Improved ejection fraction after exercise training in obesity is accompanied by reduced cardiac lipid content.

Improved Ejection Fraction after Exercise Training in Obesity Is Accompanied by Reduced Cardiac Lipid Content

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Context: Skeletal muscle and cardiac lipid accumulation are associated with diminished insulin sensitivity and cardiac function, respectively. In skeletal muscle, physical activity paradoxically increases fat accumulation, despite improvement in insulin sensitivity. Whether cardiac muscle responds similarly remains unknown.

Objective: The objective of the study was to investigate cardiac lipid content and cardiac function after a 12-wk training program.

Design: This was an intervention study with pre/postmeasurements.

Setting: The study was conducted at Maastricht University Medical Center.

Participants: Participants included 14 healthy, male overweight/obese subjects (age 58.4 $\pm$ 0.9 yr, body mass index 29.9 $\pm$ 0.01 kg/m^2).

Intervention: Intervention included a supervised 12-wk training program with three sessions per week (endurance and strength training).

Main Outcome Measures: Maximal whole-body oxygen uptake, fasting plasma parameters, systolic function (by CINE-magnetic resonance imaging), and cardiac lipid content (by proton magnetic resonance spectroscopy) were measured.

Results: Maximal whole-body oxygen uptake increased (from 2559 $\pm$ 131 to 2702 $\pm$ 124 ml/min after training, $P = 0.05$). Plasma concentrations of glucose decreased (from 6.3 $\pm$ 0.2 to 5.7 $\pm$ 0.2 mmol/liter, $P < 0.001$); plasma triacylglycerols and (free) fatty acids did not change. Also, body weight (from 94.2 $\pm$ 3.6 to 92.9 $\pm$ 3.6 kg, $P = 0.10$) and fat percentage (from 33.6 $\pm$ 1.7 to 32.5 $\pm$ 2.0%, $P = 0.14$) was unchanged. Left ventricular ejection fraction improved (from 52.2 $\pm$ 1.3 to 54.2 $\pm$ 1.2%, $P = 0.02$), and cardiac lipid content in the septum was decreased after training (0.99 $\pm$ 0.15 to 0.54 $\pm$ 0.04%, $P = 0.02$).

Conclusions: Twelve weeks of endurance/strength training significantly reduced cardiac lipid content in overweight subjects and was paralleled by improved ejection fraction. This is in line with a lipotoxic action of (excess) cardiac lipids on cardiac function, although a causal relationship cannot be derived from this study. Further research is needed to clarify the clinical relevance of cardiac lipid content in the etiology of cardiovascular complications. **(*J Clin Endocrinol Metab* 95: 1932–1938, 2010)**

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Abbreviations: ECG, Electrocardiogram; FA, fatty acid; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; MVC, maximal voluntary contraction; VO_2max, maximal oxygen uptake.

The prevalence of obesity has reached epidemic proportions and is still increasing (1). Obesity is a major risk factor for the development of cardiovascular diseases (2, 3) and an independent risk factor for the development of heart failure (4). Obesity is associated with excessive storage of lipids (triglycerides) not only in adipose tissue but also in skeletal muscle, liver, and heart. Increased lipid storage in skeletal muscle and the liver is strongly associated with insulin resistance. Likewise, high intracellular lipid concentrations may also have negative effects in the heart: in animal models, there is convincing evidence that excessive lipid storage in cardiomyocytes can induce cardiomyopathy and heart failure via lipotoxic pathways. This was initially shown in various animal models of obesity (5, 6) but more recently also in lean rodent models with targeted overexpression of genes involved in lipid delivery and synthesis in the myocardium (7–11). These animals show steatosis-induced dilated cardiomyopathy and heart failure in the absence of obesity-related cardiovascular risk factors (such as hypertension or dyslipidemia) (7–11), indicating that lipid accumulation in the myocardium can cause cardiomyopathy directly. In line with this, treatments that ameliorated cardiac lipid accumulation rescued the heart from dilated cardiomyopathy in rodents (6, 11–14).

A more physiological model for human obesity-related cardiac lipid accumulation is embodied by rats on a high-fat diet. Consumption of such a high-fat diet results in cardiac lipid accumulation, paralleled by contractile dysfunction (15) and changes in intracellular signaling pathways leading to a decreased insulin responsiveness of cardiac cells (15).

