Lower Fasting Muscle Mitochondrial Activity Relates to Hepatic Steatosis in Humans

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OBJECTIVE

Muscle insulin resistance has been implicated in the development of steatosis and dyslipidemia by changing the partitioning of postprandial substrate fluxes. Also, insulin resistance may be due to reduced mitochondrial function. We examined the association between mitochondrial activity, insulin sensitivity, and steatosis in a larger human population.

RESEARCH DESIGN AND METHODS

We analyzed muscle mitochondrial activity from ATP synthase flux (fATP) and ectopic lipids by multinuclei magnetic resonance spectroscopy from 113 volunteers with and without diabetes. Insulin sensitivity was assessed from *M* values using euglycemic-hyperinsulinemic clamps and/or from oral glucose insulin sensitivity (OGIS) using oral glucose tolerance tests.

RESULTS

Muscle fATP correlated negatively with hepatic lipid content and HbA_{1c}. After model adjustment for study effects and other confounders, fATP showed a strong negative correlation with hepatic lipid content and a positive correlation with insulin sensitivity and fasting C-peptide. The negative correlation of muscle fATP with age, HbA_{1c}, and plasma free fatty acids was weakened after adjustment. Body mass, muscle lipid contents, plasma lipoproteins, and triglycerides did not associate with fATP.

CONCLUSIONS

The association of impaired muscle mitochondrial activity with hepatic steatosis supports the concept of a close link between altered muscle and liver energy metabolism as early abnormalities promoting insulin resistance.

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Insulin resistance associates with ectopic lipid deposition in muscle (intramyocellular lipid content [IMCL]) and liver (hepatocellular lipid content [HCL]), increased lipid availability, and cardiovascular complications (1,2). It has been suggested that muscle insulin resistance predisposes for atherogenic dyslipidemia and nonalcoholic fatty liver disease (NAFLD) by partitioning the postprandial flux of ingested carbohydrates away from muscle glycogen synthesis toward hepatic de

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novo lipogenesis (3). This would increase VLDL export and in turn raise circulating triglycerides (TGs) and LDL cholesterol (3). Some exercise studies support this concept in that reversal of muscle insulin resistance decreases HCL and dyslipidemia in humans at risk for type 2 diabetes (4,5).

However, the interaction between liver and muscle metabolism in the development of insulin resistance is not fully understood. Increased insulin-stimulated de novo hepatic lipogenesis after carbohydrate intake might represent an important sink for excess glucose, ultimately improving glucose homeostasis. On the other hand, steatosis may result from inadequate hepatic oxidation rates (6), subsequently leading to increased lipid flux to skeletal muscle. Indeed, insulin resistance frequently associates with accumulation of IMCL or lipid metabolites (3). As greater lipid availability does not necessarily increase IMCL (7,8), muscle lipid oxidation could be the link between liver and muscle metabolism (9,10). Muscle mitochondrial activity assessed from ATP synthetic flux rates (fATP) reflects ADP-stimulated oxidative phosphorylation under variable conditions of energy demand and insulinemia (11). Although fasting fATP can be lower in the state of insulin resistance (12), mitochondrial activity, capacity, and content do not necessarily correlate with insulin sensitivity in humans (9,13). These findings suggest that such dissociation between insulin sensitivity and mitochondrial function might depend on an interplay between muscle energy metabolism and hepatic fat storage. Of note, previous studies generally included small groups, thereby providing lower statistical power. To overcome this limitation, we analyzed data from a series of studies performed in well-phenotyped volunteers with and without type 2 diabetes using identical methodology for assessing fATP and HCL on the same magnetic resonance (MR) spectrometer.

