Published in final edited form as:
Aging Cell. 2012 August ; 11(4): 644–650. doi:10.1111/j.1474-9726.2012.00825.x.

Caloric restriction may reverse age-related autonomic decline in humans

Phyllis K. Stein[1,*], **Andreea Soare**[2,3,*], **Timothy E. Meyer**[2], **Roberto Cangemi**[2,4], **John O. Holloszy**[2], and **Luigi Fontana**[2,5]

[1]HRV Laboratory, Washington University School of Medicine, St. Louis, MO, USA

[2]Division of Geriatrics and Nutritional Science, Washington University School of Medicine, St. Louis, MO, USA

[3]Department of Endocrinology and Diabetes, University Campus Bio-Medico, Rome, Italy

[4]Department of Experimental Medicine, University of Rome 'La Sapienza', Rome, Italy

[5]Department of Medicine, Salerno University School of Medicine, Salerno and CEINGE Biotecnologie Avanzate, Napoli, Italy

Summary

Caloric restriction (CR) retards aging in laboratory rodents. No information is available on the effects of long-term CR on physiologic markers of aging and longevity in humans. Heart rate variability (HRV) is a marker for cardiac autonomic functioning. The progressive decline in HRV with aging and the association of higher HRV with better health outcomes are well established. Heart rate variability assessment is a reliable tool by which the effects of CR on autonomic function can be assessed. Time- and frequency-domain analyses compared 24-h HRV in 22 CR individuals aged 35–82 years and 20 age-matched controls eating Western diets (WD). The CR group was significantly leaner than the WD group. Heart rate was significantly lower, and virtually, all HRV values were significantly higher in the CR group than in the WD group ($P <$ 0.002). Heart rate variability in the CR individuals was comparable with published norms for healthy individuals 20 years younger. In addition, when differences in HRAUTHOR: Please define HR. and HRV between CR and WD were compared with previously published changes in HRV induced in healthy adults given atenolol, percent differences in each measure were generally similar in direction and magnitude and suggested declines in sympathetic and increases in parasympathetic modulation of HR and increased circadian variability associated with CR. These

CorrespondenceLuigi Fontana, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8031, St. Louis, MO 63110, USA. Tel.: +314 747 1485; fax: +314 362 7657; lfontana@dom.wustl.edu and Phyllis K. Stein, Washington University School of Medicine, 660 S. Euclid Avenue, Campus Box 8086, St. Louis, MO 63110, USA. Tel.: +314 286 1350; fax: +314 747 8560; pstein@dom.wustl.edu.
*These authors contributed equally to this research.

Financial disclosures
None.

Author contributions
P. K. Stein participated in the concept, design, implementation of the study, analysis and interpretation of data, and undertook plausibility testing, drafted the report, and has seen and approved the final version. A. Soare participated in the implementation of the study, analysis and interpretation of data, helped in undertaking plausibility testing, in the drafting of the report, and has seen and approved the final version. T. E. Meyer participated in the design, implementation of the study, in the revision of the manuscript, and has seen and approved the final version. R. Cangemi participated in the implementation of the study, analysis and interpretation of the data, in the revision of the manuscript, and has seen and approved the final version. J. O. Holloszy participated in the concept and design of the study, drafting of the report, and has seen and approved the final version. L. Fontana participated in the concept, design, implementation of the study, analysis and interpretation of the data, drafted the report, and has seen and approved the final version.

findings provide evidence that CR has direct systemic effects that counter the expected age-associated changes in autonomic function so that HRV indexes in CR individuals are similar to those of individuals 20 years younger eating WDs.

Keywords

calorie restriction; heart rate variability; autonomic function; parasympathetic function; aging; cardiovascular health

Introduction

Caloric restriction without malnutrition (CR) slows aging, increases maximal life span, and protects against stress in many model organisms including yeast, worms, flies, mice, and rats (Weindruch & Walford, 1988; Fontana *et al.*, 2010a,b)AUTHOR: Fontana *et al.*, 2010 has been changed to Fontana *et al.*, 2010a, 2010b so that this citation matches the Reference List. Please confirm that this is correct.. However, little is known regarding the long-term effects of CR on the age-associated deterioration of physiological parameters in humans. The autonomic nervous system (ANS) plays a key role in controlling and coordinating several important physiological functions (Llewellyn-Smith & Verberne, 2011).