Whether myocardial lipid accumulation is also of clinical relevance is less obvious. In humans, investigating cardiac lipid content and its subcellular consequences is hampered by the difficulty of obtaining cardiac tissue. Therefore, until recently, only postmortem or transplantation studies were possible (16). However, the demonstration of reliable cardiac lipid quantification with electrocardiogram (ECG)-triggered and respiratory-gated magnetic resonance spectroscopy (MRS), makes *in vivo* evaluations possible and enables interventional studies (17–19).

Physical exercise training improved diastolic function in overweight subjects and decreased end-systolic left ventricular volume in overweight subjects (20) and hence positively affects cardiovascular health. Furthermore, regular physical activity can reverse age-induced decline in cardiac energy status (21). If cardiac lipid content is indeed detrimental, it can thus be hypothesized that endurance training will decrease cardiac lipid content, thereby allowing improvement of cardiac function. On the other hand, in skeletal muscle, endurance training increases rather than decreases muscular lipid content (intramyocellular lipids) (22), probably because endurance-trained muscles need larger internal fat depots for oxidation during exercise. Therefore, endurance training is an exception to the rule that lipid storage in skeletal muscle is unhealthy.

So far, it is unknown whether in cardiac muscle, which is also heavily depending on fatty acid oxidation, also such a paradox exists. If this is the case, cardiac lipid depots would be expanded rather than reduced with physical activity training and as a consequence, cardiac lipid content would not be a good marker for cardiac lipotoxicity.

Therefore, the aim of the present study was to investigate the change in cardiac lipid content and cardiac function in response to a physical training intervention in overweight subjects. To this end, we used ^{1}H-MRS and CINE-magnetic resonance imaging (MRI) to determine cardiac lipid content and cardiac function before and after a progressive training program of 12 wk.

Subjects and Methods

Subjects

Fourteen healthy male overweight to obese individuals were included in this study. For other characteristics, see subjects' characteristics in Table 1. The study was approved by the institutional medical ethical committee and written informed consent was obtained from all participants.

Study protocol

Before inclusion, all subjects underwent a medical examination including the assessment of their medical history and a physical examination. Also an ECG was registered, blood pressure

TABLE 1. Subjects' characteristics before and after the intervention period of 12 wk

	Before training	After training	*P* value
Age (yr)	58.4 ± 0.9	n/a	n/a
Body weight (kg)	94.2 ± 3.6	92.9 ± 3.6	0.1
Fat percentage (%)	33.6 ± 1.7	32.5 ± 2.0	0.14
Body mass index (kg/m^2)	29.9 ± 0.01	29.5 ± 0.01	0.1
VO_2max (ml/kg · min)	27.2 ± 1.4	30.3 ± 1.7	0.01[a]
Glucose (mmol)	6.3 ± 0.2	5.7 ± 0.2	<0.001[a]
HbA1c (%)	5.9 ± 0.8	5.8 ± 0.1	0.01[a]
FAs (μmol/liter)	384.3 ± 37.8	364.5 ± 24.4	0.6
Triacylglycerols (mmol/liter)	1.36 ± 0.1	1.35 ± 0.1	0.9
Total cholesterol (mmol/liter)	5.63 ± 0.22	5.30 ± 0.25	0.02[a]
LDL cholesterol (mmol/liter)	3.74 ± 0.20	3.41 ± 0.23	0.01[a]
HDL cholesterol (mmol/liter)	1.28 ± 0.07	1.27 ± 0.07	0.7

n/a, Not applicable.

[a] Statistically significant changes.

was assessed, and a fasting blood sample was taken to determine glucose and glycated hemoglobin (HbA1c) concentrations. High blood pressure (systolic blood pressure >160 mm Hg or diastolic blood pressure >100 mm Hg) was an exclusion criterion as well as known cardiac disease and type 2 diabetes mellitus. Two subjects were on blood pressure-lowering drugs (one subject on a combination drug of an angiotensin-converting enzyme inhibitor and a diureticum, one subject on a selective Ca^{2+} channel blocker) and continued medication throughout the study.

As part of the experiment, the following parameters were measured before and after a 12-wk training intervention: body composition, maximal oxygen uptake, plasma concentration of (free) fatty acids (FAs), triacylglycerols, glucose, HbA1c, and cholesterol. ^{1}H-MRS and MRI were used to determine *in vivo* cardiac lipid content next to ejection fraction, cardiac index, and cardiac output as indicators of cardiac function. Heart rate during a standardized exercise protocol was registered before and after the training program as an additional indicator of cardiac function. Two subjects were taking lipid-lowering medication (statins), which was continued throughout the study with unchanged dosage. Please note that when these subjects were excluded from analysis, the training-induced effects on ejection fraction and cardiac lipid content remain statistically significant. Subjects were asked to continue their habitual diet throughout the study. The 2 d preceding the determination of cardiac lipid content, subjects were used to refrain from alcohol, adhere to a normal eating pattern, and refrain from intense exercise. The measurement was performed in the morning in the fasted state.

