Irisin Does Not Mediate Resistance Training–Induced Alterations in Resting Metabolic Rate

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BASIC SCIENCES

ABSTRACT

SCHARHAG-ROSENBERGER, F., T. MEYER, M. WEGMANN, S. RUPPENTHAL, L. KAESTNER, A. MORSCH, and A. HECKSTEDEN. Irisin Does Not Mediate Resistance Training–Induced Alterations in Resting Metabolic Rate. *Med. Sci. Sports Exerc.*, Vol. 46, No. 9, pp. 1736–1743, 2014. **Purpose**: This study aimed to investigate the effects of a 6-month preventive resistance training program on resting metabolic rate (RMR) and its associations with fat-free mass (FFM) and the newly described myokine irisin as two potential mechanistic links between exercise training and RMR. **Methods**: In a randomized controlled trial, 74 sedentary healthy male and female participants either completed 6 months of high-repetition resistance training 3 $d \cdot wk^{-1}$ in accordance with the American College of Sports Medicine recommendations (RT: $n = 37$; 47 ± 7 yr; body mass index, 25.0 ± 3.4 $kg \cdot m^{-2}$) or served as controls (CO: $n = 37$; 50 ± 7 yr; body mass index, 24.2 ± 3.2 $kg \cdot m^{-2}$). Strength (one-repetition maximum), RMR (indirect calorimetry), body fat (caliper method), and serum irisin concentration (enzyme-linked immunosorbent assay) were measured before and after 6 months of training. **Results**: Training led to an increase in strength (one-repetition maximum leg press, 16% ± 7%; $P < 0.001$). RMR increased in RT (1671 ± 356 vs 1843 ± 385 $kcal \cdot d^{-1}$, $P < 0.001$) but not in CO (1587 ± 285 vs 1602 ± 294 $kcal \cdot d^{-1}$, $P = 0.97$; group–time interaction, $P < 0.01$). Body weight (RT, −0.5 ± 2.4 kg; CO, 0.1 ± 2.3 kg), body fat percentage (RT, −1.1% ± 2.5%; CO, −0.7% ± 2.9%), and FFM (RT, 0.4 ± 2.1 kg; CO, 0.6 ± 1.9 kg) did not develop differently between groups (group–time interaction: $P = 0.29$, $P = 0.54$, and $P = 0.59$, respectively). Serum irisin concentration increased in CO (70.8 ± 83.4 $ng \cdot mL^{-1}$, $P < 0.001$) but not in RT (22.4 ± 92.6 $ng \cdot mL^{-1}$, $P = 0.67$; group–time interaction, $P < 0.01$). The change in RMR was not associated with the change in FFM ($r = -0.11$, $P = 0.36$) or irisin ($r = -0.004$, $P = 0.97$). **Conclusions**: Preventive resistance training elicits an increase in RMR. However, in contrast to currently discussed hypotheses, this increase does not seem to be mediated by training-induced changes in FFM or circulating irisin concentration, which casts doubt in the meaning of irisin for human energy balance. **Key Words:** STRENGTH TRAINING, THERMOGENESIS, RESTING ENERGY EXPENDITURE, PGC-1α REGULATED MYOKINE, LEAN BODY MASS

In the field of health promotion, resistance training has gained growing importance over the past years (40). The American College of Sports Medicine (ACSM) recommends regular resistance exercise besides endurance training, flexibility, and neuromotor exercise to improve physical fitness and health (13). One potential benefit of resistance training, which might not be achieved through recreational endurance training (30), is an increase in resting metabolic rate (RMR). An elevated RMR seems to be beneficial for weight management and contributes to the prevention of the metabolic syndrome (40). However, previous studies revealed controversial results regarding resistance training effects on RMR, ranging from no change to significant increases (6,10,12,26,27,34).

Effects of resistance training on RMR are traditionally explained through an increase in fat-free mass (FFM) (32,40). Theoretically, a 1-kg increase in trained muscle mass results in an increase of 20 $kcal \cdot d^{-1}$ in RMR because of increased muscle protein turnover (32). This explanatory model is supported by several studies reporting concurrent increases in RMR and FFM (7,10,25,26). Therewith, it is obvious that ambitious resistance training, which considerably increases FFM, has the potential to increase RMR. However, it remains unclear whether recreational resistance training within the volumes and intensities recommended for health promotion, which might not elicit a pronounced increase in FFM, affects RMR.

