria (Worman, 2012; Carboni *et al.*, 2013). In dilated cardiomyopathy, they are the aetiological cause in about 5% of the cases (Parks *et al.*, 2008; Lakdawala *et al.*, 2012). An increasing number of healthy mutation carriers are in need of follow-up as disease-causing mutations are identified in cardiomyopathy patients, and their family members are screened for them (Perrot *et al.*, 2006; Morales & Hershberger, 2013). Cardiomyopathy-causing *LMNA* mutations are thought to have a near-complete age-dependent penetrance (Van Berlo *et al.*, 2005; Pasotti *et al.*, 2010).

Spiroergometry or cardiopulmonary exercise testing is often used to study patients with heart failure to assess maximal oxygen uptake and functional capacity. Heart failure patients have a lower oxygen consumption and an increased ventilatory response indicated by an elevated $\dot{V}E/\dot{V}CO2$ slope in spiroergometry (Poggio *et al.*, 2010; Apostolo *et al.*, 2012). Both of these abnormalities suggest a poor prognosis (Nanas *et al.*, 2006; Poggio *et al.*, 2010). Changes in lung stiffness, increased dead-space ventilation due to mismatching of ventilation and perfusion, increased exhaled CO2 level, decreased anaerobic threshold and altered ventilatory regulation have been implicated in the abnormal response to exercise (Koike *et al.*, 1992; Wasserman *et al.*, 1997; Ponikowski *et al.*, 2001; Agostoni *et al.*, 2002; Apostolo *et al.*, 2012). Pulmonary oedema is also closely associated with the changes in respiratory mechanics causing reduced lung diffusion and injury to the alveolar–capillary membrane (Apostolo *et al.*, 2012).

The aim of this study was to investigate the disease progression by spiroergometric cardiopulmonary exercise test both in patients with symptomatic cardiolaminopathy and in asymptomatic *LMNA* mutation carriers during a follow-up-period of 5 years. We report the cardiopulmonary exercise test results

Table 1 Characteristics of the control group, and the asymptomatic and symptomatic *LMNA* mutation carriers at baseline.

	Control group *n* = 23	%	Asymptomatic carriers *n* = 12	%	Symptomatic carriers *n* = 14	%
Age	40·78 (± 13·9) 18–70		34·7 (± 13·7) 18–65		48·0 (± 11·8) 25–64	
Men	10	43·5	4	33·3	5	35·7
Women	13	56·5	8	66·7	9	64·3
Height	173 (± 10) 154–193[a]		172 (± 12) 158–196[a]		171 (± 9·8) 157–187[a]	
Weight	74·6 (± 12·1) 50-108[a]		70·9 (± 17·8) 51–105[a]		74·5 (± 14·0) 58–96[a]	
BMI	24·9 (± 3·5) 19·8–33·4[a]		23·8 (±3·9) 18·7–32·7[a]		25·3 (±4·3) 20·4–36·6[a]	
FEV1 (l)	3·5 (± 1·1) 2·0–5·5[a]		3·7 (± 1·0) 2·3–5·1[a]		3·2 (± 1·2) 1·6–5·5[a]	
FEV1 (%)	90·1 (± 11·8) 65–111[a]		96·8 (± 6·8) 85–108[a]		85·3 (± 16·6) 63–114[a]	
Regular leisure exercise	8	34·8	8	66·7	4	28·6
Smoker	4	17·4	1	8·3	2	14·3
Ex-smoker	3	13·0	1	8·3	3	21·4
Non-smoker	11	47·8	10	83·3	9	64·3
Smoking status NA	5	21·7				
Mutations			0		3	
Ala132Pro			2		2	
Arg190Trp			0		1	
G1493del			9		6	
Ser143Pro			1		2	
T1085Xdel						
Medication						
Betablocker	2	8·7	0		7	50
Warfarin	0	0	0		7	50
ACE inhibitor/AT blocker	2	8·7	0		7	50
Statin	2	8·7	0		1	7·1
Diabetes medication	2	8·7	0		0	0
Digoxin	0	0	0		2	14·3
Diuretic	2	8·7	0		2	14·3
Aldosterone antagonist	0	0	0		2	14·3

[a]Mean (± SD) and range are given.

of symptomatic and asymptomatic cardiomyopathy-causing *LMNA* mutation carriers in a prospective follow-up study. To our knowledge, similar studies in cardiomyopathy or laminopathy have not been carried out earlier.