Aging is associated with increasing homeostatic imbalance, and autonomic function is altered with aging (Pfeifer *et al.*, 1983; Cowen, 1993) resulting in a progressive decline in heart rate variability (HRV), which is a well-accepted index of ANS function (O'Brien *et al.*, 1986; Ferrari *et al.*, 1991; Schwartz *et al.*, 1991; Umetani *et al.*, 1998; Zulfiqar *et al.*, 2010). Heart rate variability is a composite reflection of the interactions among multiple physiologic systems (e.g., autonomic outflow and inflow and neuroendocrine function) at the cellular, tissue, and organ level. Heart rate variability decreases in various disease states, including heart disease, hypertension, obesity, and inflammatory disease, and higher HRV is generally associated with global health (Dekker *et al.*, 1997; De Meersman & Stein, 2007). However, HRV also decreases progressively with age in normal, healthy individuals (O'Brien *et al.*, 1986; Schwartz *et al.*, 1991; Umetani *et al.*, 1998; De Meersman & Stein, 2007; Zulfiqar *et al.*, 2010). This has led to the suggestion that HRV could serve as a marker of biological age, as opposed to chronological age (Corino *et al.*, 2007).

In rodents, long-term CR has been shown to improve autonomic function and, in particular, to increase the high-frequency component of the HRV spectrum, a marker for a respiration-mediated parasympathetic activity (Herlihy *et al.*, 1992; Cowen *et al.*, 2000; Mager *et al.*, 2006). The purpose of the present study was to investigate whether CR counters the age-related decline in HRV in individuals who have been practicing long-term, strict CR. To this end, we compared indexes of HRV obtained from 24-h Holter recordings in the CR with those of age-matched, healthy individuals eating usual Western diets (WD). To further put these findings into perspective, we compared HRV measures in the CR group with published data on normative values for HRV at different ages (Umetani *et al.*, 1998). We also compared differences in HRV between CR and WD with published changes in HRV induced by the administration of atenolol in healthy young adults (Cook *et al.*, 1991).

Results

Study population characteristics

Clinical, demographic, and laboratory characteristics for the entire subject sample are summarized in Table 1. Age and gender were not different, but body mass index was significantly lower in the CR than the WD group (Table 1). Total body fat, measured by

dual-energy X-ray absorptiometry, was also much lower in CR than in the WD group (Table 1).

Cardiometabolic risk factors

Serum total cholesterol and high-density lipoprotein cholesterol concentrations of the WD group (Table 1) fell close to the 50th percentile for people in their age group in the Unites States (National Heart, Lung, and Blood Institute, 2001). In contrast, the average serum total cholesterol concentrations of the CR group fell into the lowest 10% for people in their age group (National Heart, Lung, and Blood Institute, 2001) (Table 1). Unlike the decrease in high-density lipoprotein cholesterol that often occurs when individuals are placed on low-fat diets to lose weight, the CR group had high levels, in the 85th to 90th percentile range for middle-aged men in the United States. (National Heart, Lung, and Blood Institute, 2001) (Table 1). As a consequence, their total cholesterol/high-density lipoprotein cholesterol ratio was remarkably low. Blood pressure for the WD (Table 1) was also similar to the average values found in middle-aged healthy people in the United States (Burt *et al.*, 1995; National Institute of Diabetes and Digestive and Kidney Diseases, 1995); however, both systolic and diastolic blood pressures were significantly lower in the CR group ($P < 0.001$) and fell into the range found in 10-year olds (Williams *et al.*, 2002) (Table 1). Serum total cholesterol and high-density lipoprotein cholesterol concentrations and blood pressure levels of some of our CR subjects have been previously reported (Fontana *et al.*, 2004).

Nutrient intake

Caloric restriction subjects consumed a variety of nutrient-dense unprocessed foods (i.e., vegetables, fruits, nuts, egg whites, fish, poultry, low-fat dairy products, whole grains, and beans) that supplied > 100% of the recommended daily intake for all essential nutrients. Refined foods rich in empty calories, trans-fatty acids, and salt were avoided. Energy intake was 30% lower in CR (1765 ± 328 kcal day^{-1}) than in WD (2528 ± 463 kcal day^{-1}) ($P < 0.001$). The percentage of total energy intake derived from protein, carbohydrate, fat, and alcohol was: 22%, 50%, 28%, and 0.1%, respectively, in the CR and 16%, 48%, 32%, and 4% in the WD.

Twenty-four-hour, daytime and nighttime HR, and 24-h heart rate variability analysis

All subjects were in normal sinus rhythm and therefore had recordings eligible for HRV analysis. $N = 22$ recordings for CR and 20 for WD were obtained. $N = 21$ recordings for CR and 20 recording for WD were eligible for 24-h time-domain HRV analysis. For the frequency-domain analysis, 20 CR and 18 WD had eligible 24-h data. Criteria for electrocardiogram data quality sufficient for 24-h time-domain HRV analysis and for frequency-domain analysis have been described in the Experimental procedures.

