Supplementary Information (SI) for Food & Function.
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Supplementary Materials

Supplemental Table 1 Some previous examples of IVIVR establishment with extended application to small intestinal digestion data, and how they would fit in the proposed IVIVR framework in this paper. For the definition of each IVIVR level, see Table 2.1. Detailed descriptions on the *in vitro* and *in vivo* methods for each row of the table can be found in the reference given in the table.

Food	*In vitro* method	*In vivo* method	Data used for IVIVR establishment	Findings	IVIVR	Reference
Extended application to small intestinal digestion data						
Reconstituted skim milk powder	Static model – rotating incubator: • INFOGEST static digestion protocol Dynamic model - DiDGI dynamic *in vitro* digestion system: • Controlled gastric emptying rate and intragastric pH profile to follow human study data • Followed by static small intestinal *in vitro* digestion	Growing pig model (slaughter method)	• *In vitro* SDS-PAGE, peptide profile of five most abundant milk proteins • *In vivo* SDS-PAGE, peptide profile of five most abundant milk proteins across four small intestinal sections (proximal jejunum, median jejunum, distal jejunum, and ileum)	• High Spearman correlation (σ) in the peptide pattern after gastric step for *in vivo* vs. *in vitro* dynamic digestion data (σ = 0.85) and *in vivo* vs. *in vitro* static digestion data (σ = 0.81) • Highest Spearman correlation in the peptide pattern after small intestinal step for *in vivo* distal jejunum vs. dynamic *in vitro* digestion (σ = 0.51) and *in vivo* median jejunum vs. static *in vitro* digestion (σ = 0.58)	Level A	(Egger et al. 2019)
				• Comparable protein patterns between *in vivo* and *in vitro* samples	Level D	
White bread, fruit bread, muesli bar, instant mashed potatoes, canned chickpeas	Static model – stirred container: • Chewed food bolus by human subjects directly followed by small intestinal digestion	Human study (capillary blood sampling) in healthy adults	• *In vitro* glycemic glucose equivalent, calculated from glucose and fructose released during digestion • *In vivo* glucose disposal rate, calculated from glycemic response curve	• Linear correlation (R^2 = 0.93) between glycemic glucose equivalent and glucose disposal rate, after correction for non-linearity of *in vivo* data using ratios of the linear to quadratic responses to glucose	Level C	(Monro, Mishra, and Venn 2010)
Boiled potato of various cultivars + water	Static model – shaking water bath: • Modified Englyst protocol (Englyst, Kingman, and Cummings 1992)	Published data (international tables of glycemic index)	• *In vitro* starch hydrolysis • *In vivo* glycemic index (GI)	• Strong, positive correlation between *in vivo* GI values vs. *in vitro* starch hydrolysis, particularly at 90 and 120 min (r = 0.91, p < 0.01).	Level C	(Ek et al. 2014)

