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ORIGINAL PAPER

Myotoxic reactions to lipid-lowering therapy are associated with altered oxidation of fatty acids

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Abstract Despite exceptional efficacy and safety, fear of muscle toxicity remains a major reason statins are underutilized. Evidence suggests that statin muscle toxicity may be mediated by abnormalities in lipid metabolism. To test the hypothesis that myotubes from patients intolerant of lipid-lowering therapies have abnormal fatty acid oxidation (FAO) responses we compared muscle from 11 subjects with statin intolerance (*Intolerant*) with muscle from seven statin-naive volunteers undergoing knee arthroplasty (*Comparator*). Gross muscle pathology was graded and skeletal muscle cell cultures were produced from each subject. FAO was assessed following treatment with increasing statin concentrations. There was no difference in muscle biopsy myopathy scores between the groups. Basal octanoate oxidation was greater in *Intolerant* than in *Comparator* subjects ($P = 0.03$). Lovastatin-stimulated palmitate oxidation tended to be greater for *Intolerant* compared to *Control* subjects' myotubes ($P = 0.07$ for 5 μM and $P = 0.06$ for 20 μM lovastatin). In conclusion abnormalities in FAO of *Intolerant* subjects appear to be an intrinsic characteristic of these subjects that can be measured in their cultured myotubes.

Keywords Statins · Myotubes · Complications · Lipids · Fatty acids

Abbreviations

AT	Anaerobic threshold
BSA	Bovine serum albumin
CK	Creatine kinase
CPT	Carnitine palmityl transferase
CPX	Cardiopulmonary exercise testing
FAO	Fatty acid oxidation
FFA	Free fatty acid
IOA	Index of abnormality pathology score
MI	Metabolic index pathology score
PPAR	Peroxisome proliferator-activated receptor
RER	Respiratory exchange ratio
VO_2 max	Maximal oxygen consumption
VE/VCO_2	Ventilatory efficiency
TSH	Thyroid stimulating hormone
BMI	Body mass index

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Introduction

Despite randomized trials demonstrating significant reductions in atherosclerotic endpoints without serious morbidity in over 100,000 patients, 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors or statins are prescribed for less than half of the patients who should receive this therapy [1–4]. Ignorance of the mechanism of statin-induced myotoxicity and inordinate fear of rare but serious muscle toxicity remain major impediments to the appropriate use of statins.

Most lipid-lowering therapies including statins, fibrates, and niacin may cause muscle toxicity [4–8]. Muscle pathology and abnormal excretion of urinary organic acids in patients who are intolerant of lipid-lowering drugs suggest altered fat metabolism [9, 10]. Such abnormal fatty acid oxidation (FAO) may be identified using the respiratory exchange ratio (RER) where increased fasting RER indicates reduced fat oxidation [11]. In fact, fasting RER is increased in patients with statin-associated muscle toxicity, further suggesting abnormalities in FAO [12, 13]. However, systematic studies to assess fat utilization in patients intolerant of lipid-lowering therapy are lacking.

Human skeletal myocytes grown and differentiated in culture demonstrate the biochemical and metabolic characteristics of mature skeletal muscle [14]. These myotubes retain the in vivo metabolic defects of the subjects from whom they are cultured including reduced fatty acid oxidation in myotubes from type 2 diabetic subjects [15–17]. The FAO responses of cultured skeletal myotubes from statin-intolerant patients were compared with those of a separate comparator population to test the hypothesis that fat metabolism would be different in the two groups.

Methods

Subjects

Intolerant subjects were sequentially enrolled as they were referred for muscle biopsy due to their inability to take one or more lipid-lowering therapies because of muscle pain or weakness. Their RER or S1-slope (see below) was required to be greater than 0.80 at cardiopulmonary exercise testing (CPX). Subjects with abnormal thyroid stimulating hormone (TSH) or diabetes mellitus or who were taking medications known to interact with lipid-lowering therapy were excluded. *Comparator* muscle was obtained from seven sequential, non-diabetic subjects undergoing knee arthroplasty and who agreed to incidental muscle biopsy. No *Comparator* had a history of myopathy and none had received prior lipid-lowering therapy. Subjects in both groups gave informed consent to protocols which were approved by the institutional review board.

