Cardiorespiratory Fitness and Insulin Sensitivity in Overweight or Obese Subjects May Be Linked Through Intrahepatic Lipid Content

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OBJECTIVE—Low cardiorespiratory fitness (CRF) predisposes one to cardiovascular disease and type 2 diabetes in part independently of body weight. Given the close relationship between intrahepatic lipid content (IHL) and insulin sensitivity, we hypothesized that the direct relationship between fitness and insulin sensitivity may be explained by IHL.

RESEARCH DESIGN AND METHODS—We included 138 overweight to obese, otherwise healthy subjects (aged 43.6 ± 8.9 years, BMI 33.8 ± 4 kg/m^2). Body composition was estimated by bioimpedance analyses. Abdominal fat distribution, intramyocellular, and IHL were assessed by magnetic resonance spectroscopy and tomography. Incremental exercise testing was performed to estimate an individual's CRF. Insulin sensitivity was determined during an oral glucose tolerance test.

RESULTS—For all subjects, CRF was related to insulin sensitivity ($r = 0.32$, $P < 0.05$), IHL ($r = -0.27$, $P < 0.05$), and visceral ($r = -0.25$, $P < 0.05$) and total fat mass ($r = -0.32$, $P < 0.05$), but not to intramyocellular lipids ($r = -0.08$, NS). Insulin sensitivity correlated significantly with all fat depots. In multivariate regression analyses, independent predictors of insulin sensitivity were IHL, visceral fat, and fitness ($r^2 = -0.43$, $P < 0.01$, $r^2 = -0.34$, and $r^2 = 0.29$, $P < 0.05$, respectively). However, the positive correlation between fitness and insulin sensitivity was abolished after adjustment for IHL ($r = 0.16$, NS), whereas it remained significant when adjusted for visceral or total body fat. Further, when subjects were grouped into high versus low IHL, insulin sensitivity was higher in those subjects with low IHL, irrespective of fitness levels.

CONCLUSIONS—Our study suggests that the positive effect of increased CRF on insulin sensitivity in overweight to obese subjects may be mediated indirectly through IHL reduction. ***Diabetes*** **59:1640–1647, 2010**

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Low cardiorespiratory fitness (CRF) predisposes one to metabolic disease and increases cardiovascular morbidity and mortality in women and men (1–3). Insulin resistance, an early event in the pathogenesis of both type 2 diabetes and cardiovascular disease, is more pronounced in people with reduced CRF (4). CRF is also reduced in type 2 diabetic patients compared with healthy subjects (5). Insulin resistance is more prevalent in individuals with excess body fat than in normal-weight people (6,7). Yet, fit individuals have lower rates of cardiometabolic diseases than their unfit counterparts, regardless of total body fat (3,8). Thus, "fitness" and "fatness" affect metabolic and cardiovascular risk in part independently. The mechanisms mediating the adiposity-independent effect of CRF on metabolic disease are not fully understood. Direct influences on peripheral insulin sensitivity through improved muscular oxidative metabolism (9) and fat redistribution may be involved. The latter mechanism may be important because visceral adipose tissue (VAT) accumulation and increased muscular and hepatic intracellular fat deposition contribute to insulin resistance (10–13). However, intrahepatic lipid accumulation (IHL) appears to be particularly important in this regard (14,15). Furthermore, hepatic fat accumulation increases circulating triglyceride-rich lipoproteins (16,17). Hypertriglyceridemia is common in obesity and responds to physical exercise (18). Although IHL is intimately related to insulin resistance and dyslipidemia, previous studies on the interaction between CRF and metabolism did not assess intrahepatic fat. We applied magnetic resonance spectroscopy to test the hypothesis that in obesity the beneficial effect of CRF on metabolism is related to intrahepatic fat. Given the strong sex effect on metabolic and cardiovascular regulation (19–21), we analyzed women and men separately.

RESEARCH DESIGN AND METHODS

We investigated 138 overweight/obese but otherwise healthy volunteers (107 women and 31 men) in our study. All subjects completed a comprehensive medical evaluation including a dietary record for 7 consecutive days before study participation. Volunteers reported <2 h of physical activity per week and were not taking medications that could affect metabolism or liver function. Subjects consuming >20 g/day of alcohol, with diagnosis of type 2 diabetes, with acute or chronic infections, with any diseases that required treatment, or with known drug abuse were excluded. Our institutional review board approved the study, and written informed consent was obtained before entry. Volunteers were advised to continue their current physical activity level and lifestyle throughout the study.

