Substrate Concentration and Metabolism in Left and Right Muscles of Rats

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Skeletal muscle fiber heterogeneity among muscle groups is well known; however, laterality of muscle metabolism has not been addressed. In the present studies, metabolite concentrations in left and right gastrocnemius, tibialis anterior, quadriceps, and soleus muscles and their response to exogenous insulin have been compared in fasted awake rats. The results indicated that the concentrations of muscle free glycerol ($P > .4$), glycerol 3-phosphate ($P > .1$) nonesterified fatty acids (NEFA) ($P > .6$) and intramyocellular triglycerides (imcTG) ($P > .08$) are comparable between left and right of the same muscle, and are similar among mixed glycolytic-oxidative muscles. The concentration of free glycerol in soleus responded to exogenous insulin in a pattern distinct to that seen for the mixed muscles. The results support interchangeable use of left and right side of same muscles, and probably among different muscles of similar fiber type, but not muscles of different fiber types.

SKELETAL MUSCLE is the largest tissue (40% of total body weight) in mammals and thus its metabolism plays an important role in whole body metabolism. However, the study of skeletal muscle is somewhat difficult compared to other tissues such as adipose tissue because the collection of muscle sample or biopsy is associated with greater pains and thus stress. Repeated sampling in same muscles is not feasible, and can potentially affect results from the later samples due to stress, paracrine/neural refluxes, or other factors. Thus, the most common paradigm used in studies of muscle metabolism, for both humans and animals, is that only one sample is taken from a muscle of each side. The first biopsy from one side is used as the control or baseline, the results from which are applied to those from the other sample from the other side for calculations of metabolic parameters.[1] In doing so, the underlying assumption is that the metabolites and their metabolism on two sides are identical (concentration, kinetics, etc), However, limb laterality and muscle heterogeneity[2] may be a limitation to such approach. This is because laterality/heterogeneity will introduce errors proportional to the discrepancy between 2 sides. Even greater discrepancy becomes likely if different muscle groups are sampled and used in the same manner. This is the case, for example, when more than one sample is collected from different locations on one side and assumed to be metabolically same. This is especially true for small animals whose muscles are small and much greater stress is likely.

Muscle fiber type-related heterogeneity has been extensively studied.[3-6] In contrast, studies of muscle metabolic heterogeneity related to laterality are very limited. Lexell and Taylor did most of the work comparing fiber morphology of left and right leg muscles of humans,[7] but studies on metabolic laterality of muscle are lacking. Even more so, studies of muscle metabolic laterality in animals are essentially absent. It appears true that animals are of less laterality, but it is also true that to assume they are free of laterality is probably improper. This is exemplified by the finding that chicken breast meets at left and right are different.[8] Experiments are, therefore, justified to test muscle metabolic laterality in laboratory animals directly.

To determine the absence or presence, and the degree thereof, of metabolic laterality of skeletal muscle in laboratory animals, we conducted the present studies to compare laterally the concentrations of several metabolites involved in lipid metabolism (muscle free glycerol, glycerol 3-phosphate, intramyocellular nonesterified fatty acids [NEFA], and intramyocellular triglycerides [imcTG]) using rat as a model.

MATERIALS AND METHODS

Male Sprague-Dawley rats (100 g) were purchased from Harlan Sprague Dawley (Indianapolis, IN). The rats were fed a high-fat diet (55% calories as lard) until 400 to 500 g when they were studied as described below. Porcine insulin used in insulin infusion experiments was purchased as powder from Sigma Chemical (St Louis, MO). The protocols were approved by Mayo Institutional Animal Care and Use Committee.

Experimental Procedures

After an overnight fast, a flexible catheter for blood collection was placed in the ventral tail artery under local anesthesia with lidocaine. After the catheter installation, the rats were allowed to relax in a spacious metabolic cage (1 cubit foot) for at least 30 minutes. Then an intravenous infusion line was inserted into a lateral tail vein by venopuncturing. Saline (n = 5) or porcine insulin at 50 (n = 5) or 100 (n = 5) pmol/kg/min were then infused for 2 hours. During the entire infusion, the rats were awake in the metabolic cage with the tail exposed outside for infusion and blood collection, while the body part inside could move about and drink water. During insulin infusion, glucose was infused to maintain euglycemia. Glycerol, free fatty acids, and triglycerides (Intralipid without heparin) are simultaneously infused at predetermined rates to maintain basal levels. At the end of infusion, rats were anesthetized by pentobarbital (50 mg/kg) and gastrocnemius, tibialis anterior, quadriceps, and soleus muscles were collected from both sides and immediately saved in liquid nitrogen. Later the muscle biopsies were transferred to a freezer (−80°C) until analyzed.