RESEARCH DESIGN AND METHODS

Participants

Data were collected from six clinical experimental studies, published between 2007 and 2011, including 113 individuals who underwent assessment of muscle fATP, IMCL, and HCL by one single method applied by one research group (7,9,14–17). All participants were sedentary according to Baecke et al. (18) in all studies. Female subjects were postmenopausal or studied between days 5–9 of their menstrual cycle. For 3 days prior to every study day, participants refrained from any physical exercise, consumed an isocaloric diet, and then fasted for 12 h before the start of the experiments. The number of individuals, their glucose tolerance, and the methods for measuring glucose, insulin, and C-peptide concentrations and for assessing insulin sensitivity have been described in detail in the studies summarized in Table 1. The parameters age, sex, BMI, fATP, and either oral glucose insulin sensitivity (OGIS) or *M* value had to be available for all individuals. Individuals with type 1 diabetes and with endocrine diseases other than type 2 diabetes were excluded from this analysis. Data were obtained at baseline, i.e., in the fasted state without or before any planned interventions. The selection process of the studies is described in a flowchart (Supplementary Fig. 1). The local institutional ethics board approved all study protocols, and all subjects gave written informed consent after the nature and possible consequences of the studies had been explained to them.

Measures of Insulin Sensitivity

For the oral glucose tolerance tests (OGTTs), participants drank a solution containing 75 g of glucose dissolved in water and underwent sampling of venous blood before (zero time) and at timed intervals for 2 h. Measurements of plasma glucose and insulin at zero time, 90 min, and 120 min were used for calculating the oral glucose insulin sensitivity (OGIS) (19). OGIS is a measure of glucose clearance and represents an index of whole-body insulin sensitivity, which has been validated against the euglycemic-hyperinsulinemic clamp (20). Euglycemic (~5.5 mmol/L)-hyperinsulinemic (~500 pmol/L) clamps were performed as previously reported using a primed-continuous insulin infusion (40 mU · m^{-2} body surface area · min^{-1}) and a variable 20% dextrose infusion. Whole-body insulin sensitivity was assessed from glucose infusion rates (*M*) during the last 30 min of the clamp (9). For comparison of measures of insulin sensitivity, we checked the available files comprising data from both OGTT and euglycemic-hyperinsulinemic clamps in the same people regardless of their glucose tolerance or underlying pathology. The resulting data sets allowed the development of an equation to obtain a reconstructed *M* value (*Mr*) from OGIS. After testing several different models, a simple linear regression finally yielded the best result ($R = 0.75$, $P = 0.0001$) (Fig. 1*A*) as given by the equation *Mr* (mg · min^{-1} · kg^{-1}) = 0.023[OGIS (mL · min^{-1} · m^2)] – 4.1. The equation was validated in several datasets from other studies, from which euglycemic-hyperinsulinemic clamps and OGTT were available (data not shown). This equation was then applied for the first time to the data of the present analysis to derive *Mr* values from OGTT data, which yielded a tight correlation ($R = 0.67$, $P < 0.0001$) between *Mr* and measured *M* values.

In Vivo MR Spectroscopy

All measurements were performed by MR spectroscopy (MRS) experts with

Table 1—Baseline characteristics (mean ± SD) of all participants, showing the number of participants per subgroup, sex, mean age, and glucose tolerance: normal glucose tolerance/impaired fasting glucose or impaired glucose tolerance/type 2 diabetes

n (male/female)	Age (years)	Diabetes status	Assessment of insulin sensitivity	Reference
7 (4/3)	45 ± 11	3/4/0	OGTT	17
7 (7/0)	26 ± 2	7/0/0	Clamp	7
31 (19/12)	47 ± 16	21/0/10	Clamp	9
5 (5/0)	27 ± 1	5/0/0	OGTT/clamp	14
36 (17/19)	39 ± 12	28/8/0	OGTT	15
27 (0/27)	36 ± 5	25/2/0	OGTT/clamp	16