Volunteers visited the laboratory on 2 separate days for anthropometric, metabolic, and cardiovascular evaluations and exercise testing. After a 10- to

12-h overnight fast, we measured body weight, height, and waist circumference in a standardized fashion. After a resting period of at least 5 min in the seated position, we determined blood pressure and heart rate with an automated blood pressure cuff (Dinamap; Critikon, Tampa, FL). During an oral glucose load (75 g glucose/500 ml), we obtained blood samples at baseline and 15, 30, 45, 60, 90, and 120 min after glucose ingestion to measure glucose and insulin. We assessed lean body and fat mass by bioimpedance analysis (BIA 5 series; Denner, Feldmeilen, Switzerland). Glucose, insulin, and blood lipids were measured by standard laboratory procedures in a certified clinical chemistry laboratory. During the second visit, we quantified visceral and subcutaneous abdominal fat mass and IHL and intramyocellular lipid (IMCL) content by magnetic resonance tomography and spectroscopy. CRF was determined during an exhaustive incremental exercise test on a cycle ergometer. We assessed nutrition including the kind and amount of alcohol consumption using standardized protocols over a 7-day period. On the basis of these records, we estimated the average amount of alcohol consumption in grams per day.

Magnetic resonance imaging and spectroscopy. Abdominal fat imaging and fat spectroscopy of the lower leg musculature were performed on a 1.5-tesla magnetic resonance scanner (Magnetom Avanto and Sonata; Siemens Medical Solutions, Erlangen, Germany). An axial T1-weighted and water-suppressed gradient echo technique (TR 80, TE 6.11, FA 80, FOV 500 × 500 mm, 512 × 512 matrix, slice thickness 10 mm, interslice gap 10 mm) was applied for abdominal imaging. During repetitive breath-holds, consecutive datasets were acquired, covering the abdomen from the diaphragm to symphysis. Images were analyzed for the amount of VAT and subcutaneous adipose tissue (SAT) by semiautomated image segmentation software that employs a contour-following algorithm (Vitom, University Duisburg-Essen, Germany). Total abdominal adipose tissue was calculated as the sum of VAT and SAT. IHL was assessed by ^{1}H magnetic resonance spectroscopy of a single voxel located in segment 7 of the liver (TR 7,000, TE 30, voxel size 30 × 30 × 20 mm, number of averages 24, acquisition during repetitive breath-holds). A fat-to-water ratio was calculated after postprocessing of the spectra including a standard line-fitting procedure and integration of the hepatic triglycerides from 0.5 to 2.8 ppm. The unit of measurement is the ratio of the signal from fat (f) to total signal from fat (f) and water (w) (f/[f + w]%). Furthermore, IMCL content was quantified by ^{1}H single-voxel spectroscopy of the tibialis anterior muscle. High-resolution T1 weighted spin-echo images of the calf allowed identification of an appropriate voxel position in the tibialis anterior, thereby minimizing spectral contamination from extramyocellular lipids from adipose tissue attached to the muscular fasciae. A spin-echo single-voxel spectroscopy sequence (TR 3,000, TE 30, voxel size 11 × 11 × 20 mm, number of averages 64) with frequency-selective water suppression was applied. After baseline and constant phase correction, a standard line-fitting procedure using prior knowledge about the resonance peaks was performed for quantification. IMCL values were calculated as the area under the curve of the IMCL methylene line normalized to the creatine–CH_3 signal and corrected for differences in T1 and T2, resulting in a dimensionless value.

Incremental exercise test. Subjects underwent a stepwise incremental exercise test on a bicycle ergometer (VIAsprint 150P; Ergoline, Bitz, Germany) until volitional exhaustion. Exercise was performed in a temperature-controlled room (21–22°C) ~2 h after subjects had ingested a standardized breakfast (containing ~520 kcal: 24% fat, 68% carbohydrate, and 8% protein). Alcohol and caffeine were not permitted 48 h before the exercise test. After 3 min in the seated position, resting measurements were recorded. Exercise was then started at a workload of 25 W. Workload was increased every 2 min by 25 W until the subjects could not maintain the requested 60 rpm pedal frequency. We monitored gas exchange continuously during the test to assess oxygen uptake and power output. Using an open spirometric system (Vmax Spectra model 229D analyzer; SensorMedics, Yorba Linda, CA), the time course of oxygen uptake and carbon dioxide production was recorded breath-by-breath and averaged in 10-s intervals. Heart rate was recorded by an electrocardiogram (GE Medical Systems, Waukesha, WI) throughout the exercise test. We assumed that subjects had reached maximal oxygen uptake ($V\text{O}_{2\text{max}}$) when at least two of the following criteria were met: *1*) respiratory exchange ratio > 1.10, *2*) $V\text{O}_2$ leveling off despite increase in power output, and *3*) heart rate within 10 beats × min^{-1} of the predicted maximum heart rate. To consider the individual differences in body weight, oxygen uptake was expressed as kilograms of body weight ($V\text{O}_2$: ml^{-1} × min^{-1} × kg^{-1}).

