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ARTICLE

The contribution of muscle, kidney, and splanchnic tissues to leucine transamination in humans

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Abstract: The first steps of leucine utilization are reversible deamination to α-ketoisocaproic acid (α-KIC) and irreversible oxidation. Recently the regulatory role of leucine deamination over oxidation was underlined in rodents. Our aim was to measure leucine deamination and reamination in the whole body, in respect to previously determined rates across individual organs, in humans. By leucine and KIC isotope kinetics, we determined whole-body leucine deamination and reamination, and we compared these rates with those already reported across the sampled organs. As an in vivo counterpart of the "metabolon" concept, we analysed ratios between oxidation and either deamination or reamination. Leucine deamination to KIC was greater than KIC reamination to leucine in the whole body ($p = 0.005$), muscles ($p = 0.005$), and the splanchnic area ($p = 0.025$). These rates were not significantly different in the kidneys. Muscle accounted for ≈60% and ≈78%, the splanchnic bed for ≈15% and ≈15%, and the kidney for ≈12% and ≈18%, of whole-body leucine deamination and reamination rates, respectively. In the kidney, percent leucine oxidation over either deamination or reamination was >3-fold greater than muscle and the splanchnic bed. Skeletal muscle contributes by the largest fraction of leucine deamination, reamination, and oxidation. However, in relative terms, the kidney plays a key role in leucine oxidation.

Key words: leucine, transamination, BCAA, ketoacids, skeletal muscle, kidney.

Résumé : La désamination réversible de l'acide α-céto-isocaproïque (α-KIC) et l'oxydation irréversible constituent les premières étapes de l'utilisation de la leucine. On a récemment souligné chez les rongeurs l'importance du rôle de régulation de la désamination de la leucine par rapport à l'oxydation. Nous avions comme objectif de mesurer la désamination et la réamination de la leucine dans le corps entier, en regard de taux établis antérieurement dans des organes donnés chez l'humain. À l'aide de la cinétique d'isotopes de leucine et du KIC, nous avons établi la désamination et la réamination de la leucine à l'échelle du corps entier, et nous avons comparé ces taux à ceux qui ont déjà été rapportés dans les organes échantillonnés. Nous avons considéré les ratios entre l'oxydation et la désamination ou la réamination comme étant une contrepartie in vivo du concept de « métabolon », et nous les avons analysés. La désamination de la leucine en KIC était plus importante que la réamination du KIC en leucine dans le corps entier (p = 0,005), dans les muscles (p = 0,005) et dans le lit splanchnique (p = 0,025). Ces taux n'étaient pas nettement différents dans les reins. Les muscles comptaient pour environ 60 et 78 % dans les taux respectifs de désamination et de réamination de la leucine dans le corps entier, le lit splanchnique dans environ 15 et 15 % du total et les reins dans 12 et 18 % du total. Le pourcentage d'oxydation de la leucine par rapport à sa désamination ou à sa réamination était plus de trois fois supérieur dans les reins par rapport aux muscles et au lit splanchnique. Le muscle squelettique contribue dans la plus grande mesure à la désamination, à la réamination et à l'oxydation de la leucine. Cependant, tout étant relatif, les reins jouent un rôle clé dans l'oxydation de la leucine. [Traduit par la Rédaction]

Mots-clés : leucine, transamination, acides aminés ramifiés, cétoacides, muscles squelettiques, reins.

Introduction

The control of the catabolic flux of most amino acids takes place at the first metabolic steps, i.e., at the transamination/deamination reactions (Nelson and Cox 2015). The term "transamination" refers to the overall process of amino group transfer from a donor amino acid to an accepting ketoacid, to form a daughter amino acid. Conversely, "deamination" refers just to loss of an amino group by a given amino acid, whereas "reamination" refers to the capture of an amino group by a ketoacid to form an amino acid.