CPX protocol

Our fasting CPX protocol has been described previously [13]. *Intolerants* underwent the fasting canopy study followed by breath by breath gas exchange analysis during a ramped exercise treadmill protocol. *Comparator* subjects underwent the thirty minute canopy study in the resting fasted state but they did not undergo full exercise testing due to their orthopedic limitations. Aerobic indices of maximal oxygen consumption (VO_2 max), anaerobic threshold (AT), and ventilatory efficiency (VE/VCO_2) were calculated by standard techniques [18, 19]. The S1-slope, an index of muscle substrate selection, was determined as the slope of the VO_2 versus VCO_2 plot before AT [20]. These results were compared to *Comparator* values (RER) or to accepted normal ranges (S_1-slope, VO_2, VCO_2, AT, and VE/VCO_2).

Muscle biopsy protocol

Muscle biopsies of the vastus lateralis were performed percutaneously with a Bergstrom needle in the *Intolerant* subjects and by open biopsy of the same muscle at the time of knee surgery in *Comparator* subjects. Muscle tissue for cell culture was immediately placed in chilled Ham's F-10 medium for transport. Tissue for pathological analysis was snap-frozen in liquid nitrogen-cooled isopentane and prepared according to standard techniques [21]. Muscle biopsies from both groups were scored for both myopathy and for metabolic changes using a scoring system developed by one of us (MAV) and detailed in Table 1.

Myocyte cell culture and free FAO protocols

The techniques of skeletal muscle satellite cell isolation and growth have been described previously [14]. After growth to ~90% confluency, media were changed to a less enriched differentiation medium. Fusion and differentiation to mature myotubes, as determined by the extent of multinucleation (~90% of cells present) and protein expression of sarcomeric-specific alpha actin, was complete within 4 days. At that point myotubes were exposed to lovastatin (0–20 μM) in the media for 48 h. The extent of differentiation did not change during the treatment period and was similar in cells from both groups. The lovastatin concentrations used range from the therapeutic to the toxic concentrations realized in patients on statin therapy [22, 23]. Baseline cells were treated with vehicle. After removal of the media and extensive washing, fatty acid metabolism was assayed by following the oxidation of both long chain (palmitate) and medium chain (octanoate) fatty acids. The procedure for assaying FAO is that described by Rognstad for adherent cells [24]. Briefly, cells

Table 1 Muscle scoring: The Index of Abnormality (IOA) is presented as a single number composed of two components representing the non-specific myopathic features and those features relating to lipid storage and mitochondrial dysfunction

Morphological descriptor (% fibers)	Grade 0	Grade 1	Grade 2	Grade 3
Internal nucleation per mm^2[a]	0–3	4–7	8–15	>15
Angular fiber Atrophy[a]	0	1–5	6–10	>10
Sarcolemmal nuclear aggregate (morula) formation[a]	0–1	2–5	6–10	>10
Degenerative/regenerative fibers[a]	0	1–2	3–5	>5
Lipid accumulation[a,b]	Very fine droplet, negative in type II fiber	Mild lipid increase type I > type II Rare positive single fiber	Type I ≫ type II Numerous hyperactive fibers, occasional large droplets	Hyperactive, globular and coalexcent lipid droplets, subsarcolemmal aggregation
NADH tetrazolium reductase/SDH[a,b]	Fiber type differentiation, no hyperactive change	Rare architectural change, <3%	Lipid droplet accentuation, architectural change 3–10% fibers	Mitochondrial lysis/aggregation, abnormal subsarcolemmal reactivity, lipid globule
Cytochrome C oxidase (Cox) -negative[a,b]	0	1–2	3–5	>5
Ragged red fibers[a,b]	0	1	2–5	>5
Biopsy size (mm^2) =				
Type 1 fiber diameter (mean, range) =				
Type 2 fiber diameter (mean, range) =				
Type 1 fiber coefficient of variation (SD/mean) =				
Type 1 fiber coefficient of variation (SD/mean) =				