Food	*In vitro* method	*In vivo* method	Data used for IVIVR establishment	Findings	IVIVR	Reference
White bread, spaghetti, rice, biscuits, lentils, chickpeas, beans, peas, boiled potatoes, crisp potatoes	Static model – shaking water bath: • Incubation in excess gastric fluid (gastric phase) followed by pH adjustment and excess small intestinal fluid addition (small intestinal phase)	Published data (international tables of glycemic index)	• *In vitro* hydrolysis index evaluated at 30 to 180 min • *In vivo* glycemic index	• Correlation coefficient (*r*) for *in vivo* glycemic index vs. *in vitro* HI evaluated at different endpoints ranged between 0.84 to 0.91 (90-min endpoint) • Regression equation with the highest correlation was defined as a model to estimate *in vivo* GI from *in vitro* HI	Level C	(Goñi, Garcia-Alonso, and Saura-Calixto 1997)
				• Similar trend between *in vitro* and *in vivo* data: legume products had low results and cereal products had high results	Level D	
Field peas of different varieties (ground, screen size = 2.38 mm)	Static model – shaking water bath: • Pepsin-pancreatin digestion	Adult pig model (cannulated at the terminal ileum)	• *In vitro* degree of starch hydrolysis (the endpoint of gastric digestion) • *In vivo* coefficient of apparent ileal starch digestibility (CAID)	• Linear correlation (not 1:1 correlation) between *in vitro* and *in vivo* data, adjusted $R^2 = 0.755$	Level C	(Montoya and Leterme 2012)
Breakfast cereal with milks (3.1% or 9.3%-wt protein) with normal (80:20) or modified (60:40) casein:whey ratio The cereal was milled and sieved (1.5 mm)	Semi-dynamic model – rheometer equipped with jacketed beaker: • Controlled gastric and duodenal phase pH using a pH-stat titrator • Addition of the entire gastric or duodenal fluids in the beginning of the respective phase	Human study (fingerprick blood sampling and blood sampling from venous catheter) in healthy young adults	• *In vitro* digesta apparent viscosity, total amino acids concentration, and reducing sugar concentration at the end of gastric (60 min) duodenal (120 min) digestion • *In vivo* plasma total amino acids (TAA), blood glucose concentration, gastric emptying (measured as plasma paracetamol concentration) at the end of gastric and duodenal digestion	• Possible association between *in vivo slower* gastric emptying vs. *in vitro* digesta viscosity based on observed trends in *in vitro* viscosity and *in vivo* paracetamol concentration • Similar trends between *in vivo* 90-min blood glucose concentration and *in vitro* reducing sugar concentration at the end of *in vitro* duodenal digestion • Similar trends between *in vivo* plasma total amino acids (TAA) and *in vitro* TAA concentration after gastric digestion	Level D	(Kung et al. 2019)
Semi-synthetic diets containing faba bean, pea,	Static model – stirred flask: • *In vitro* digestion protocol for protein and	Growing pig model (cannulated in the ileum)	• Aparent ileal digestibility of protein and four amino acids	• Linear correlations between *in vitro* and *in vivo* apparent ileal digestibility of protein and the amino acids ($0.43 \leq r^2 \leq$	Level C	(Święch and Buraczewska 2001)

Food	*In vitro* method	*In vivo* method	Data used for IVIVR establishment	Findings	IVIVR	Reference
and lupin of different varieties	amino acids ileal digestibility prediction (Boisen and Fernández 1995)		(cystine, lysine, methionine, threonine)	0.94). Highest correlation ($r^2 \geq 0.89$)for cystine and methionine, lower correlation for lysine, and poor correlation for threonine		
Commercial tofu and soya milk, with standardized amount of protein, fat, and calories	• Dynamic in vitro digestion (DiDGI) consisted of a gastric compartment, and two compartments of small intestine (duodenum, jejunum + ileum)	Mini pig model (cannulated around the gastric corpus, the pyloric sphincter, or the distal ileum)	• Dry matter content (duodenal and ileal) • Proteolysis (gastric and duodenal) • Molecular weight determination by HPSEC (gastric) • Protein digestibility	• Same range of *in vitro* and *in vivo* values for nitrogen digestibility when the supernatant fraction of *in vitro* ileal digesta was considered as the absorbed fraction • No clear *in vivo-in vitro* similarity for results from the gastric phase • Agreement between *in vivo* and *in vitro* trend in the duodenal global kinetics of proteolysis and ileal nitrogen digestibility	Level D	(Reynaud et al. 2021)

Supplemental Table 2 Data used for identifying data points that deviate from 1:1 line in the Level A IVIVR plots of case study 2. Data with %*in vitro-in vivo* difference greater than 50% are identified as deviating data points and shown in red fonts. In vivo data were obtained from Roy et al. (2022), *in vitro* data were obtained from Roy et al. (2021). Bias and MAPE were calculated using Eqn. (3) and (4), respectively.