The transamination of leucine, valine, and isoleucine (branched-chain amino acids; BCAA) plays a key role in nitrogen distribution among nonessential amino acids and in nitrogen shunting either to urea formation or to protein synthesis (Nelson and Cox 2015). At variance with most amino acids, which are predominantly utilized by the liver, BCAAs are thought to be mainly catabolized by extrahepatic tissues (Nelson and Cox 2015; Cohen and Hekhuis 1941; Ichihara 1985; Miller 1961), above all skeletal muscle (Miller 1961; Adibi 1976; Brosnan and Brosnan 2006) and adipose tissue (Brosnan and Brosnan 2006). Nevertheless, the relative role of BCAA transamination vs. oxidation is incompletely defined. Recent observations suggest that transamination plays a more important role than that previously assumed, on the control of BCAA metabolism. Mitochondrial BCAA transaminases (BCAA-Tm) are ubiquitary in animal tissues (Taylor and Jenkins 1966; Suryawan et al. 1998). Furthermore, it has been recently demonstrated that

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mitochondrial BCAA-Tm and branched chain α-ketoacid dehydrogenase (BCKD) enzyme complexes associate to form a supra molecular entity (Islam et al. 2007), of the type originally defined as "metabolon" (Robinson and Srere 1985), that provides a coordinated control of the BCAA metabolic flux and oxidation. According to this concept, the tissue-specific level of BCKD dehydrogenase, and the phosphorylation state of the BCKD complex, regulate both BCAA-carbon irreversible loss (i.e., oxidation) and rates and direction of reamination/deamination. In BCAA-Tm knock-out mouse, it was concluded that BCAA-Tm actually plays a key role in BCAA signaling and anabolism, beyond that "classically" played by oxidation (Joshi et al. 2006).

Among the 3 BCAAs, leucine plays a pivotal role both as an important anabolic signal and a regulator of protein turnover (Buse and Reid 1975; Kimball and Jefferson 2004). In addition, leucine kinetic is commonly taken as an index of whole-body and organ protein turnover (Matthews and Bier 1983; Tessari 1994). So far, however, only a few studies have provided data on whole-body and organ leucine transamination and oxidation in humans. Matthews et al. (1981) proposed a model of leucine deamination and reamination at whole-body level, while Cheng et al. (1985) reported estimates of these rates across the human forearm. More recently, we reported the rates of leucine deamination, reamination, and oxidation across the leg, the splanchnic area, and the kidney (Tessari et al. 1996). However, in that study, we did not report some additional data, which were not analyzed at that time yet, on whole-body leucine deamination and reamination, to which organ rates could be compared.

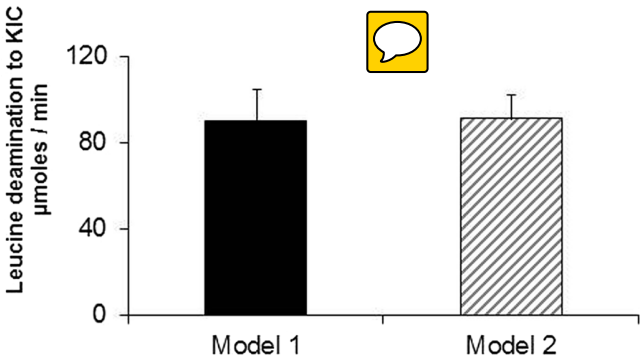
This study aims to complete the picture of leucine deamination, reamination, and oxidation rates across 3 major organs in humans, in comparison with the corresponding ones determined at whole-body level. In addition, this study reports on a new model to measure whole-body leucine deamination, based on the infusion of an independent ketoisocaproic acid (KIC) tracer, conceptually similar to that previously used to estimate first pass splanchnic leucine uptake and deamination (Biolo and Tessari 1997). The contributions of skeletal muscle, the splanchnic bed, and the kidneys, to whole-body leucine deamination, reamination, and oxidation, are also presented in the light of the "metabolon" concept, based on a combined analysis of leucine transamination and oxidation.

Material and methods

Subjects were enrolled between September 1993 and July 1994. The subjects' clinical characteristics, the inclusion criteria, and the experimental design, had been previously reported (Tessari et al. 1996), except for an additional subject (male gender, age 56 years, BMI 25.8 kg·m^{-2}), whose data became available after the publication of the original manuscript (Tessari et al. 1996). In addition to the previously reported tracers, all subjects had also been infused with the [$^2H_3$]-α-ketoisocaproic acid stable isotope tracer ([α-D_3-KIC] (>98% purity, obtained from Tracer Technologies, Somerville, Massachusetts, USA). All isotopes were dissolved in sterile saline and proven to be sterile and pyrogen-free before use. Studies were performed in the post-absorptive state. At 0800, primed-continuous infusions of the leucine tracers were started and carried out for 4 h (Tessari et al. 1996). The D_3-KIC infusion rate was 0.0343 ± 0.0051 (mean ± SE) μmol·kg^{-1}·min^{-1}. Isotope priming doses were 30× the continuous infusion rates per minute.