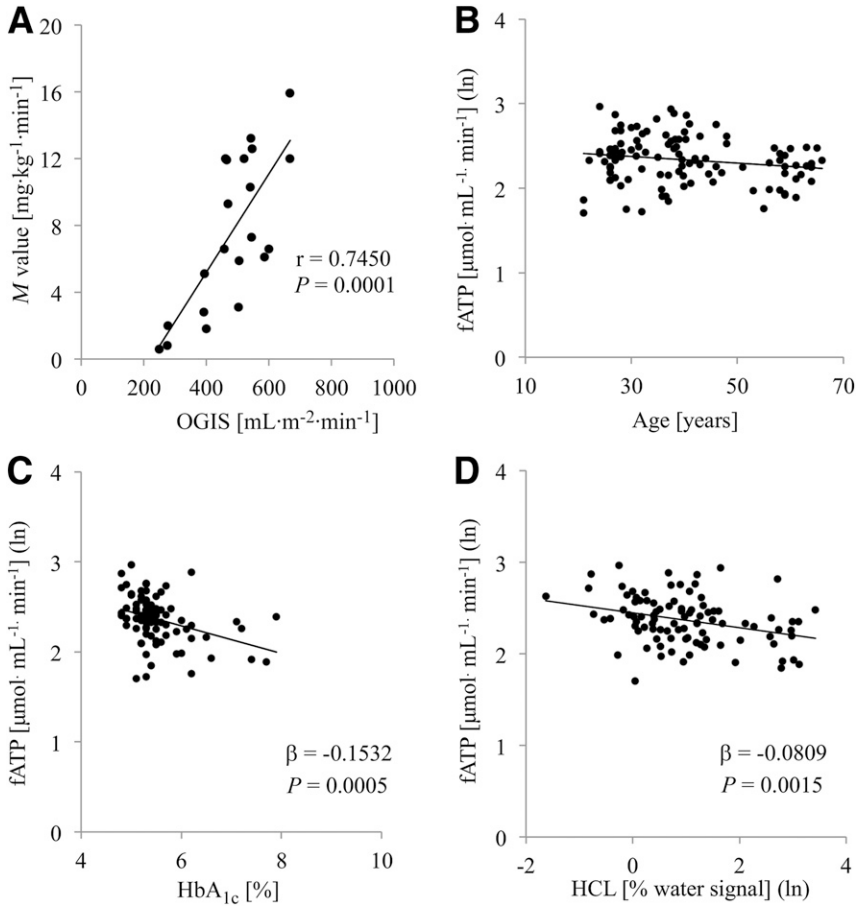

Figure 1—Relationship between OGIS and *M* value (n = 21 who underwent both euglycemic-hyperinsulinemic clamps and OGTT) (*A*), and associations between flux through ATP synthase during fasting (fATP [ln]) and age (n = 113) (*B*), HbA_{1c} (n = 86) (*C*), and HCL (n = 95) (*D*).

participants lying supine inside a 3 Tesla Medspec MR spectrometer (Bruker Biospin, Ettlingen, Germany). ^{31}P-MRS was used with a surface coil positioned ~2 cm into the medial head of the right gastrocnemius muscle and the saturation transfer experiment to measure fATP from the exchange between Pi and ATP as described earlier (7,9,12). ^{1}H-MRS was used to quantify IMCL in soleus muscle (7) and HCL as described previously (21).

Analytical Procedures

Plasma glucose was measured by the glucose oxidase method (Glucose Analyzer II; Beckman Coulter). Plasma free fatty acids (FFAs) were assayed microfluorometrically in samples containing orlistat to prevent in vitro lipolysis (Wako USA) (7). Plasma lactate was determined enzymatically (Roche) (7). Plasma insulin and C-peptide were determined by commercial double antibody RIA (7).

Statistical Analyses

Data are presented as mean ± SD. We applied univariate and multivariate linear regression analyses to evaluate the relationships between fATP and insulin sensitivity, parameters of glucose, and lipid metabolism.

Because of their skewed distributions, fATP, *M* value, HCL, fasting plasma C-peptide, a surrogate marker of β-cell function, insulin, FFA, TG, and the fasting TG-to-HDL cholesterol ratio (TG/HDL-C), which has been used as a surrogate of insulin resistance, were transformed to their natural logarithms (ln) before further analyses. In order to estimate the predictive power of individual variables, we performed regression analyses with multiple adjustments for parameters potentially confounding collinear associations with the dependent variable fATP. The multiple regression analysis for the dependent variable log-transformed flux rates through ATP synthase (fATP [ln]) included the following parameters: age, BMI, insulin sensitivity (*M* value or OGIS-derived reconstructed *Mr* value), HbA_{1c}, plasma FFAs, HCL, and IMCL (ln) as independent variables. Multiple regression with many predictor variables might serve as extension of linear regression with two predictor variables. Of note, the inclusion of a bundle of independent variables automatically induces the selection of a specific subgroup of our study population.

