Electronic Supplementary Material (ESI) for Food & Function.

Supplement A: TIM-1

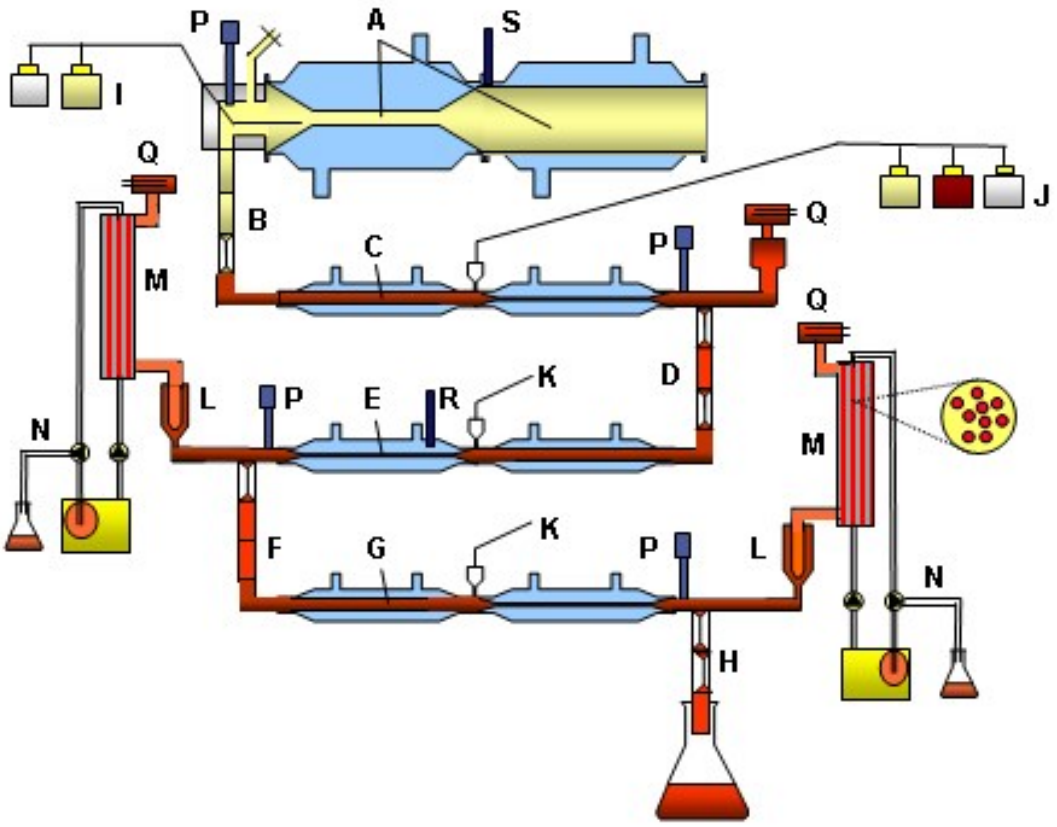

Figure S1. Schematic diagram of the dynamic, multi-compartmental TNO in vitro model of the stomach and small intestine (TIM-1). A. stomach compartment; B. pyloric sphincter; C. duodenum compartment; D. peristaltic valve; E. jejunum compartment; F. peristaltic valve; G. ileum compartment; H. ileocecal sphincter; I. stomach secretion; J. duodenum secretion; K. jejunum/ileum secretion; L. pre-filter; M. semi-permeable membrane; N. water absorption; P. pH electrodes; Q. level sensors; R. temperature sensor; S. pressure sensor. Reprinted from Keller et al. *(2017) with permission*[44].