The newly described messenger substance irisin (5) has attracted great attention as another potential mechanistic link

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between exercise training and RMR (17,23,38). Irisin is supposed to be released from the skeletal muscle and to act on the adipose tissue, inducing a "browning" of white or beige adipocytes, respectively (9). This transformation leads to an uncoupling of mitochondrial respiration and, thereby, an increase in energy expenditure or RMR, respectively (5). In more detail, irisin is a proteolytic hormone derivate of fibronectin type III domain containing 5 gene (FNDC5), and the expression of FNDC5 is increased by the activation of the gene transcriptional coactivator PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1α) after exercise (5,9,19,38). However, it should be noted that this link between exercise training, irisin, and elevated energy expenditure is based on a mouse model (5). In humans, several studies investigated the effect of exercise training on circulating irisin, muscle irisin messenger RNA, or the irisin precursor FNDC5. They revealed different mean results ranging from a significant increase (5) to no training-induced change (3,14,15,21,24,35) to a decrease (22) and also demonstrated considerable individual variation in irisin response. However, no study investigated the association between exercise training, irisin and RMR in humans, although an increase in energy expenditure is an important proposed health benefit of irisin.

The effects of a 6-month health-oriented resistance training program on RMR and its associations with changes in FFM and circulating irisin concentration were therefore investigated in initially untrained middle-age subjects. It was hypothesized that 1) the resistance training program elicits an increase in RMR and 2) this increase is associated with increases in FFM and serum irisin concentration.

METHODS

General design. This study is part of the SAusE trial, a four-arm randomized, controlled exercise intervention trial (clinicaltrials.gov ID NCT01263522), of which two arms were designed to investigate resistance training effects on RMR. Subjects in one arm preformed 6 months of progressive health-oriented resistance training (RT), whereas subjects in the other arm maintained their sedentary lifestyle for 6 months and served as controls (CO). Dietary intake was not modified, and dietary records served to compare energy intake at the beginning and at the end of intervention. The main outcome variable was a change in RMR. Changes in FFM and serum irisin concentration were evaluated as potential explanatory factors for changes in RMR. Posttraining RMR measurements and blood sampling were performed on separate occasions 2–7 d (≥48 h) after completion of the training program between 8:00 and 10:00 after a restful night's sleep and an overnight (≥12 h) fast. Strength tests in RT served to confirm efficacy of the resistance training intervention.

Participants. A total of 112 sedentary healthy men and women were recruited for participation. They were stratified for sex and baseline fitness level and randomly assigned to either RT or CO. Participants fulfilled the following inclusion criteria: 1) age 30 to 60 yr, 2) <1 $h \cdot wk^{-1}$ of regular physical activity for at least 1 yr, 3) body mass index (BMI) <30 $kg \cdot m^{-2}$, 4) nonsmokers, and 5) absence of major cardiovascular, metabolic, or orthopedic diseases or disorders. All participants gave a written informed consent to take part in the study, which had been approved by the local ethics committee (approval number, 148/10). A total of 38 participants dropped out of the study. Reasons for dropout were discontinuation for personal reasons (RT, $n = 7$; CO, $n = 4$), lack of adherence to the training/control protocol (RT, $n = 6$ with attendance rate <70% as defined *a priori*; CO, $n = 1$ who did not maintain sedentary lifestyle, which was assessed by regular telephone interviews), illnesses or injuries not related to the study (RT, $n = 4$; CO, $n = 1$), and missing data due to technical problems or violation of standard operating procedures (RT, $n = 5$; CO, $n = 10$). Finally, 74 participants were analyzed. Their characteristics were as follows: RT ($n = 37$ (17 males and 20 females); age, 47 ± 7 yr; height, 173 ± 10 cm; BMI, 25.0 ± 3.4 $kg \cdot m^{-2}$) and CO ($n = 37$ (12 males and 25 females); age, 50 ± 7 yr; height, 169 ± 8 cm; BMI, 24.2 ± 3.2 $kg \cdot m^{-2}$). Baseline age, height, and BMI were not significantly different between groups ($P = 0.19$, $P = 0.07$, and $P = 0.32$, respectively).

