Habitual Physical Activity Is Associated With Intrahepatic Fat Content in Humans

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OBJECTIVE — Fatty liver may be involved in the pathogenesis of type 2 diabetes. Physical exercise is a tool to improve insulin sensitivity, but little is known about its effect on intrahepatic fat (IHF) content. The purpose of this study was to examine the association of habitual physical activity, insulin resistance, and adiponectin with IHF content.

RESEARCH DESIGN AND METHODS — Participants were 191 (77 female and 114 male) apparently healthy, nonalcoholic individuals (aged 19–62 years; BMI 17.0–35.5 kg/m^2). IHF content was assessed in a quantitative fashion and noninvasively as a continuous variable by means of ^{1}H magnetic resonance spectroscopy (MRS), and habitual physical activity was assessed by means of a questionnaire. Fatty liver was defined as IHF content of >5% wet weight, and insulin sensitivity was estimated using the computer homeostasis model assessment (HOMA)-2 indexes.

RESULTS — A reduced prevalence of fatty liver in the quartile of the most physically active individuals (25, 11, 25, and 2% in quartile 1, 2, 3, and 4, respectively; $\chi^2 = 15.63$; $P = 0.001$) was found along with an inverse correlation between the physical activity index and the IHF content when plotted as continuous variables (Pearson's $r = -0.27$; $P < 0.000$). This association was not attenuated when adjusted for age, sex, BMI, HOMA-2, and adiponectin (partial correlation $r = -0.25$; $P < 0.001$).

CONCLUSIONS — This study demonstrated that a higher level of habitual physical activity is associated with a lower IHF content and suggested that this relationship may be due to the effect of exercise per se.

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Alanine aminotransferase and γ-glutamyltransferase are associated with type 2 diabetes risk (1–3), and it is thought that the link is represented by the intrahepatic fat (IHF) content. Ectopic fat accumulation within the liver, in fact, has been reported in association with impairment of insulin-stimulated glucose metabolism, of suppression of endogenous glucose production, and of whole-body lipolysis in nondiabetic individuals with nonalcoholic fatty liver disease (NAFLD) (4,5). Additional results also demonstrated that decreased levels of circulating adiponectin in NAFLD are related to hepatic insulin sensitivity and to the IHF content, suggesting that hypoadiponectinemia may be involved in excessive hepatic fat accumulation (6).

Physical exercise was found to be associated with a reduced risk of development of type 2 diabetes (7,8) and is a well-recognized tool to improve insulin sensitivity at the level of the skeletal muscle (9). However, whether physical exercise may affect insulin sensitivity and diabetes risk via an effect on the IHF content and adiponectin remains unknown.

The IHF content may be assessed as a continuous variable by means of ^{1}H magnetic resonance spectroscopy (MRS) (10), and recently this technique was found to be a sensitive, quantitative, and noninvasive method also when applied to a large population (11) without the need for the use of a more invasive approach such as liver biopsy. The purpose of this study was, therefore, to examine the association of habitual physical activity, insulin resistance, and plasma adiponectin concentration with the IHF content in a population of 191 nonalcoholic, healthy individuals using a cross-sectional approach.

RESEARCH DESIGN AND METHODS

One-hundred and ninety-one individuals were recruited via a survey performed to assess the prevalence of fatty liver among the employees of the San Raffaele Scientific Institute. These individuals were recruited in the outpatient service of the Center of Nutrition/Metabolism of the San Raffaele Scientific Institute. Their body weight had to be stable for at least 6 months for inclusion; exclusion criteria included a history of hepatic disease, substance abuse, or daily consumption of >1 alcohol drink (<20 g/day) or the equivalent in beer and wine. Normal or higher than normal IHF content was set at 5% wet weight as suggested by the American Association for the Study of Liver Diseases (AASLD) (12). The anthropometric characteristics of the subjects are summarized in Table 1. Subjects were in good health as assessed by medical history, physical examination, hematological analysis, and urinalysis. Recruited subjects gave their informed written consent after explanation of the

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Abbreviations: FFA, free fatty acid; HOMA, homeostasis model assessment; HOMA2-%B, HOMA2-derived index of β-cell insulin sensitivity; HOMA2-%S, HOMA2-derived index of insulin sensitivity; IHF, intrahepatic fat; MRS, magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; TSH, thyroid-stimulating hormone.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—*Anthropometric and laboratory features of individuals with fatty liver (IHF content >5% wet weight) and normal subjects (IHF content <5% wet weight)*