Methods

Patients and controls

The study included 26 carriers of five different *LMNA* mutations known to cause cardiomyopathy (Table 1). The cardiac presentation varied ranging from asymptomatic carriers with no signs of heart disease in clinical assessment, ECG or echocardiography to end-stage dilated cardiomyopathy and heart failure. Due to this variation, we divided the study population into two groups according to their phenotype. Patients with atrial fibrillation, sustained ventricular tachycardia, cardiac pacemakers or ICDs, or dilated cardiomyopathy were included in the symptomatic group. The asymptomatic group comprised of individuals without clinically significant manifestations of their *LMNA* mutation. The subjects did not switch groups during the follow-up. The patients were recruited and studied annually at Helsinki and Kuopio university hospitals between 2005 and 2010.

Twenty-three control individuals, without known heart disease, were matched for the whole study population for age, sex and body mass index.

Methods

The patients were evaluated on an annual basis by clinical examination, 12-lead electrocardiogram, echocardiography and spiroergometry. Echocardiographic data were gathered either from study visits or from hospital records. In addition, data concerning the clinical cardiac follow-up and treatments were collected from hospital records. The 23 control subjects underwent ECG and spiroergometry once. Seventeen of them were also studied with echocardiography.

Cardiorespiratory capacity was measured by a work-conducted maximal exercise test with gas exchange analysis (spiroergometry) using an electrically braked bicycle ergometer (Ergoselect ERG Ergometer; Marquette Hellige, Marquette Medical Systems, Germany) and a breath-by-breath gas exchange analysis system (V_{max} Encore, Sensormedics, Yorba Linda, CA, USA) with the test subject sitting upright. The initial workload was normally 40 W for women and 50 W for men. The work load was then increased by 40 or 50 W, respectively, at 3-min intervals until level 17–19/20 on the Borg scale for perceived exertion was reached and respiratory quotient RQ ($=\dot{V}CO_2/\dot{V}O_2$) >1·0. Ventilatory anaerobic threshold was assessed at the slope change of $\dot{V}CO_2$ exceeding $\dot{V}O_2$, increase of $\dot{V}E/\dot{V}O_2$ compared to $\dot{V}E/\dot{V}CO_2$ and increase of $PetO_2$ versus $PetCO_2$ (partial pressures of O_2 and CO_2 in expiratory air) (Balady *et al.*, 2010). Blood pressure was measured manually using a stethoscope and a sphygmomanometer (Erka, Germany). Measurements were taken from the left arm before exercise, at each exercise level, and 4 and 6 min after exercise. A 12-lead ECG (Mason–Likar) was continuously monitored and recorded during the exercise test using a computerized device (CardioSoft version V6·5, GE Medical systems, Milwaukee, WI, USA). For measurement of respiratory gases, a tightly attached face mask (Rudolph series 7910, Hans Rudolph, Kansas City, MI, USA) with a dead space of 185 ml was used. Arterial O_2 saturation was assessed non-invasively with two pulse oxymeters (Datex-Ohmeda 3900 and Datex-Ohmeda 3800; Datex-Ohmeda, Louisville, CO, USA). One sensor was attached to the earlobe and one to the left middle finger. A mass flow sensor of the gas exchange device (Sensormedics) was used to measure forced expiratory volume in 1 second (FEV1) before exercise and to assess tidal volume and minute ventilation during exercise. From the breath-by-breath recordings, ventilatory and gas exchange variables were averaged at 30-s intervals using computer-assisted equipment.

Echocardiography data from study visits or hospital records from clinical follow-up visits were used. The study visit echocardiography protocol included M-mode, two-dimensional and Doppler analyses (Vivid 7; GE Medical Systems, Horten, Norway). Individuals with left ventricular ejection fraction <45% and surface area–normalized LVEDD >27 mm m^{-2} were diagnosed with DCM (Kärkkäinen *et al.*, 2004).

The protocol (SPSS Inc, Chicago, IL, USA) was designed and performed according to the principles of the Declaration of Helsinki and was approved by the Ethical Committee of the Helsinki University Central Hospital (decision number: Dnro 322/E5/03).

Statistical methods

IBM SPSS version 20.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analyses. Analysis of covariance with age, gender, weight, height and the use of beta-blockers as covariates was used to compare the results of the symptomatic and asymptomatic patients to the controls for parameters concerning oxygen uptake, heart rate or working capacity. For echocardiography parameters and those spiroergometry parameters measuring ventilation, beta-blockers were not included as a covariate. Most of the variables were normally distributed. For those few parameters that were not normally distributed, the comparisons of the patients to the control group giving statistically significant differences in covariance analysis were confirmed using the nonparametric Mann–Whitney U-test.

Paired *t*-test was used to compare the results within the symptomatic and asymptomatic *LMNA* mutation carrier groups during the follow-up. Bivariate correlation tests were performed between some cardiologic and gas exchange variables, Pearson correlation was used for normally distributed parameters, and Spearman correlation if the parameters were not normally distributed.