RMR measurements. Participants minimized physical activity after awaking and refrained from strenuous physical activity ≥24 h before the RMR measurements. Measurements took place in a silent, slightly darkened room with ambient temperature of 22°C–25°C. Experimental conditions were exactly standardized for each participant using a checklist. Participants rested in a supine position for 30 min while gas exchange was being measured using a mixing chamber system (Cortex MetaMax II; Cortex Biophysik, Leipzig, Germany). The system was calibrated before each test according to the instructions of the manufacturer. Oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$), and respiratory exchange ratio (RER) were averaged for the 5-min period, with the lowest values and a stability of ±10% for $\dot{V}O_2$ and $\dot{V}CO_2$ and ±5% for RER (8). RMR was calculated using the Weir equation (39). A correlation of the time that elapsed between the last exercise bout and the RMR measurement with the change in RMR served to rule out any effect of long-term excess postexercise oxygen consumption (40).

Determination of anthropometric data. Height and body weight (BW) were measured in light sportswear without shoes. Body fat percentage was assessed by always the same experienced investigator with a Harpenden caliper using the 10-site skinfold method (11). FFM was calculated from BW and body fat percentage. Waist and hip circumference were measured halfway between the lowest rip and iliac crest and the widest site of the hip, respectively. Waist-to-hip ratio (WHR) was calculated as the quotient of waist and hip circumference.

Blood sampling and irisin analysis. Blood samples were taken from the antecubital vein after a 10-min supine resting period. Samples were centrifuged and serum aliquots were frozen at −20°C until analysis. Serum aliquots of 71 of 74 participants were available for irisin analysis (RT, $n = 37$; CO, $n = 34$). Serum irisin concentrations were determined

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threefold using a commercial enzyme-linked immunosorbent assay kit (Phoenix Pharmaceutics, Burlingame, CA) and a Sunrise microplate reader (Tecan, Männerdorf, Switzerland) according to the lot-specific protocol of the manufacturer. To minimize differences in the handling of the wells, a semiautomatic 96-channel pipette (Selma; CyBio AG, Jena, Germany) was used. Sample values were determined by extrapolation to a standard curve (determined for each measurement) using curve fitting in Matlab (MathWorks, Ismaning, Germany). All samples were analyzed in one analytical run immediately after completion of the study. Because irisin concentrations demonstrated an inverse association with storage time (range, 17–758 d), values were corrected for storage time using the slope of the regression line of all baseline values and follow-up values of CO (0.184 $ng \cdot mL^{-1} \cdot d^{-1}$), as described in detail elsewhere (14). Uncorrected and corrected values are presented.

Strength tests and training intervention. The resistance training program was designed to be in accordance with the current ACSM recommendations (1,2,13). The training program was preceded by four familiarization sessions to ensure appropriate lifting techniques and introduce high exercise intensities in preparation for the one-repetition maximum (1RM) test. In the beginning of the first training session, after 15 repetitions with low intensity for warm-up, the 1RM was determined on the leg press and on the chest press as measure of maximum strength. Furthermore, the 20RM was determined on all training machines as a measure of muscular endurance and to derive training loads. The 20RM was redetermined every 6 wk, and training load was adapted for each 6-wk cycle. The 1RM tests were repeated in the beginning of the last training session at the same time of the day. Machine adjustments were exactly standardized for each subject.

Participants trained 3 $d \cdot wk^{-1}$ for 24 wk. Each training session consisted of eight machine-based exercises for the major muscle groups: back extension and crunch (Dr. Wolff, Arnsberg, Germany), pulldown machine, seated row machine, seated leg curl machine, seated leg extension machine, seated chest press machine, and lying leg press machine (Gym80 International, Gelsenkirchen, Germany). Participants performed two sets of 16 repetitions in weeks 1 and 2, 18 repetitions in weeks 3 and 4, and 20 repetitions in weeks 5 and 6 of each 6-wk cycle. Exercise intensity was the 20RM with 1 min of rest between sets. The 20RM on the leg press corresponded to 64% ± 7% 1RM in the beginning and 71% ± 6% 1RM at the end of the training intervention. All training sessions were recorded using training logs, and one of three training sessions per week was completely supervised. From the weight and the number of repetitions for each exercise, the total training load of each training session was calculated. In a subsample of eight participants, blood lactate concentration, HR, and RPE on a 6-to-20 scale (4) were determined at the end of the second set in a training session around week 12.