	Individuals with fatty liver	Normal subjects	*P* value
Sex (female/male)	31 (4/27)	160 (73/87)	0.001*
Age (years)	36 ± 8	34 ± 9	0.52
Height (cm)	173 ± 7	170 ± 16	0.35
Weight (kg)	82 ± 15	70 ± 14	0.0001
BMI (kg/m^2)	27.4 ± 3.8	23.7 ± 3.6	0.0001
Systolic blood pressure (mmHg)	128 ± 10	117 ± 10	0.0001
Diastolic blood pressure (mmHg)	83 ± 8	77 ± 8	0.0001
Total cholesterol (mmol/l)	5.25 ± 1.24	4.65 ± 0.80	0.002
HDL cholesterol (mmol/l)	1.22 ± 0.31	1.53 ± 0.39	0.0001
Triglycerides (mmol/l)	1.66 ± 0.92	0.86 ± 0.36	0.0001
FFAs (mmol/l)	0.62 ± 0.16	0.57 ± 0.23	0.22
Creatinine (μmol/l)	74 ± 17	74 ± 15	0.86
TSH (mU/l)	1.26 ± 0.97	1.18 ± 0.91	0.77
Fasting glucose (mmol/l)	5.11 ± 0.61	4.72 ± 0.50	0.0001
Fasting insulin (pmol/l)	97 ± 37	70 ± 32	0.0001
HOMA2-%B	153 ± 43	142 ± 58	0.15
HOMA2-%S	56 ± 24	83 ± 38	0.0001
Adiponectin (μg/ml)	5.3 ± 2.0	8.2 ± 3.7	0.0001
PAI work	2.60 ± 0.50	2.59 ± 0.53	0.92
PAI sport	2.06 ± 0.69	2.61 ± 1.06	0.006
PAI leisure time	3.03 ± 0.65	3.10 ± 0.60	0.58
PAI total	7.69 ± 1.23	8.30 ± 1.41	0.02

Data are means ± SD. Independent-samples *t* test (two tailed). The range of possible scores for the total physical activity index (PAI) is 3–15; the lowest value corresponds to the level of physical activity of a clerical worker who plays a light sport (energy expended is <0.76 MJ/h; e.g., bowling) and who participates in sedentary activities during leisure time. The highest value corresponds to the level of physical activity of a person who is very physically active at work (e.g., a construction worker), who plays heavy sports (energy expended is at least 1.76 MJ/h; e.g., boxing, basketball, football, or rugby), and who is very physically active during leisure time (e.g., walking >1 h/day or biking >45 min/day). *Pearson χ^2 test.

purpose, nature, and potential risks of the study.

Subjects were instructed by a registered dietitian to consume an isocaloric diet containing at least 250 g of carbohydrates and 70–90 g of protein/day and to abstain from exercise activity for 3 days before the study. They were studied after an 8- to 10-h overnight fast by means of ^{1}H-MRS for the assessment of IHF content. Blood samples were collected for measurement of serum insulin, plasma glucose, free fatty acids (FFAs), the lipid profile, and biochemical parameters.

Experimental protocol

Assessment of habitual physical activity. Habitual physical activity was assessed using a validated questionnaire that had been developed for the various socioeconomic classes in the general population (13). Briefly, three meaningful factors can be distinguished within habitual physical activity: *1*) occupational physical activity (precoded according to three levels of physical activity at work); *2*) sport during leisure time subdivided in three levels and a sport score calculated from a combination of the intensity of the sport (low level, 0.76 MJ/h: sailing, bowling, billiards, and golf; middle level, 1.26 MJ/h: cycling, dancing, swimming, and tennis; and high level, 1.76 MJ/h: basketball, football, and rowing), the amount of time per week (from 0.5 to >4 h), and the proportion of the year (from <1 to >9 months) during which the sport was played regularly; and *3*) other physical activity during leisure time (this specifically relates to watching television, walking, and cycling during leisure time). The test-retest reliability of this questionnaire, validated in a Dutch population, was tested for Italian individuals in our laboratory in previous studies; the questionnaire was administered twice 2 weeks apart and the coefficients of variation (CVs) of the work, sport, and leisure time indexes were 2, 3, and 8%, respectively.