Sample processing and analytical methods were performed as described by Tessari et al. (1996) and Schwenk et al. (1984). We employed a compartmental model in the analysis of the regional leucine metabolic steps, as previously reported (Tessari et al. 1995, 1996) (see Supplementary Methods and Supplementary Fig. S1[1]). Fn2

Fig. 1. Rates of whole-body leucine deamination to ketoisocaproic acid (KIC) (i.e., of the KIC derived from leucine) calculated using either Model 1 (from Matthews et al. 1993) or from Model 2 (eqs. 4 to 6 of the compartmental model). Data are means ± SE of 10 subjects. The deamination data calculated with the 2 models were virtually identical ($p > 0.85$ by paired t test).



Arterial and venous concentrations and enrichments (Supplementary Fig. S2[1]) of leucine, KIC, and CO_2 remained stable, indicating that tracers and tracees were at steady state throughout the study.

Statistical analysis

The data were reported as means ± SE. Organ as well as whole-body kinetics data were normalized per 1.73 m^2 of body surface (Tessari et al. 1996). The Wilcoxon test for paired data was used to compare the rates of leucine deamination and reamination within each organ as well as in the whole body, using the Statistical Software (Version 7.1, StatSoft Italia). In addition, the data were analyzed also using the two-tailed Student's t test for paired data. To compare the percent contribution of each organ with whole-body deamination or reamination rates, as well as the fraction of leucine oxidation over either leucine deamination to KIC or KIC reamination to leucine among the different organs, we employed the one-way ANOVA followed by the Newman–Keuls post hoc test. A p value less than 0.05 was considered statistically significant.

Results

Leucine deamination and KIC reamination

The rates of whole-body leucine deamination to KIC were quite similar using either Model 1 as reported by Matthews et al. (1981) or Model 2 (based on the data calculated from the infusion of the independent KIC tracer (Fig. 1). F1

Leucine deamination to KIC was greater t IC reamination to leucine at whole-body level (p = 0.005), in skeletal muscle (p = 0.005), and in the splanchnic area (p = 0.025), whereas these rates were not significantly different from each other in the kidneys (Fig. 2A), as previously reported (Tessari et al. 1996). F2

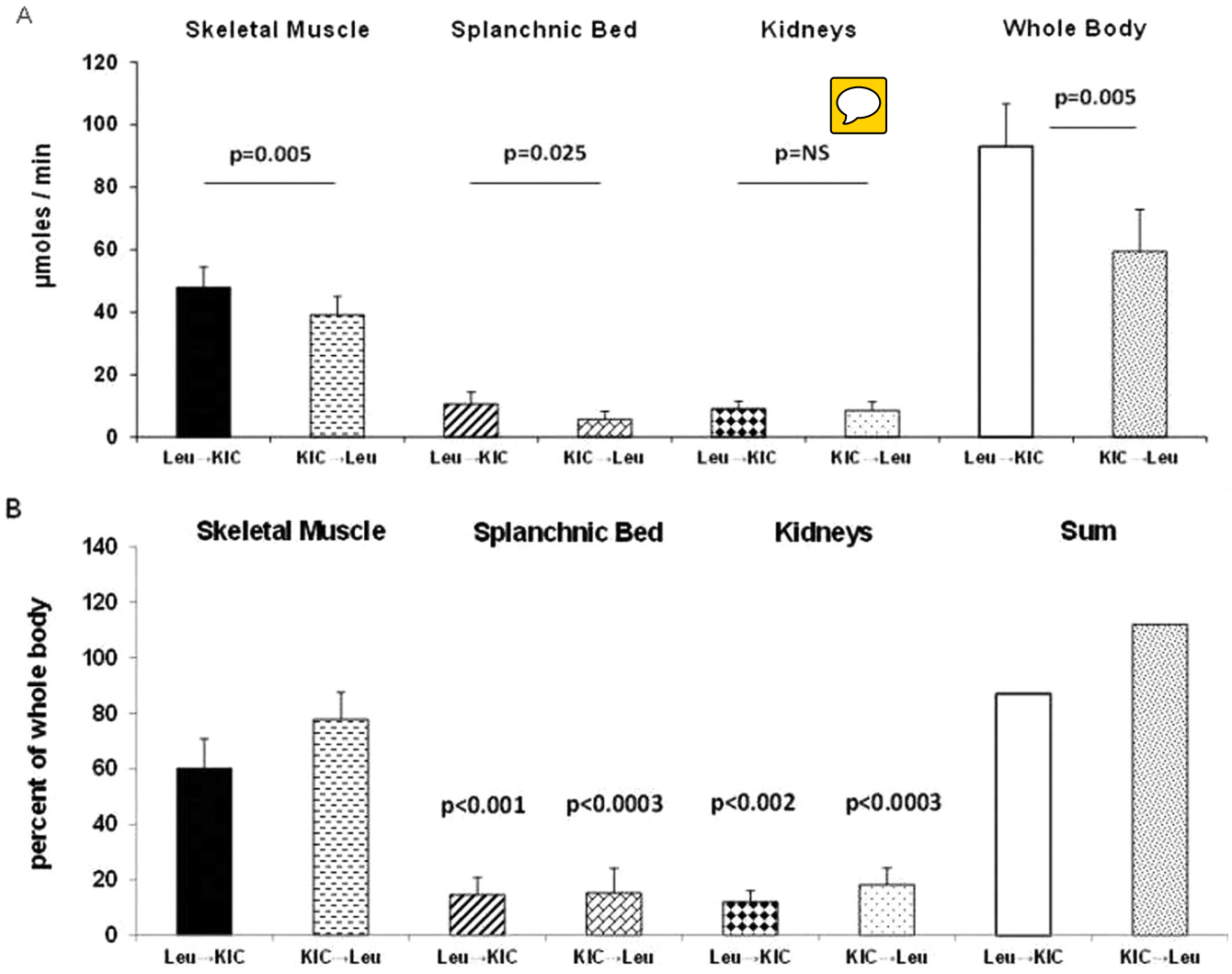
When expressed as percentage of whole-body rates, tota tal muscle accounted for ≈60% of leucine deamination and ≈78% of leucine reamination, the splanchnic bed for ≈15% and ≈15%, and the kidneys for ≈12% and ≈18%, respectively (Fig. 2B). The sum of the percent contributions by the 3 organs, to body leucine deamination to KIC was ≈87%, while that of KIC reamination to leucine was ≈112%.