Dietary records. The participants kept prospective written dietary records over 3 d (two weekdays and one weekend day) at the beginning and at the end of the intervention period. Fifty-five participants handed in complete records (RT, $n = 30$; CO, $n = 25$). The records were based on a food frequency list of 127 items supplemented with free text. Energy intake in kilocalories per day was calculated from the records based on the German food composition database.

Statistics. Data were checked for normality by visual inspection of normal probability plots and the Shapiro–Wilk test. Outcome measures were mainly normally distributed, except for serum irisin concentration (uncorrected and corrected values), which was partly skewed and, therefore, log transformed for statistical analyses. Differences in age, height, and BMI between groups were tested with unpaired *t*-tests. Changes in strength within RT were tested using paired Student's *t*-tests. For the other outcome measures, two-way repeated-measures ANOVA were applied (factor 1, group; factor 2, time). To test for baseline differences between RT and CO, the main effect for group was considered and Dunnett tests were applied *post hoc*. The group–time interaction effect served to test for intervention effects. To test for within-group changes, the main effect for time was considered *post hoc* using Tukey tests. In addition, ANCOVA were performed to adjust for baseline values, sex, and age (37). Pre- and postdifferences of the outcome measure served as the dependent variable, group served as the independent variable, and baseline values, sex, and age served as covariates. To examine associations between RMR and FFM, RMR and irisin, as well as the change in RMR and the time that elapsed since the last exercise bout, Pearson correlations were used. Furthermore, a multiple linear regression analysis, with the change in RMR as the dependent variable and the changes in irisin and FFM as independent variables, was performed. Data are presented as means ± SD. $P < 0.05$ was considered to be significant.

RESULTS

Training and strength changes. RT performed 2.8 ± 0.1 training sessions per week on average over the 6-month intervention period (94% ± 5% attendance rate). The training load increased from 9037 ± 1907 kg per session at the end of the first 6-wk training cycle to 12,024 ± 2246 kg per session at the end of the last 6-wk training cycle ($P < 0.001$, $n = 37$). Blood lactate concentration, HR, and RPE at the end of a training session averaged 8.8 ± 1.4 $mmol \cdot L^{-1}$, 80% ± 6% HR_{max}, and 17.6 ± 0.9, respectively ($n = 8$). No adverse events occurred over the entire training period. The 1RM increased by 16% ± 7% on the leg press ($P < 0.001$) (Fig. 1A) and 25% ± 9% on the chest press ($P < 0.001$). The 20RM increased by 29% ± 12% on the leg press ($P < 0.001$) and 26% ± 12% on the chest press ($P < 0.001$, $n = 37$).

RMR. Absolute RMR is given in Figure 1B, and RMR relative to BW and FFM is given in Table 1. There were no significant baseline differences between groups ($P = 0.76$ for absolute RMR, $P = 0.49$ for RMR relative to BW, and $P = 0.23$ for RMR relative to FFM). Over the 6-month training period, RMR developed differently between groups (ANOVA