Results

The clinical characteristics of the mutation carriers and the control individuals are reported in Table 1. The mean follow-up times were 44 months for the entire study group, 51 (0–63) months for the asymptomatic group and 38 (0–66) months for the symptomatic group. Figure 1 plots $FetCO_2$ and $\dot{V}E/\dot{V}CO_2$ slope against left ventricular ejection fraction in all mutation carriers at baseline. Figure 2 shows the $\dot{V}E/\dot{V}CO_2$ slope values and Fig. 3 the $FetCO_2$ values of symptomatic and asymptomatic mutation carriers in follow-up.

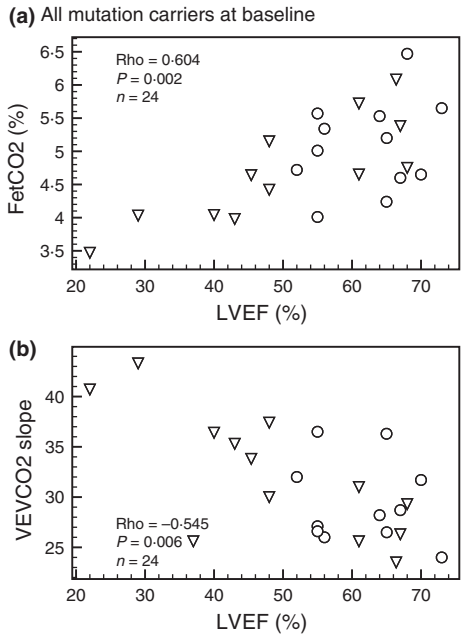

Figure 1 $FetCO_2$ at maximal exercise (a) and $\dot{V}E/\dot{V}CO_2$ slope (b) over left ventricular ejection fraction (LVEF) at the baseline examination. The asymptomatic LMNA mutation carriers are indicated with circles and the symptomatic with triangles.

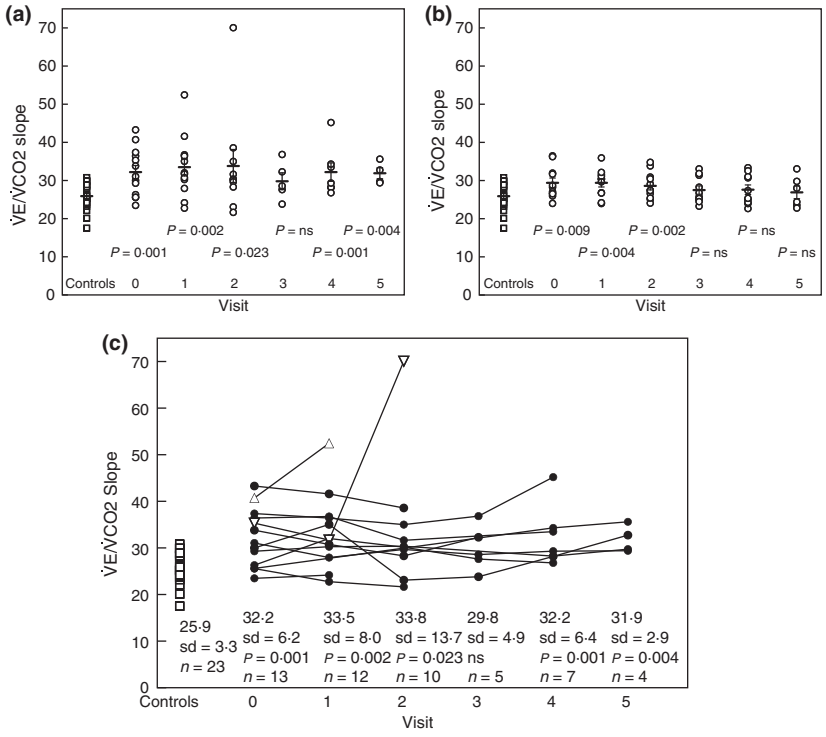

Figure 2 (a,b) $\dot{V}E/\dot{V}CO_2$ slope of the symptomatic (a) and asymptomatic (b) *LMNA* mutation carriers and the control group. (c). The progression of $\dot{V}E/\dot{V}CO_2$ slope in follow-up of the individual symptomatic mutation carriers. The two individuals marked by triangles received heart transplants after 3 and 6 months of the last follow-up visit they attended.

Symptomatic *LMNA* mutation carriers

The results of the symptomatic *LMNA* mutation carriers are reported in Table 2. The maximal heart rate during exercise was lower in the symptomatic *LMNA* mutation carriers compared to the control group throughout the follow-up, but the difference was not statistically significant at all visits. The symptomatic mutation carriers had a lower maximal working capacity (per cent of predicted value) than the control group, but the difference was not statistically significant at follow-up visits 3 and 5. No further reduction from baseline in maximal working capacity within the symptomatic *LMNA* mutation carrier group was seen. Anaerobic threshold was lower in this group than in the control group, but the difference was not statistically significant.