Biochemical measurements and calculations. Glucose (millimoles per liter), insulin (microunits per milliliter), and alanine aminotransferase (units per liter) were determined by standard methods in a certified clinical chemistry laboratory. Insulin resistance was estimated by homeostasis model assessment index (HOMA) derived from fasting glucose and insulin concentrations. HOMA was calculated from fasting insulin and glucose by (insulin [microunits per milliliter] × glucose [millimoles per liter])/22.5) (22). Areas under the curve for insulin (AUC_{INS}) and glucose (AUC_{GLU}) were assessed using the trapezoid method. Insulin sensitivity was calculated by the composite insulin-sensitivity index (C-ISI) (23): C-ISI = 10,000/$\sqrt{}$[(FPG × FPI) × (G × I)], where FPG and FPI are fasting plasma glucose (milligrams per deciliter) and fasting plasma insulin (microunits per milliliter), respectively, and G (milligrams per deciliter) and I microunits per milliliter) are the mean glucose and mean insulin concentration during the 2-h oral glucose tolerance test, respectively. Serum high sensitivity C-reactive protein (hs-CRP) (in micrograms per milliliter), total adiponectin as well as low-, middle-, and high-molecular-weight adiponectin multimers (all in micrograms per milliliter), fetuin-A (in nanograms per milliliter), and retinol-binding protein 4 (RBP4) (in micrograms per milliliter) were measured by sandwich enzyme-linked immunosorbent assay with the following characteristics: hs-CRP (RH961CRP01HR; BioVendor, Heidelberg, Germany), intra-assay coefficient of variation 3.8% and interassay coefficient of variation 5.2%; adiponectin (47-ADPHU-E01; ALPCO Immunoassays, Salem, NH), intra-assay coefficient of variation between 5.1 and 9.8% for the different multimers and interassay coefficient of variation between 4.8 and 6.5%; fetuin-A (RD191037100; BioVendor), intra-assay coefficient of variation 4.9% and interassay coefficient of variation 5.7%; and RBP4 (AG-45A-0011EK-KI01; AdipoGen, Seoul, Korea#), intraassay coefficient of variation 3.9% and interassay coefficient of variation 8.1%.

Statistical analysis. Data were first tested for normal distribution and variance homogeneity with Kolmogorov-Smirnov test and the Levene test, respectively. Pearson correlation coefficients were used to determine the relationship between CRF and insulin sensitivity. Partial correlations were used to control for total body fat mass, VAT, SAT, and intrahepatic lipid content. To specify the effect of CRF on insulin sensitivity, anthropometric and metabolic risk marker subjects were categorized into CRF tertiles ($V\text{O}_{2\text{max}}$). Then a one-way ANOVA was performed to examine differences in subject characteristics across fitness levels. When the ANOVA result was significant, a Tukey post hoc comparison test was used to identify specific between-group differences. To further analyze influence of fat redistribution, we subgrouped subjects among CRF tertiles on the basis of sex-specific VAT levels (<2.9 or ≥2.9 kg for men and <1.5 or ≥1.5 kg for women), IHL content (<7.4 or ≥7.4% for men and <4.6% or ≥4.6% for women), and total body fat mass (<29.8 or ≥29.8 kg for men and <33.6 or ≥33.6 kg for women). Finally, a stepwise multivariate regression analysis was performed to identify predictors of insulin sensitivity. All statistical analyses were performed with SPSS 16 (SPSS, Chicago, IL). Significance was accepted at $P < 0.05$. Values are given as means ± SD.

RESULTS

Of 213 screened subjects, 172 subjects met the inclusion criteria of our study. Fourteen women and 2 men smoked regularly between 5 and 20 cigarettes per day. Nineteen women and 2 men did not meet the criteria for valid $V\text{O}_{2\text{max}}$ estimation. MR studies were unsuccessful in 11 subjects due to claustrophobia ($n = 7$), equipment failure ($n = 1$), waist circumference exceeding the magnetic resonance scanner limits table ($n = 1$), and poor magnetic resonance image quality ($n = 2$). Two subjects dropped out for personal reasons. Anthropometric and metabolic characteristics of the remaining 138 overweight and obese women and men, classified into CRF tertiles, are given in Table 1. $V\text{O}_{2\text{max}}$ was 25.4 ± 4.1 ml/min/kg in men and 21.1 ± 3.5 ml/min/kg in women ($P < 0.05$). Furthermore, VAT mass and IHL content were greater in men (VAT 3.49 ± 1.06 vs. 1.63 ± 0.79 kg, $P < 0.01$; IHL 11.63 ± 4.7 vs. 7.9 ± 3.6%, $P < 0.05$), whereas insulin sensitivity was lower than in women (C-ISI 4.8 ± 2.7 vs. 6.1 ± 2.4, $P < 0.05$; HOMA 2.1 ± 1.1 vs. 1.6 ± 1.2, $P = 0.69$). Alanine aminotransferase was higher in men (21.7 ± 8.1 vs. 36.1 ± 11.2 units/l, $P > 0.05$). Average alcohol consumption was 6.6 ± 4.8 g/day in women and 9.5 ± 5.2 g/day in men.