Food	Time (min)	Averaged value		(y-x)/y *100	Absolute (y-x)/y*100
		In vivo (y)	*In vitro* (x)		
A. Intragastric liquid pH					
Cow milk	30	5.94	5.31	11%	11%
	90	4.55	3.72	18%	18%
	150	3.23	2.8	13%	13%
	210	3.00	2.06	31%	31%
Goat milk	30	5.96	5.77	3%	3%
	90	4.37	4.27	2%	2%
	150	3.30	2.85	14%	14%
	210	2.17	2.02	7%	7%
Sheep milk	30	5.76	5.22	9%	9%
	90	4.46	3.76	16%	16%
	150	3.43	2.85	17%	17%
	210	3.00	1.95	35%	35%
			Average difference	Bias = 15%	MAPE = 15%
B. Curd dry matter (DM) retention					
Cow milk	30	0.65	0.60	2%	2%
	90	0.43	0.49	-15%	15%
	150	0.40	0.41	-1%	1%
	210	0.26	0.31	-19%	19%
Goat milk	30	0.58	0.47	19%	19%
	90	0.35	0.45	-27%	27%
	150	0.23	0.43	-90%	90%
	210	0.15	0.42	-185%	185%
Sheep milk	30	0.55	0.61	-11%	11%
	90	0.37	0.56	-48%	48%
	150	0.28	0.50	-75%	75%
	210	0.14	0.45	-227%	227%
			Average difference	Bias = -56%	MAPE = 60%
C. Curd protein retention					
Cow milk	30	0.77	0.79	-3%	3%
	90	0.60	0.63	-4%	4%
	150	0.58	0.54	6%	6%
	210	0.41	0.39	5%	5%
Goat milk	30	0.70	0.69	2%	2%
	90	0.49	0.67	-37%	37%
	150	0.33	0.66	-100%	100%
	210	0.23	0.64	-179%	179%
Sheep milk	30	0.58	0.77	-33%	33%

Food	Time (min)	Averaged value		(y-x)/y *100	Absolute (y-x)/y*100
		In vivo **(y)**	***In vitro*** **(x)**		
	90	0.43	0.72	-69%	69%
	150	0.37	0.66	-78%	78%
	210	0.18	0.61	-239%	239%
			Average difference	Bias = -61%	MAPE = 63%
D. Curd fat retention					
Cow milk	30	0.80	0.90	-13%	13%
	90	0.52	0.73	-41%	41%
	150	0.48	0.59	-22%	22%
	210	0.33	0.46	-39%	39%
Goat milk	30	0.77	0.80	-4%	4%
	90	0.49	0.76	-55%	55%
	150	0.34	0.73	-114%	114%
	210	0.22	0.70	-215%	215%
Sheep milk	30	0.61	0.82	-35%	35%
	90	0.42	0.75	-80%	80%
	150	0.36	0.68	-86%	86%
	210	0.17	0.59	-253%	253%
			Average difference	Bias = -80%	MAPE = 80%

Supplemental Table 3 *In vivo-in vitro* linear regression coefficients (slope, intercept), correlation coefficient (r), and the significance of the correlation (p) for the digestion parameters examined in case study 2 (Section 3.3.2), examined at individual milk type level. Significant correlation is present when $p < 0.05$.

	Cow milk	**Goat milk**	**Sheep milk**
pH			
Slope	0.96	0.97	0.87
Intercept	0.86	0.32	1.15
r / R^2	0.99/0.97	1.00/0.99	0.99/0.98
p	0.013	0.004	0.008
Curd DM retention			
Slope	1.14	8.21	2.45
Intercept	-0.09	-3.29	-0.96
r / R^2	0.98/0.96	0.99/0.97	0.99/0.98
p	0.018	0.014	0.009
Curd protein retention			
Slope	0.87	9.56	2.21
Intercept	0.08	-5.93	-1.14
r / R^2	0.99/0.98	0.97/0.93	0.97/0.94
p	0.011	0.034	0.032
Curd fat retention			
Slope	1.00	5.22	1.83
Intercept	-0.14	-3.45	-0.91
r / R^2	0.97/0.93	0.98/0.97	0.98/0.97
p	0.035	0.015	0.016

Supplemental Table 4 Data used for identifying data points that deviate from 1:1 line in the Level A IVIVR plots of case study 3. Data with %*in vitro-in vivo* difference greater than 30% are identified as deviating data points and shown in red fonts. In vivo data were obtained from Nadia et al. (2021), *in vitro* data were obtained from Nadia et al. (2022) at digestion condition of 0 min proximal phase followed by up to 180 min distal phase. Bias and MAPE were calculated using Eqn. (3) and (4), respectively.