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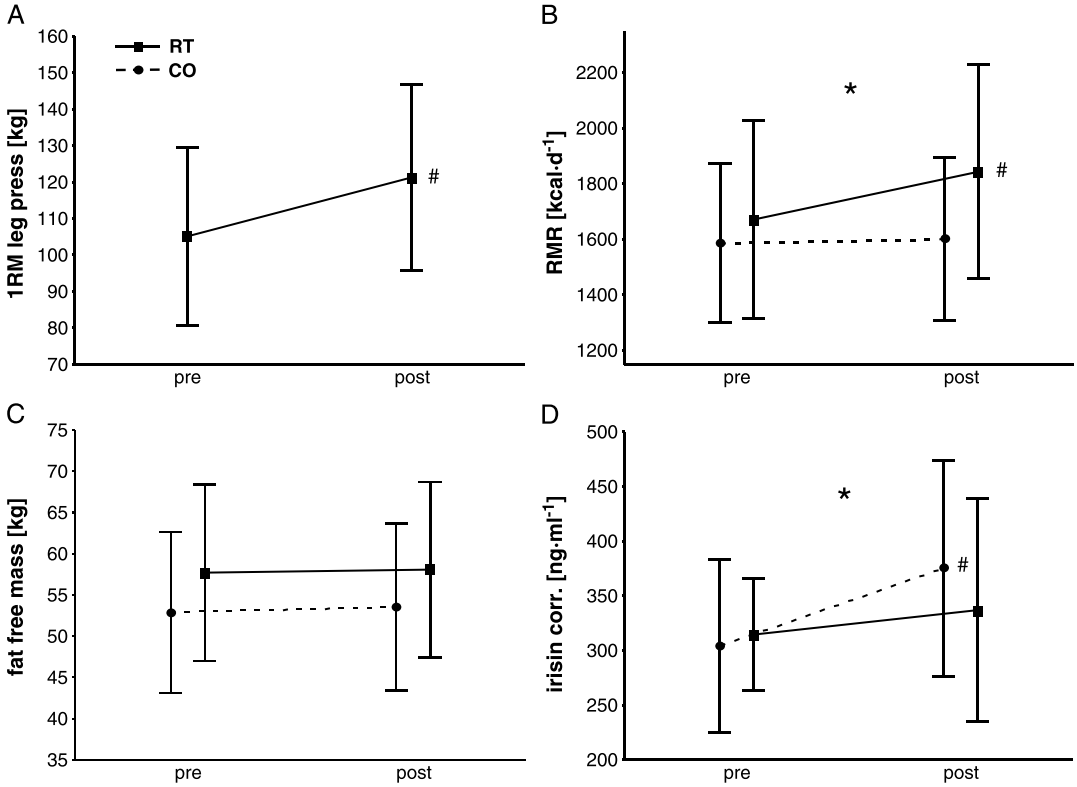

FIGURE 1—A, 1RM on the leg press in resistance training (RT) before and after 6 months of resistance training (n = 37). B, RMR before and after 6 months of resistance training (n = 37 in each group). C, FFM before and after 6 months of resistance training (n = 37 in each group). D, Serum irisin concentration corrected for storage time before and after 6 months of resistance training (CO, n = 34; RT, n = 37). *Asterisks* indicate significant group–time interaction, and *hashes* indicate significant changes within the group over time (means ± SD).

group–time interaction, $P < 0.01$ for absolute RMR, RMR relative to BW, and RMR relative to FFM). RT demonstrated a significant increase in RMR when expressed in absolute terms, relative to BW and relative to FFM, whereas no change was observed in CO. The differences in changes in RMR between groups held true when adjusted for baseline RMR, sex, and age (ANCOVA: $P < 0.01$ for absolute RMR and RMR relative to BW and $P < 0.05$ for RMR relative to FFM; n = 37 in each group). There was no association between the change in RMR and the time that elapsed between the last exercise bout and the RMR measurement ($r = -0.03$, $P = 0.84$, $n = 74$).

Body composition. BW, body fat percentage, and WHR are displayed in Table 2. FFM is presented in Figure 1C. There were no significant baseline differences between RT and CO for BW, body fat, and FFM ($P = 0.10$, $P = 0.33$, and $P = 0.05$, respectively). WHR was significantly higher in RT at baseline ($P = 0.02$). None of the parameters developed differently over time (ANOVA group–time interaction: $P = 0.29$, $P = 0.52$, $P = 0.59$, and $P = 0.07$, respectively). When adjusted for baseline level, sex, and age, there were also no significant differences in the changes in anthropometric data between groups (ANCOVA: $P = 0.31$, $P = 0.45$, $P = 0.59$, and $P = 0.13$, respectively; n = 37 in each group).

Irisin. Serum irisin concentrations are presented in Table 2 and Figure 1D. Baseline serum irisin concentration was not significantly different between groups (uncorrected values, $P = 0.81$; values corrected for storage time, $P = 0.56$). Uncorrected values demonstrated a significant group–time interaction in the ANOVA ($P < 0.01$). They increased over time in both groups, with bigger increases in CO. The change in irisin remained significantly different between groups when adjusted for baseline level, sex, and age (ANCOVA, $P < 0.01$). Values corrected for storage time also demonstrated a significant group–time interaction in the ANOVA ($P < 0.01$). Irisin increased in CO, whereas there was no change in RT. The change in irisin values corrected for storage time also remained significantly different between groups when adjusted for baseline level, sex, and age (ANCOVA, $P < 0.01$; RT, n = 37; CO, n = 34).