Leucine flux through oxidation, deamination, and reamination

The sum of leucine deamination (the F7 model parameter) and leucine oxidation (F9), as well as the sum of reamination (F8) and

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjpp-2017-0439.

Fig. 2. (A) Rates of leucine deamination to α–ketoisocaproate, KIC (Leu→KIC), and of KIC reamination to leucine (KIC→Leu), across total skeletal muscle (n = 10), the splanchnic bed (n = 8), the kidneys (n = 7), as well as in the whole body. Rates are expressed as μmol·min^{-1}. Levels of the statistically significant difference between deamination and reamination (by the Wilcoxon paired t test) within each organ, as well as in the whole body, are reported. Data are shown as means ± SE. (B) Percent contributions to the whole body, of leucine deamination to α-ketoisocaproate, KIC (Leu→KIC), and of KIC reamination to leucine (KIC→Leu), across total skeletal muscle (n = 10), the splanchnic bed (n = 8) and the kidneys (n = 7), as well as the sum of the percentages of the 3 organs. The reported levels of statistical significance indicate the differences (by the one-way ANOVA and the Newman–Keuls post hoc test) between rates in either the splanchnic bed or the kidneys, and the corresponding ones in skeletal muscle. The sum of rates from the 3 organs are reported as gross means (i.e., without SE), because not all subjects were studied across all the 3 organs. The sum of either deamination or reamination are not significantly different from 100% (p > 0.7). Data are shown as means ± SE. NS, not significant.



oxidation, expressed both as μmol·min^{-1} and as percentage of whole-body rates, are reported in Table 2. In absolute terms, skeletal muscle accounted for the largest portion of these rates in respect to those of the whole body, about 4-fold greater than those of either the splanchnic bed or the kidneys. In relative terms, the sum of the 3 sampled districts accounted for ≈80% (for F7+F8), and for 93% (for F8+F9), of the corresponding whole-body rates.

The ratios of leucine oxidation (F9) to deamination (F7), and that of leucine oxidation to reamination (F8), are reported in Fig. 3. These ratios indicate the fraction of leucine irreversible loss (through oxidation), over either leucine deamination to KIC (Fig. 3A), or KIC reamination to leucine (Fig. 3B), and they can approximate the "metabolon" concept in vivo across the 3 sampled organs. In the kidneys, percent leucine oxidation over either deamination (55% ± 16%) or reamination (119% ± 47%), was >3-fold greater than the

Table 1. Whole-body leucine kinetics either derived from Matthews et al. (1981) or determined in the present study.