^{1}H-MRS. Hepatic ^{1}H-MRS was performed at rest and with patients in the supine position with the use of a 1.5-T whole-body scanner (Gyroscan Intera Master 1.5 MR System; Philips Medical Systems, Best, Netherlands) using a conventional circular superficial coil as described previously (14). First, coronal and transverse images of the liver were obtained for all patients. Next, T1 in-phase and out-of-phase sequences were obtained to look for a potential loss of signal on out-of-phase images, indicating the presence of IHF accumulation. Then an 8-cm^3 spectroscopic volume of interest was positioned within the right lobe, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. The voxel shimming was executed to optimize the homogeneity of the magnetic field within the specific volume of interest. Two ^{1}H spectra were collected from the hepatic parenchyma with the same prescanning conditions using a PRESS pulse sequence (interpulse delay TR = 3,000 ms, spin-echo time TE = 25 ms, 1,024 data points over a 1,000-Hz spectral width and 64 acquisitions) with and without suppression of the water signal, respectively. Area of resonances from protons of water (4.8 ppm) and methylene groups in fatty acid chains of the hepatic triglycerides (1.4 ppm) were obtained with a time-domain nonlinear fitting routine using commercial software (VARPRO-MRUI; http://www.mrui.uab.es). The percent IHF was calculated by dividing the integral of the methylene groups in fatty acid chains of the hepatic triglycerides (obtained from the water-suppressed spectrum) by the sum of methylene groups and water (obtained from the nonwater-suppressed spectrum) × 100. The CV of the IHF content assessed using the above-described setting in our laboratory is 4.7% in individuals with <5% wet weight and 3.1% in individuals with an IHF content >5% wet weight, and the CV was calculated using the row data obtained from two consecutive acquisitions performed using the same volume of interest and the same prescanning procedures.

Analytical determinations

Glucose (Beckman Coulter, Fullerton, CA), FFAs, triglycerides, total cholesterol, HDL cholesterol, and serum creatinine were measured as described previously (14). Plasma levels of insulin (sensitivity 2 μU/ml; intra- and interassay CVs of <3.1 and 6%, respectively) were measured with a radioimmunoassay (Linco Research, St. Charles, MO). Serum adiponectin was measured, as described

Table 2—*Characteristics of study subjects by quartiles of physical activity index (PAI)*

	Quartile 1 (4.63–7.25)	Quartile 2 (7.26–8.00)	Quartile 3 (8.01–9.08)	Quartile 4 (9.09–13.06)	P value
Sex (female/male)	23/29	20/24	18/30	16/31	0.63*
Age (years)	34 ± 7	35 ± 7	35 ± 9	35 ± 11	0.82
BMI (kg/m^2)	24.9 ± 4.3	24.2 ± 4.6	25.1 ± 3.4	23.1 ± 2.8	0.036
Systolic blood pressure (mmHg)	119 ± 13	119 ± 11	121 ± 11	118 ± 8	0.35
Diastolic blood pressure (mmHg)	77 ± 9	79 ± 7	80 ± 9	76 ± 7	0.15
Total cholesterol (mmol/l)	4.76 ± 0.80	4.68 ± 0.70	5.02 ± 1.14	4.58 ± 0.88	0.09
HDL cholesterol (mmol/l)	1.42 ± 0.39	1.45 ± 0.41	1.50 ± 0.44	1.55 ± 0.36	0.37
Triglycerides (mmol/l)	1.13 ± 0.79	1.05 ± 0.90	1.01 ± 0.45	0.76 ± 0.30	0.06
Fasting glucose (mmol/l)	4.77 ± 0.56	4.77 ± 0.56	4.83 ± 0.55	4.72 ± 0.56	0.85
Fasting insulin (pmol/l)	85 ± 37	67 ± 27	73 ± 34	66 ± 32†	0.03
HOMA2-%B	157 ± 58	131 ± 35	137 ± 39	139 ± 68	0.11
HOMA2-%S	69 ± 24	81 ± 22	80 ± 30	88 ± 29‡	0.01
Adiponectin (μg/ml)	6.8 ± 2.8	7.2 ± 4.1	8.4 ± 3.7	8.4 ± 3.6	0.08
IHF content (% wet weight)	5.1 ± 6.5	2.8 ± 3.6	4.9 ± 7.1	1.5 ± 1.0§	0.0001
Fatty liver	13/52 (25)	5/44 (11)	12/48 (25)	1/46 (2)	0.001*
PAI work	2.2 ± 0.4‖	2.6 ± 0.5§	2.8 ± 0.4	2.8 ± 0.6	0.000
PAI sport	1.7 ± 0.4‖	2.2 ± 0.5	2.4 ± 0.7	3.8 ± 1.1‖	0.000
PAI leisure time	2.7 ± 0.5¶	2.9 ± 0.5¶	3.3 ± 0.5	3.5 ± 0.5	0.000

Data are means ± SD or n (%). *Pearson χ^2 test. †$P < 0.05$ vs. quartile 1; ‡$P < 0.05$ vs. quartile 1 and quartile 2; §$P < 0.02$ vs. quartile 1 and quartile 3; ‖$P < 0.001$ vs. all; ¶$P < 0.001$ vs. quartiles 3 and 4, Bonferroni post hoc analysis.

previously (15), with an enzyme-linked immunosorbent kit (B-Bridge International, Sunnyvale, CA) with a sensitivity of 25 pg/ml. The intra- and interassay CVs were <3.7 and <6%, respectively. Thyroid-stimulating hormone (TSH) was measured by an immunofluorometric method as described previously (16).