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Table 1. Comparisons of Muscle Free Glycerol Concentrations in Left and Right Muscle (nmol/g wet weight)

	Gn		TA		Qc	
	Left	Right	Left	Right	Left	Right
Mean ± SEM (n = 11)	311 ± 32	332 ± 30	356 ± 25	360 ± 28	427 ± 37	429 ± 24
P values						
L *v* R	.45		.79		.98	
v Qc	.001		.001			
v TA	.2					

NOTE. Except comparison of left and right, other comparisons used the average of left and right of same muscles.
Abbreviations: L *v* R: left *v* right; Gn, gastrocnemius; TA; tibialis anterior; Qc, quadriceps.

Sample Processing and Analyses

Muscle samples were microdissected under a stereo microscope to remove any fat cells both outside and inside.[9] The dissected samples were then homogenized on ice and extracted by Folch method.[10] The upper phase was used to determine free glycerol concentration. The lower phase was dried down under N_2 and the lipid classes were separated by thin-layer chromatography (TLC) with hexane:ether:acetic acid (70:30:1, vol/vol) as the solvent system. The triglycerides (TG) band was scraped and TG was extracted using chloroform:methanol (2:1, vol/vol). The scraped TLC plate was rerun in the same solvents to separate NEFA from diglycerides. The NEFA band was scraped and extracted using chloroform:methanol (2:1, vol/vol) before determined enzymatically. TG was hydrolyzed in 2.5% H_2SO_4 at 100°C for 2 hours. TG-glycerol, free glycerol, and glycerol 3-phosphate concentrations were determined enzymatically.[11]

To assess the intramuscular variability of metabolite concentration, additional gastrocnemius and tibialis anterior muscles were each divided into 3 equal aliquots and from each aliquot the same metabolites were purified and analyzed as described above.

Data Analysis

All values are mean ± SEM. Statistical comparisons were made between left and right by paired Student *t* test, and among 3 muscles using 1-way analysis of variance (ANOVA). Intramuscular variability is expressed as the coefficient of variation (CV). If the results from 0, 50, and 100 pmol/kg/min insulin treatments were not statistically different as determined by ANOVA ($P > .05$), the data for same muscles were pooled, left and right side separately.

RESULTS

Intramuscular Variability of Metabolite Concentration

The average CVs of concentrations of muscle free glycerol, NEFA, TG, and glycerol 3-phosphate were 1.5% ± 0.4%, 3.7% ± 0.6%, 7.0% ± 0.6%, and 11.6% ± 3.2%, respectively (n = 6).

Comparisons of Metabolite Concentrations Between Left and Right and Among Muscles

Table 1 shows the concentrations of free glycerol in left and right gastrocnemius, tibialis anterior, and quadriceps muscle. No significant differences were observed between the left and right sides in any of these muscles ($P = .45$ to .98). However, the left-right average glycerol concentration in quadriceps was significantly higher than that of gastrocnemius or tibialis anterior (both $P = .001$).

Fig 1 shows the means and standard errors of concentration differences between left and right muscles (absolute values used to convert all values to positive). The left-right differences from individual animals for glycerol 3-phosphate and imcTG varied more than that for NEFA and glycerol. However, the CV for imcTG was still relatively small (<15%), and that for glycerol 3-phosphate was up to 26%. CVs for NEFA and glycerol were less than 5%.

Table 2 shows the concentrations of glycerol 3-phosphate in left and right side of the same muscles as in Table 1. Again, there were no statistically significant differences in glycerol 3-phosphate concentration between left and right for gastrocnemius ($P = .51$), tibialis anterior ($P = .11$), and quadriceps ($P = .38$), and among them ($F = 0.38$) (left-right average).

As seen in Table 3, the concentrations of NEFA in left and right gastocnemius, tibalis anterior, and quadriceps were almost identical. There were no significant differences ($F = 0.26$) among these 3 muscles (left-right average). In contrast, NEFA concentrations in soleus were about twice as high as in other muscles ($P < .001$).

The concentration of imcTG (Table 4) tended, but not significantly, to be different between left and right side of gastrocnemius ($P = .08$), tibialis anterior ($P = .1$), and quadriceps ($P = .09$). There were no significant differences among gastrocnemius, tibialis anterior, and quadriceps ($F = 0.08$) (left and right average). In contrast, as repeatedly reported previously,[3,6] the left-right average concentration of imcTG in soleus was more than twice that in the other 3 muscles, and all of the differences were highly significant ($P = .0001$).