Table 2 compares 24-h, daytime and nighttime heart rate, and 24-h HRV measures between CR and WD. Twenty-four-hour, daytime, and nighttime heart rates were significantly lower in CR than in WD ($P < 0.001$). At the same time, virtually all 24-h HRV measures were significantly higher ($P < 0.005$) in CR vs. WD. The exception was low-frequency power where the difference in ln-transformed measures was of borderline significance ($P = 0.06$). Differences in the ratio of HRV measures were smaller between groups but remained significant ($P < 0.02$).

Comparison of caloric restriction-associated with age-related changes in heart rate variability Figure 1 shows plots of selected HRV measures by age taken from published norms (Umetani *et al.*, 1998). Filled diamonds indicate mean values for CR, and filled circles indicate mean values for WD. As can be seen in the figure, mean 24-h standard deviation of normal-to-normal (N-N) intervals in ms (SDNN), 24-h SD of 5-min-averaged

N-N intervals (SDANN), 24-h average of SD of 5-min N-N intervals (SDNNIDX), and root mean square successive difference of N-N intervals in ms (rMSSD) values of the CR group were similar to values found in healthy adults aged 20–30 year, whereas individuals in the WD group had HRV values consistent with published norms for their age (Umetani *et al.*, 1998).

Comparison of heart rate variability differences between the caloric restriction and control groups with changes induced by beta-blockade

Cook *et al.* (1991) gave 50 mg of atenolol four times a day to 16 healthy adults (mean age 32 ± 7 years) in a randomized, double-blind, placebo-controlled trial to determine the effect of beta-blockade on 24-h Holter-based heart rate and HRV. We compared the percent change in these parameters induced by atenolol with percent differences in comparable HRV parameters between WD and CR. As can be seen in Fig. 2(A), the effect of atenolol on the heart rate was similar in magnitude and direction to differences between CR and WD, except for nighttime heart rate that was 8% lower than placebo with atenolol and 24% lower in the CR than in WD. Figure 2(B) compares the effect of atenolol administration on 24-h time-domain HRV with the difference in these parameters between WD and CR. Atenolol had no effect on SDNN, but SDNN in CR was 31% higher than in WD. Atenolol was associated with a 61% increase in rMSSD, similar to differences seen between CR and WD (57%). There was a 69% increase in percent of successive N-N intervals differences > 50 ms (pNN50) with atenolol administration and an almost doubled difference in CR (140%). Finally, in Fig. 2(C), increases in total power and low-frequency power appeared to be similar between atenolol and CR/WD, but, consistent with findings for rMSSD, difference in high-frequency power were greater for CR vs. WD than for atenolol vs. placebo.

Discussion

Prospective data on the effects of long-term CR on ANS function, as assessed by 24-h HRV in healthy lean humans are not yet available. In this cross-sectional study, research-quality HRV assessment was performed in 22 men and women who had been on self-imposed CR for 3–15 years and compared with HRV in 20 age-matched healthy controls eating typical WD. Our study is the first, to our knowledge, to demonstrate that long-term CR individuals have markedly lower heart rate and a markedly better HRV profile than healthy people of the same age eating standard WD.

Caloric restriction has been shown to slow aging and prevent or delay several chronic diseases in rodents (Weindruch & Walford, 1988; Fontana *et al.*, 2010a,b). Caloric restriction also protects against diabetes, cancer, and cardiovascular disease in Rhesus monkeys (Colman *et al.*, 2009). In humans, CR is associated with metabolic changes that protect against these age-related pathologies and against left ventricular diastolic dysfunction (Fontana *et al.*, 2004, 2006, 2010a,b; Meyer *et al.*, 2006; Fontana & Klein, 2007; Cangemi *et al.*, 2010; Soare *et al.*, 2011). In particular, we have previously shown that long-term CR in humans protects against obesity, insulin resistance, hypertension, inflammation, and atherosclerosis and is associated with many of the same hormonal changes that are thought to mediate the anti-aging effects of CR in rodents (Fontana *et al.*, 2004, 2006, 2010a,b; Meyer *et al.*, 2006; Fontana & Klein, 2007; Cangemi *et al.*, 2010; Soare *et al.*, 2011).