Calculations

Insulin resistance was determined by updated computer homeostasis model assessment (HOMA)-2 indexes (17) available from http://www.OCDEM.ox.ac.uk. The percent IHF was calculated by dividing the integral of the methylene groups in fatty acid chains of the hepatic triglycerides by the sum of methylene groups and water × 100. Signal decay due to spin-spin relaxation was calculated using mean T2 relaxation times for water and fat of 50 and 60 ms, respectively, and the exponential relaxation equation $I_m = I_0 \exp(-Te/T2)$, where I_m is the measured signal intensity obtained at the selected echo-time Te, I_0 is the signal intensity immediately after the 90° pulse, and T2 is the spin-spin relaxation time. Average T2 relaxation times were used for these calculations (10,18) as previously performed (11). These values represent a relative quantity of water and hepatic triglyceride fatty acid chain protons in the volume of interest. To convert these values to absolute concentrations expressed as percent fat by weight of volume, we used equations validated by Longo et al. (10). A liver fat content >50 mg/g (5% by wet weight and equivalent to 6.5% of the ratio of methylene to methylene + water × 100 in our setting) is diagnostic of hepatic steatosis (12), and study subjects could be segregated into a group of individuals with normal (<5% wet weight) or higher than normal IHF content (>5% wet weight).

Statistical analysis

Data in text and tables are means ± SD. Analyses were performed using the SPSS software (version 10.0; SPSS, Chicago, IL). Variables with skewed distribution assessed using the Kolmogorov-Smirnov test of normality (IHF content, HDL cholesterol, triglycerides, systolic and diastolic blood pressure, insulin, TSH, HOMA2-derived index of insulin sensitivity [HOMA2-%S], and HOMA2-derived index of β-cell insulin sensitivity [HOMA2-%B]) were log transformed before the analysis. One-way ANOVA or a Kruskal-Wallis nonparametric test was used when appropriate to compare variables between subjects with and subjects without fatty liver (Table 1) or among quartiles of the physical activity index (Table 2). The Bonferroni post hoc test was used. $P < 0.05$ was considered to be significant. The relationship between IHF content and the physical activity index was examined by a two-tailed Pearson's correlation. Partial correlation was used to examine these relationships independently of age, sex, BMI, adiponectin, and HOMA2-%S.

RESULTS

Anthropometric and laboratory features of study subjects with or without fatty liver

A higher than normal IHF content (>5% wet weight) was found in 31 individuals (16%) with a higher prevalence in men than in women (Table 1). These individuals were characterized by a higher BMI, systolic and diastolic blood pressure, total cholesterol, and triglycerides along with a reduced HDL cholesterol (Table 1). Plasma FFAs, serum creatinine, and TSH were not different between individuals with or without fatty liver. Two volunteers had impaired fasting glucose (one man with 6.1 mmol/l and fatty liver and one woman with 6.3 mmol/l and normal IHF content) and were included in the study. Fasting plasma glucose and insulin concentrations were increased in individuals with fatty liver compared with normal subjects (Table 1) ($P < 0.0001$); in association, the HOMA2-%S was reduced in subjects with than in those without fatty liver ($P < 0.0001$), whereas the HOMA2-%B was not different (Table 1). The serum adiponectin concentration was reduced in individuals with fatty liver more than in the normal subjects ($P < 0.0001$). The score for the total physical activity index was reduced in individuals with fatty liver more than in the normal subjects (Table 1) ($P < 0.02$), and this difference was due exclusively to physical exercise during sport activities ($P < 0.006$), whereas scores for physical activity during work ($P = 0.92$) and leisure time ($P = 0.58$) were not different.