Breathing reserve did not differ from that of the control groups. The ventilatory equivalents for O_2 and CO_2 ($\dot{V}EO_2$ and $\dot{V}ECO_2$) of the symptomatic carriers were significantly higher than in the control group throughout the follow-up. Similarly, $FetCO_2$ was significantly lower in the symptomatic carrier group than in the control group during the entire follow-up. $\dot{V}E/\dot{V}CO_2$ slope was continuously higher in the symptomatic group compared to the control group, and the difference was statistically significant at all visits except follow-up visit 3. The progression of $\dot{V}E/\dot{V}CO_2$ slope in each individual symptomatic mutation carrier is plotted in Fig. 2. Left ventricular ejection fraction was lower in the symptomatic group than in the control group, the difference being statistically significant at baseline and follow-up visits 1 and 3.

Asymptomatic *LMNA* mutation carriers

The results of the asymptomatic *LMNA* carriers are reported in Table 3. The asymptomatic group had a very similar overall performance in spiroergometry compared to the controls throughout the follow-up. Similarly, there was no significant difference between the asymptomatic *LMNA* carriers and the control group in LVEF measured by echocardiography.

As the follow-up progressed, the maximal oxygen uptake and oxygen pulse improved within the asymptomatic carrier group. Using paired t-test, a statistically significant improvement from baseline was seen towards the end of the follow-up. However, comparing these parameters to the control group did not show statistically significant differences.

The ventilatory equivalents of the asymptomatic carriers did not systematically differ from the controls, whereas $\dot{V}E/\dot{V}CO_2$ slope was significantly higher in the asymptomatic carriers compared to the control group from baseline to follow-up visit 2 (Fig. 2). The $FetCO_2$ level was significantly lower in the asymptomatic carriers compared to the control group from follow-up visits 1–4.

Dropouts

Only 4 of the initial 14 symptomatic carriers attended the last follow-up visit. Two patients dropped out from follow-up due to heart transplants. Two patients were unable to continue

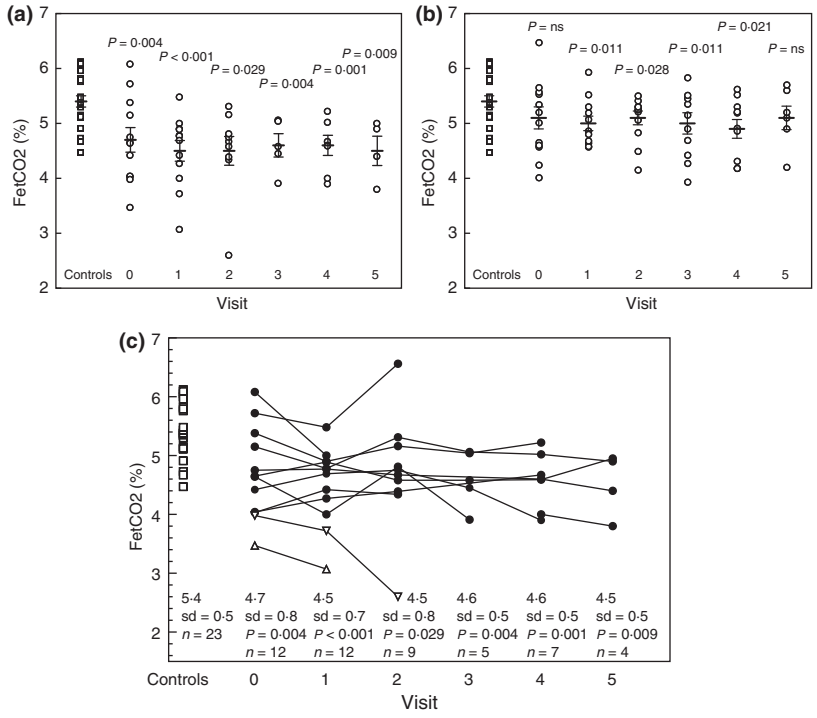

Figure 3 (a,b) The fraction of end-tidal CO_2 level at maximal exercise ($FetCO_2$) of the symptomatic (a) and asymptomatic (b) *LMNA* mutation carriers and the control group. (c) The progression of $FetCO_2$ in follow-up of the individual symptomatic mutation carriers. The two individuals marked by triangles received heart transplants after 3 and 6 months of the last follow-up visit they attended.

the follow-up due to ICDs. Three patients were lost to follow-up due to the progression of their dilated cardiomyopathy. Three patients finished the follow-up early because they were enrolled to the study at a later stage. One of them underwent spiroergometry three times, one-four times and one-five times.