The Metabolic Index (MI) is the sum of the grades of the morphological abnormalities reflective of a metabolic abnormality: lipid index, NADH/SDH, COX-negative, ragged red fibers. The IOA equals the sum of the MI score plus grades for internal nucleation, angular fiber atrophy, sarcolemmal nuclear aggregates, and degeneration/regeneration. An IOA $\geq$ 3 or MI $\geq$ 2 is defined as abnormal

[a] Comprise the IOA, [b] Comprise the MI

were incubated in serum-free α-MEM media containing substrate ([9,10 − ^{3}H] palmitic acid or [8 −^{3}H] octanoic acid, 0.2 μCi to a final concentration of 5 μM) in a 95% O2: 5% CO2 incubator at 37°C for 3 h. These concentrations were selected so that the free fatty acid (FFA) entry into the cell would not be rate-limiting; oxidized FFA represented less than 10% of FFA taken up by cells (data not shown). Stock solutions of FFAs were prepared by mixing, under N_2, with fatty acid-free bovine serum albumin (BSA) to obtain a FFA:BSA ratio of 5:1, as described by Corkey et al. [25]. Concentrations of the stock solutions were determined by a colorimetric FFA assay (WAKO NEFA-C kit, WAKO Chemical Industries, Osaka, Japan). After incubation, an aliquot (100 μl) of the culture medium was placed over an ion-exchange resin and the column was washed twice with 0.75 ml of water. Intact FFA (charged state) was retained by the resin, while the ^{3}H released during oxidation is incorporated into water, which passes freely through the resin column and was collected. All results were adjusted for total cellular protein content determined by the Bradford method [26].

Biochemical analyses

CK (peak on statin and off therapy) and TSH were measured by standard techniques in commercial laboratories.

Statistical analyses

Intolerant and *Comparator* subjects were compared on clinical parameters: age, gender, fasting RER, and BMI. The muscle biopsies were compared for their myopathy scores. An index of abnormality (IOA) ≥3 or metabolic index (MI) ≥2 was defined as abnormal. The amount of FAO for palmitate and octanoate in cell cultures was compared between *Intolerant* and *Comparator* groups at basal level and each lovastatin dose. Wilcoxon and Fisher's exact tests were used. The amount of FAO was additionally analyzed with two mixed effect ANOVA models. Palmitate and octanoate oxidation measures were square-root transformed to symmetrize the distribution and modeled as a function of group (*Intolerant* versus *Comparator*), statin (presence regardless of the dose versus absence) and their interaction. A random (subject-specific) intercept was fitted to adjust for within-patient dependence.

Results

Eleven *Intolerant* subjects who fulfilled entry criteria were enrolled sequentially at the time of muscle biopsy. Seven statin-naïve subjects consenting to donate muscle at the time of knee surgery constitute the *Comparator* group.

Clinical data

The clinical characteristics of the *Intolerant* and *Comparator* subjects are presented in Tables 2 and 3. Age, BMI, and sex distribution were balanced between the groups.

CPX results

Fasting RER was abnormally increased in all *Intolerant* subjects as a criterion for inclusion and was significantly greater than the *Comparator* RER (0.85 ± 0.06 vs. 0.746 ± 0.02; $P < 0.002$) (Table 3). The S1-slope was increased in three of the eleven *Intolerants* (overall mean 0.73 ± 0.08, normal = 0.75 ± 0.06; NS). VO_2 max and AT were normal off of lipid-lowering therapy (22.9 ± 9 and 14.8 ± 3.0 ml/kg/min, respectively). The VE/VCO_2 was impaired in five of the eleven *Intolerants* but not for the group overall (30.4 ± 4.7, normal = 26–29, NS).