Decreased HRV is associated with senescence in rodents, as well as in healthy humans (Pfeifer *et al.*, 1983; O'Brien *et al.*, 1986; Ferrari *et al.*, 1991; Schwartz *et al.*, 1991; Herlihy *et al.*, 1992; Cowen, 1993; Umetani *et al.*, 1998; Cowen *et al.*, 2000; Mager *et al.*, 2006; Corino *et al.*, 2007; Zulfiqar *et al.*, 2010). Mager *et al.* (2006) have shown that, in rats, several markers of ANS function were altered by CR. In particular, CR rats exhibited

decreased low-frequency power in diastolic blood pressure variability and increased high-frequency power in HRV, suggesting that CR results in decreased sympathetic activity and augmented parasympathetic activity (Mager *et al.*, 2006). Interestingly, the HRV differences we found in our CR subjects were similar to those found in long-lived CR rats, strongly suggesting that long-term CR also retards the age-associated deterioration of ANS function in humans.

Many bodily systems that deteriorate with aging (e.g., cardiovascular, gastrointestinal and neuroendocrine functions, regulation of body temperature, energy homeostasis, metabolism, and tissue defense) are dependent on the healthy functioning of the ANS (Llewellyn-Smith & Verberne, 2011). Novel insights into how long-term CR affects ANS function in healthy human individuals are provided by our study. First, CR individuals demonstrated profoundly increased parasympathetic activity as measured by rMSSD and high-frequency power. Moreover, lower levels of vagally modulated HRV have been demonstrated to be associated with increased inflammation and cardiovascular morbidity and mortality in the elderly (Tsuji *et al.*, 1996; Dekker *et al.*, 1997). In contrast, activation of the parasympathetic nervous system, through stimulation of the efferent vagus nerve, decreases systemic inflammation in several experimental models of acute systemic inflammation (Tracey, 2007). Furthermore, predicted values of rMSSD and pNN50 by age developed by Umetani *et al.* (1998) indicate that rMSSD and pNN50 levels found in the CR group would be expected to be seen in someone under the age of thirty, whereas rMSSD and pNN50 in the WD group was consistent with their being in the mid-fifties, which they were. Thus, as CR is associated with levels of ANS functioning seen in a younger cohort, and there is no evidence that individuals who choose CR already have better ANS function, our results suggest that CR reverses the age-associated decline in ANS function.

Atenolol is a beta-blocker and has been shown to both reduce sympathetic and increase relative parasympathetic control of heart rate. The HRV changes associated with atenolol administration, as shown by Cook *et al.* (1991), are consistent with these autonomic effects. Our data suggest that CR is associated with very similar autonomic effect on heart rate, because the direction of heart rate and HRV changes are generally consisted with those associated with atenolol administration. Interestingly, CR appears to be associated with even greater reduction in nighttime heart rate and greater increase in measures of parasympathetic activity, despite the study population being, on average, 20 years older.

In conclusion, results of this study on subjects following a strict CR diet for an average of 7 years provide the first evidence in humans that long-term CR is associated with similar effect to those already documented in animal studies, that is, better autonomic function than in matched controls. Our data suggest that long-term may, as has been show in animals, attenuate the deterioration of multiple HRV parameters associated with primary aging in humans. Prospective studies are needed to verify the causal relationship of CR with these markers. Findings would need to be verified in different age groups and in those with established clinical cardiovascular disease risk factors. In addition, the dose-response relationships for the anti-aging benefits of CR would be of great clinical interest.

Experimental procedures

Study participants

Twenty-two individuals practicing long-term CR for an average of 7 years (3–15 years) were recruited, mainly through the Caloric Restriction Society. Two were from the St. Louis area and the others came to the Washington University Medical Center from other cities in the United States, Canada, and UK. Their motivation for practicing CR is the strong desire to live as long as possible in good health and the belief, based on the findings on CR rodents

and other species, that CR will markedly prolong their healthy life span. Their average age was 51.5 ± 10.8 years (range 35–82 years). Four CR subjects were women. Twenty individuals eating a conventional US diet, matched with the CR group in terms of age, gender, and socioeconomic status, were the comparison group. None of the subjects had evidence of chronic diseases, including cardiovascular, lung, gastrointestinal and autoimmune diseases, type 2 diabetes or cancer. None were smokers. In addition, none of the subjects were taking lipid-lowering or antihypertensive agents, or other medications that could have affected cardiometabolic or HRV measures. Subjects were enrolled after undergoing a physical examination, medical history, and laboratory evaluation that revealed no evidence of any health problems. All study participants were weight stable (i.e., < 2 kg weight change in the preceding 6 months) and did not perform more than 20 min of vigorous exercise twice per week. This study was approved by the Human Studies Committee of Washington University School of Medicine, and all subjects gave informed consent before their participation.