Table 2 shows Pearson correlation coefficients between CRF and selected cardiometabolic risk markers for women and for men before and after adjustment for either VAT or IHL. CRF correlated significantly negatively with BMI, percent body fat, systolic blood pressure, subcutaneous fat mass, and total abdominal fat mass. Diastolic blood pressure correlated significantly negatively to CRF in

TABLE 1
Anthropometric and metabolic characteristics of subjects classified into tertiles based on CRF

	Women			Men		
	Low tertile	Middle tertile	High tertile	Low tertile	Middle tertile	High tertile
n		107			31	
$V_{O_{2max}}$ (ml/min/kg)	15.6 ± 2.5	20.7 ± 1.3*	26.3 ± 2.9*†	18.8 ± 1.5	25.4 ± 1.7*	30.8 ± 2.7*†
Age (years)	45.7 ± 8.1	42.9 ± 9.6	42.1 ± 9.1	47.8 ± 6.4	43.9 ± 10.1	45.1 ± 9.2
BMI (kg/m^2)	34.7 ± 4.1	34.1 ± 3.8	31.2 ± 3.6*†	38.3 ± 4.8	36.1 ± 3.6	32.9 ± 4.7*
Body fat mass (%)	38.7 ± 3.6	37.9 ± 5	32.5 ± 5.9*†	34.8 ± 5.3	27.1 ± 9.1	26.9 ± 7.1*
Systolic blood pressure (mm/Hg)	127.9 ± 11.7	122.2 ± 12.1	116.5 ± 9.9*	139.2 ± 13.8	129.6 ± 8.1	132.6 ± 8.1
Diastolic blood pressure (mm/Hg)	75.7 ± 6.3	71.9 ± 6.4	69.4 ± 5.3*	79.4 ± 10.5	74.1 ± 8.3	75.2 ± 9.9
Cholesterol (mmol/l)						
Total	5.06 ± 0.73	4.76 ± 0.75	4.52 ± 0.41	4.98 ± 0.71	5.08 ± 0.87	4.38 ± 0.63†
HDL	1.39 ± 0.31	1.45 ± 0.65	1.44 ± 0.75	1.06 ± 0.31	1.19 ± 0.12	1.29 ± 0.34
LDL	3.18 ± 0.69	2.93 ± 0.73	2.83 ± 0.78	3.25 ± 0.71	3.06 ± 0.64	2.61 ± 0.61
Triglycerides	1.18 ± 0.41	1.11 ± 0.55	1.09 ± 0.51	1.45 ± 0.64	1.51 ± 0.77	1.02 ± 0.29†
Adipose tissue mass (kg)						
Total abdominal	12.6 ± 2.7	12.6 ± 3.3	10.4 ± 2.1*†	15.7 ± 3.2	13.6 ± 4.6	10.4 ± 2.2*
Visceral	1.69 ± 0.63	1.64 ± 0.78	1.27 ± 0.71	3.79 ± 0.87	3.52 ± 1.41	3.21 ± 1.42
Subcutaneous	10.9 ± 2.5	11 ± 3.1	9.1 ± 2.7*	11.9 ± 3.4	10.1 ± 4.2	7.2 ± 1.6*†
IMCL	4.49 ± 1.42	5.37 ± 2.08	4.89 ± 1.86	5.1 ± 0.92	4.82 ± 1.57	4.16 ± 0.74
IHL (f/f + w) (%)	8.6 ± 6.4	8.9 ± 8.3	6.9 ± 5.3	16.9 ± 9.1	11.6 ± 8.1	7.6 ± 7.2*
Indices of insulin sensitivity						
AUC glucose (mmol/l × min)	1,048 ± 180	1,010 ± 176	1,011 ± 146	1,151 ± 96	1,042 ± 154	985 ± 160
AUC insulin (μU/ml × min)	6,781 ± 2,410	6,541 ± 3,260	6,097 ± 2,565	12,305 ± 6,102	6,386 ± 3,221*	6,477 ± 4,150*
HOMA (insulin resistance)	1.51 ± 0.6	1.49 ± 1.14	1.26 ± 0.71	4.32 ± 2.91	2.03 ± 0.53	1.47 ± 1.28*
C-ISI	5.79 ± 2.1	5.98 ± 1.99	6.95 ± 3.41	2.45 ± 1.16	4.78 ± 2.41	6.98 ± 2.96*

Data are means ± SD. CRF is given as maximum oxygen uptake ($V_{O_{2max}}$) expressed per kg body wt. (ml × min^{-1} × kg^{-1}). *Significantly different from low tertile. †Significantly different from middle tertile.

women only. In men, VAT tended to inversely correlate with CRF. In women, we observed a trend for an inverse correlation between IHL content and CRF. The inverse correlation between IHL and CRF in men was mainly driven by the particularly low IHL content in the highest fitness tertile (Table 1).