Muscle biopsy results

The pathological interpretation and myopathy scores for *Intolerant* and *Comparator* specimens are listed on Tables 2 and 3. Seven of nine *Intolerant* biopsies graded by the myopathy score were abnormal. Three of the five *Comparator* biopsies that were graded were abnormal ($P = 0.58$ for IOA and $P > 0.99$ for MI). The pathology among the *Intolerant* subjects was heterogeneous with six suggesting myopathy and four suggesting neurogenic atrophy. The abnormal *Comparator* biopsies showed a variety of morphological changes that may have been due to age or to atrophy secondary to their orthopedic conditions.

FAO results

The release of ^{3}H from labeled FFAs into water was taken as a measure of β-oxidation of fatty acids in the myocyte cultures [24]. The dose-response curves for both *Comparator* and *Intolerant* groups are presented in Fig. 1. The basal (untreated) FAO of *Intolerant* myotubes was significantly higher than *Comparator* for octanoate (19.5 ± 8.6 vs. 9.5 ± 4.8 nmoles/mg, $P = 0.03$), but not for palmitate (38.3 ± 22.4 vs. 30.1 ± 32.5 nmoles/mg, $P = 0.18$). The significant difference in basal octanoate oxidation between *Intolerant* and *Comparator* myotubes did not persist after lovastatin exposure. For palmitate, lovastatin increased oxidation more in *Intolerant* than in *Comparator* cells so that there was a trend to greater palmitate oxidation in *Intolerant* cells for both 5 μM lovastatin (44.3 ± 33.9 vs. 24.1 ± 24.1, $P = 0.07$) and 20 μM lovastatin (46.0 ± 48.6 vs. 22.7 ± 25.6, $P = 0.06$). The ANOVA analysis showed an overall trend-level difference between *Intolerant* and *Comparator* groups, across all statin doses, on both

Table 2 Clinical characteristics of *Intolerant* subjects (NA = not measured)

Intolerant subject number	Drug intolerance (dose, reaction) C = cramps, P = muscle pain, F = fatigue, W = weakness	CK (IU/L) (peak, off therapy) normal = 0–195	Muscle biopsy results Index of Abnormality (IOA) and Metabolic Index (MI) are defined in the appendix
1	Pravastatin 40 W, P Atorvastatin 20 W, P Fluvastatin ? W, P Lovastatin/Niacin ? W, P Cerivastatin ? W, P Gemfibrozil ? W, P Simvastatin 20 W, P Ezetimibe 10 W, P	123, 64	Myopathy with fiber size variation, increased internal nuclei and pyknotic nuclear clumps IOA = 3 MI = 0
2	Atorvastatin 10 W, P Niacin 1000 W, P Ezetimibe 10 W, P	451, 437	Minor myopathic abnormality with increased internal nuclei and cox-negative fibers IOA = 3 MI = 1
3	Atorvastatin 80 P Pravastatin 40 P Rosuvastatin 10 P Ezetimibe 10 P	135, NA	Normal IOA = 0 MI = 0
4	Atorvastatin 10 P Lovastatin 40 P	589, 511	Minor myopathic abnormality with generalized hypertrophy, increased subsarcolemmal staining, increased internal nuclei and split fibers IOA = 3 MI = 0
5	Atorvastatin 10 P, C	274, 117	Increased lipid in marginated subsarcolemmal pattern, fiber type grouping IOA = 7 MI = 3
6	Niacin 2000 P Simvastatin 20 P Cerivastatin ? P Ezetimibe 10 P	274, 211	Myopathy with fiber size variation, increased internal nuclei, increased lipid staining and concentric laminated mitochondrial inclusions IOA = 4 MI = 2
7	Atorvastatin 20 F, C	815, 740	Neurogenic atrophy with secondary myopathic changes including pyknotic nuclear clumps and increased internal nuclei IOA = 7 MI = 0
8	Simvastatin 20 W	816, 428	Neurogenic atrophy with secondary myopathic changes including fiber size variation, pyknotic nuclear clumps and two "nonspecific" rimmed vacuoles. IOA = 8 MI = 0
9	Pravastatin 60 C	127, NA	Type II fiber atrophy IOA = 0 MI = 0
10	Simvastatin 10 P, W	356, 264	Type II fiber atrophy with modest fiber size variation IOA = NA MI = NA
11	Atorvastatin 20 P, W Niacin 1000 P,W Rosuvastatin 10 P,W	198, 151	Mitochondrial cytopathy with abnormal mitochondria and paracrystalline inclusions. Fiber size variation and ragged red fibers. IOA = NA MI = NA