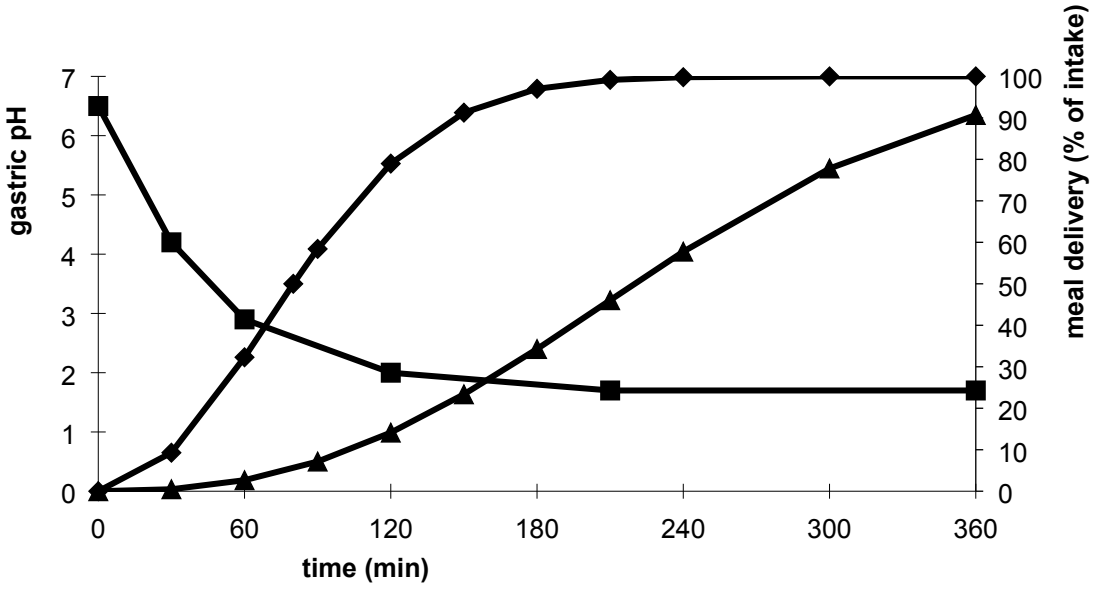

Figure S2. Gastric delivery (♦), ileal delivery (▲) [both expressed as percentage of the ingested meal], and the gastric pH (■) curves as mimicked in TIM-1 over time, representing adults.

Supplement B: Details on method of analysis for protein-bound and free dAGEs

In this study, every sample was analysed in both the hydrolysed (total dAGEs, i.e. free + protein-bound dAGEs) and unhydrolysed form (free dAGEs). The sample preparation is based on an earlier studies [1, 16] and similar to our previous study[3]. Briefly, sodium borate (0.33 M; pH 9) and sodium borohydride (2 M) were added to an aliquot of the samples (equivalent to ~1 mg protein) to obtain a sodium borohydride concentration of 0.2 M in the samples. These sample solutions were incubated for 4 h at room temperature. Subsequently, 1 mL of a chloroform/methanol (2/1 (v/v)) mix was added, and the samples were centrifuged for 10 min at 12,000 rpm. The chloroform phase was discarded and the remaining solution containing proteins was hydrolysed in 6 M HCl at 110°C for 18 h. The solutions were evaporated until dryness at 50°C under a stream of nitrogen and dissolved in 1 v/v% aqueous TFA (1 mL). Prior to UPLC-MS/MS analysis, internal standards and MilliQ were added to 50 µL of these analyte solutions to achieve a final volume of 200 µL. The free dAGE content in the samples was analysed by omitting the hydrolysis step in the sample preparation procedure, thereby circumventing the release of protein-bound dAGEs.