Anthropometrics and body composition

Height was measured without shoes to the nearest 0.1 cm. Body weight was obtained on a balance scale in the morning after a 12-h fast. Body mass index was calculated by dividing body weight (in kograms) by the square of height (in meters). Total body fat mass and fat-free mass were determined by dual-energy X-ray absorptiometry (QDR 1000/w; Hologic, Waltham, MA, USA).

Cardiometabolic risk factors

Blood pressure was measured with a mercury sphygmomanometer, with the participant in the sitting position after 5 min of rest in a quiet environment, according to the recommendations of the American Hypertension Society. Four measurements of systolic and diastolic blood pressure were made at ≈5-min intervals and averaged. A venous blood sample was taken to determine lipid concentrations after subjects had fasted for at least 12 h. Measurement of serum lipid concentrations was performed in the Core Laboratory for Clinical Studies at Washington University. Total cholesterol was measured by automated enzymatic commercial kits (Miles/Technicon, Tarrytown, NY, USA). High-density lipoprotein cholesterol was measured in plasma after precipitation of apolipoprotein B-containing lipoproteins by dextrane sulfate (50 000 molecular weight) and magnesium (Warnick *et al.*, 1982).

Dietary assessment

Subjects were instructed by a research dietician to record all food and beverages consumed, preparation methods, and approximate portion sizes for seven consecutive days. To assist with portion size determinations, measuring spoon and cup sets were provided to all participants, and all food diaries had a ruler imprinted on the back cover. Food records were analyzed by using the Nutrition Data System for Research programAUTHOR: Please give manufacturer information for ‘Nutrition Data System for Research program’: company name, town, state (if USA), and country. (version 4.03_31) from the Nutrition Coordinating Center at the University of Minnesota.

Heart rate variability data collection

All subjects underwent 24-h ambulatory Holter electrocardiogram monitoring. Electrocardigrams were recorded on DMS 300-3M digital Holter recorders at a sampling rate of 250 Hz and downloaded to a personal computer for analysis using CARDIO SCAN software (V12.0; DMS Holter, Stateside, NV, USA). Recordings were processed at the Washington University School of Medicine Heart Rate Variability Laboratory. After the scanner

automatically detected and labeled all QRSAUTHOR: Please define QRS. complexes, beat labels were edited using standard research Holter analysis procedures. The CARDIO SCAN software also displayed a beat-by-beat heart rate tachogram, and clicking on a specific segment brought up the associated electrocardiogram strip. This permitted detection of missed beats, missed ectopic beats, and other outliers that could then be corrected. All Holter analyses were reviewed in detail by PKS and AS with special attention paid to ensuring that only N-N beats with uniformly detected onsets were included in the HRV analysis. The longest and shortest true N-N intervals were identified for each recording and intervals outside of these limits, including blocked atrial premature contractions as well as ectopic or inserted beats, were excluded from all calculations. After editing, the labeled QRS data stream was transferred to a Sun workstation (Sun Microsystems, Palo Alto, CA, USA) for HRV analysis. For recording to be accepted for time-domain analysis (which is less sensitive to missing data), ≥ 18 h of data with at least 50% N-N interbeat intervals in each 5-min segment were required. For a recording to be used for frequency-domain analyses, a ≥ 80% N-N interval in each 5-min segment was required. Heart rate and HRV were calculated for the entire recording, for daytime (08:00–20:00) and nighttime (00:00–06:00).

Time-domain analysis of heart rate variability

Time-domain indices of HRV are derived from statistical calculations performed on the set of N-N intervals. A detailed definition for the HRV indices tested in the analysis can be found in legend for Table 2. Average heart rate was computed from N-N intervals only. Standard deviation of normal-to-normal (N-N) intervals in ms and SDANN are primarily influenced by circadian rhythms (Kleiger *et al.*, 1992). SDNNIDX reflects intermediate-term HRV. Short-term HRV indices like pNN50 and rMSSD reflect beat-to-beat changes in heart rate, mediated by changes in parasympathetic activity (Kleiger *et al.*, 1992).