Thus, we performed model analyses using different models (M1–M5) for each dependent variable and controlled for a potential influence of the study group, examining investigators, and time point of investigation by adjusting for the study effect (regression models M2–M5). Model 1 (M1) was not adjusted, whereas M2 was adjusted for study effects, M3 for study effects, age, and sex, and M4 for study effects, age, sex, and BMI. The last model (M5) was further adjusted for glucose tolerance status (i.e., normal glucose tolerance, impaired fasting glucose/glucose intolerance, and type 2 diabetes). Because of the previous reports on positive associations between *M* value and muscle fATP and negative associations between *M* value and HCL, we also examined the influence of HCL on the relationship between fATP and *M* value. To this end, the analysis of fATP and *M* value in M6 was adjusted for study effects, age, sex, BMI, glucose tolerance, and, additionally, HCL.

As we aimed to compare the effect of independent variables, all of which were expressed in different units, on the dependent variable, we report standardized coefficients rather than correlation coefficients. All analyses were performed using SAS for Windows version 9.2 software (SAS Institute, Cary, NC).

RESULTS

Baseline Characteristics

The study population comprised 113 (52 male and 61 female) participants with mean age 40 ± 13 years and mean BMI 25.0 ± 3.5 kg/m^2, of whom 87 were glucose tolerant, 16 were prediabetic,

i.e., having impaired fasting glucose and/or glucose intolerance, and 10 had overt type 2 diabetes (Table 1). The individuals were rather insulin sensitive (*M* value and *Mr* value: 7.5 ± 4.6 and 7.0 ± 2.7 mg · min^{-1} · kg^{-1}) and metabolically well controlled (HbA_{1c} = 5.52 ± 0.62% [37 ± 4 mmol/mol], TG = 95 ± 49 mg/dL, FFA = 462 ± 206 μmol/L).

Fasting Muscle Mitochondrial Activity, Age, and Glucose Metabolism

Regression analyses were performed with fATP (ln) as dependent variable and the following independent variables: age, insulin sensitivity, fasting C-peptide (ln), fasting insulin (ln), fasting glucose, and HbA_{1c} (Table 2). Also, parameters potentially influencing the associations were identified and subsequently used for adjusting the regression analyses. The fATP (ln) correlated negatively with age when adjusted for study effects only, but not in M4 or M5 (Fig. 1*B*). The fATP (ln) correlated positively with insulin sensitivity (*M* value [ln] or *Mr* value [ln]) upon adjustment in M2, M3, M4, and M5. The correlation between fATP (ln) and *M* value (ln) remained upon additional adjusting for HCL (M6: $P = 0.043$, $\beta = 0.225$, $n = 95$). Fasting plasma C-peptide levels (ln) related negatively to fATP (ln) in M2, M3, M4, and M5. Whereas fasting plasma glucose and insulin did not associate with fATP (ln), HbA_{1c} correlated negatively with fATP in M1, M2, and M3 (Fig. 1*C*), but not in M4 and M5.

Fasting Muscle Mitochondrial Activity and Fat Distribution

Regression analyses were performed with fATP (ln) as dependent variable and BMI, HCL (ln), and IMCL (ln) as independent variables (Table 3). BMI as well as IMCL (ln) did not associate with fATP (ln). fATP (ln) correlated negatively with HCL in all tested models (Fig. 1*D*). In a multiple stepwise regression model, HCL was also confirmed as the only independent predictor of fATP (standardized coefficient, B: −0.339, $P = 0.003$). Of note, IMCL did not relate to fATP (ln) in any of the models.

