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Letters to the editor

Despite similar rates of alanine release, fasting and diabetes affect de novo alanine synthesis differently

Dear Sir,
Diabetes and fasting have long been known to induce a substantial release of alanine from muscle whereas the intramuscular alanine pool remains unchanged [1, 2]. The substantial alterations of alanine metabolism in fasting and diabetes are not completely understood, especially with regard to the contribution of leucine as nitrogen donor in alanine de novo synthesis. Alanine remains an important gluconeogenic precursor in humans [3, 4], although glutamine was recently reported as a major source of carbon for gluconeogenesis in diabetic or postabsorptive humans [5, 6]. For this reason, we recently assessed, by using [^{15}N]leucine, the capacity of skeletal muscle to synthesise alanine de novo through leucine transamination in experimental diabetic rats. Indeed, few studies have addressed either the direct effect of leucine supply on both synthesis (within the muscle) and release of alanine (from the muscle) or the contribution of proteolysis and de novo synthesis to alanine production in muscle. For this purpose, we used ^{15}N/^{1}H NMR combined with GC-MS. We have previously demonstrated that streptozotocin-induced diabetes in growing rats is associated with: 1) an increase in nitrogen exchange between leucine and alanine leading to newly synthesised [^{15}N]alanine; and 2) an increase in total alanine release from muscle originating from both proteolysis and de novo synthesis [7]. In the present letter, we would like to report some data obtained with extensor digitorum longus muscles from fasted rats in order to compare nitrogen exchange between leucine and alanine in 48 h-fasting and in experimental diabetes.

We observed that the size of [^{15}N]alanine pool within the muscle was smaller in fasted than in diabetic and control extensor digitorum longus muscles (Table 1). Yet, in order to evaluate the true nitrogen transfer between leucine and alanine, both the labelled intramuscular alanine pool (M_L) and the labelled alanine released (R_L) at the end of experiment should be taken into account. For this reason, we calculated the sum of these two components under each experimental condition (see Table 1). Surprisingly, nitrogen exchange was less intense in fasted than in diabetic rats. Moreover, it should be pointed out that the intramuscular total alanine pool was severely depressed by 48 h-fasting. This could be related to the extensive alanine utilisation in the whole body during the period of fasting before the superfusion experiment.

By contrast, a similar increase in total alanine release from muscle of fasted and streptozotocin-diabetic rats (Table 1) was observed. Even though 48 h-fasting had a greater effect on proteolysis than did streptozotocin-diabetes (as reflected by the measurement of net tyrosine release, e.g. 292 ± 25 vs 186 ± 12 nmol tyrosine $\cdot$ g^{-1} $\cdot$ 2 h^{-1}), the percentage of alanine release originating from proteolysis was the same in fasted muscle as it is in streptozotocin-diabetic extensor digitorum longus muscles (approximatively 60 %). Consequently, leucine contributed to approximately 40 % of alanine α-amino N released in both control and diabetic rats. Only a concomitant increase in proteolysis and de novo synthesis can explain the increase in alanine released from fasted extensor digitorum longus muscles, as previously reported in experimental diabetes [7].

In conclusion, these results confirm the interest of studying both muscle and medium superfusion compartments to better understand alanine regulation within the muscle of fasted rats. Surprisingly, leucine appears less efficient as a donor of α-amino N for the synthesis of alanine in skeletal muscle from fasted than in streptozotocin-diabetic rat even though leucine transamination has been previously described as being increased to a similar extent by fasting or diabetes [8]. These data confirm that alanine synthesis within the muscle may be limited by the availability of amino group acceptors [2]. Whereas α-ketoglurate is added to the medium of superfusion (3 mmol/l) regardless of the animals conditions, glucose is presumably extensively oxidized in superfused extensor digitorum longus muscles from fasted rats, as reflected by the high pH value (7–7.3) in comparison to fed muscles [9]. Consequently, decreased availability of pyruvate may be a limiting factor in glucose-superfused extensor digitorum longus muscles from fasted rats, explaining a lower rate of alanine synthesis in fasted rats than in diabetic rats.