Food	Time (min)	Averaged value		(y-x)/y *100	Absolute (y-x)/y*100
		In vivo (y)	*In vitro* (x)		
Moisture content, dry basis (g H_2O/g DM)					
Couscous	30	3.36	3.76	-12%	12%
	60	3.86	4.07	-5%	5%
	120	4.12	4.32	-5%	5%
Rice couscous	30	2.93	3.70	-26%	26%
	60	3.32	3.71	-12%	12%
	120	3.82	3.80	1%	1%
Rice grain	30	2.98	2.78	7%	7%
	60	3.01	3.05	-1%	1%
	120	3.40	3.33	2%	2%
Pasta	30	2.77	2.57	7%	7%
	60	3.12	3.01	4%	4%
	120	3.44	2.81	18%	18%
Rice noodle	30	2.80	3.19	-14%	14%
	60	3.23	3.44	-6%	6%
	120	3.64	3.52	3%	3%
			Average difference	Bias = -3%	MAPE = 8%
Normalized hardness					
Couscous	30	0.15	0.31	-104%	104%
	60	0.06	0.13	-120%	120%
	120	0.05	0.08	-61%	61%
Rice couscous	30	0.02	0.31	-1630%	1630%
	60	0.01	0.32	-4017%	4017%
	120	0.04	0.26	-572%	572%
Rice grain	30	0.58	0.94	-62%	62%
	60	0.46	0.82	-77%	77%
	120	0.53	0.57	-8%	8%
Pasta	30	0.72	1.00	-38%	38%
	60	0.53	0.71	-34%	34%
	120	0.51	0.64	-26%	26%
Rice noodle	30	0.50	0.86	-71%	71%
	60	0.29	0.78	-169%	169%
	120	0.39	0.59	-54%	54%
			Average difference	Bias = -470%	MAPE = 470%
Dry matter retention (DMt/DM0)					
Couscous	30	0.89	0.91	-2%	2%

Food	Time (min)	Averaged value		(y-x)/y *100	Absolute (y-x)/y*100
		***In vivo* (y)**	***In vitro* (x)**		
	60	0.73	0.89	-22%	22%
	120	0.63	0.87	-38%	38%
Rice couscous	30	0.84	0.73	14%	14%
	60	0.72	0.72	1%	1%
	120	0.59	0.69	-16%	16%
Rice grain	30	0.94	0.94	0%	0%
	60	0.84	0.95	-13%	13%
	120	0.69	0.95	-38%	38%
Pasta	30	0.84	0.97	-16%	16%
	60	0.77	0.90	-17%	17%
	120	0.73	0.97	-34%	34%
Rice noodle	30	0.88	0.95	-9%	9%
	60	0.81	0.98	-21%	21%
	120	0.72	1.02	-42%	42%
			Average difference	Bias = -17%	MAPE = 19%

Supplemental Table 5 *In vivo-in vitro* linear regression coefficients (slope, intercept), correlation coefficient (*r*), and the significance of the correlation (*p*) for the digestion parameters examined in case study 3 (Section 3.3.3), examined at individual food type level. Significant correlation is present when $p < 0.05$.

	Couscous	Pasta	Rice couscous	Rice grain	Rice noodle
Moisture content of solid fraction					
Slope	1.38	0.88	8.08	0.77	2.35
Intercept	-1.79	0.66	-26.85	0.79	-4.72
r / R^2	0.99/0.99	0.57/0.32	0.93/0.87	0.91/0.82	0.97/0.94
p	0.070	0.617	0.237	0.276	0.164
Normalized hardness (Ht/H0)					
Slope	0.46	0.62	-0.42	0.08	0.25
Intercept	0.01	0.11	0.14	0.46	0.21
r / R^2	0.99/0.99	1.00/0.99	-0.97/0.95	0.25/0.06	0.32/0.10
p	0.076	0.052	0.149	0.839	0.793
DM retention					
Slope	6.28	0.08	5.80	-23.53	-2.54
Intercept	-4.84	0.70	-3.40	23.15	3.30
r / R^2	0.98/0.97	0.06/0.004	0.97/0.94	-0.94/0.88	-1.00/1.00
p	0.117	0.960	0.155	0.225	0.040

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