Associations between irisin, FFM, and RMR. At baseline, RMR was significantly correlated with FFM

TABLE 1. RMR before and after 6 months of resistance training (*post hoc* test results are given for significant time effects, means ± SD, n = 37 in each group).

		Before Training	After Training	Time Effect	Group–Time Interaction
RMR (kcal·d^{-1}·kg^{-1} BW)	RT	22.4 ± 3.2	24.9 ± 3.6	$P < 0.001$	$P < 0.01$
	CO	23.1 ± 3.6	23.2 ± 3.6	$P = 0.99$	
RMR (kcal·d^{-1}·kg^{-1} FFM)	RT	29.1 ± 4.0	31.9 ± 4.4	$P < 0.01$	$P < 0.01$
	CO	30.3 ± 4.6	30.2 ± 4.1	$P = 0.99$	

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TABLE 2. Anthropometric data (n = 37 in each group) and serum irisin concentrations (uncorrected values and values corrected for storage time; RT, n = 37; CO, n = 34; means ± SD) before and after 6 months of resistance training (*post hoc* test results are given for significant time effects).

		Before Training	After Training	Time Effect	Group–Time Interaction
BW (kg)	RT	75.2 ± 14.3	74.7 ± 14.7	P = 0.51	P = 0.29
	CO	69.7 ± 13.6	69.8 ± 13.6		
Body fat (%)	RT	23.0 ± 4.8	21.9 ± 4.9	P = 0.08	P = 0.54
	CO	23.9 ± 5.1	23.2 ± 4.6	P = 0.40	
WHR	RT	0.84 ± 0.09	0.85 ± 0.09	P = 0.99	P = 0.07
	CO	0.79 ± 0.10	0.81 ± 0.10	P < 0.05	
Irisin (ng·mL^{-1})	RT	199.5 ± 50.7	257.0 ± 103.4	P < 0.01	P < 0.01
	CO	187.6 ± 75.8	293.4 ± 99.6	P < 0.001	
Irisin, corrected (ng·mL^{-1})	RT	314.6 ± 51.1	337.0 ± 102.1	P = 0.67	P < 0.01
	CO	304.2 ± 79.2	375.0 ± 98.6	P < 0.001	

(r = 0.71, P < 0.001, n = 74), but not with serum irisin concentration (uncorrected values: r = 0.02, P = 0.90; values corrected for storage time: r = 0.008, P = 0.95; n = 71). Correlations between changes in RMR and FFM as well as irisin are presented in Figure 2. Changes in RMR were not significantly associated with changes in FFM (r = −0.11, P = 0.36, n = 74). Furthermore, they were not significantly associated with changes in serum irisin concentration (uncorrected values: r = 0.006, P = 0.96; values corrected for storage time: r = −0.004, P = 0.97; n = 71). Multiple linear regression, with the change in RMR as the dependent variable and the changes in FFM and irisin as independent variables, was not significant either (R^2 = 0.01 and P = 0.65 for uncorrected irisin values; R^2 = 0.01 and P = 0.66 for irisin values corrected for storage time; n = 71).

Diet. Energy intake in RT (2021 ± 469 kcal·d^{-1} at the beginning and 2151 ± 664 kcal·d^{-1} at the end of the study, n = 30) and CO (1995 ± 594 kcal·d^{-1} at the beginning and 2045 ± 716 kcal·d^{-1} at the end of the study, n = 25) was not significantly different at baseline (P = 0.99) and did not develop differently over time (ANOVA group–time interaction, P = 0.60). When adjusted for baseline level, sex, and age, there was also no significant difference in the changes in energy intake between groups (ANCOVA, P = 0.73).

DISCUSSION

The present study demonstrates that 6 months of preventive high-repetition resistance training leads to an increase in strength and RMR. However, in contrast to current assumptions and our hypothesis, the training-induced increase in RMR was not associated with an increase in FFM or the newly identified myokine irisin. This association between training-induced changes in RMR and irisin was investigated for the first time in humans.