Response to Insulin

Fig 2 depicts the changes in muscle free glycerol concentration in response to insulin infusion. Distinct patterns were

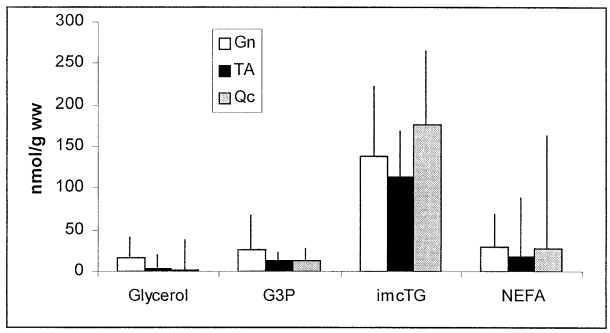

Fig 1. Means and deviations (SEM) of the difference (in absolute values) in metabolite concentration between left and right gastrocnemius (Gn), tibialis anterior (TA), and quadriceps (Qc).

Table 2. Comparisons of Muscle Glycerol 3-Phosphate Concentrations in Left and Right Muscle (nmol/g wet weight)

	Gn		TA		Qc	
	Left	Right	Left	Right	Left	Right
Mean ± SEM (n = 11)	63 ± 10	98 ± 15	78 ± 19	62 ± 16	80 ± 16	63 ± 14
P value						
L *v* R	.51		.11		.38	
Among muscles	$F = 0.38$					

NOTE. *F* value is from comparison of glycerol 3-phosphate concentrations for Gn, TA, and Qc.

observed between soleus and gastrocnemius or tibialis anterior, while that of the latter two was almost identical. Insulin at 50 pmol/kg/min tended to increase free glycerol concentration in gastrocnemius ($P = .06$), and to a lesser extent in tibialis anterior ($P = .13$). Increasing insulin infusion to100 pmol/kg/min did not further increase glycerol in either muscle. In contrast, glycerol concentration in soleus was progressively suppressed by increasing insulin infusion rate. At 100 pmol/kg/min, glycerol in soleus was significantly lower than the control value ($P = .048$).

DISCUSSION

The present results demonstrated that the concentration and metabolism of some key metabolites in lipid metabolism in mixed glycolytic-oxidative skeletal muscles (gastrocnemius, tibialis anterior, and quadriceps) are similar between left and right. This similarity in metabolite concentration and metabolism appears to suggest that metabolic laterality is not present in these rat muscles and therefore it is probably appropriate to interchangeably use the left and right sides of these muscles in metabolic studies. Presumably, although not tested in this study, it is possible that this lateral similarity is likely true for other muscles as well, such as soleus (due to size limit, left and right soleus were combined for assay and thus laterality was not tested). The finding is similar to that from one previous human study showing that there were no left-right differences in fiber type and size in human vastus lateralis muscle.[7] Other aspects of muscle laterality (eg, electromyography) have been reported,[12,13] but no data are currently available on lateral comparison of metabolites or metabolism of skeletal muscle. The present study appears to be the first to examine metabolic laterality of skeletal muscle.

Intramuscular variability for concentration of muscle free glycerol, NEFA, TG, and glycerol 3-phosphate is small, especially for glycerol and NEFA (CV $< 5\%$). This agrees with our experience that the results for these 2 metabolites are consistently reproducible. Intramuscular variability for TG is also relatively low. Therefore, the data suggest that intramuscular variability did not confound the results as described above. On the other hand, the variability for glycerol 3-phophate (CV = 12%) is much greater and this is likely due to the fact that this compound is labial so that substantial autohydrolysis must have occurred during muscle processing (<5 minutes). However, the variability is still much smaller than the interanimal variability (CV up to 25%) as shown in Table 2. Therefore, the conclusion for this metabolite should remain valid, but, in light of the greater analytical variability, further study on this compound seems warranted.

Another novel finding from the present results is that the concentrations of all 4 metabolites examined were comparable among gastrocnemius, tibialis anterior, and quadriceps, all mixed glycolytic-oxidative muscles. The implication is that these muscles can probably be interchangeably used in the studies of these compounds. For example, more than one sample from these muscles on one side may be possible in rats (from one muscle is almost impossible because the later sample will be severely affected) if the stress can be minimized. On the other hand, it should be cautious to do so for TG because of the difference of approaching significance among these muscles. In contrast, the concentrations of NEFA and imcTG in soleus are distinctly higher, as widely recognized,[3,4,5,6,14] suggesting that oxidative muscles are more active than mixed muscles in lipid metabolism.