μmol·kg^{-1}·h^{-1}	Matthews et al. (1981)	Present data
Leu C Ra	120	122
Leu N Ra	155	174
Leu Ox	21	23
Leu to KIC (= deamination)	56	87
KIC to Leu (= reamination)	35	58

Note: KIC, ketoisocaproic acid.

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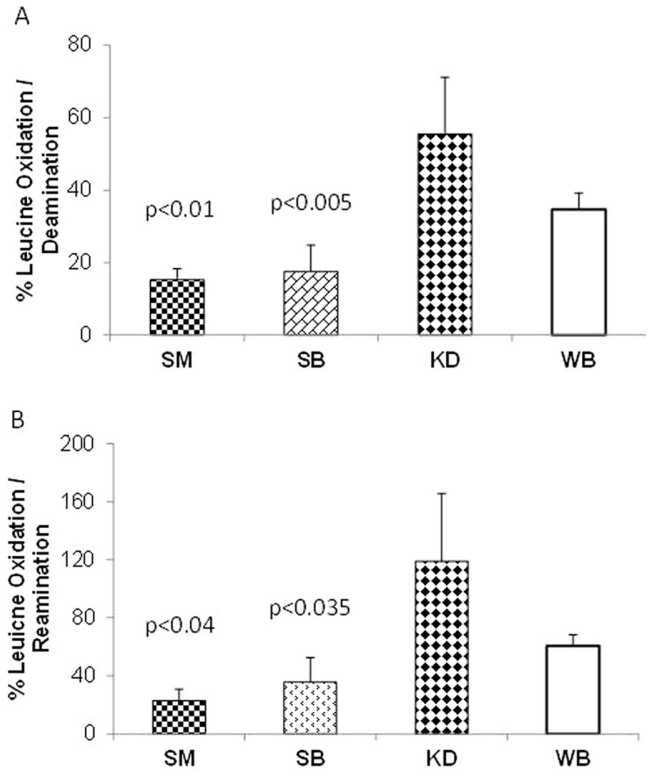
Table 2. The sum of leucine deamination (F7 model parameter) and of leucine oxidation (F9 model parameter) (in μmol·min⁻¹), as well as of leucine reamination (F8) and oxidation (F9), across each of the 3 sampled districts and in the whole body, and their percentage contribution to whole-body rates.

	Total skeletal muscle (n = 10)	Splanchnic area (n = 8)	Kidneys (n = 7)	Whole body (n = 10)
Leu Deam. (F7) + Ox (F9)	55.1±7.7	13.8±5.1	13.6±2.3	125.1±15.6
(F7+F9) as % whole body	53.1±10.9	14.0±6.3	12.9±3.2	≈80%
Leu Ream. (F8) + Ox (F9)	46.4±6.7	8.9±3.5	13.1±2.3	91.5±17.4
(F8+F9) as % whole body	62.1±10.5	14.2±7.3	17.3±3.8	≈93%

Note: Values are means ± SE.

Fig. 3. (A) The percentage of leucine oxidation (F9 model parameter) vs. deamination (F7) of each organ as well as in the whole body (WB). The reported p values indicate the level of the significant differences between either total skeletal muscle (SM, n = 10), or the splanchnic bed (SB, n = 7) and the kidneys (KD, n = 6), calculated by the one-way ANOVA and the Newman–Keuls post hoc test). Whole body ratios (n = 10) are also reported. Data are shown as Means ± SE. (B) The percentage of leucine oxidation (F9 model parameter) vs. reamination (F8) of each organ as well as in the WB. The reported p values indicate the level of the significant differences between either total SM (n = 10), or the SB (n = 7) and the KD (n = 7), calculated by the one-way ANOVA and the Newman–Keuls post hoc test). WB ratios (n = 10) are also reported. Data are shown as means ± SE.



corresponding values calculated across both the skeletal muscle (15% ± 3% and 23% ± 8% for deamination and reamination, $p < 0.04$ and $p < 0.01$, respectively) and the splanchnic bed (17% ± 7% and 36% ± 17%, $p < 0.005$ and $p < 0.04$, respectively) (Figs. 3A and 3B). These data indicate that, in the kidneys, leucine is preferentially oxidized, to a much larger extent than that observed in either the skeletal muscle or the splanchnic area.