RMR increased in response to the strength training program. This finding is in accordance with some previous studies (7,10,12,18,25,26) but not with others (6,10,16,27, 31,34). One study reported that higher training intensities elicit more pronounced increases in RMR (12), but it is not possible to derive an intensity threshold necessary for changes in RMR from this or other studies. The present study demonstrates that high-repetition strength training at 64%–71% 1RM, classified as moderate to vigorous and recommended to novices by ACSM (13), is sufficient to increase RMR. Furthermore, previous studies reveal conflicting information about whether strength training effects on RMR depend on age (6,10,12,18,25,34) or gender (7,10,26,27,34). The present study indicates that strength training may elicit an increase in RMR in healthy middle-age male and female participants who are a typical target group for health promotion. Altogether, health-oriented preventive resistance training can increase RMR.

Regarding the mechanistic link between exercise training and RMR, the newly described myokine irisin is currently under discussion. Since Boström et al. (5) published the first work on irisin in *Nature* in 2012, comments in *Science* (17),

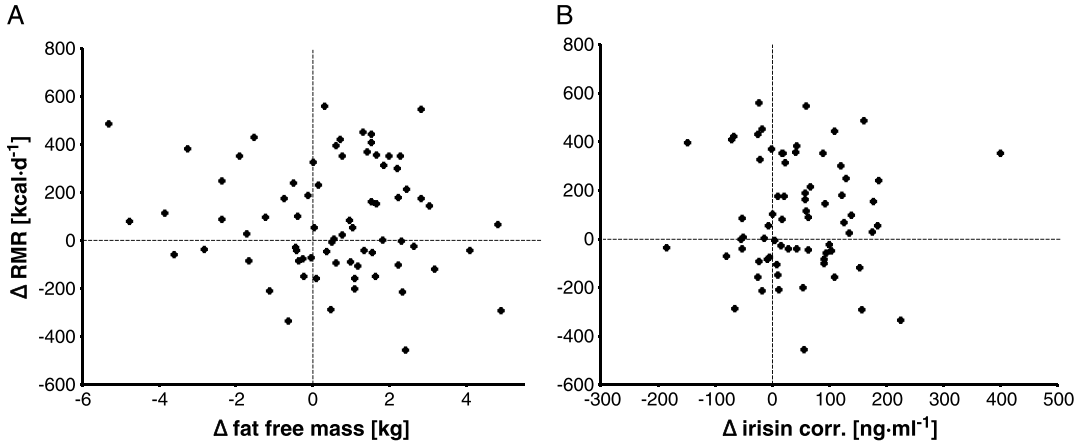

FIGURE 2—A, Correlation between changes in RMR and FFM (n = 74). B, Correlation between changes in RMR and serum irisin concentration corrected for storage time (n = 71).

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The New England Journal of Medicine (23), and others (9,28,38) took up its great potential and even speculated about the development of an "exercise pill." However, the link between exercise training, circulating irisin concentrations, and energy expenditure had never been investigated in humans. One cross-sectional study investigated the association between irisin and 24-h energy expenditure in overweight-to-obese women between 50 and 70 yr of age and found no overall correlation (33). However, a subsample of subjects whose energy expenditure was greater than expected demonstrated a significant correlation between irisin and 24-h energy expenditure. Previous exercise training studies in humans investigated training effects on irisin but not on RMR. One of them revealed an increase in circulating irisin after 10 wk of endurance training (5). Several other studies failed to demonstrate training-induced mean effects on circulating irisin, muscle irisin messenger RNA, or the irisin precursor FNDC5 after training programs of different types and durations (3,14,15,21, 24,35). Finally, in one study, circulating irisin concentration decreased after 12 wk of combined endurance and resistance training (22). The present study revealed neither a training-induced increase in irisin nor a correlation between changes in RMR and irisin after 6 months of high-repetition resistance training in healthy middle-age men and women. Therefore, currently available data create doubt in the meaning of irisin in humans.

Furthermore, the present study is the first exercise training study on irisin in humans that included a nontraining control group. Only Pekkala et al. (24) presented data of two control subjects versus a total of 18 subjects in two training groups. The control group of the present study demonstrated a significant increase in irisin. This increase is not attributable to a change in energy intake, which was verified by dietary records. However, macronutrient intake might have influenced irisin concentration, which needs evaluation in future studies. Effects of season, which might be associated with ambient temperature and shivering, can be ruled out because recruitment was evenly spread over the year. Altogether, little is still known about influencing factors on irisin production. Non-exercise control groups are needed in future human training studies to investigate the course of irisin concentration over time and to ensure that potentially observed changes in irisin in the training group are attributable to the training stimulus.