Characteristics of study subjects by quartiles of the physical activity index

To assess in a cross-sectional fashion the impact of habitual physical activity, study subjects were segregated in subgroups of quartiles of the score of total physical activity index (cutoffs 7.25, 8.00, and 9.08) as summarized in Table 2. The subgroups were not different per sex and age; for BMI, one-way ANOVA showed that it was different even if the Bonferroni post hoc analysis did not reveal a significant difference among quartiles. Blood pressure, total cholesterol ($P = 0.09$), HDL cholesterol, triglycerides ($P = 0.06$), and fasting plasma glucose were not different. In contrast, fasting plasma insulin was lower in the most physically active (quartile 4) compared with the least active (quartile 1) individuals. β-Cell insulin sensitivity was not different among quartiles, whereas HOMA2-%S (Table 2) was higher in quartile 4 compared with quartiles 1 and 2. The fasting serum adiponectin concentration was not different among quartiles ($P = 0.08$) (Table 2). Finally, IHF content was lower in the quartile of the most physically active (quartile 4) individuals compared with those in quartiles 1 and 3 ($P < 0.02$) (Table 2 and Fig. 1*A*), and this finding was confirmed by analyzing the prevalence of fatty liver, which was the lowest (2%) in the quartile of the most physically active individuals (Pearson χ^2 test: $P < 0.001$).