The muscle type distinction is so clear in the response of muscle glycerol concentration to exogenous insulin infusion. Unlike gastrocnemius and tibialis anterior, which responded to insulin by sizable increases in glycerol concentration, albeit insignificantly, soleus responded by a progressive decrease in the concentration. At the higher insulin concentration (100 pmol/kg/min), glycerol concentration in soleus had declined by

Table 3. Comparisons of Nonesterified Fatty Acids Concentrations in Left and Right Muscles and in Combined (average) Soleus (μmol/g wet weight)

	Gn		TA		Qc		So
	Left	Right	Left	Right	Left	Right	Average
Mean ± SEM (n = 7)	0.44 ± 0.02	0.46 ± 0.02	0.41 ± 0.04	0.43 ± 0.05	0.36 ± 0.07	0.38 ± 0.07	0.85 ± 0.10
P value							
L *v* R	.6		.8		.8		
v So	.0001		.0002		.0002		
Among muscles	$F = 0.26$						

NOTE. *F* value is from comparison of NEFA concentrations for Gn, TA, and Qc.
Abbreviation: So, soleus

Table 4. Comparisons of Intramyocellular Triglyceride Concentrations in Left and Right Muscles and in Combined (average) Soleus (μmol/g wet weight)

	Gn		TA		Qc		So
	Left	Right	Left	Right	Left	Right	Average
Mean ± SEM (n = 11)	1.06 ± 0.09	0.91 ± 0.03	1.15 ± 0.09	1.26 ± 0.07	1.02 ± 0.09	1.20 ± 0.1	3.1 ± 0.2
P value							
L *v* R	.08		.1		.09		
v So	.0001		.0001		.0001		
			$F = 0.08$				

NOTE. *F* value is from comparison of TG concentrations for Gn, TA, and Qc.

30% from the baseline values (Fig 2). This disparate pattern of response to insulin further highlights the distinction between soleus and other muscles. In contrast, the remarkable similarity in metabolite response to insulin by gastrocnemius and tibialis anterior (Fig 2) echoed the similarity in metabolite concentration between these 2 muscles.

This muscle type-related difference in metabolism stressed the importance not to use muscles of different types to represent each other. In practice, it means that the site of muscle samples from 2 sides must be identical, at least as close as possible, in order to minimize the errors introduced by including different muscle types. In this regard, open muscle biopsy procedure[3] or perfused muscle studies[15] appear to be advantageous where muscles are directly visible and thus the site of sampling can be precisely controlled. However, this perhaps presents a technical challenge for studies that take more than one muscle sample from one side because this paradigm mandates the involvement of muscles at different anatomical locations and thus likely of different muscle types. If too close, the later sample is likely affected. This may be more so for humans because of greater muscle heterogeneity. However, no data are available and direct assessment seems needed.

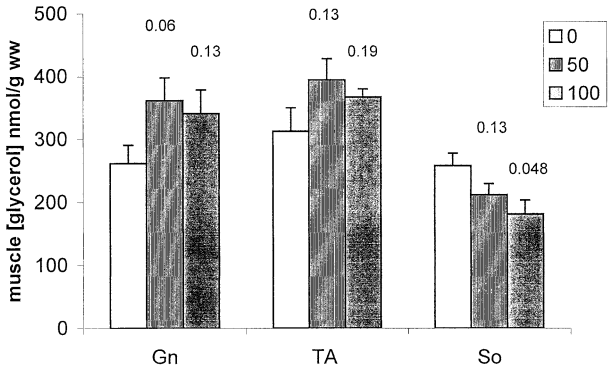

Fig 2. Response of muscle free glycerol concentration in 3 muscles to insulin infusion at 0, 50, or 100 pmol/kg/min. The infusion lasted 120 min during which glucose was infused to maintain euglycemia. At the end of the infusion, muscles were biopsied. Five rats were used for each level of insulin. *P* values are comparisons between 50 or 100 pmol/kg/min insulin against the control (without insulin infusion). Gn, gastrocnemius; TA, tibialis anterior; So, soleus.

It is encouraging to note that the total variability for complex lipid triglycerides (Table 4) was comparable to that for simple compound NEFA and glycerol. Considering the classic great variability of this lipid pool,[6,16,17] the present data suggest that careful microdissection of skeletal muscle samples before lipid extraction is critical and effective and therefore a necessary step for studies of skeletal muscle lipid metabolism. The data provide further support to our previous conclusion that the removal of extramyocellular fat cells from muscle samples is not only possible, but also practical in routine experiments.[16] It also suggests that detailed microdissection of skeletal muscle samples to remove adipocytes does not involve noticeable autolipolysis (low variability for NEFA concentration).

In conclusion, the present results support the use of the left and right of same muscles interchangeably in lipid studies involving fatty acids, glycerol, and TG. Gastrocnemius and tibialis anterior muscles are similar in metabolite profile and response to insulin, raising a possibility of using these muscles interchangeably.

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