Frequency-domain analysis of normal-to-normal intervals

Frequency-domain or power spectral analysis partitions the variance in heart rate (i.e., length of N-N interbeat intervals) signal into underlying frequencies. A full definition for the frequency-domain HRV measures tested in this study will be found in the Legend for Table 2. Total power is the sum of the variance of all of the components (Kleiger *et al.*, 2005). Very-low-frequency power quantifies the variance in heart rate occurring at cycles of 25-s to 5 min and is unaffected by beta-blockade and abolished by atropine, suggesting that it reflects parasympathetic control of heart rate (Taylor *et al.*, 1998). In addition, it is affected by angiotensin-converting enzyme inhibition, suggesting an influence of the renin-angiotensin system (Taylor *et al.*, 1998). Thermoregulatory and peripheral vasomotor influences have also been suggested (Akselrod *et al.*, 1985; Pomeranz *et al.*, 1985). In addition, ratio measures of HRV, normalized low- and normalized high-frequency power, which have been suggested as rough surrogates of autonomic balance, were compared between groups (Pomeranz *et al.*, 1985).

Statistical analysis

T-tests compared clinical and demographic factors, heart rate, and HRV between CR and WD. Frequency-domain HRV measures are highly skewed and are generally ln transformed to permit parametric comparisons and that was done in this case. A $P < 0.05$ was considered statistically significant. International Business Machines, statistical package for the social science 19 (SPSS, Inc, Chicago, IL, USA) software was used for these analyses.

Acknowledgments

Supported by grants from the National Center for Research Resources (UL1 RR024992; a component of the National Institutes of Health and NIH Roadmap for Medical Research), the National Institute of Diabetes And

Digestive And Kidney Diseases (P30DK056341), the Longer Life Foundation (an RGA/Washington University Partnership), and a donation from the Bakewell Foundation and the Scott and Annie Appleby Charitable Trust. The funding agencies had no role in the analysis or interpretation of the data or in the decision to submit the report for publicationAUTHOR: Please check text under the heading 'Acknowledgments'..

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Daytime HR, average HR between 08:00 and 20:00; Ln, natural logarithm; min HR, 60 000/minimum instantaneous N-N; max HR, 60 000/maximum instantaneous N-N; N-N, normal-to-normal RR intervals; nighttime HR, average HR between 00:00 and 06:00; pNN50, percent of successive N-N intervals differences > 50 ms in %; rMSSD, root mean square successive difference of N-N intervals in ms; SDNN, 24-h standard deviation of N-N intervals in ms; SDANN, 24-h SD of 5-min-averaged N-N intervals; SDNNIDX, 24-h average of SD of 5-min N-N intervals; total power, total spectral power of HRV; very-low-frequency power, 24-h average of power between 0.003 and 0.04 Hz; 24-h HR, 60 000/average N-N for 24-h in bpm.

Low-frequency power (24-h average of 5-min power between 0.04 and 0.15 Hz); high-frequency power (24-h average of 5-min power between 0.15 and 0.4 Hz); normalized low-frequency power (24-h average of the proportion of total power in each 5-min interval accounted for by the low-frequency component); normalized high-frequency power (24-h average of the proportion of total power in each 5-min interval accounted for by the high-frequency component).

Values are expressed as mean ± SD or median [IRQ].

