# **Supplementary material**

# Title: Postprandial protein metabolism during aging: raw data and calculations using KIC as a precursor

Authors: Martial Dangin, Christelle Guillet, Clara Garcia-Rodenas, Pierre Gachon, Corinne Bouteloup-Demange, Kristel Reiffers-Magnani, Jacques Fauquant, Olivier Ballèvre and Bernard Beaufrère

DOI: 10.1113/jphysiol.2002.036897

Supplementary material The online version of this paper can be found at: http://www.jphysiol.org/cgi/content/full/XXX/X/XXX and contains material entitled: Postprandial protein metabolism during aging: raw data and calculations using KIC as a precursor.

Contents: 1 PDF file.

#### Electronic journal archives (part 1: results)

**Tracer enrichments:** In all studies, plasma [<sup>13</sup>C] leucine MPE (*i.v.* tracer) reached a plateau before meal ingestion with no significant effect of age and of the type of meal (Fig. 6A, Tab. 2, electronic journal archive). Thereafter, those enrichments decreased differently among meals (Y<sub>max</sub>: P<0·01, T<sub>1/2</sub>: P<0·001, AUC: P<0·05) with no effect of age. In both age groups, the maximal variations induced by WP-iN were more pronounced (Y<sub>max</sub>: P<0· 001), but with a lower AUC (P<0.05) and a longer T<sub>1/2</sub> than with CAS (P<0.001). In young men, comparison between WP-iL and CAS led to the same conclusions. By contrast, in the older group only  $T_{1/2}$  was longer (WP-iL vs. CAS: P<0. 001). The oral tracer ([<sup>2</sup>H<sub>3</sub>] leucine MPE, Fig. 6B, Tab. 2, electronic journal archive) appeared rapidly in the plasma, with differences related to the meals ingested (P<0 01). With similar dietary protein enrichment, WP-iL had faster absorption than CAS in both age groups ( $T_{1/2}$ , P < 0.01), a higher peak (P < 0.01) and a lower AUC (P<0. 01). With WP-iN (lower dietary protein enrichment), Y<sub>max</sub> and AUC were lower (P<0.001) but T<sub>1/2</sub> (P<0.05) was again shorter than with CAS. There was an effect of age since the differences between the WP meals and CAS (WP-iL minus CAS and WP-iN minus CAS) were smaller in the old group ( $Y_{max}$ : P < 0.01;  $T_{1/2}$ : P < 0.001) than in the young one. Plasma  $[^{13}C]$  and  $[^{2}H_{3}]$  KIC MPE paralleled the pattern of plasma  $[^{13}C]$  and  $[^{2}H_{3}]$  leucine. respectively (Fig. 7A, B, electronic journal archive).

Time dependent evolutions of <sup>13</sup>CO<sub>2</sub> production rate (µmol kgMM<sup>-1</sup> min<sup>-1</sup>) were shown in the electronic journal archive (Fig. 6C and in Tab. 2). <sup>13</sup>CO<sub>2</sub> production rate responded differently to meals. Indeed, in both age groups, WP-iL and WP-iN induced higher  $Y_{max}$  (*P*<0· 05) a, shorter  $T_{1/2}$  (*P*<0· 05, except WP-iN *vs.* CAS in the older group, *P*=NS). Age affected the differences between WP meals and CAS on  $Y_{max}$ ,  $T_{1/2}$  and AUC (Tab. 2), the differences being smaller in elderly subjects than in young men (*P*<0· 01).