Training protocol

All subjects were engaged in a highly controlled exercise program for 12 wk, consisting of a combination of aerobic and resistance exercise. The aerobic exercise was carried out on a cycling ergometer twice a week for 30 min at 55% of their predetermined maximal workload. After 6 wk maximal performance was reassessed and the intensity of the training adjusted. Resistance exercise was performed once a week and consisted of one series of eight repetitions at 55% of their maximal voluntary contraction (MVC) and two series of eight repetitions at 75% MVC. A series of eight exercises concentrating on large muscle groups were performed (chest press, leg extension, lat pull-down, leg press, triceps curls, biceps curls, abdominal crunches, horizontal row). All training sessions were preceded by a 5 min warm-up and ended with 5 min of cooling down on the cycle ergometer at 45% of maximal workload. Every 4 wk, MVC was reassessed, and the exercise intensity was adjusted if necessary. All exercise sessions were carried out in small groups of four subjects, supervised by a coach.

Maximal oxygen uptake (VO_2max; whole body oxidative capacity)

A routine incremental cycling test was used to determine the maximal aerobic capacity as described previously (23). Briefly, after a warming-up period of 5 min, the intensity was increased every 2.5 min until exhaustion. Oxygen consumption was measured continuously throughout the test using indirect calorimetry (Oxycon β, Mijnhardt, The Netherlands) to determine VO_2max.

Heart rate at submaximal exercise

The heart rate was determined during a submaximal work load before and after the training intervention at 125 W (64% of average maximal performance before training) and at 150 W (77% of average maximal performance before training). To this end, a heart rate monitor was employed (Polar Electro Inc., Kempele, Finland) with a transmitter fastened around the chest.

Hydrostatic weighing

Hydrostatic weighing with simultaneous measurement of lung volume was used to determine whole body fat percentage in the morning in the fasted state. The equation of Siri (24) was used to calculate fat percentage.

Blood sample analysis

Blood samples were collected in EDTA-containing tubes and immediately centrifuged at high speed. Plasma FAs and glucose were measured with enzymatic assays automated on a Cobas Fara/Mira (FAs: Nefa C test kit; Wako Chemicals, Neuss, Germany) (glucose: hexokinase method; Roche, Basel, Switzerland).

Total cholesterol (Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase method; Roche Diagnostics Systems, Hoffmann-La Roche, Indianapolis, IN), high-density lipoprotein (HDL) cholesterol (Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase method; Roche Diagnostics Systems, Hoffmann-La Roche) after precipitation of apolipoprotein-B-containing lipoproteins by adding phosphotungstic acid and magnesium ions (precipitation method; Monotest cholesterol; Roche Molecular Biochemicals, Indianapolis, IN), and triacylglycerol (GPO-Trinder; Sigma Diagnostics, St. Louis, MO) were analyzed in serum enzymatically. All samples from one participant were analyzed within one analysis at the end of the study using a semiautomatic ABX Pentra 400 (Horiba ABX, Montpellier, France). The Friedewald equation (25) was used to calculate serum low-density lipoprotein (LDL) cholesterol.

MRI

Measurements were performed on a clinical whole-body MRI scanner (Intera, 1.5T; Philips Healthcare, Best, The Netherlands) with a dedicated, five-element cardiac radiofrequency coil. An electrocardiographically triggered turbo field echo sequence was used to acquire images in two- and four chamber views and image the whole heart in the short-axis orientation during breath holds (24 frames per heart cycle). Due to anxiety in one case and technical problems in another case, the imaging protocol had to be shortened and functional parameters could not be determined in one subject before training, and in another subject after training. Therefore, the comparison of cardiac function is based on 12 subjects.

Image processing

All images were analyzed quantitatively using dedicated software (CAAS; Pie Medical Imaging, Maastricht, The Netherlands) to determine left ventricular ejection volume, cardiac output, and cardiac index.