For all subjects, alanine aminotransferase was related to BMI ($r = 0.27$, $P < 0.01$), IHL ($r = 0.47$, $P < 0.001$), VAT ($r = 0.36$, $P < 0.01$), fetuin-A ($r = 0.20$, $P < 0.05$), and C-ISI ($r = -0.32$, $P < 0.001$) and tended to do so for CRF ($r = -0.17$, $P = 0.076$). Critical serum parameters (fetuin-A, adiponectin, hs-CRP, and RBP4) and their relation to adiposity and fat distribution are given in Table 4. Insulin sensitivity was positively related to total ($r = 0.36$, $P < 0.01$) and high-molecular-weight adiponectin ($r = 0.37$, $P < 0.01$) and negatively related to fetuin-A ($r = -0.19$, $P < 0.05$) and hs-CRP ($r = -0.20$, $P < 0.05$) but not to RBP4 ($r = 0.04$). $V_{O_{2max}}$ showed a correlation to hs-CRP

TABLE 2
Pearson correlation coefficients (*r* values) between CRF with anthropometric and metabolic variables before and after adjustment with either VAT and IHL

	Women			Men		
Variables	Unadjusted	Adjusted for VAT	Adjusted for IHL	Unadjusted	Adjusted for VAT	Adjusted for IHL
C-ISI	0.18†	0.09	0.01	0.51*	0.37*	0.19
HOMA	−0.17*	−0.10	−0.03	−0.54*	−0.32*	−0.18
BMI (kg/m^2)	−0.34*	−0.17	−0.28	−0.36*	−0.34*	−0.18
Body fat (%)	−0.37*	−0.29*	−0.35*	−0.37*	−0.47*	−0.52*
Systolic blood pressure (mm/Hg)	−0.35**	−0.32*	−0.39*	−0.36*	−0.23	−0.11
Diastolic blood pressure (mm/Hg)	−0.28*	−0.28*	−0.36*	−0.14	−0.09	−0.10
Cholesterol (mmol/l)						
Total	−0.11	−0.14	−0.15	−0.21	−0.08	0.04
HDL	0.09	0.05	0.06	0.12	0.05	−0.05
LDL	−0.08	−0.07	−0.08	−0.22	−0.06	0.07
Triglycerides	−0.11	−0.07	−0.01	−0.20	−0.14	0.08
Adipose tissue mass (kg)						
Total abdominal	−0.33*	−0.15	−0.27	−0.45*	−0.47*	−0.40*
Visceral	−0.22*		−0.23*	−0.27†		−0.06
Subcutaneous	−0.29*	−0.15	−0.26	−0.51**	−0.52**	−0.42**
IMCL	−0.09	0.02	0.04	−0.19	−0.17	−0.18
IHL (f/f + w) (%)	−0.19†	−0.13		−0.43*	−0.44*	

†$P < 0.10$ and >0.05; *$P < 0.05$; **$P < 0.01$.

TABLE 3
Multivariate regression analyses with insulin sensitivity as dependent variable

	Women				Men			
Independent variable	β-Coefficient	*P* value	Model r^2	Model *P* value	β-Coefficient	*P* value	Model r^2	Model *P* value
Model 1								
VAT	−0.38	<0.05	0.33	0.04	−0.34	<0.05	0.39	0.02
IHL	−0.41	<0.01			−0.48	<0.01		
CRF	0.15	0.08			0.45	<0.01		
Model 2								
% body fat	0.14	0.11	0.36	0.02	−0.23	0.11	0.41	<0.01
VAT	−0.34	<0.05			−0.36	<0.05		
IHL	−0.38	<0.01			−0.43	<0.01		
CRF	0.14	0.09			0.44	<0.01		
Model 3								
% body fat	−0.13	0.11	0.38	0.02	−0.22	0.13	0.42	<0.01
VAT	−0.31	<0.05			−0.33	<0.05		
SAT	−0.14	0.26			−0.18	0.19		
IHL	−0.38	<0.01			−0.43	<0.01		
CRF	0.15	0.07			0.42	<0.01		
Model 4								
BMI	−0.09	0.37	0.41	<0.01	0.12	0.65	0.44	<0.01
% body fat	−0.17	0.09			−0.23	0.10		
VAT	− 0.31	<0.05			− 0.33	<0.05		
SAT	− 0.13	0.24			− 0.15	0.20		
IHL	− 0.42	<0.01			− 0.46	<0.01		
CRF	0.23	<0.05			0.43	<0.01		
Model 5								
Age	−0.04	0.63	0.41	<0.01	0.01	0.73	0.45	<0.01
BMI	−0.07	0.38			−0.09	0.64		
% body fat	− 0.16	0.09			− 0.29	0.12		
VAT	−0.36	<0.01			− 0.31	<0.05		
SAT	−0.11	0.27			−0.11	0.37		
Triglycerides	−0.08	0.38			− 0.12	0.21		
IHL	−0.41	<0.01			−0.46	<0.01		
CRF	0.21	<0.05			0.37	<0.01		

CRF is given as maximum oxygen uptake (Vo_{2max}) expressed per kg body wt. (ml × min^{-1} × kg^{-1}).

($r = -0.23$, $P < 0.05$) but not to fetuin-A, adiponectin multimers, or RBP4. Alcohol consumption (inclusion criteria <20 g/day) showed no significant relation to IHL content ($r = -0.05$) or measures of liver function (alanine aminotransferase $r = 0.12$).