Fasting Muscle Mitochondrial Activity and Lipid Metabolism

Further regression analyses were performed with fATP (ln) as dependent variable and HDL-C, TG, TG/HDL-C, and plasma FFA as independent variables (Table 3). Only fasting plasma FFA (ln) negatively related to fATP (ln), when adjusted for study effects in M2. This association disappeared either without adjustments (M1) or in M3, M4, and M5.

Table 2—Associations between fasting muscle mitochondrial activity, age, and glucose metabolism

			Model				
Independent variable	*n*		M1	M2	M3	M4	M5
Age	113	β	−0.0039	**−0.0052**	**−0.0052**	−0.0045	−0.0033
		P value	0.0522	**0.0200**	**0.0245**	0.543	0.1869
Insulin sensitivity (ln)	113	β	0.0672	**0.2264**	**0.1905**	**0.1814**	**0.1984**
		P value	0.2434	**0.0015**	**0.0141**	**0.0331**	**0.0430**
Plasma glucose	113	β	−0.002	−0.0016	0.0029	−0.0003	0.0038
		P value	0.0719	0.1821	0.2184	0.8121	0.1182
HbA_{1c}	86	β	**−0.1532**	**−0.1382**	**−0.1088**	−0.0986	−0.0791
		P value	**0.0005**	**0.0037**	**0.0489**	0.0687	0.3196
Plasma C-peptide (ln)	84	β	−0.0793	**−0.3937**	**−0.3629**	**−0.3271**	**−0.3085**
		P value	0.1375	**0.0001**	**0.0003**	**0.0051**	**0.0167**
Plasma insulin (ln)	113	β	−0.0513	−0.00003	−0.0116	0.0190	0.0157
		P value	0.2579	0.9996	0.8439	0.7660	0.8060

Linear regression analyses with log-transformed flux rates through ATP synthase (fATP [ln]) as dependent variable and insulin sensitivity (*M* value or OGIS-derived reconstructed *Mr* value), fasting concentrations of plasma glucose, C-peptide (ln), insulin (ln), and HbA_{1c} as independent variables. All statistically significant results are represented in boldface. M1, not adjusted; M2, adjusted for study effects; M3, adjusted for study effects, age, and sex; M4, adjusted for study effects, age, sex, and BMI; M5, adjusted for study effects, age, sex, BMI, and glucose tolerance.

CONCLUSIONS

The main finding of this study is the strong and independent correlation between fasting muscle mitochondrial activity and ectopic lipid deposition in the liver. Second, this analysis, derived from a larger study population, supports previous reports on a positive relationship of fATP with age and insulin sensitivity in sedentary humans. Third, fATP relates positively to glycemia as assessed from HbA_{1c} and negatively with fasting plasma FFA. Finally, in this study population of adults with different glucose tolerance status, fATP did not relate to any other parameter of lipid metabolism or intramyocellular lipid storage.

The observed tight and significant association between muscle fATP and HCL was not detected previously. This is likely due to the lower statistical power of the individual studies, although some of the included studies at least suggested that individuals with high HCL may have lower fATP (9,16,17). Whereas steatosis and NAFLD are known to relate to insulin resistance, and even predict type 2 diabetes (6,21,22), the interaction between HCL and muscle energy metabolism is less clear. According to one theory, decreased nonoxidative storage of ingested carbohydrates in skeletal muscle represents a primary abnormality in insulin-resistant states. This would redirect glucose to the liver to serve as substrate for hepatic de novo lipogenesis and subsequently cause hyperinsulinemia, dyslipidemia, and steatosis (4). In the present analysis, muscle fATP was measured during fasting, suggesting that muscle energy metabolism may also interfere with liver energy storage in the postabsorptive state. In this context, a recent study reported higher muscle complex I activities but lower mitochondrial content in morbidly obese NAFLD patients undergoing bariatric surgery (23). This suggests that increased activities of electron transport chain components reflect adaptation of muscle mitochondria to fat overload and mitochondrial damage. Indeed, muscle complex I activity gradually increased with progressing steatosis and fibrosis in that study. Another study