Discussion

BCAA metabolism in humans is tightly regulated to maintain levels sufficiently high to support major functions, such as protein synthesis, provision of nitrogen for the synthesis of nonessential amino acids and hormone-like signals, but at the same time excess levels are prevented by the upregulation of irreversible disposal pathways (Suryawan et al. 1998). Four major observations can be drawn from this study. First, in post-absorptive human beings, skeletal muscle accounts for the largest fraction of body BCAA leucine deamination and reamination, even larger than that expected from tissue BCAA-Tm distribution (Suryawan et al. 1998). Second, a relevant fraction (≈30%–35%) of these rates is accounted for by visceral organs (splanchnic bed and kidneys). Third, skeletal muscle and visceral organs together virtually account for body total leucine deamination and reamination. Fourth, in the kidneys, leucine is preferentially channeled towards oxidation rather than to reamination.

Overall, our in vivo data demonstrate that skeletal muscle plays a major role in overall leucine metabolism. The high percentage of body leucine deamination and oxidation accounted for by skeletal muscle, in agreement with the concept that the BCAAs are predominantly catabolized by extrahepatic tissues, as also suggested by their low first-pass splanchnic extraction (Biolo et al. 1992; Matthews et al. 1993; Stoll et al. 1998). Also leucine oxidation took place to a large extent in skeletal muscle (≈37% of whole-body rates), as previously reported (Tessari et al. 1996). Of note, the high rates of leucine deamination and reamination in muscle can provide nitrogen for the synthesis of nonessential amino acids such as alanine and glutamine, the key nitrogen carriers from the periphery (skeletal muscle) to the liver.

We observed that in the whole body, leucine deamination is 6- to 7-fold faster than oxidation (Tessari et al. 1996), a finding similar to what was previously observed for valine also (Staten et al. 1984). A similar leucine deamination/oxidation ratio is observed in skeletal muscle and in splanchnic organs. These data suggest that in muscle and splanchnic organs the transamination step may regulate the free levels of the individual BCAA's, while decarboxylation limits their catabolism. Contrariwise, the leucine deamination and oxidation rates are similar in the kidney.

A new finding of this study is that the contribution by visceral organs to whole-body leucine deamination and reamination rates is substantial (about one third of total), and almost equally shared by kidneys and splanchnic organs. In the fetal kidney, BCAA transport is necessary for early nutrition and development (Guetg et al. 2015). The adult kidney, in particular in the medullary thick ascending limb, is rich in all the enzymatic machinery involved in leucine catabolism and (or) oxidation. Oxidation of leucine in this nephron segment may provide energy to sustain active ion transport (Tring-Trang-Tan et al. 1988).

The liver is thought to be the primary site for the oxidation of branched-chain keto acid (BCKA) but not BCAA (Brosnan and Brosnan 2006). In this study, the contribution of splanchnic or-

gans to whole-body leucine deamination and reamination rate was greater than expected (~15%). However, we could not evaluate the oxidation of KIC to beta-hydroxy-beta-methylbutyrate by the enzyme KIC dioxygenase, a reaction which takes predominantly in the liver. In liver rats, it has been observed that a minor percentage (about 5%) of daily leucine metabolism is channeled through the dioxygenase pathway (Van Koevering and Nissen 1992).

Sites other than muscle and visceral organs, such as adipose tissue, brain, heart, and lung (that could not be sampled in our study), should contribute minimally to leucine metabolism. However, concerning body fat, it should be considered that the data derived from skeletal muscle (i.e., leg) catheterization also include the contributions of both intramuscular and subcutaneous adipose tissue (Frick et al. 1988).