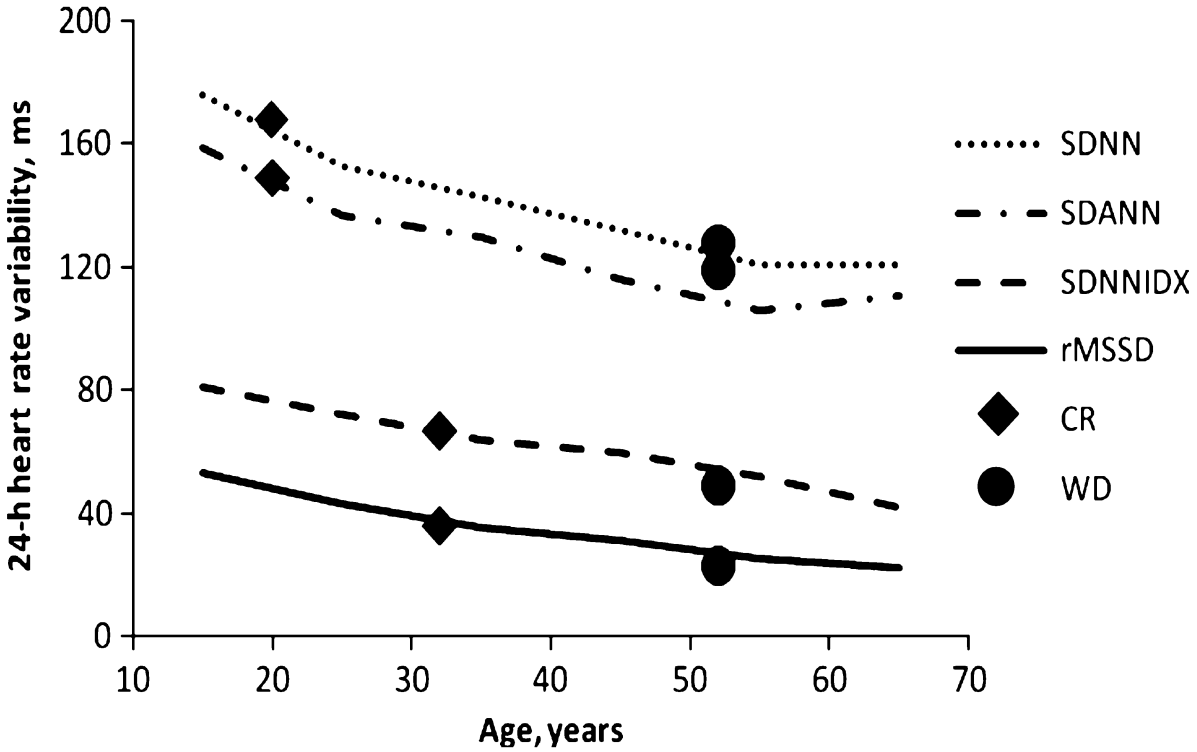

Fig. 1.

Mean heart rate variability (HRV) in caloric restriction (CR) (age, 51.5 ± 10.8 years) and Western diets (WD) (age, 52 ± 8.9 years) compared with previously published age-related norms. Curves show age-related norms for 24-h standard deviation of normal-to-normal (N-N) intervals in ms (SDNN), 24-h SD of 5-min-averaged N-N intervals (SDANN), 24-h average of SD of 5-min N-N intervals (SDNNIDX), and root mean square successive difference of N-N intervals in ms (rMSSD) (Umetani *et al.*, 1998). Mean CR HRV values are indicated by filled diamonds and mean WD values by filled circles. See Table 2 legend for HRV definitions.

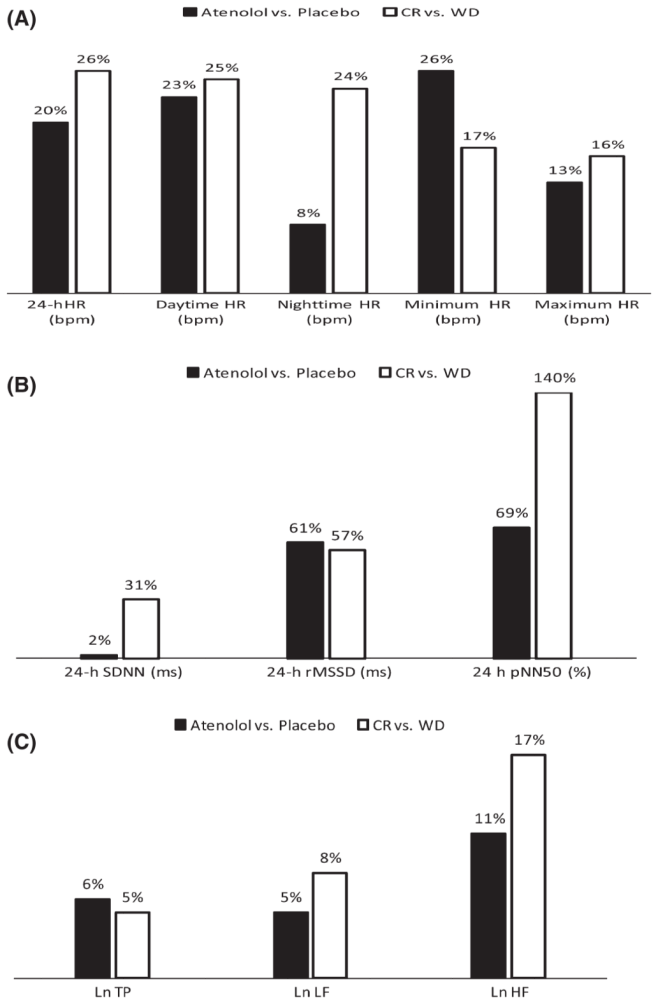

Fig. 2.

