CORRESPONDENCE

Muscle glutamine production in burn patients: the physiological meaning of tracer estimates

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In a previous issue of Clinical Science, Biolo et al. [1] report data showing that hypercatabolic burn patients have a decreased release of glutamine from the leg muscles. This observation is of clinical relevance since muscle is considered to be the main glutamine producing tissue in man and because the glutamine released from muscle supports the function of the gut and the immune system [2,3]. In healthy controls glutamine is the main amino acid exported from muscle [3–5]. However, in the burn patients an excessive rate of glycolysis appears to increase muscle pyruvate concentrations and, therefore, alanine production [1–3,6] at the expense of glutamine production. A decreased release of glutamine from the leg muscles of burn patients [1] suggests that these patients may have an increased need for glutamine supplementation.

The message from this study [1] seems to be in striking contrast to a recent paper from the same group using stable isotope tracers of glutamine [7]. The title of that study was: ‘Accelerated glutamine synthesis in critically ill patients cannot maintain normal intramuscular free glutamine concentration’. Four of the five patients investigated in [7] also were hypercatabolic burn patients with comparable clinical characteristics to the patients in [1]. The main difference between the two studies [1,7] is the method used to investigate glutamine metabolism. In the most recent study [1], only the mass balance of glutamine has been measured (arterio-venous concentration difference × leg blood flow). This variable indicated that the leg muscles of burn patients released less glutamine than those of control subjects. In the study by Mittendorfer et al. [7] a [5-15N]glutamine tracer has also been infused in order to measure several intramuscular components of glutamine metabolism. The authors made use of the so-called ‘three pool model’ for the calculation of intramuscular glutamine kinetics [8]. This model, apart from the estimate of the mass balance, permits calculation of the rate of inward transport of glutamine into the muscle, the rate of outward transport of glutamine from the muscle into the circulation, and the rate of intracellular synthesis and rate of intracellular utilization of glutamine. Both the rate of intracellular synthesis and intracellular utilization of glutamine were 3-fold higher in burn patients than in controls, according to this tracer model. However, the mass balance, which can be calculated from other variables reported in [7], indicated that the net amount of glutamine released from the leg was decreased to the same extent as that observed in the larger population in [1]. Apart from the fact that the titles of the two papers [1] versus [7] are confusing, the question as to what the physiological meaning of the tracer estimate of glutamine synthesis and glutamine utilization is, can be raised. It can simply mean that there is a high rate of futile cycling between glutamine and glutamate (with the amide-N being removed by the action of glutaminase and being incorporated again by the action of glutamine synthetase). It could also mean that glutamine synthesis and oxidation in the tricarboxylic acid cycle (via the subsequent action of glutaminase, alanine aminotransferase and α-ketoglutarate dehydrogenase) occur simultaneously. Independent of the enzymic reactions that occur, the end result still is that the net mass balance (the amount of glutamine released from the leg muscles) is decreased in the patients and that, therefore, the amount of glutamine available to support the gut and immune system is decreased. So, for the clinician who has to judge whether a patient may be in need of glutamine supplementation, the tracer dilution method does not seem to provide relevant or meaningful physiological information.

An additional complication is that the validity of the three pool model [8] for the calculation of intramuscular glutamine kinetics has recently been questioned [9,10]. Van Acker et al. [9] have shown that it takes more than 20 h for the intramuscular glutamine pool to reach a steady state (constant enrichment). This is the consequence of the enormous size of the intramuscular glutamine pool in human muscle [9,10]. Theoretical calculations [10] have shown that an earlier claim of Biolo et al. [8] that a steady state was reached in four healthy subjects already at 5 h after the start of the glutamine tracer infusion is highly unlikely, and were not confirmed by experimental data in 20 patients [9]. Failure to freeze dry and clean the muscle biopsies from contamination visible only under a dissection microscope (the recommended procedure in Scandinavian muscle physiology laboratories) may give errors in the estimates of glutamine concentration and enrichment and this may...
be the reason for the discrepancy between the data of Biolo et al. [8] and that of Van Acker et al. [9]. The three pool model should, therefore, only be used in studies where firm evidence has been given for the presence of a steady state. Failure to reach a steady state, as a consequence of an infusion period that is too short, will lead to the apparent disappearance of tracer in the muscle and to artifactual increases in the tracer estimate of both the rate of intramuscular glutamine synthesis and the rate of intramuscular glutamine utilization.