Six of the initial 12 asymptomatic carriers attended the last follow-up visit. Three asymptomatic carriers were enrolled at a later stage of the study and underwent follow-up until follow-up visit 4. One asymptomatic carrier could not attend the last spiroergometry due to a bone fracture, and another one due to pregnancy. One participant was enrolled at such a late stage that she only underwent spiroergometry once.

Discussion

We present the results of a 5-year follow-up study of symptomatic and asymptomatic *LMNA* mutation carriers. The symptomatic mutation carriers showed lower oxygen uptake, a non-significantly lower anaerobic threshold and signs of increased ventilation during exercise. A previously undescribed finding in our study was that also the asymptomatic carriers presented with signs of increased ventilation during exercise, namely, elevated $\dot{V}E/\dot{V}CO_2$ slope and decreased $FetCO_2$.

Our study population of 26 *LMNA* mutation carriers is clinically thoroughly studied. Fourteen patients were symptomatic with dilated cardiomyopathy, arrhythmias and/or conduction defects necessitating pacemakers, or atrial fibrillation. Twelve mutation carriers were asymptomatic and showed no clinically relevant abnormalities in echocardiography, nor hemodynamically significant arrhythmias. As expected, signs of increased ventilation during exercise typical for heart failure were found in the symptomatic *LMNA* mutation carriers (Buller & Poole-Wilson, 1990; Francis *et al.*, 2000; Mezzani *et al.*, 2009; Apostolo *et al.*, 2012). However, the asymptomatic *LMNA* mutation carrier group also showed an increased ventilatory response to exercise in terms of a decreased $FetCO_2$ and increased $\dot{V}E/\dot{V}CO_2$ slope.

A decreased oxygen uptake during cardiopulmonary exercise testing is a known predictor of clinical outcome in heart failure (Myers & Froelicher, 1991; Cahalin *et al.*, 2013; Chase *et al.*, 2013). This is easily understandable as oxygen uptake is a product of cardiac output and the difference between arterial and venous oxygen (Myers & Froelicher, 1991). More recently established variables with predictive power in heart failure outcome are those measuring excessive ventilation during exercise, namely the ventilatory equivalents, $\dot{V}E/\dot{V}CO_2$ slope and $FetCO_2$ (Francis *et al.*, 2000; Cahalin *et al.*, 2013; Chase *et al.*, 2013). The combination of an early anaerobic threshold associated with a poor overall condition, increased dead-space ventilation caused by increased overall ventilation, reduced pulmonary perfusion causing ventilation-perfusion mismatching and an altered control of ventilation caused by enhanced chemosensitivity are likely mechanisms to the increased ventilatory response to exercise (Buller & Poole-Wilson, 1990; Koike *et al.*, 1992; Sovijärvi *et al.*, 1992; Chua *et al.*, 1997; Wasserman *et al.*, 1997; Ponikowski *et al.*, 2001; Agostoni *et al.*, 2002; Apostolo *et al.*, 2012).

As the symptomatic group comprised mainly patients with dilated cardiomyopathy and systolic heart failure, an increased

Table 2 The spiroergometry and echocardiography results of the symptomatic *LMNA* mutation carriers and the control group. Mean and standard deviation are given.