One original contribution of our study is the investigation of the relative roles of leucine deamination, reamination, and oxidation, both in the whole body and in selected organs in humans. By such data presentation, we intended to depict a sort of leucine "metabolon" in humans. Nevertheless, we are fully aware that the transfer of the "metabolon" concept into the in vivo human setting is rather complex, and perhaps not entirely appropriate. One example and (or) limitation of such a transfer is given by the precursor substrate(s) of renal leucine oxidation. Because in the kidneys leucine deamination and reamination rates were not statistically different from each other, leucine oxidation in the kidneys (≈4.5 μmol·min^{-1}, unreported data) should have predominantly derived from other, unaccounted sources: one would likely be KIC itself. Indeed, as reported previously (Garibotto et al. 2002), there was a net KIC uptake by the kidneys, of ≈3.3 μmol·min^{-1} (using plasma data), a figure that, added to the net (albeit insignificant) difference between deamination and reamination (≈0.5 μmol·min^{-1}), yields a total of 3.8 μmol·min^{-1}, that would account for ≈85% of renal total leucine oxidation. Therefore, should leucine oxidation in the kidneys predominantly derive from the KIC taken up (i.e., not from leucine deamination itself), thus directly entering the mitochondria for oxidation, this other oxidation route may not be strictly considered under the "metabolon" concept. On the other hand, the limitation of our model and (or) approach is physically linked to the complexity of the studies in humans.

Another feature of BCAAs is the role of leucine as an anabolic nutrient signal to stimulate protein synthesis by activation of mTORC1, an effect reinforced by physical exercise and protein feeding. Several conditions causing wasting, such as sarcopenia of aging and inflammation, blunt the leucine-induced mTORC1 1 activation (Ham et al. 2014). A limitation of our study is that we evaluated leucine deamination only in the post-absorptive, basal state. In addition, our patients were studied at rest, and the effects of physical exercise on leucine metabolism have not therefore been addressed.

The understanding of BCAA/Tm-BCKD activity in individual organs and in the whole body in disease is of major importance in several clinical fields. Elevated levels of BCAAs are implicated in obesity, insulin resistance, and type 2 diabetes (Adams 2011). An emergent hypothesis is that in obese, insulin-resistant state or in type 2 diabetes, raised BCAA and BCKA levels reflect reduced BCAA/Tm-BCKD activity in a variety of metabolically relevant tissues such as liver, WAT, and possibly muscle (Adams 2011). However, this hypothesis still needs to be confirmed in human studies. It is interesting that in animal models, liver BCKD activity can be modulated by protein intake (Brosnan and Brosnan 2006). Should this take place also in humans, changes in the amounts or quality of ingested protein could influence BCKD activity in different tissues to correct the alterations in BCAA/BCKA pattern in insulin-resistant states.

Further studies are required to understand mechanism and mediators that regulate deamination and reamination rates in sepsis. During sepsis, accelerated protein degradation is associated with increased transamination and oxidation of amino acids in skeletal muscle (Woolf et al. 1979). Reamination of KIC to leucine has also been shown to be enhanced in the liver of starved and endotoxin-treated rats (Holecek et al. 2001).

BCAA deamination and transamination is also a potential major topic in patients with chronic kidney disease (CKD). Our finding of preferential leucine/KIC degradation in the human kidney suggests that progressive CKD may be associated with reduced requirement for leucine. This observation may explain, at least in part, the good nutritional status achieved even with very low protein diets in non-dialyzed patients with CKD (Bellizzi et al. 2016). Of note, supplemented very low protein diets (0.28–0.40 g·kg^{-1}) containing BCKAs are offered to CKD patients to provide EAA precursors without the nitrogen load from EAAs. These supplemented very low protein diets appear to generate less toxic metabolic products than similar amounts of protein from LPDs (Gao et al. 2010) and have proven to be effective and safe when postponing dialysis treatment in elderly CKD patients (Bellizzi et al. 2016). However, the optimal doses of keto-acids and the muscle and systemic adaptations to keto acid supplementation are still unresolved.

In conclusion, this study provides estimates of leucine deamination, reamination, and oxidation in the skeletal muscle, the splanchnic bed, and the kidneys, as well as in the whole body in the post-absorptive state, in humans. Whereas in absolute terms, the most relevant contributions are provided by skeletal muscle; in relative terms, the kidney plays a remarkable role particularly in leucine oxidation. The data here presented could be of help in the understanding of whole-body as well as organ leucine metabolism, and they can ultimately lead to a better modeling of amino acid metabolism. They may also be important in the calculation of the amount of leucine effectively delivered to tissues, both from a nutritional standpoint and for the associated signaling effect.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

Acknowledgements

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Author contributions: G.G. and P.T. designed the protocol; recruited the subjects; and contributed to study performance, data and statistical analyses, and the overall data evaluation. P.T. developed the original model of whole-body leucine deamination. D.V. and M.V. performed the laboratory analyses and critically reviewed the data. G.G. and P.T. wrote the manuscript and had the primary responsibility for the final content. All authors read and approved the final manuscript.

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