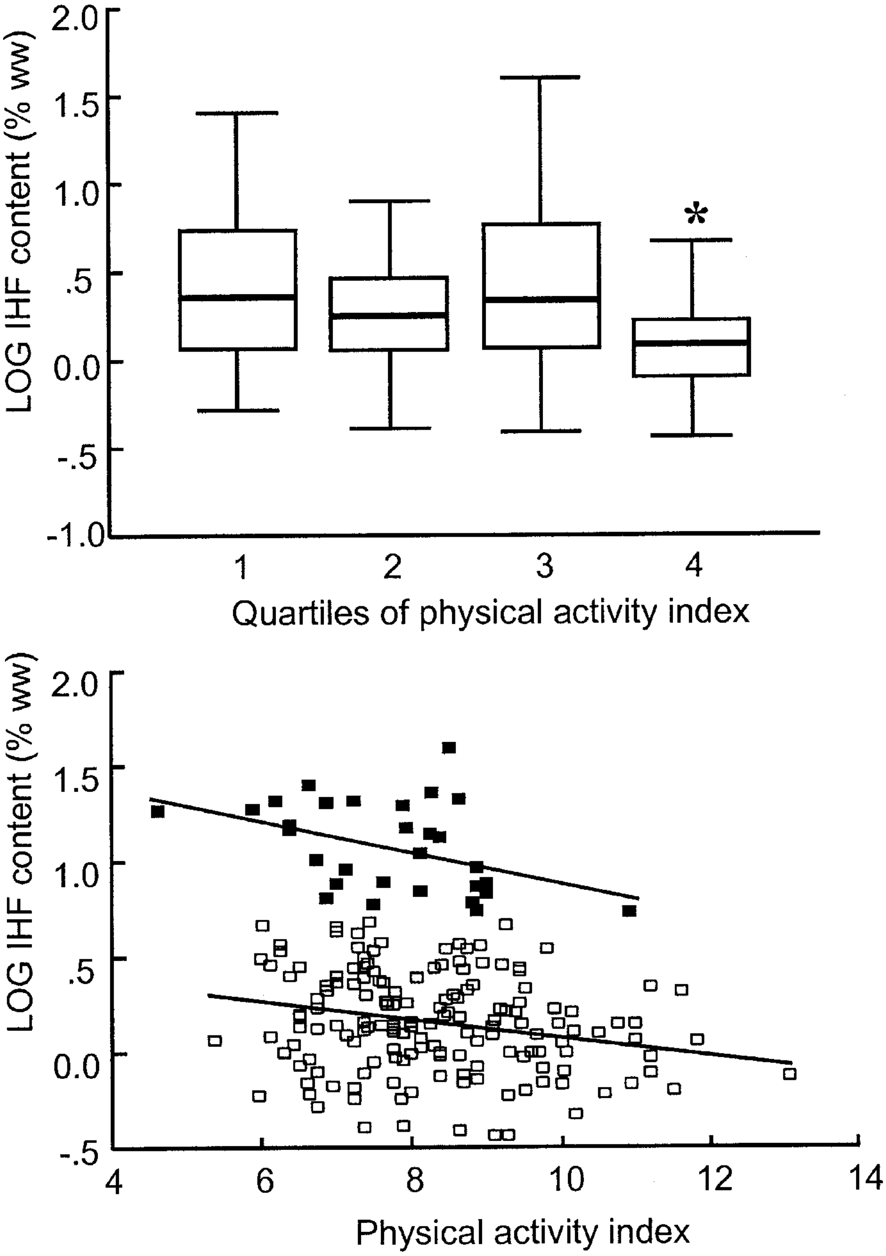

Figure 1—A*: IHF content by quartiles of physical activity index. Box plot of the log IHF content in individuals within quartiles of physical activity index showing that the higher level of habitual physical activity (quartile 4) is associated with lower IHF content.* *$P < 0.02$ *versus quartiles 1 and 3, one-way ANOVA and Bonferroni post hoc analysis.* B*: Correlation between the IHF content and physical activity index. Scatter plot of the log IHF content and the score of total physical activity.* ■*, individuals with fatty liver (>5% wet weight);* □*, individuals with normal IHF content (<5% wet weight). Pearson's correlation analysis showed that the variables were significantly associated in the entire population* ($r = -0.27$; $P < 0.000$*, regression line not shown) and also in the two subgroups of individuals separately* ($r = -0.21$, $P < 0.008$ *in the 160 subjects with normal IHF content and* $r = -0.39$, $P < 0.03$ *in the 31 subjects with fatty liver).*

Correlative analysis

Based on the working hypothesis, we tested whether the IHF content correlated with the total score of the physical activity index ($r = -0.27$; $P < 0.000$), with the sport index ($r = -0.26$; $P < 0.000$), and with the leisure time physical activity index ($r = -0.17$; $P < 0.02$) but not with the work physical activity index ($r = 0.08$; $P = 0.92$) in the entire population. The same findings were reproducible when the correlative analysis was performed separately in individuals with or without fatty liver. In particular, the total score of the physical activity index was significantly associated with individuals with normal IHF content and with fatty liver (Fig. 1*B*). The IHF content correlated strongly also with the BMI ($r = 0.54$; $P < 0.000$), HOMA2-%S ($r = -0.31$; $P < 0.000$), and adiponectin ($r = -0.45$; $P < 0.000$). We therefore performed a correlative analysis between the IHF content and the total score of the physical activity index, controlling for age, sex, BMI, HOMA2-%S, and adiponectin, and found that it was not attenuated (partial correlation $r = -0.25$; $P < 0.001$). The adjusted correlative analysis remained unaffected also when it was performed separately in the two subgroups: partial correlation factor from -0.21 to -0.29 ($P < 0.001$) in the subgroup of individuals with normal IHF content and from -0.39 to -0.33 ($P < 0.05$) in the subgroup of individuals with fatty liver.

CONCLUSIONS — The present study is based on the hypothesis that part of the well-known beneficial impact of physical exercise on the prevention and treatment of insulin resistance and type 2 diabetes might be mediated by a depleting effect on ectopic fat accumulation within the liver. In support of this hypothesis we demonstrated in this study, using a cross-sectional approach, that a higher level of habitual physical activity is associated with lower IHF content in humans; in addition, the results suggested that this association is detectable regardless of other key factors involved in the pathogenesis of type 2 diabetes: age, sex, obesity, insulin resistance, and circulating adiponectin levels.

In support of this conclusion, we found in a population of 191 nonalcoholic, apparently healthy individuals, in which the prevalence of fatty liver was found to be 16%, that *1)* those segregated in the quartile of the highest score of physical activity were characterized by the lowest IHF content when analyzed as a continuous variable (Fig. 1*A*), *2)* they were characterized by the lowest prevalence of fatty liver (Table 2) when analyzed as a categorical variable (IHF content >5% wet weight), and *3)* the physical activity index was inversely associated with the IHF content within the entire population and within each subgroup of individuals with or without fatty liver (Fig. 1*B*). These results are in agreement with previous cross-sectional studies showing an association of physical activity with indirect markers of fatty liver: Lawlor et al. (19) showed an independent association of the level of physical activity with alanine aminotransferase and γ-glutamyltransferase, whereas Church et al. (20) showed an association between physical fitness and the prevalence of NAFLD. An additional study reported a significant correlation between cardiorespiratory fitness and a semiquantitative, computed tomography–derived value obtained as a ratio of the liver-to-spleen attenuation signal in men (21). With respect to these studies, the importance of our own report was *1)* to provide for the first time a direct and absolute quantification of the IHF content using a reliable, highly sensitive and specific in vivo technique and *2)* to obtain the determination of additional variables strongly involved in the pathogenesis of type 2 diabetes and in the development of ectopic fat accumulation. Studies have pointed to insulin resistance as pathogenic factors in NAFLD and fatty liver (22,23). On the basis of the measurement of fasting plasma glucose and insulin concentration, we estimated insulin sensitivity using the computer HOMA2 indexes and showed that, not surprisingly, the individuals segregated in the quartile of highest score of physical activity were characterized by higher insulin sensitivity (Table 2) and the IHF content and HOMA2-%S were strongly correlated. Similarly, it was suggested that adiponectin was independently associated with fatty liver (6), and also in our own population the IHF content strongly correlated with adiponectin. Because exercise training may influence both insulin sensitivity and circulating levels of adiponectin, it might be that the association between lower IHF content and higher habitual physical activity was due to an effect mediated by the fitness status on insulin sensitivity and adiponectin rather than being a direct effect on liver storage. Against the hypothesis of an indirect effect of physical activity mediated by the modulation of insulin sensitivity or adiponectin, as well as age, sex, and obesity, the correlation analysis adjusted for all these variables revealed that the association between IHF content and the physical activity score was not lessened, suggesting that the relationship between the two variables was an independent one. Taking into account the fact that excessive fatty liver accumulation appeared to be peculiarly associated with hepatic insulin resistance (5) and that reduction of the IHF content due to a moderate weight reduction was associated with improvement of hepatic insulin sensitivity (24), the potential beneficial effect of physical exercise would represent an additional tool to improve the metabolic profile of patients type 2 diabetes. The precise mechanisms by which physical exercise may reduce hepatic steatosis remains unknown and needs to be extensively explored, even if it was suggested that it would stimulate lipid oxidation and inhibit lipid synthesis in liver through the activation of the AMP-activated protein kinase pathway (25).