Yours sincerely,
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Table 1. Nitrogen transfer from leucine to alanine in superfused extensor digitorum longus (EDL) muscles from fasted and diabetic rats

Group	Intramuscular pool (μmol/g wet wt muscle)		EDL rate of release ($\mu mol \cdot 2\ h^{-1} \cdot g^{-1}$)		N transfer from Leu to Ala ($\mu mol \cdot 2\ h^{-1} \cdot g^{-1}$)
	Total alanine (M_T)	[15N]alanine (M_L)	Total alanine (R_T)	[15N]alanine (R_L)	[15N]alanine ($M_L + R_L$)
Control	2.46 ± 0.01	0.79 ± 0.05	1.80 ± 0.07	0.74 ± 0.04	1.53 ± 0.04
Diabetes	2.73 ± 0.14	1.10 ± 0.07[a]	2.15 ± 0.10[a]	0.88 ± 0.14	1.97 ± 0.15[a,c]
Fasting	1.11 ± 0.14[a,b]	0.55 ± 0.03[a,b]	2.44 ± 0.10[a]	0.94 ± 0.14	1.49 ± 0.15

Values are given as means ± SEM ($n = 4$ to 8, no. of experiments/treatment group); [a] $p < 0.05$ significantly different from control rats, [b] $p < 0.05$ significantly different from diabetic rats, [c] $p < 0.05$ significantly different from fasted rats. $^{15}N/^{1}H$ NMR, GC-MS and aminoacid measurements were made at the end of 2 h-superfusion experiments. M_T, intramuscular pool of total alanine; M_L, intramuscular pool of labelled alanine; R_T, release of total alanine from muscle; R_L, release of labelled alanine from muscle. N transfer from Leu to Ala was calculated as the sum of intramuscular [^{15}N]alanine (M_L) and the rate of [^{15}N]alanine released from muscle (R_L)

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Hyperexcitability to sulphonylurea in MODY3

Dear Sir,

The genotypes of maturity-onset diabetes of the young (MODY) have, to some extent, specific phenotypic expression. Mutations in glucokinase (MODY2) are associated with impaired glucose tolerance or mild diabetes mellitus [1]. Mutations in the hepatocyte nuclear factor-1alpha gene (MODY3) [2] are, on the other hand, associated with a high prevalence of overt diabetes, resulting in chronic vascular complications [3]. Several years ago we described a large Norwegian family with MODY, in which there was a particular high frequency of severe eye complications. Five patients were blind, two showed proliferative retinopathy, and one patient had simplex retinopathy and cataract [4]. Whereas the diabetic subjects of this family were characterized by an almost abolished insulin response to oral glucose, several patients showed a remarkable sensitivity to sulphonylurea, with symptoms of hypoglycaemia 60–90 min after as little as 0.5 mg glibenclamide orally. The response to glibenclamide was not tested in glucose-tolerant subjects of the family. These observations were striking enough to be taken as part of the phenotypic expression in this family. At the time of publication the gene defect was unknown. Genotyping of this family now reveals that the mutated gene is HNF-1 alpha (MODY3), with mutation P291 fsinsC in exon 4 (the mutational hot spot), see Figure 1. This is the first MODY3 family observed in Norway. Recently, Hansen et al. [5] presented evidence of hyperexcitability to tolbutamide and glucagon in a glucose-tolerant subject with a MODY3 mutation. The authors speculated that such a hyperexcitability might be characteristic of the early stages in the pathogenesis of MODY3. It should be noted that the sulphonylurea hypersensitivity observed by us, was present in diabetic subjects after they had had the disease for several years. More work is obviously needed to establish if an abnormally sensitive sulphonylurea receptor is an element of the pathogenesis in some families with MODY3.

Yours sincerely

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