In partial correlation analysis for both sexes, we observed that the direct, sex-independent relationship between CRF and insulin sensitivity ($r = 0.32$, $P < 0.05$) was abolished after controlling for IHL ($r = 0.14$, NS), shown in Fig. 1, whereas for the adjustment of percent body fat ($r = 0.28$, $P < 0.01$), VAT ($r = 0.25$, $P < 0.05$), and SAT ($r = 0.29$, $P < 0.01$), the correlation between CRF and insulin

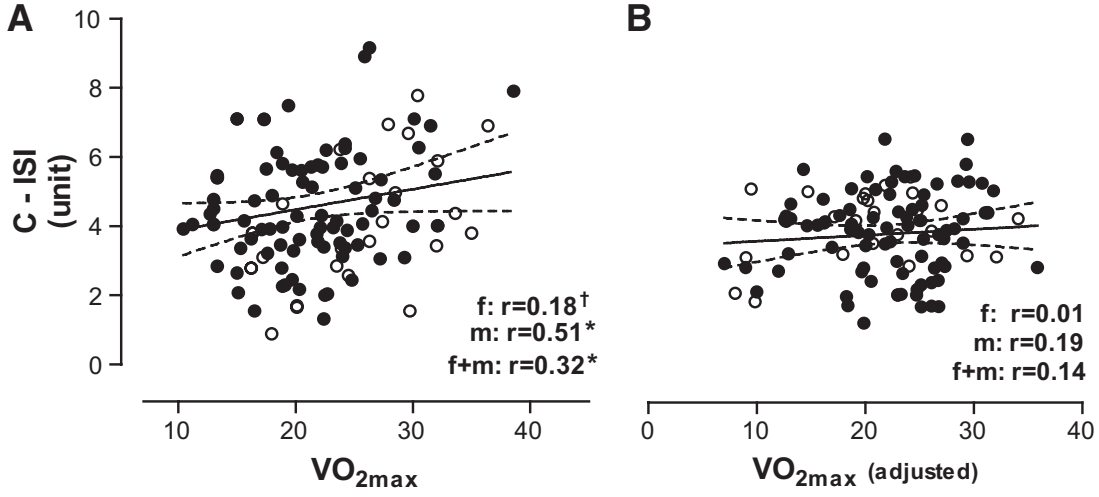

FIG. 1. Correlation between C-ISI and Vo_{2max} before (*A*: $r = 0.32$, $P < 0.05$) and after adjustment for intrahepatic lipid content (*B*: $r = 0.14$, NS); ●, women; ○, men. *$P < 0.05$; †$P < 0.1$ and >0.05.

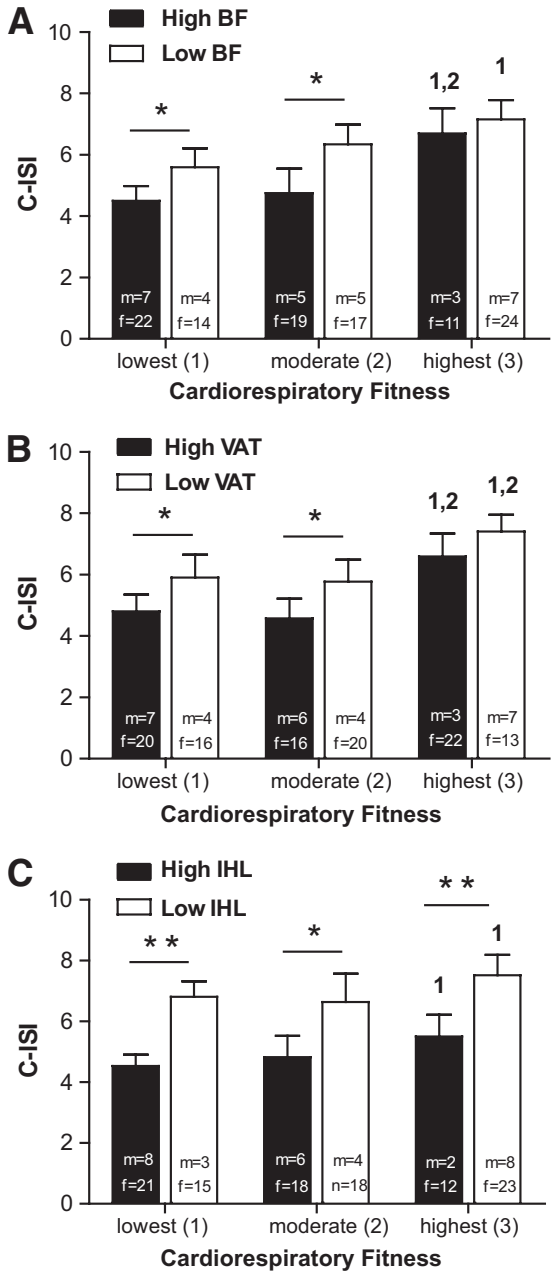

FIG. 2. C-ISI in men (m) and women (f) classified into tertiles of CRF with sex-specific subgroups of either low or high body and fat mass (*A*), low or high VAT mass (*B*), and low or high IHL content (*C*). 1,2, significantly different from corresponding subgroup in other tertile(s). Data are means ± SEM. **P* < 0.05; ***P* < 0.01, significantly different among the same tertile. BF, body fat.

sensitivity was attenuated but remained significant. Adjustment for alanine aminotransferase ($r = 0.19$, NS) and hs-CRP ($r = 0.17$, NS) also abolished the direct correlation between CRF and insulin sensitivity.