The score of habitual physical activity was based on three factors: occupational physical activity, sport activity, and physical activity during leisure time. The study population was rather homogeneous in terms of occupational physical activity because these individuals were employees in our institute and were similarly involved in mild occupational activity. Consequently, it was not surprising that the occupational physical activity was not different between groups (Table 1). Also physical activity during leisure time was not apparently different between the individuals with or without fatty liver despite the observation that when plotted as a continuous variable, it correlated with IHF content even if in a weaker fashion than the sport physical activity. Therefore, we should emphasize that among the three components of the physical activity index, the habit of playing a sport regularly was the most relevant in the correlation with IHF content (Table 1).

The strength of this work is based on the highly sensitive and specific absolute quantification of IHF content using a noninvasive technique, controlling for anthropometric, metabolic, and endocrine variables; on the other hand, some major limitations need to be stated. One limitation is the cross-sectional nature of the study; unfortunately, we are not aware of any study in which the effect of acute exercise or of an exercise training program on IHF content was assessed. Only data in animal models are available, and they showed that physical exercise performed for 8 weeks in rats during the administration of a high-fat diet (26) or introduced midway through a 16-week period (27) largely reduced fat accumulation. Therefore, studies aimed to assess longitudinally the effect of physical exercise are warranted and need to be performed in the near future. In addition, diet habits may affect IHF content (28), but we did not have valuable dietary data for this population to assess its potential influence and interplay with habitual physical activity. These limitations are not trivial issues; in fact we can say that, in general, there exists a consensus that lifestyle changes and increasing physical activity through exercise are cornerstones to therapy of insulin-resistant states and fatty liver, and these sorts of interventions are typically part of initial recommendations. However, the efficacy of this common sense approach remains to be established, and the relative merits of different levels of diet and exercise on IHF metabolism remain to be defined. This is confirmed by the fact that on the basis of the results of the present work, the impact of habitual physical activity, even if significant, explained only 8% of the variance of the IHF content, suggesting that the amount of IHF is mainly regulated by a factor other than habitual physical activity.

Thus, it may be concluded that by using a cross-sectional approach in the present work, we provided evidence that habitual physical activity is associated with a lower IHF content in humans. However, it is possible that physical exercise may only modulate the severity of the degree of hepatic fat accumulation.