MRS

Cardiac lipid content was determined *in vivo* by MRS. Signal acquisition was restricted to a volume of interest of 6 cm^3 ($10 \times 20 \times 30$ mm) in the septum of the heart (PRESS sequence; spec-

tral band width = 1000 Hz; points acquired = 512; echo time = 26 msec; repetition time = 4 sec), signal acquisition was ECG triggered to end systole (310 msec after R-top). Respiratory gating and tracking was performed with a pencil beam navigator placed on the diaphragm (19). High efficiency of the navigator-gated acquisition was achieved by instructing subjects to breath in the 4s rhythm of the measurement. A spectrum with and without water suppression was acquired as reported earlier for skeletal muscle (22). Overall acquisition time (imaging and spectroscopy) was 90 min.

Spectral postprocessing

Postprocessing of the spectra was performed with the jMRUI software (26) as reported earlier for skeletal muscle; however, only three lipid peaks were fitted with imposing relative ratios of the amplitudes and line widths, using the AMARES algorithm (27) (see Fig. 1). The results are displayed as ratios of the amplitudes of the CH_2 peak relative to the water peak (not corrected for T1 and T2 relaxation).

Statistics

All data are presented as mean ± SEM. Statistical analyses were performed using SPSS 16.0 for Mac (SPSS Inc., Chicago, IL). The effect of the intervention was determined by a two-sided, paired Student *t* test. To correct/adjust changes in cardiac lipid content for changes in body weight and fat percentage, a linear regression analysis was performed with the change in cardiac lipid content as dependent variable and the changes in body weight and fat percentage as independent variables. $P < 0.05$ was considered significant.

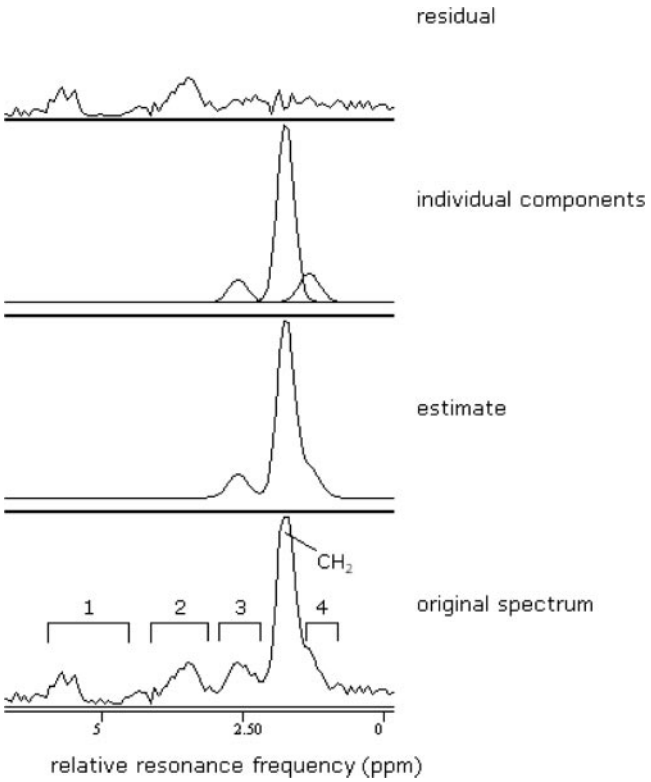

FIG. 1. Typical spectrum. A typical proton magnetic resonance spectrum, acquired from the septum of the heart. The peak originating from the CH_2 group is depicted. Region 1 depicts the residual water signal, region 2 shows other cardiac metabolites (creatine and trimethylammonium), region 3 depicts a lipid peak originating from chemically diverse protons in the neighborhood of oxygen atoms and double bonds, and region 4 corresponds to the CH_3 peak of cardiac lipids.

Results

VO_2max

Maximal whole-body oxygen uptake improved with training (from 2559 ± 131 to 2702 ± 124 ml/min after training, $P < 0.05$).

Body weight and whole-body fat percentage

Body weight and fat percentage did not change with training (body weight from 94.2 ± 3.6 to 92.9 ± 3.6 kg, $P = 0.10$; fat percentage from 33.6 ± 1.7 to 32.5 ± 2.0% ($P = 0.14$), see Table 1].

Plasma parameters

The training program resulted in a significant decrease in fasting plasma glucose concentration (from 6.3 ± 0.2 to 5.7 ± 0.2 mmol/liter, $P < 0.001$). Similarly, HbA1c plasma concentration decreased with training from 5.9 ± 0.1 to 5.8 ± 0.1% ($P = 0.012$).