Serum irisin concentration demonstrated an inverse association with storage time, which has not been described before in other studies. Although the 10-wk training intervention investigated by Boström et al. (5) was shorter than the 6-month intervention in the present study, it seems tenable that the observed increase in irisin in the Boström study might have been an effect of storage time, particularly as no control group data were presented. Future training studies should therefore consider intermediate blood analyses with extra aliquots to control for the effect of storage time to overcome this problem.

A potential limitation of all studies on irisin in humans is that the kinetics of circulating irisin concentration after acute and chronic exercise is unknown. Huh et al. (15) found significantly elevated irisin levels 30 min after an acute exercise bout. However, this increase diminished after 8 wk of chronic exercise training. In contrast, Pekkala et al. (24) did not find elevated irisin concentration at different time points after a single heavy exercise bout, 3 h after a low-intensity endurance exercise bout or 3 h after the last bout of a 21-wk heavy endurance or combined endurance and resistance training program. In the present study, ≥48 h elapsed between the last training session of the 6-month training period and blood sampling. It cannot be ruled out that irisin is secreted during or acutely after unaccustomed exercise but not chronically after long-term training programs. Future training studies should therefore investigate the kinetics of circulating irisin concentration at multiple points after a single exercise bout in the beginning of the study and chronic exercise training at the end of the study.

The traditional explanation for training-induced increases in RMR is an increase in FFM (36). However, in the present study, RMR increased despite lack of change in FFM, which is surprising at first glance. Numerous previous resistance training studies found either concurrent increases in RMR and FFM (7,10,18,25,26) or no change in both parameters (16,27,34). However, a closer look at the literature reveals that an increase in FFM is not always associated with an increase in RMR (6), that RMR can also increase when expressed per kilogram of FFM (26), and that the correlation between increases in RMR and FFM can be unconvincingly weak ($r = 0.37$, $P = 0.08$) (18). The latter results and the present study suggest that there must be further mechanistic links between resistance training and RMR. Because no adipose tissue biopsies were conducted in the present study, it cannot be ruled out that a "browning" of white adipocytes occurred despite lack of change in circulating irisin. Increased thermogenesis through changes in adipose tissue is therefore possible. The activity of myocytes might also have increased and contributed to the increase in RMR. Finally, it seems possible that other systems involved in energy supply might have increased their activity. Further studies are needed to systematically address this question.

In contrast to the majority of previous studies, the strength training program did not elicit changes in anthropometric data (7,10,18,25,26). This might be attributable to the training stimulus. In the present study, a high-repetition workout at moderate-to-vigorous intensity (16–20 repetitions at 64%–71% 1RM) was performed. High-repetition workout improves muscular endurance rather than eliciting hypertrophy and, therewith, an increase in FFM (13). In other studies, fewer repetitions at higher intensities were prescribed (6–15 repetitions at 80%–90% of 1RM or 90% of 3RM) (10,18,25,26), which are more likely to elicit muscle hypertrophy (13). Another possible explanation for missing changes in body composition is that the caliper method was not sensitive enough to show small effects. Other studies used more sensitive methods like dual energy x-ray absorptiometry or computed tomography. It might be questioned whether the present training program was highly effective in health promotion if

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no distinct changes in anthropometric data occurred. However, the training program led to an increase in RMR, which may have contributed to long-term stability of BW, body fat content, and WHR in the intervention group, which is a favorable training effect. Therefore, preventive resistance training within the volumes and intensities recommended for novices (13) seems to be effectively promoting health, as demonstrated before for preventive endurance training (20,29).

CONCLUSIONS

The present study demonstrates that 6 months of preventive resistance training 3 $d \cdot wk^{-1}$ (eight machine-based exercises, two sets of 16–20 repetitions at 64%–71% 1RM) leads to an increase in strength and RMR in healthy middle-age men and women. Circulating irisin concentration (≥48 h postexercise) did not increase in response to the training program, and changes in RMR were not associated with changes in irisin. Therefore, the training-induced increase in RMR does not seem to be mediated by the myokine irisin in humans, which was investigated for the first time in the present study. The increase in RMR was also not associated with an increase in FFM or accompanied by changes in body composition. However, it might have contributed to stable anthropometric data in the intervention group, which is a favorable training effect. The mechanisms linking exercise training and RMR need further investigation.

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