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References

1. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, Tataranni PA: High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. *Diabetes* 51:1889–1895, 2002
2. Sattar N, Scherbakova O, Ford I, O'Reilly DS, Stanley A, Forrest E, Macfarlane PW, Packard CJ, Cobbe SM, Shepherd J; West of Scotland Coronary Prevention Study: Elevated alanine aminotransferase predicts new-onset type 2 diabetes independently of classical risk factors, metabolic syndrome, and C-reactive protein in the west of Scotland coronary prevention study. *Diabetes* 53:2855–2860, 2004
3. Perry IJ, Wannamethee SG, Shaper AG: Prospective study of serum γ-glutamyltransferase and risk of NIDDM. *Diabetes Care* 21:732–737, 1998
4. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50: 1844–1850, 2001
5. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H: Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity an normal men. *J Clin Endocrinol Metab* 87:3023–3028, 2002
6. Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, Gentilcore E, Natale S, Cassader M, Rizzetto M, Pasquali R, Marchesini G: Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab* 90:3498–3504, 2005
7. Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG, Willett WC: Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* 345: 790–797, 2001
8. Hu G, Qiao Q, Silventoinen K, Eriksson JG, Jousilahti P, Lindstrom J, Valle TT, Nissinen A, Tuomilehto J: Occupational, commuting, and leisure-time physical activity in relation to risk for type 2 diabetes in middle-aged Finnish men and women. *Diabetologia* 46:322–329, 2003
9. Perseghin G, Price TB, Petersen KF, Roden M, Cline GW, Gerow K, Rothman DL, Shulman GI: Increased glucose transport/phosphorylation and muscle glycogen synthesis after exercise training in insulin resistant subjects. *N Engl J Med* 335:1357–1362, 1996
10. Longo R, Ricci C, Masutti F, Vidimari R, Croce LS, Bercich L, Tiribelli C, Dalla Palma L: Fatty infiltration of the liver quantification by ^{1}H localized magnetic resonance spectroscopy and comparison with computed tomography. *Invest Radiol* 28:297–302, 1993
11. Szczepaniak LS, Nurenberg P, Leonard D, Browning JD, Reingold JS, Grundy S, Hobbs HH, Dobbins RL: Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab* 288:E462–E468, 2004
12. Neuschwander-Tetri BA, Caldwell SH: Nonalcoholic steatohepatitis: summary of an AASLD single topic conference. *Hepatology* 37:1202–1219, 2003
13. Baecke JAH, Burema J, Frijters JER: A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 36:936–942, 1982
14. Perseghin G, Lattuada G, De Cobelli F, Esposito A, Costantino F, Canu T, Ragogna F, Scifo P, De Taddeo F, Maffi P, Secchi A, Del Maschio A, Luzi L: Reduced intra-hepatic fat content is associated with increased whole body lipid oxidation in patients with type 1 diabetes. *Diabetologia* 48:2615–2621, 2005
15. Perseghin G, Lattuada G, De Cobelli F, Ntali G, Esposito A, Burska A, Belloni E, Canu T, Ragogna F, Scifo P, Del Maschio A, Luzi L Serum resistin and intra-hepatic fat content in non-diabetic individuals. *J Clin Endocrinol Metab* 81:5122–5125, 2006
16. Perseghin G, Bonfanti R, Magni S, Lattuada G, De Cobelli F, Canu T, Esposito A, Scifo P, Ntali G, Costantino F, Bosio L, Ragogna F, Del Maschio A, Chiumello G, Luzi L: Insulin resistance and whole body energy homeostasis in obese adolescents with fatty liver disease. *Am J Physiol* 291: E697–E703, 2006
17. Wallace TM, Levy JC, Matthews DR: Use and abuse of HOMA modeling. *Diabetes Care* 27:1487–1495, 2004
18. Thomsen C, Becker U, Winkler K, Christoffersen P, Jensen M, Henriksen O: Quantification of liver fat by using magnetic resonance spectroscopy. *Magn Reson Imaging* 12:487–495, 1994
19. Lawlor DA, Sattar N, Smith GD, Ebrahim S: The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. *Am J Epidemiol* 161:1081–1088, 2005
20. Church TS, Kuk JL, Ross R, Priest EL, Biltoff E, Blair SN: Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology* 130:2023–2030, 2006
21. Nguyen-Duy TB, Nichaman MZ, Church TS, Blair SN, Ross R: Visceral fat and liver fat are independent predictors of metabolic risk factors in men. *Am J Physiol* 284: E1065–E1071, 2003
22. Marchesini G, Brizi M, Morselli Labate AM, Bianchi G, Bugianesi G, McCullough AJ, Forlani G, Melchionda N: Association of non-alcoholic fatty liver disease to insulin resistance. *Am J Med* 107:450–455, 1999
23. Lewis GF, Carpentier A, Adeli K, Giacca A: Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. *Endocr Rev* 23: 201–229, 2002
24. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI: Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 54:603–608, 2005
25. Lavoie JM, Gauthier MS: Regulation of fat metabolism in the liver: link to non-alcoholic hepatic steatosis and impact of physical exercise. *Cell Mol Life Sci* 63: 1393–1409, 2006
26. Gauthier MS, Couturier K, Latour JG, Lavoie JM: Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol* 94:2127–2134, 2003
27. Gauthier MS, Couturier K, Charbonneau A, Lavoie JM: Effects of introducing physical training in the course of a 16-week high-fat diet regimen on hepatic steatosis, adipose tissue fat accumulation, and plasma lipid profile. *Int J Obes Relat Metab Disord* 28:1064–1071, 2004
28. Westerbacka J, Lammi K, Hakkinen A-M, Rissanen A, Salminen I, Aro A, Yki-Jarvinen H: Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 90:2804–2809, 2005