Table 3—Associations between fasting muscle mitochondrial activity, fat distribution, and lipid metabolism

			Model				
Independent variable	*n*		M1	M2	M3	M4	M5
BMI	113	β	−0.0119	−0.0131	−0.0099		−0.0087
		P value	0.1120	0.0968	0.2189		0.2769
HCL (ln)	95	β	**−0.0809**	**−0.0805**	**−0.0662**	**−0.0684**	**−0.0759**
		P value	**0.0015**	**0.0025**	**0.0185**	**0.0264**	**0.0232**
IMCL (ln)	112	β	0.0270	0.0614	0.0691	0.0752	0.0728
		P value	0.5591	0.2321	0.1731	0.1371	0.1542
Plasma TG (ln)	105	β	−0.0350	−0.0425	−0.0192	−0.0047	−0.0067
		P value	0.5323	0.4502	0.7427	0.9389	0.9135
Plasma HDL-C	98	β	0.0759	0.0911	0.1504	0.1430	0.1372
		P value	0.3872	0.3008	0.1199	0.1641	0.1836
TG/HDL-C (ln)	91	β	−0.0604	−0.0674	−0.0587	−0.0628	−0.0629
		P value	0.2375	0.1950	0.2904	0.3016	0.3060
Plasma FFA (ln)	104	β	−0.0981	**−1.2135**	−0.0930	−0.0989	−0.0831
		P value	0.0759	**0.0379**	0.1291	0.1090	0.1883

Linear regression with fATP (ln) as dependent variable and BMI, HCL (ln), and IMCL (ln) as independent variables. Further linear regression analyses include parameters of systemic lipid profile, namely, fasting plasma concentrations of TG (ln), HDL-C, TG/HDL-C, and plasma FFAs as independent variables. All statistically significant results are represented in boldface. M1, not adjusted; M2, adjusted for study effects; M3, adjusted for study effects, age, and sex; M4, adjusted for study effects, age, sex, and BMI; M5, adjusted for study effects, age, sex, BMI, and glucose tolerance.

found that lifestyle intervention resulting in greater muscle glucose disposal also improved steatosis and NAFLD (5). Although muscle mitochondrial function was not measured in that study, muscle mitochondrial adaptation could be one mechanism to modulate hepatic fat loading during the development of type 2 diabetes.

On the other hand, liver mitochondria could exhibit a similar abnormality as muscle mitochondria of people at risk for type 2 diabetes, which would reduce hepatic fat oxidation and thereby favor TG deposition. In support of this contention, lower liver ATP levels (21), fATP (6), and ATP recovery upon fructose challenge (24) provide evidence for lower hepatic mitochondrial function, at least in insulin-resistant groups such as severe obesity or type 2 diabetes. Furthermore, impaired hepatic energy metabolism could subsequently raise plasma FFA via lipolysis of VLDL (3) and in turn induce lipid-mediated muscle insulin resistance (25). Our observation of an independent correlation between HCL, fasting plasma FFA, insulin resistance, and lower muscle fATP is in line with this concept. Nevertheless, these cross-sectional studies do not allow definite conclusions, and, of course, potentially coincident but significant relationships do not imply any causality.