	Controls $n = 23$	Symptomatic carriers baseline $n = 14$	Control visit 1 $n = 12$	Control visit 2 $n = 10$	Control visit 3 $n = 5$	Control visit 4 $n = 7$	Control visit 5 $n = 4$
Maximal heart rate (1 per min)	172·1 ± 19·3	136·6 ± 35·3*	135·9 ± 40·3	126·2 ± 46·2*	133·8 ± 51·6	145·6 ± 36·4	111·8 ± 39·9*
Maximal heart rate (% of predicted)	93·2 ± 8·3	75·1 ± 18·0*	75·0 ± 20·5	69·8 ± 24·5*	71·5 ± 29·3	81·7 ± 18·7	63·5 ± 22·6*
Maximal working capacity/3 min (W_{max} per 3 min) (W)[a]	175·7 ± 65·1	122·6 ± 60·0	120·3 ± 62·9	107·5 ± 74·2	116·2 ± 89·4	103·4 ± 69·4	79·3 ± 29·0
Maximal working capacity/3 min (% of predicted)	95·0 ± 17·5	64·7 ± 18·2*	62·8 ± 22·7*	61·5 ± 28·0*	63·9 ± 28·1	62·7 ± 21·1*	63·5 ± 26·4
Maximal oxygen uptake ($\dot{V}O_2$) (l per min)	2·4 ± 0·9	1·7 ± 0·7*	1·6 ± 0·8	1·6 ± 1·0	1·8 ± 1·3	1·7 ± 0·9	1·3 ± 0·3
Maximal oxygen uptake kg^{-1} (ml min^{-1} kg^{-1})	32·8 ± 11·2	22·4 ± 8·0*	20·4 ± 9·3	20·5 ± 10·4	25·1 ± 15·2	22·3 ± 10·1	18·1 ± 6·2
Oxygen pulse ($\dot{V}O_2$/HR) (ml)	13·9 ± 4·7	12·1 ± 3·1	11·1 ± 2·8**	12·2 ± 4·6	13·6 ± 6·4	11·2 ± 4·5	12·9 ± 4·4
Anaerobic threshold (AT) (ml min^{-1})	1343 ± 479	990 ± 373	928 ± 474	1002 ± 427	1024 ± 383	1092 ± 777	822 ± 249
AT % of estimated maximal $\dot{V}O_2$	56·8 ± 14·9	41·7 ± 10·9	38·1 ± 13·3	43·5 ± 11·7	44·9 ± 10·0	49·3 ± 21·1	46·6 ± 13·9
Breathing reserve ((MVV–VE)/MVV) × 100) where MVV = 38 × FEV1 and VE = maximal minute ventilation	34·3 ± 20·3	39·9 ± 10·1	40·7 ± 11·4	43·1 ± 16·0	42·4 ± 11·6	37·0 ± 8·4	42·0 ± 4·6
Ventilatory equivalent for $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$)	34·0 ± 4·4	41·6 ± 7·5*	46·0 ± 8·2*·**	43·8 ± 10·6*	40·0 ± 5·7*	41·1 ± 8·0*	42·0 ± 9·1
Ventilatory equivalent for $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$)	29·7 ± 3·2	35·4 ± 6·8*	38·2 ± 7·5*	37·4 ± 10·3*	34·4 ± 2·4*	35·4 ± 4·9*	35·3 ± 4·6*
Respiratory quotient (RQ)	1·14 ± 0·07	1·18 ± 0·06	1·21 ± 0·06*	1·18 ± 0·10	1·16 ± 0·10	1·15 ± 0·07	1·2 ± 0·1
Fraction of end-tidal CO_2 ($FetCO_2$) (%)	5·4 ± 0·5	4·7 ± 0·8*	4·5 ± 0·7*	4·5 ± 0·8*	4·6 ± 0·5*	4·6 ± 0·5*	4·5 ± 0·5*
W_{max}/VO_{2max} (Mechanical efficiency (%))	21·2 ± 2·6	20·6 ± 2·4	21·8 ± 2·9	19·6 ± 3·1	17·7 ± 2·7	17·4 ± 1·6*·**	16·8 ± 2·7**
$\dot{V}E/\dot{V}CO_2$ slope	25·9 ± 3·3	32·2 ± 6·2*	33·5 ± 8·0*	33·8 ± 13·7*	29·8 ± 4·9	32·2 ± 6·4*	31·9 ± 2·9*
Dead-space ventilation/tidal volume (VD/VT)	0·13 ± 0·05	0·17 ± 0·06	0·18 ± 0·07	0·18 ± 0·08	0·13 ± 0·08**	0·15 ± 0·07	0·17 ± 0·02
Ejection fraction in echocardiography (%)	60·4 ± 4·9	48·9 ± 14·9*	46·5 ± 13·7*	49·4 ± 16·1	49·5 ± 7·6*	52·4 ± 14·0	53·9 ± 9·0
Left ventricular end-diastolic diameter in echocardiography (mm)	48·1 ± 4·9	55·4 ± 4·7*	53·5 ± 5·4*·**	55·1 ± 6·2*	49·0 ± 6·0	51·7 ± 3·3	49·4 ± 2·0**

*P<0·05; patients compared to controls using covariance analysis.

**P<0·05; within-group comparisons, control visit results compared to baseline results.

[a]W_{max}/3 min was defined as the mean workload during the last 3 min of exercise (Nordesjö & Landelius, 1975).

Table 3 The spiroergometry and echocardiography results of the asymptomatic *LMNA* mutation carriers and the control group. Mean and standard deviation are given.