(A) Comparison of the effect of atenolol vs. calorie restriction on heart rate. Comparison of % decreases in 24-h, daytime, and nighttime heart rate between atenolol and placebo and long-term caloric restriction (CR) vs. Western diets (WD). (B) Effect of atenolol vs. calorie restriction on time-domain heart rate variability (HRV). Comparison of % increases in time-domain HRV between atenolol vs. placebo and long-term CR vs. WD. SDNN, 24-hour standard deviation of normal-to-normal (N-N) intervals in ms; rMSSD, root mean square successive difference of N-N intervals in ms; pNN50, percent of successive N-N intervals differences > 50 ms in %. (C) Effect of atenolol vs. calorie restriction on frequency-domain HRV. Comparison of % increases in frequency-domain HRV between atenolol and placebo and long-term CR vs. WD. LnTP, normalized total power; LnLF, normalized low-frequency power; LnHF, normalized high-frequency power.

Table 1

Clinical and Demographic Characteristics of Study Population

	CR (n=22) μ±SD	WD (n=20) μ±SD	p-value
Age (years)	51.5±10.8	52±8.9	NS
Gender (M/F)	18/4	16/4	NS
Height (m)	1.74±0.1	1.79±0.1	NS
Weight (kg)	57±5.9	80.1±13.4	<0.001
Body mass index (kg/m^2)	18.8±1.1	25±3.1	<0.001
Lean mass (kg)	49±6.7	56.8±11.3	0.009
Total body fat (%)	9.9±4.7	24.3±8.7	<0.001
Systolic blood pressure (mmHg)	99±9	125±12	<0.001
Diastolic blood pressure (mmHg)	61±6	79±10	<0.001
Total cholesterol (mg/dl)	164±35	196±42	0.003
High-density lipoprotein cholesterol (mg/dl)	63±18	53±14	NS
Total cholesterol/high-density lipoprotein cholesterol ratio	2.7±0.5	4.0±1.2	<0.001

Table 2

Comparison of heart rate and 24-hr HRV between long-term CR and WD

	CR μ±SD	WD μ±SD	p-value
Average HR for 24 hr (bpm)	57±6	76±9	< 0.001
Average daytime HR (bpm)	61±7	81±10	< 0.001
Average nighttime HR (bpm)	50±5	67±10	< 0.001
Maximum instantaneous HR (bpm)	110±22	129±14	0.001
Minimum instantaneous HR (bpm)	37±4	45±10	0.002
SDNN (ms)	168±26	128±46	0.001
SDANN (ms)	149±20	119±38	0.005
SDNNIDX (ms)	67±15	49±15	<0.001
rMSSD (ms)	36±12	23±11	0.001
pNN50 (%)	12±8	5±5	0.001
Total power (ms^2, Median[IQR])	26764[9456]	14578[16665]	
Ln total power	10.2±0.3	9.6±0.6	0.002
Very-low-frequency power (ms^2, Median[IQR])	2971[1709]	1138[1111]	
Ln very-low-frequency power	7.8±0.4	7.1±0.6	<0.001
Low-frequency power (ms^2, Median[IQR])	986[1026]	508[652]	
Ln low-frequency power	6.8±0.7	6.3±0.8	0.06
High-frequency power (ms^2, Median[IQR])	284 [232]	110 [165]	
Ln high-frequency power	5.7±0.7	4.9±1.0	0.004
Normalized low-frequency power (%)	64±9	71±9	0.020
Normalized high-frequency power (%)	26±7	20±9	0.017

Ln= natural logarithm; N-N= normal-to-normal RR intervals; 24-hr HR= 60,000/average N-N for 24-hrs in bpm; daytime HR= average HR between 08:00 and 20:00; nighttime HR= average HR between 00:00 and 06:00; min HR= 60,000/minimum instantaneous N-N; max HR= 60,000/maximum instantaneous N-N; SDNN= 24-hr standard deviation of N-N intervals in ms; SDANN=24-hr SD of 5-min-averaged N-N intervals; SDNNIDX= 24-hr average of SD of 5-min N-N intervals; rMSSD= root mean square successive difference of N-N intervals in ms; pNN50= percent of successive N-N intervals differences > 50 ms in %; total power= total spectral power of HRV; very-low-frequency power = 24-hr average of power between 0.003-0.04 Hz; low-frequency power (24-hr average of 5-min power between 0.04-0.15 Hz); high-frequency power (24-hr average of 5-min power between 0.15-0.4 Hz); normalized low-frequency power (24-hr average of the proportion of total power in each 5-min interval accounted for by the low-frequency component); normalized high-frequency power (24-hr average of the proportion of total power in each 5-min interval accounted for by the high-frequency component). Values are expressed as mean ± SD or median [IRQ].