Furthermore, the positive correlation between whole-body insulin sensitivity and muscle fATP is in agreement with data of one of our smaller group studies, reporting a correlation of *M* value with fasting fATP in patients with type 2 diabetes (9). Lower fATP in insulin-resistant compared with insulin-sensitive healthy humans has also been observed in other studies during fasting (9,12,16,17), during hyperinsulinemia (9,26), after exercise (15), and during acute lipid-induced insulin resistance (7), all without reporting an association of fATP with insulin sensitivity. In vivo mitochondrial oxidative capacity as assessed from postexercise muscle phosphocreatine repletion was also reduced in patients with type 2 diabetes but did not correlate with insulin sensitivity (27). Likewise, ex vivo measures of mitochondrial function, such as mitochondrial content (28), oxidative enzyme capacity (29,30), and mitochondrial morphology (31), were lower in insulin-resistant humans, but again without association with insulin sensitivity. However, only a few cross-sectional studies found a correlation between insulin sensitivity and markers of mitochondrial function in untrained, metabolically well-controlled humans with and without type 2 diabetes (9,32,33). On the other hand, some lifestyle intervention studies found associations between improvement of insulin sensitivity and various parameters of mitochondrial function and suggest that raising glucose and lipid oxidation rates could underlie reduction of insulin resistance (34). However, aerobic exercise training can consistently improve muscle mitochondrial response in all age-groups, whereas only younger participants also improved their insulin sensitivity (35). In this context, the present analyses reported a weak negative correlation of fATP with age in sedentary humans, which underlines the predominant role of physical activity as the major determinant of mitochondrial biogenesis and function (35). These previous findings may be explained by the small size of the studied groups; however, dissociation between mitochondrial function and insulin sensitivity was also suggested. Although the individuals participating in the present combined analysis were rather insulin sensitive, lean, or overweight and mostly glucose tolerant and metabolically well controlled, fATP correlated with insulin sensitivity also upon adjustment for age, sex, BMI, and glucose tolerance. Of note, even HCL did not disrupt the relationship between fATP and insulin sensitivity. Thus, analyzing this much larger study population revealed that reduced mitochondrial activity might indeed be an early abnormality occurring during the development of insulin resistance.

Nevertheless, impairment of mitochondrial function can result from chronic lipid- and glucose-mediated increases in oxidative substrate flux rates causing oxidative stress and thereby damaging mitochondrial proteins (11). Of note, the present analysis identified HbA_{1c} as a strong predictor of fATP and fasting plasma FFA as the only lipid parameter correlating (negatively) with fATP. Increase of mitochondrial oxidative capacity conferred by lipid-induced stimulation

of the peroxisome proliferator–activated receptor γ coactivator 1-α (10,36) might coincide with lipid-induced insulin resistance in skeletal muscle. This might explain why others did not find a correlation of mitochondrial function and insulin sensitivity. On the other hand, hyperinsulinemia increases ATP synthesis (9,26), whereas this effect is blunted in insulin-resistant humans (9,26) and during lipid exposure (7). Accordingly, C-peptide levels, as surrogate of insulin secretion, related negatively to fATP. Thus, insulin deficiency and insulin resistance at the level of mitochondria and poor metabolic control, comprising impaired adaptation to prevalent metabolic conditions rather than insufficient oxidative capacity, might limit oxidation rates (11). In accordance, some (1,29), but not all, studies found higher IMCL in patients with type 2 diabetes and no correlation with insulin resistance or fasting fATP (27), which is in line with our finding.

This analysis of a number of studies, consistently acquiring data on the same MR scanner, benefits from the application of one single methodology for quantification of muscle ATP production as a measure of mitochondrial activity to a metabolically well-characterized larger study population. The applied saturation transfer method for assessing fATP by means of ^{31}P-MRS has been validated in a variety of in vitro systems and in human muscle biopsy samples, as reviewed recently (37). The limitations of this approach are that this method provides a measure of ATP synthesis/hydrolysis cycle at rest driven by energy demands rather than maximal oxidative capacity and that differences in individual mitochondrial content are not taken into account. Nevertheless, fATP associates with changes in mitochondrial content, coupling, and oxygen consumption (38) and tightly correlates with postexercise muscle phosphocreatine (39), indicating that skeletal muscle with higher maximal oxidative ATP synthetic rates is also metabolically more active at rest. An increased prevalence of insulin resistance in Asian-Indian men was associated with a twofold increase in HCL compared with Caucasian men. These data demonstrate important ethnic differences in the pathogenesis of insulin resistance and steatosis (40). One further limitation of the study might be that only Caucasian participants of identical ethnic background have been included.

In conclusion, the association of impaired muscle mitochondrial activity with hepatic steatosis supports the concept of a close link between altered muscle and liver metabolism as early abnormalities promoting insulin resistance.

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