Table 3 Clinical, pathological, and FAO characteristics of *Intolerant* and *Comparator* subjects (IOA = index of abnormality, MI = metabolic index)

Characteristic	*Intolerant* ($N = 11$)	*Comparator* ($N = 7$)	P-value *Intolerant* versus *Comparator*
Age	66 ± 13	68 ± 10	0.56
Sex	8 M, 3 F	2 M, 5 F	0.14
Body mass index (kilograms/meters)	29.1 ± 5	26.3 ± 5	0.47
Fasting RER	0.85 ± 0.06	0.76 ± 0.02	0.002
Pathology score			
IOA ≥ 3	7 of 9	3 of 5	0.58
MI ≥ 2	2 of 9	1 of 5	1.0
Fatty acid oxidation (nM/mg protein)			
Octanoate at 0 μM lovastatin	19 ± 9	9.5 ± 4.8	0.03
Octanoate at 5 μM lovastatin	16 ± 10	8.5 ± 6.1	0.11
Octanoate at 20 μM lovastatin	19 ± 16	8.7 ± 7.4	0.22
Palmitate at 0 μM lovastatin	38 ± 22	30 ± 32	0.18
Palmitate at 5 μM lovastatin	44 ± 34	24 ± 24	0.07
Palmitate at 20 μM lovastatin	46 ± 49	23 ± 26	0.06

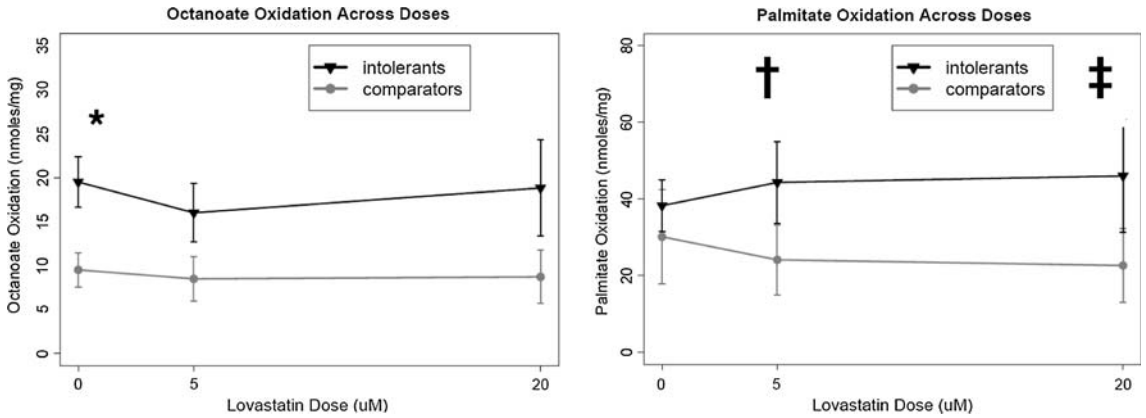

Fig.1 Fatty acid oxidation (FAO) measured as nmoles per mg of myotube protein is plotted for *Intolerant* (solid symbols) and *Comparator* (open symbols) subjects at 0, 5, and 20 μM lovastatin exposure. *Intolerant* myotubes have significantly more basal or unstimulated FAO for octanoate than *Comparator* (* $P = 0.03$). At 5 and 20 μM lovastatin there is a trend for lovastatin-stimulated *Intolerant* myotubes to have greater palmitate oxidation than *Comparator* myotubes († $P = 0.07$, ‡ $P = 0.06$)

palmitate oxidation ($P = 0.09$) and octanoate oxidation ($P = 0.06$). In both cases the *Intolerant* group had higher oxidation. The statin effect (presence versus absence) was not significant, and there was no significant group/statin interaction for either palmitate or octanoate.