Figure 2 presents C-ISI values in women and in men classified into sex-specific CRF tertiles and then further subdivided into groups with high versus low VAT mass, IHL content, and body fat mass based on the sex-specific median for each measurement. For VAT levels, there was a significant difference in the lowest and moderate CRF tertile, with lower insulin sensitivity among subjects with high levels of VAT. However, in the high CRF tertile, there was no difference between the high versus low VAT subgroup. We observed a similar pattern between subgroups classified on the basis of total body fat mass. Whereas in the low and moderate CRF tertiles insulin sensitivity was lower among men and women in the subgroup with high body fat, there was no influence of body fat on the relationship between CRF and insulin sensitivity in the highest fitness tertile. The influence of VAT and body fat on insulin sensitivity across fitness levels was unchanged when we excluded men from the analysis. However, in subjects with high IHL content, insulin sensitivity was lower compared with that in subjects with low IHL content, irrespective of fitness level. The relationship remained significant when men were excluded from the analysis ($P < 0.05$).

The correlation of insulin sensitivity with percent body fat, VAT and SAT mass, and IHL content were similar between men and women (% body fat: women $r = -0.19$, NS, men $r = -0.16$, NS; VAT: women $r = -0.46$, $P < 0.05$, men $r = -0.51$, $P < 0.05$; SAT: women $r = -0.21$, NS, men $r = -0.25$, NS; IHL: women $r = -0.44$, $P < 0.01$, men $r = -0.58$, $P < 0.01$), whereas a correlation between C-ISI and IMCL was observed in women only ($r = -0.29$, $P < 0.05$).

To assess determinants of insulin sensitivity in more detail, we conducted a multivariate regression analysis with insulin sensitivity as the dependent variable. In women, the significant direct correlation for insulin sensitivity with visceral fat mass and IHL persisted even after inclusion of age, BMI, percent body fat, subcutaneous fat mass, triglycerides, and $V\text{O}_{2\text{max}}$ as covariables (Table 3). Also, $V\text{O}_{2\text{max}}$ (CRF) became a weak but significant predictive variable. The model that included age, BMI, percent body fat, visceral and subcutaneous fat mass, triglycerides, IHL, and $V\text{O}_{2\text{max}}$ as independent variables explained 41% of the variation in insulin sensitivity. In men, multivariate regression with the same variables included (Table 3) revealed IHL, visceral fat mass, and $V\text{O}_{2\text{max}}$ as significant predictors of insulin sensitivity and explained 45% of the variation in insulin sensitivity. When men and women were analyzed together, IHL ($r^2 = -0.43$, $P < 0.01$), VAT ($r^2 = -0.34$, $P < 0.01$), and CRF ($r^2 = 0.29$, $P < 0.05$) were observed as independent predictors of insulin sensitivity. IHL, VAT, and CRF explained 33 and 39% of the variation in insulin sensitivity in women and men, respectively (Table 3, model 1). When we excluded IHL from the multivariate regression model 5 (Table 3), the predictive power of CRF on insulin sensitivity was stronger, both in women (from $r = 0.21$, $P < 0.05$ to $r = 0.34$, $P < 0.01$) and in men (from $r = 0.37$, $P < 0.01$ to $r = 0.43$, $P < 0.001$).

DISCUSSION

The novel finding of our study is that the positive relationship between CRF and insulin sensitivity in overweight and obese subjects was no longer present after controlling for intrahepatic fat content. In contrast, the relationship between CRF and insulin sensitivity was largely unaffected after adjustment for total body, abdominal subcutaneous, abdominal visceral, and intramyocellular fat. Thus, our study suggests that the positive effect of increased CRF in overweight on insulin sensitivity to obese subjects may be mediated indirectly through IHL reduction. Moreover, high CRF is "protective" in patients with excessive visceral fat but does not negate the metabolic effect of increased IHL.

We obtained multislice whole-abdomen images providing an accurate estimate of visceral and subcutaneous fat.

TABLE 4
Biochemical parameters and their sex-independent association (Pearson correlation coefficients) to adiposity and body fat distribution

	Women	Men	BMI	% Body fat	VAT	SAT	IHL
n	107	31					
Fetuin-A (ng/ml)	253 ± 71	268 ± 79	0.26**	0.09	0.22*	0.20*	0.35**
Adiponectin (μg/ml)							
Total	6.4 ± 2.3	5.1 ± 1.5*	−0.16†	−0.03	−0.25**	−0.04	−0.28**
High molecular weight	3.2 ± 1.6	2.2 ± 1*	−0.18*	−0.08	−0.25**	−0.02	−0.27**
Middle molecular weight	1.3 ± 0.4	0.9 ± 0.3*	−0.08	0.02	−0.20*	0.02	−0.21*
Low molecular weight	2 ± 0.5	1.9 ± 0.4	−0.10	−0.01	−0.14†	−0.07	−0.22*
hs-CRP (μg/l)	1.6 ± 0.1	1.5 ± 0.2	0.29**	0.20*	0.15	0.23*	0.24*
RBP4 (μg/ml)	74.8 ± 29.7	83.8 ± 26.2	0.03	−0.02	0.19*	0.04	0.25*

Data are means ± SD. Group comparison by *t* test for unpaired samples: †$P < 0.1$; *$P < 0.05$, **$P < 0.01$.