Mean FA concentration after an overnight fast was not affected by training [384.3 ± 37.8 to 364.5 ± 24.4 μmol/liter, before and after training, respectively ($P = 0.64$)]. Mean total cholesterol decreased (from 5.63 ± 0.22 to 5.29 ± 0.22 mmol/liter, $P = 0.02$), and LDL cholesterol decreased (from 3.74 ± 0.20 to 3.41 ± 0.23 mmol/liter, $P = 0.012$). Mean HDL cholesterol did not change (1.28 ± 0.07 before training and 1.28 ± 0.07 mmol/liter after training ($P = 0.9$)]. Mean plasma triacylglycerol concentrations did not change [1.36 ± 0.10 before training and 1.35 ± 0.11 mmol/liter after training ($P = 0.9$)].

Cardiac function

Heart rate during a submaximal cycling protocol at a workload of 125 W decreased from 127.9 ± 3.6 beats/min (bpm) to 121.2 ± 3.3 bpm ($P = 0.05$), and at a workload of 150 W, it decreased from 141.3 ± 4.1 bpm to 131.4 ± 3.5 bpm ($P = 0.03$), indicating a training-induced increase in stroke volume. In line with this, multislice CINE-MRI revealed that ejection fraction was improved after training [from 52.2 ± 1.3 to 54.2 ± 1.2% ($P = 0.02$), see Fig. 2]. Cardiac index and cardiac output were not significantly changed after training [cardiac index: from 3.00 ± 1.48 to 3.01 ± 2.08 liters/min · m^2 ($P = 0.8$) and cardiac output from 6.1 ± 0.3 to 5.8 ± 0.3 liters/min ($P = 0.2$)]. Changes in ejection fraction correlated negatively with changes in HDL cholesterol ($P = 0.736$, $P =$

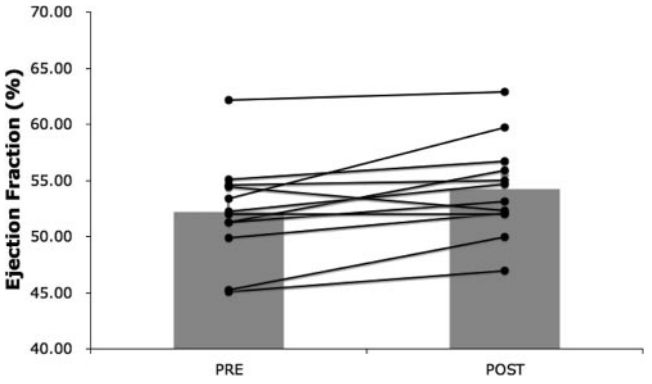

FIG. 2. Improved systolic function. Left ventricular ejection fraction improved with the training intervention ($P = 0.02$, n = 12).

0.006). Changes in ejection fraction did not correlate with changes in VO_2max ($P = 0.3$).

Cardiac lipid content

Cardiac lipid content significantly decreased after training (from 0.99 ± 0.15 to 0.54 ± 0.04%, $P = 0.02$, see Fig. 3). In two individuals, cardiac lipid content was very high before the intervention. Please note that the training-induced decrease in cardiac lipid content remains statistically significant after removal of the two outliers (from 0.79 ± 0.08 to 0.54 ± 0.05%, $P = 0.02$). The decrease in cardiac lipid content was still significant after correction for changes in body weight and fat percentage by linear regression ($P = 0.04$). Changes in cardiac lipid content did not correlate with changes in VO_2max ($P = 0.9$).

Discussion

The primary finding of the present study is that cardiac lipid content is decreased with physical exercise training in healthy overweight subjects and that this is paralleled by improved systolic function. It is well known that regular physical exercise is beneficial to cardiovascular health, and accordingly, the American Heart Association advises regular physical activity (20–30 min sessions, at least three to five times per week, depending on the intensity) (28). Using multislice CINE-MRI, which is considered the gold standard for determination of ejection fraction, we demonstrated here that after 12 wk of supervised progressive exercise training, ejection fraction is improved in obese male subjects. It has been demonstrated in some, but not all studies before, that physical exercise programs lead to improvements in cardiac function (20, 29). Here we present evidence that indeed, even after a relatively short period of 12 wk, cardiac function adapts positively, reflected by improved ejection fraction and a decreased heart rate during submaximal exercise. Most importantly, however, we show here for the first time that cardiac lipid content can be markedly lowered in healthy obese subjects by regular exercise training. Cardiac lipid content has been linked to the development of cardiomyopathy in obese subjects and diabetic patients. Our results show that even a relatively short (3 months) training intervention program, without major body weight loss, is already able to markedly lower cardiac lipid content. Even though the improvement in cardiac function was only modest in the present study, it can be expected that the training-induced reduction in cardiac lipid content will be beneficial with regard to reducing the risk on cardiomyopathy in the longer term, although future studies are needed to prove this concept.