Biochemical analyses

Three of the eleven *Intolerant* subjects had normal CK and another three had minimally elevated CK (<300). Five subjects' CK was significantly elevated (300–900), but none exceeded the accepted "ten times normal" indication to stop statin therapy [4]. Preoperative CK was not measured in *Comparator* subjects.

Discussion

This work provides in vitro support for previous clinical, physiological, and biochemical observations suggesting abnormal FAO characteristics in patients with intolerance to lipid-lowering therapy. *Intolerant* FAO was different from *Comparator* in several respects. First, basal FAO of medium chain fatty acids (octanoate) was greater for *Intolerant* than for *Comparator* myotubes. Second, there was a trend to greater palmitate oxidation for 5 and 20 μM lovastatin exposure in *Intolerant* myotubes compared to *Comparator* myotubes. Overall (across all levels of lovastatin) FAO of both palmitate and octanoate was greater for Intolerant than for Comparator myotubes at a trend level of significance (slightly stronger for octanoate).

Graded muscle biopsies did not distinguish the subject groups as well as FAO characteristics did. This may be attributable in part to the fact that *Comparator* subjects were orthopedic patients that demonstrated some muscle pathology related to their underlying conditions. Muscle biopsies from *Intolerant* subjects often showed evidence of metabolic toxicity but pathology was also heterogeneous and some biopsies were normal (Table 1). Previous work has suggested significant heterogeneity among patients who are intolerant of lipid-lowering therapies [9, 13]. However, a consistent feature among these distinct toxic phenotypes appears to be abnormalities in both basal and statin-stimulated FAO compared to *Comparator* subjects. It is likely that lipid-lowering therapies alter fat substrate availability or transport in patients with a variety of disorders resulting in these heterogeneous presentations. It is an important analogy that patients with a tendency to rhabdomyolysis without the provocation of lipid-lowering therapy often have similar metabolic defects [27].

It is not clear how *Intolerant* subjects' elevated fasting RER, suggesting reduced fat oxidation, relates to their increased basal octanoate oxidation or the trend to greater statin-induced palmitate oxidation in this myotube model. We have previously noted that cytochrome oxidase activity was depressed in statin-intolerant subjects [28]. However, the activity measured in the subjects' cultured cells represents the actual intrinsic capacity of that subject's skeletal muscle to oxidize fatty acids, independent of the influence of the in vivo environment. Meanwhile, RER measured in the intact subject is affected by circulating hormones, substrates (especially the hyperlipidemia characteristic of the *Intolerant* subjects), and other factors including the contribution of non-muscle tissues. Cultured skeletal muscle cells from type 2 diabetic subjects retain defects in FAO that are reflective of their in vivo FAO [17]. Similarly, the abnormal FAO responses demonstrated here and their relationship to in vivo factors may be a unique feature of statin-intolerant individuals. Preliminary work studying the effects of statins on FAO in vivo is contradictory. Some investigators find a 20–25% reduction in FFA in statin-treated subjects [29]. Other work shows no change in FFA flux in patients who are asymptomatic on statins [30]. The results of the myocyte culture studies presented here require further validation with FAO studies in patients with statin myopathy in vivo.