A third variable which is sometimes used to indirectly estimate the muscle glutamine production in patients is the whole body appearance rate of glutamine in the plasma pool (plasma Ra). It is estimated from the dilution observed in plasma of a glutamine tracer administered by continuous infusion [7,9]. This variable is also increased in burn patients [7,11], but again its physiological meaning is not entirely clear. The plasma Ra is an estimate of the release of unlabelled glutamine from all tissues of the body into the plasma compartment. It is clear by now, from cannulation studies of tissues with tracers [12], that most tissues of the human body not only release glutamine, but that they also take up glutamine at the same time. However, when we want to judge whether the amount of glutamine that is released by tissues and that is available to the cells of the gut and the immune system is sufficient then we are interested in the net balance (release–uptake). Therefore, the plasma Ra also does not seem to provide the right information. The plasma Ra in healthy individuals [7,9,11,12] is 6–20-fold higher than the estimated glutamine mass balance released from the combined human skeletal muscles into the circulation [1,4,5,12,13]. Therefore, the relation between the muscle production and the plasma Ra is not clear and may differ in each and every pathological condition. Increased intracellular recycling (e.g. between glutamine and glutamate) will increase the plasma Ra and have no effect on the glutamine mass balance across the muscle.

The conclusion, therefore, is that the best variable at this moment in time to estimate whether a patient has a decreased glutamine release from the muscle, is the glutamine mass balance across a forearm or leg. The conclusion from [1] is that burn patients have a decreased release of glutamine from the muscle, and that they may, therefore, require glutamine supplements in their enteral and/or parenteral nutrition in order to support the gut and immune system. The functional benefits of glutamine supplementation in hypercatabolic patients still have not been firmly established and it remains an area of continuing research in many clinical research centres.

REFERENCES


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Muscle glutamine production in burn patients: the physiological meaning of tracer estimates: authors’ reply

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Our response to the Correspondence of Dr A. J. M. Wagenmakers is as follows. Dr Wagenmakers is not commenting on our recently published paper in *Clinical Science* [1], but rather is criticizing a tracer technique used in a previous publication [2]. The criticism he makes in his letter is repeating that made in an earlier publication by Van Acker et al. [3].

Dr Wagenmakers feels that isotopic equilibrium was not achieved in the intramuscular pool in our previous study [2], because the size of the muscle glutamine pool is too large. However, in that study, the isotopic enrichment of the intramuscular glutamine pool was greater in relation to plasma enrichment in normal volunteers than in the patients, yet the intramuscular pool of unlabelled glutamine was four times larger in the normal volunteers than in the patients. This is exactly the opposite of what would be expected if the size of the intramuscular pool determined the time required to reach equilibrium. In fact, there is no reason to expect that pool size alone would play a role in the time required to reach an isotopic equilibrium. It is the turnover time of the pool that determines this factor. This is explained in [4].

The results of Van Acker et al. [3] have little relevance to our study. In that paper, an insufficient priming dose was used and we would predict many hours would be required to reach equilibrium using their approach. In our study, the priming dose, which was three times that used by Van Acker et al. [3], was based on both theoretical considerations [4] and empirical experience. In essence, our method was criticized based on the results of a study in which our method was not followed.

It is indeed possible that reaching equilibrium in the intramuscular glutamine pool in muscle is difficult in critically ill patients. Contrary to the reasons suggested by Dr Wagenmakers, the most likely reason would be impaired inward transport of glutamine. In this case, we would have overestimated the extent of cycling between glutamine synthesis and cycling in the patients reported previously [2]. We will address this issue in a subsequent publication.

With regard to our recent publication [1], this issue is moot, because our conclusions were based entirely on net balance data, and isotopic data are not mentioned. The apparent discrepancy between the titles of our earlier paper [2] and our more recent paper [1] stems from reference in the earlier paper to the isotopically determined value of total glutamine synthesis, whereas the recent paper [1] refers to net glutamine production. As Dr Wagenmakers points out, the net balance data of the two papers are in agreement. The use of contradictory titles is unfortunate but, in self defence, we would say that our attempt to distinguish between total and net glutamine production in the title was a casualty of the editing process.

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