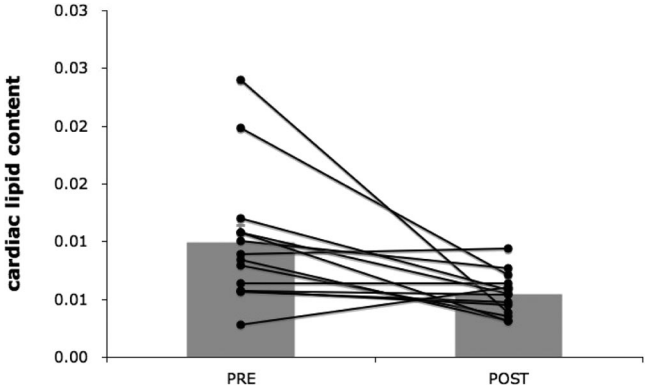

FIG. 3. Cardiac lipid content. Cardiac lipid content before and after the training intervention. Cardiac lipid content is expressed as the relative intensity of the CH_2 peak, compared with the unsuppressed water resonance. Cardiac lipid content decreased after the training intervention ($P = 0.02$). Please note that the training effect persists after removal of the two outliers ($P = 0.02$).

The decreased lipid content in the heart after exercise training is opposite to observations in skeletal muscle. In skeletal muscle, lipid content is increased in response to physical exercise training, we have shown previously that 2 wk of endurance training was already sufficient to increase intramyocellular lipids content by 42% in healthy lean subjects (22). Therefore, whereas exercise training is increasing the storage capacity for intracellular substrate in skeletal muscle, the present results might indicate that the reliance on intracellular substrate during exercise is less pronounced for cardiac muscle. The increased lipid content with exercise training in skeletal muscle makes it difficult to use skeletal muscle lipid content as an early marker of developing insulin resistance because physical activity can be a confounding factor. In the present study, we show that for cardiac muscle, this is not the case. This may have the important implication that cardiac lipid content could be used as a risk marker for cardiac deterioration.

Another important finding is that although total body fat percentage and body weight was unaffected by the current physical activity training program, it resulted in partitioning of FAs away from possibly unfavorable stor-

age sites in cardiac muscle. Therefore, these results underscore the power of physical activity in improving health in obesity, and the present findings should encourage people that may be disappointed by the lack of cosmetic changes by physical activity. A limitation of the current study is that only male subjects were investigated; therefore, it is yet unknown whether women respond in the same way.

The mechanism underlying the decrease in cardiac fat deposition is still under investigation. A decreased availability of plasma lipids after training may play a role. Although in the present study, plasma FAs and triacylglycerol concentrations were not (yet) decreased, we do report lower total and LDL cholesterol concentrations after training, which represents a more favorable lipid profile.

Next to a decreased systemic availability of FAs, an increased capacity for FA oxidation in the myocardium may underlie the current findings of lowered cardiac lipid content. In the present study, oxidative capacity of the heart could not be determined, but the training program did improve whole-body maximal oxygen uptake. In that regard, it is interesting to note that in rats, maximal oxygen uptake of isolated cardiomyocytes was indeed higher after an endurance training intervention (30).

The beneficial effect of training on systolic and diastolic function has been linked to alterations of the Ca^{2+} regulatory systems involved in the excitation-contraction coupling and relaxation processes (30, 31). Interestingly, an increased supply of long chain fatty acids also may influence excitation-contraction coupling as well as other processes linked to cellular Ca^{2+} handling (32, 33). These mechanisms may link the exercise induced decrease in lipid content to the improvement in systolic function. In line with this reasoning, whereas exercise training decreases cardiac lipid content and improves cardiac function, an increased cardiac lipid content due to high-fat diets in rodents leads to contractile dysfunction and altered intracellular signaling (15). However, although the results of the current study are in line with a lipotoxic action of cardiac lipids on cardiac function, a causal relationship cannot be proven, and it cannot be excluded that endurance training influences cardiac function and lipid content independently.

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