This work provides some insight into screening for statin myopathy. Since over half of the *Intolerant* subjects had normal or near normal CK, this parameter does not appear to be an adequate screen for these disorders. Although an abnormal RER did not distinguish between muscle pathology in the *Intolerant* and *Comparator* groups in this study, this may be due to the *Comparator* group being selected from an orthopedic population who might be expected to have muscle changes. Nonetheless, since the abnormal RER used for eligibility in this study correlated with abnormal muscle pathology in 8 of 11 of *Intolerant* subjects and with the abnormal basal octanoate oxidation reported above this may prove to be a reasonable screen. An abnormal RER in these subjects seems to be a reliable predictor of abnormalities in basal FAO responses that may account for symptoms in some subjects intolerant of lipid-lowering therapy.

While the effect of statins on protein synthesis was not studied directly in this project, there was a decline in the protein content of the myocytes with statin exposure (data not shown). The FAO was indexed to protein content to account for this effect. Recent work indicates that atrogin-1 and protein catabolism are critical mediators of statin muscle toxicity [31]. The accelerated proteolysis of fasting and other disorders that cause muscle atrophy is due to activation of the ubiquitin proteasome pathway [32]. Atrogin-1 is a ubiquitin protein ligase that is significantly induced by these conditions [33]. Atrogin-1 is also induced in subjects with normal CK myopathy and with statin-induced rhabdomyolysis [31]. It is not clear if statin toxicity induces atrogin-1 by shifts in prenylated proteins downstream to HMG-CoA reductase or if it is a non-specific response to the substrate deficiency caused by reduced serum lipids. Neither option is excluded by the current investigation. This study raises the possibility that some subjects with disordered lipid metabolism are more likely to induce atrogin-1 when stressed by statin-induced muscle changes. Further studies comparing the atrogin-1 of *Intolerant* myocytes with and without statin exposure to that of *Comparator* myocytes are needed to clarify this relationship.

Perhaps the most important finding here is that the FAO defect anticipated in *Intolerant* subjects appears to be present in their cultured skeletal myotubes. This implies this problem is genetically mediated and is not likely determined solely by the pharmacokinetic interactions usually offered as an explanation for statin myotoxicity [4, 34].

Limitations

This study does not address the mechanism by which basal octanoate oxidation and statin-induced palmitate oxidation in *Intolerant* subjects are greater than *Comparator* subjects. Peroxisome proliferator-activated receptor alpha (PPARα) activity modulates FAO in muscle and cultured muscle cells [35]. Statins act as PPARα agonists and would be expected to increase FAO in normal muscle [36, 37]. Some have suggested PPARα activity itself may mediate the myotoxicity associated with lipid-lowering therapy [38, 39]. PPARα expression, carnitine palmityl transferase and other mediators of FAO were not measured in this

preliminary work and warrant further evaluation as possible mediators of intolerance of lipid-lowering therapy.

This study does not address how often subjects with muscle complaints on lipid-lowering therapy and a normal fasting RER might also demonstrate abnormalities in FAO as only those with abnormal fasting RER were studied.

An alternate control group might have been selected among age-matched statin-treated subjects who have no complaints of muscle pain or weakness. However, we have seen many patients who are asymptomatic on statin but who evidence statin toxicity by muscle pathology and by abnormal gas exchange parameters. Therefore "asymptomatic" subjects on statins do not represent a reliable control group and we chose to study statin-naïve rather than "asymptomatic" statin-treated elderly subjects. This choice means that the lipid values of *Intolerant* and *Comparator* groups would not be matched if compared. The Intolerant subjects were selected based on the severity and reproducibility of their reactions. Such reactions would be expected to occur in only a small percentage of patients taking lipid-lowering therapy.

Conclusions

Clinical, physiological, and pathological abnormalities in patients who are intolerant of lipid-lowering therapy suggest abnormalities in fatty acid oxidation. Skeletal myotubes from these patients express an intrinsic difference in basal FAO and in the FAO response to statin that is retained in vitro with increased basal octanoate oxidation and trends to greater palmitate oxidation with statin exposure compared to *Comparator* subjects. While there is heterogeneity among these subjects, the abnormal response of FAO displayed by their myotubes suggests that abnormalities in fat oxidation are a common feature in patients intolerant of lipid-lowering therapy with basal RER >0.80.

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