The sample extracts were analysed using an Acquity UPLC system (Waters, Milford, MA, USA) equipped with a BEH C18 analytical column (100 x 2.1 mm, 1.7 µm particle size, Waters, Milford, MA, USA)). The mobile phases were 5 mM NFPA in water (A) and acetonitrile (B), and the flow was set at 0.4 mL/min. The mobile phase gradient was as follows: a linear increase from 100% A (t = 0.5 min) to 70% A (t = 5 min.) followed by a linear increase to 100% B (0% A) at 8.5 min, keeping 100% B to t=10.3 min and returning back to initial conditions again (100% A), allowing a 2 min equilibration at these conditions. The column was kept at a constant temperature of 35°C. A Qtrap 6500 triple quadrupole mass spectrometer (Sciex) was connected to the UPLC was operated in positive electrospray ionization (ESI+). The capillary voltage was set to 4.5 kV, and the source temperature was 500°C, respectively. The curtain gas was set at 35 and the (instrument specific) gas 1&2 were set at 60. The injection volume was 5 µL. Quantification was performed using an internal standard approach and nine-point calibration curves (matrix matched standards). Quantification was performed using the precursor-product ion multiple reaction monitoring (MRM) transitions reported in below Table S1. The accuracy of the analysis was monitored by spiking each sample with a dAGEs standard. The average accuracies ranged from 75-133% (see Table S2), demonstrating that no severe losses occurred during sample preparation, and no signal enhancements or suppression occurred during UPLC-MS/MS analysis. Ion ratios between the quantifier ion and qualifier ion were monitored. In case ion ratios deviated more than 20% from the ratio observed in the standard, then the identity of the peak could not be confirmed. In the case of MG-H1 and G-H1, ion-ratio deviations were observed in GC, ginger cookies and apple juice. A brief investigation into this issue revealed that isomers of MG-H1 (i.e. MG-H2 and MG-H3) and G-H1 (i.e. G-H2 and G-H3) may co-exist in the samples. Additional explorative experiments showed that these may co-elute and alter the ion-ratios (data not shown). Future work is needed to explore this work further, but with the isomers issue in consideration, deviating ion ratios were accepted for MG-H1 and G-H1. It is expected that this also played a role in the slightly elevated (or lower) accuracies for MG-H1 and G-H1 (Table S2). The limit of quantification (LOQ) is provided in Table S3. The LOQ for the protein-bound dAGEs was 125 µg/L and 10 µg/L for the free dAGEs (Table S3). This 12.5 fold difference can be explained by omitting the acid hydrolysis step, which introduces a dilution of the sample. By omitting this step, the sample is not diluted, resulting in lower LOQs.

Table S1. MS/MS settings for the UPLC analysis of dAGEs in glycated casein, ginger biscuit and apple juice.

dAGE	Precursor > product ion (m/z)	Collision cell exit potential (CXP)	Collision energy (CE) (eV)	Declustering potential (V)
CML	205 > 130 205 > 84	6 14	17 25	51 51
CEL	219 > 130 219 > 84	14 10	19 27	60 60
MG-H1	229 > 114 229 > 70	8 10	21 37	50 50
G-H1	215 > 152 215 > 116	14 8	20 19	50 50

Table S2. Average (+/- SD) accuracies of spiked dAGEs standards in glycated casein, ginger biscuit and apple juice.

Sample		CML (%)	CEL (%)	MG-H1 (%)	G-H1 (%)
Glycated casein	Total dAGEs	106 (13)	114 (7)	107 (21)	103 (23)
	Free dAGEs	100 (4)	105 (4)	75 (32)	92 (12)
Ginger cookie	Total dAGEs	100 (5)	96 (5)	133 (50)	96 (8)
	Free dAGEs	98 (4)	103 (4)	91 (37)	91 (12)
Apple juice	Total dAGEs	99 (11)	104 (10)	106 (13)	106 (13)
	Free dAGEs	102 (6)	101 (6)	116 (14)	101 (11)

Table S3. Limit of quantification of the dAGEs in this study

Sample		CML	CEL	MG-H1	G-H1
Glycated casein	Total dAGEs	125	125	125	125
	Free dAGEs	10	10	10	10
Ginger cookie	Total dAGEs	125	125	125	125
	Free dAGEs	10	10	10	10
Apple juice	Total dAGEs	125	125	125	125
	Free dAGEs	10	10	10	10