In previous studies, abdominal fat area was measured from a single-slice computed tomography scan at the level of the fourth or fifth vertebra (8,10,11,24,25) or the umbilicus (26). Moreover, we measured both IHL and IMCL in the same subjects. Thus, we were able to assess the individual contribution of fat distribution and organ fat on glucose metabolism in obese subjects.

Increased intrahepatic and intramyocellular lipids are associated with hyperinsulinemia, impaired glucose tolerance, and hepatic insulin resistance in diabetic and nondiabetic subjects (9,13,14). Recent studies reported that intrahepatic fat has a stronger impact on insulin sensitivity than VAT (27–29). Furthermore, the detrimental influence of intrahepatic lipid accumulation on metabolic function appears to be independent of VAT mass (27). In stepwise regression analysis, IHL content was a stronger predictor of insulin sensitivity than visceral fat mass or IMCL content, independent of sex, age, or BMI, underscoring the central role of the liver in the pathogenesis of obesity-associated metabolic disease. Furthermore, the finding suggests IHL as a prime candidate for explaining the relationship between CRF and glucose metabolism.

In our study, individuals with high CRF were more insulin sensitive than less fit individuals. Similarly, previous studies identified low CRF as a strong and independent predictor of incident metabolic syndrome. Moderate to high CRF lowered the risk of all-cause and cardiovascular mortality independent of BMI (1,2,30). These studies focused on VAT accumulation when evaluating the relationship between CRF levels and metabolic risk markers. Some investigators observed an influence of VAT on the association of CRF with metabolic risk (10,25). We and others failed to show such a relationship (8). Differences in adiposity and sex distribution among studies could contribute to the discrepancy. Men in the latter study were older, and the prevalence of obesity was higher than that for men in the study by Arsenault et al. (10). Only one study quantified VAT in overweight and obese women to assess mechanistic links between CRF and metabolism (25). Yet, obese women are prone to develop metabolic disease (20,31). Given the discrepancy among studies, we suggest that VAT may not be the crucial factor linking CRF and metabolism.

The positive relationship between CRF and insulin sensitivity in our study was no longer significant after controlling for intrahepatic fat content or alanine aminotransferase, as an indicator of liver function (32). This finding suggests that the relationship between insulin sensitivity and CRF in overweight to obese subjects is mediated by IHL content rather than total body, abdominal, or intramyocellular fat accumulation. Moreover, in subjects in the highest CRF tertile, insulin sensitivity was unaffected by VAT mass or total body fat. In contrast, insulin sensitivity was consistently impaired in subjects with increased IHL regardless of physical fitness. Therefore, the beneficial effect of high CRF on insulin sensitivity appears to be limited to individuals with low intrahepatic fat content.

Potential mechanisms linking CRF and IHL include factors regulating hepatic lipid oxidation (33–36). Substrate oxidation is tightly coupled to mitochondrial oxidative capacity (37,38). Mitochondria occupy ~18% of the liver cell volume (39). Mitochondrial function, a strong determinant of fitness, (40) could conceivably affect hepatic lipid oxidation. In fact, variation in the genes encoding peroxisome proliferated–activated receptor (PPAR) δ, PPAR coactivator 1α, and PPARγ affects mitochondrial function, responsiveness to physical training (33), and liver fat content (41). Very recent experiments in rats showed that low aerobic fitness causes reduced hepatic mitochondrial oxidative capacity, which increased susceptibility to hepatic steatosis and liver injury (42). Plasma biomarker analysis revealed that hs-CRP could also contribute, at least partly, to the observed association between CRF, insulin sensitivity, and IHL accumulation. hs-CRP is elevated in liver disease and predicts the incidence of type 2 diabetes in humans (43,44). Our findings underscore the importance of IHL in obesity-associated insulin resistance and type 2 diabetes (12,45).

The strength of the relationship between fat distribution, CRF, and insulin sensitivity differs between men and women. The sex difference may be explained in part by relatively low CRF (46) and IHL content in our women.

In conclusion, our study suggests mechanisms through which CRF improves cardiovascular and metabolic risk factors independently of body weight. The interaction between CRF and insulin sensitivity seems to be mediated by hepatic lipid content rather than the amount of total, visceral, subcutaneous, or intramyocellular fat. Regular physical activity improves whole-body and abdominal fat mass as well as IHL accumulation and insulin sensitivity (47–49). Physical fitness noticeably improves metabolic risk and contributes independently to metabolic health even without body fat reduction (8,47,50). Optimization of nutritional, exercise, and pharmacological interventions such that lipid mobilization from the liver is maximized may be particularly beneficial in terms of metabolic risk

reduction. Our study provides a strong impetus to test this hypothesis in prospective studies.

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