## Electronic journal archives (part 2: Table)

		Young ( <i>n</i> =6)			Elderly (n=9)			Age-effect
		WP-iN	CAS	WP-iL	WP-iN	CAS	WP-iL	$\Delta \text{WP}$ vs. CAS
	Ymax	1· 88±0· 12*	0. 69±0.05	1· 36±0· 05*	1· 37±0· 05*	0· 91±0· 08	1· 11±0· 12	+
Exo Leu Ra	T <sub>1/2</sub>	129±5*	197±6	108±5*	145±7*	171±9	141±12* <sup>*</sup>	+
	AUC	342 <u>+</u> 27*	189±5	200±9	275±14*	199±7	197±14	
Endo Leu Ra	Basal	2· 04±0· 09	2· 21±0· 08	2· 13±0· 10	2· 00±0· 08	2· 14±0· 10	2· 06±0· 11	
	Ymax	1· 66±0· 10	1· 74 <u>±</u> 0· 06	1· 75±0· 10	1·48±0·04	1· 53±0· 13	1· 61±0· 11	
	T1/2	197±2	204±2	199±2	192±5*	209±2	200±1*	+
	AUC	777±46	807±27	811±34	739±23	768±43	754±45	
NOLD	Basal	1· 86±0· 08	1· 91±0· 06	1· 83±0· 06	1· 70 <u>±</u> 0· 08	1· 78±0· 08	1· 75 <u>±</u> 0· 09	
	Ymax	2· 79±0· 14*	2· 31±0· 09	2· 63±0· 11	2· 78±0· 20*	2· 25±0· 09	2· 26±0· 09	
	T1/2	181±2*	204±3	183±2*	184±5*	199 <del>±</del> 2	191±3	+
	AUC	772±31	767 <u>±</u> 25	740±22	696±31	705±38	680±28	

Table 2 Kinetics of	fluxes calculated	lusing KIC a	anrichment after	ar meal indestion
Table Z. Kinelics of	nuxes calculated	i using kic e	ennchment alte	er meai ingestion.

Meals ingested contained casein (CAS) or two different amounts of whey proteins: WP-iN isonitrogenous with CAS or WP-iL that provided the same amount of leucine as CAS. Results are means  $\pm$ S.E.M. Basal: baseline values. Y<sub>max</sub>: zenith or nadir value ; T<sub>1/2</sub>: time to reach half area under the curve (in min). AUC: postprandial area under the curve. Exo Leu Ra: Exogenous (dietary) Leucine Rate of appearance. Endo Leu Ra: Endogenous Leucine Rate of appearance, i.e. proteolysis. NOLD: Non Oxidative Leucine Disposal, i.e. protein synthesis. Leu Ox: Total Leucine Oxidized. Flux values are in µmol kgFMM<sup>-1</sup> min<sup>-1</sup> except Exo Leu Ra (µmol kg<sup>-1</sup> min<sup>-1</sup>). Statistical analyses were performed by ANOVA to assess the differences related to the type of meals within age-group (<sup>\*</sup>:*P*<0· 05) and to age (†:*P*<0· 05).

### Electronic journal archives (part 3: figures)

#### FIGURES LEGENDS

**Fig. 6.** Evolution of plasma leucine enrichment (panels *A* and *B*) and [<sup>13</sup>C]CO<sub>2</sub> production rate (panel C) after ingestion of CAS, WP-iL and WP-iN by young or elderly subjects (upper and lower graphs, respectively). Results are means±S.E.M. MPE: mol percent Excess. FFM: Fat Free Mass (kg).

**Fig. 7.** Evolution of plasma ketoisocaproate (KIC) enrichment (panels *A* and *B*) after ingestion of CAS, WP-iL and WP-iN by young or elderly subjects (upper and lower graphs, respectively). Results are means±S.E.M. MPE: mol percent Excess.

**Fig. 8.** Fluxes calculated using KIC enrichments. Exogenous leucine rate of appearance (Exo Leu Ra, panel *A*) endogenous leucine rate of appearance, i.e. proteolysis (Endo Leu Ra, panel *B*) and NOLD, i.e. protein synthesis (panel *C*) after ingestion of CAS, WP-iL and WP-iN by young or elderly subjects (upper and lower graphs, respectively). Results are means±S.E.M. FFM: Fat Free Mass (kg).



Fig. 6







Fig. 8