	Controls $n = 23$	Asymptomatic carriers baseline $n = 12$	Control visit 1 $n = 11$	Control visit 2 $n = 11$	Control visit 3 $n = 10$	Control visit 4 $n = 10$	Control visit 5 $n = 6$
Maximal heart rate (1 per min)	172·1 ± 19·3	175·3 ± 20·6	175·7 ± 17·3	172·6 ± 16·3	172·6 ± 12·4	168·7 ± 16·1	170·0 ± 19·5
Maximal heart rate (% of predicted)	93·2 ± 8·3	93·2 ± 8·9	93·3 ± 8·5	91·8 ± 7·5	92·1 ± 7·0	90·3 ± 7·8	91·7 ± 9·4
Maximal working capacity/3 min (W_{max}/3 min) (W)[a]	175·7 ± 65·1	165·0 ± 57·8	175·7 ± 63·6	175·3 ± 63·8	175·0 ± 60·8	166·9 ± 64·9	165·3 ± 57·6
Maximal working capacity/3 min (% of predicted)	95·0 ± 17·5	89·1 ± 17·9	89·4 ± 13·5	92·1 ± 12·1	85·8 ± 14·2	87·4 ± 14·8	90·8 ± 17·4
Maximal oxygen uptake ($\dot{V}O_2$) (l per min)	2·4 ± 0·9	2·2 ± 0·8	2·2 ± 0·8	2·3 ± 0·9	2·6 ± 0·9	2·5 ± 0·9	2·4 ± 0·8**
Maximal oxygen uptake kg^{-1} (ml min^{-1} kg^{-1})	32·8 ± 11·2	29·1 ± 5·9*	30·2 ± 5·0	32·1 ± 7·2	32·6 ± 6·6	34·4 ± 5·1**	31·4 ± 3·9**
Oxygen pulse ($\dot{V}O_2$/HR) (ml)	13·9 ± 4·7	11·9 ± 4·1	12·4 ± 4·3	13·4 ± 4·8	14·5 ± 5·8**	14·7 ± 5·8**	14·6 ± 6·0**
Anaerobic threshold (AT) (ml min^{-1})	1343 ± 479	1129 ± 398	1221 ± 551	1373 ± 427**	1152 ± 349	1216 ± 473	1117 ± 297
AT % of estimated maximal of $\dot{V}O_2$	56·8 ± 14·9	46·8 ± 11·2*	49·8 ± 15·2	57·4 ± 14·5**	47·4 ± 10·1	50·4 ± 14·7	48·3 ± 6·2
Breathing reserve ((MVV−VE)/MVV) × 100) where MVV = 38 × FEV1 and VE = maximal minute ventilation	34·3 ± 20·3	44·6 ± 12·6	43·8 ± 8·0	40·1 ± 13·4	40·6 ± 8·4	37·6 ± 12·1	35·0 ± 16·9
Ventilatory equivalent for $\dot{V}O_2$ ($\dot{V}E/\dot{V}O_2$)	34·0 ± 4·4	37·8 ± 6·7	37·9 ± 4·7*	37·2 ± 6·3	36·7 ± 7·5	36·6. ± 4·1	34·3 ± 4·8**
Ventilatory equivalent for $\dot{V}CO_2$ ($\dot{V}E/\dot{V}CO_2$)	29·7 ± 3·2	32·7 ± 4·3	32·0 ± 3·0*	31·8 ± 3·1*·**	31·5 ± 4·2	31·6 ± 3·1	30·3 ± 4·2**
Respiratory quotient (RQ)	1·14 ± 0·07	1·15 ± 0·08	1·18 ± 0·06*	1·17 ± 0·10	1·15 ± 0·09	1·15 ± 0·06	1·15 ± 0·04
Fraction of end-tidal CO_2 ($FetCO_2$) (%)	5·4 ± 0·5	5·1 ± 0·7	5·0 ± 0·4*	5·1 ± 0·4*	5·0 ± 0·6*	4·9 ± 0·5*	5·1 ± 0·5
W_{max}/VO_{2max} (Mechanical efficiency (%))	21·2 ± 2·6	22·7 ± 2·6	23·2 ± 1·4	21·9 ± 2·5	20·5 ± 1·3**	19·4 ± 1·0**	19·6 ± 1·0**
$\dot{V}E/\dot{V}CO_2$ slope	25·9 ± 3·3	29·4 ± 4·2*	29·4 ± 3·6*	28·6 ± 3·5*	27·5 ± 3·4	27·6 ± 3·9	26·9 ± 4·1
Dead-space ventilation/tidal volume (VD/VT)	0·13 ± 0·05	0·14 ± 0·07	0·13 ± 0·07	0·13 ± 0·07**	0·09 ± 0·05*·**	0·10 ± 0·05*·**	0·11 ± 0·05
Ejection fraction in echocardiography (%)	60·4 ± 4·9	62·1 ± 7·1	61·1 ± 7·6	59·5 ± 6·8	57·6 ± 8·3	61·5 ± 8·5	61·7 ± 9·1
Left ventricular end-diastolic diameter in echocardiography (mm)	48·1 ± 4·9	49·1 ± 5·3	50·7 ± 5·9	51·0 ± 5·7	50·9 ± 4·8	50·2 ± 5·6	50·7 ± 6·3

*P<0·05; patients compared to controls using covariance analysis.

**P<0·05; within-group comparisons, control visit results compared to baseline results.

[a]W_{max}/3 min was defined as the mean workload during the last 3 min of exercise (Nordesjö & Landelius, 1975).

 37, 1, 8–16

ventilatory response to exercise was foreseeable. The similar ventilatory response to exercise of the asymptomatic group, however, was somewhat unexpected. The asymptomatic carriers did not have increased dead-space ventilation, nor decreased anaerobic threshold. Thus, increased chemosensitivity or incipient ventilation-perfusion mismatch might offer a feasible mechanism. If a ventilation-perfusion mismatch was present, it was slight because no hypoxaemia was present. The mean LVEDD in the asymptomatic carriers was slightly, non-significantly larger than in the controls, suggesting that subclinical cardiomyopathy might also be a basis for the finding.

$\dot{V}E/\dot{V}CO2$ slope levels > 30–34 have been associated with an increased risk of events and mortality in heart failure patients (Chua *et al.*, 1997; Francis *et al.*, 2000; Gitt *et al.*, 2002; Arena *et al.*, 2004; Balady *et al.*, 2010; Cahalin *et al.*, 2013), whereas values between 20 and 30 are regarded as normal (Mezzani *et al.*, 2009; Balady *et al.*, 2010). In our study, the levels of $\dot{V}E/\dot{V}CO_2$ slope were between 30 and 34 in the symptomatic mutation carrier group and between 27 and 30 in the asymptomatic mutation carrier group. However, the $\dot{V}E/\dot{V}CO_2$ slope values presented here are not directly comparable with the mortality studies quoted above as they evaluated the power of spiroergometric indices to predict events and mortality in heart failure patients after a single spiroergometry. We are not aware of other studies in which cardiomyopathy patients or heart failure patients have been tested repeatedly using spiroergometry for several years.

There has been some controversy on the measurement technique of $\dot{V}E/\dot{V}CO_2$ slope (Tabet *et al.*, 2003; Mezzani *et al.*, 2009). We measured the initial part of the slope ignoring the part turning nonlinear towards the end of exercise. The method used here gives lower values than the measurement of the final or overall $\dot{V}E/\dot{V}CO_2$ slope (Tabet *et al.*, 2003). It is also considered more physiological, which is why it is recommended in a recent American Heart Association guideline (Balady *et al.*, 2010). Furthermore, using the initial part of the slope is probably more suitable for repeated testing than measuring the final $\dot{V}E/\dot{V}CO_2$ slope, which is more dependent on reaching maximal exercise and may therefore vary in a clinical material.

Some patients in the symptomatic group dropped out of the study due to a worsening clinical condition, whereas the patients attending the entire follow-up were in better clinical condition. This explains why the results of the symptomatic carrier group appear to improve at follow-up visits 3–5.

Towards the end of the follow-up, the cardiac performance of the asymptomatic mutation carrier group improved as measured by the oxygen pulse and oxygen uptake. Dropout does not offer an explanation to this, as reaching the final 5th follow-up visit was not affected by clinical condition in this group. It may be that learning about their cardiomyopathy-causing *LMNA* mutation and regular follow-up and spiroergometric tests could have encouraged the asymptomatic carriers to exercise more. In addition, the study group got more familiar with the spiroergometry method, which might have made the testing slightly easier and diminished anxiety caused by it.

Dropout of patients caused difficulties in the statistical testing as repeated-measurement tests were not applicable. Therefore, analysis of covariance was used. The comparisons were not independent comparisons of multiple variables, but instead repeated measurements of the same subjects, which is why Bonferroni correction was not applicable here. The control group underwent clinical exercise testing only once, which is a weakness in our study.

This is a clinical prospective follow-up study on a genetically defined cardiomyopathy. A lower oxygen uptake and an enhanced ventilatory response during exercise typical to heart failure were found in the symptomatic *LMNA* mutation carrier group. Also the asymptomatic *LMNA* mutation carriers showed signs of excessive ventilation during exercise, namely increased $\dot{V}E/\dot{V}CO_2$ slope and decreased $FetCO_2$. Our findings suggest that increased ventilation during exercise may be a subclinical sign of cardiomyopathy in otherwise healthy *LMNA* mutation carriers.

Acknowledgments

We would like to thank Professor Seppo Sarna for his excellent advice concerning statistical analysis and Sini Weckström, RN, for invaluable assistance in data collection, Professors Tomi Laitinen and Esko Vanninen for spiroergometry testing performed in Kuopio University Hospital and Docent Tiina Lyyra-Laitinen for management of digital data.

Funding

Aarne Koskelo Foundation, Finnish Foundation for Cardiovascular Research, Governmental subsidy (EVO-grants), Finnish Medical Foundation, Ida Montin Foundation.

Conflict of interest

The authors have no conflicts of interest.

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