# Supplementary: computer programming of the bi-compartmental elderly or adult dynamic digestion models and further supporting results

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Development and application of *in vitro* digestion models has become a vivid field of research. To this end, increasing accessibility, method harmonization and model validation have become of great importance[1, 2]. Within such efforts, this research attempts to bring forward readily and highly bio-relevant models based on commercially available equipment and software. This supplementary material describes in detail the development of the software used to control the bioreactors and to provide additional evidence on the ability of the models to detect differences in protein breakdown.

**Computer programming and mathematical definitions.** To enable bio-relevant mirroring of the dynamic characteristics of gastro-duodenal digestion, "*BioXpert*" software was programmed. This supplementary material depicts the programming procedures of the adult model, which can be easily modified to the elderly models through adjusting the numeric values within the procedures. The set of procedures programmed to control V1 was intended to recreate the gastric mixing pattern, pH gradient and gastric emptying, as described in **Table 1** of the paper. Due to the reported contractile nature of the stomach[3], the stirrer of the gastric bioreactor was programmed to operate through the following procedure:

```
IF stirrer=0
Stirrer=200
ELSE
Stirrer=0
ENDIF
```

This programming enabled achieving an average mixing profile of one to two mixing events of 200 RPM per min which concurs with the gastric contractions measured *in vivo*[4, 5]. Post-prandial gastric

pH gradient measured in healthy adults[6] was introduced into the software through a series of set points defining time and pH during the experiment, as described in **Table I**. Gastric emptying was programmed through the following program procedure:

```
IF TIME>=5
A =2-0.0463386*power(TIME,0.7)
B =power(2,A)
C =0.562091*B
pace =C/power(TIME,0.3)
pump4 =pace/0.07487
ELSE
Pump4=0
ENDIF
```

The program was also designed to control V2 for two major digestive constituents: digesta neutralization and bile secretion, as described in detail in **Table 1** of the paper. Bile secretion has been reported to be gradual in healthy adults ingesting a liquid meal, with two distinct rates of secretion: first rapid secretion for 5 min from the beginning of the meal, i.e. at the beginning of gastric emptying, followed by a moderate secretion[7, 8]. The control program designed in the study accounted for such dynamics of bile secretion through the following procedure:

```
IF TIME<=13 AND TIME>8
pace =0.061
pump4 =pace*100
ENDIF
IF TIME>13
Pace =0.003
Pump4 =pace*100
ENDIF
```

## Implementation of the bi-compartmental models to probe protein digestibility

Once the *in vitro* adult and elderly models were set up, the proteolytic breakdown of whey protein isolate, as a realistic product, was evaluated and outcomes of adult and elderly digestion experiments are presented in **Figure 1** herein. This qualitative SDS-PAGE analysis demonstrated that the semi-continuity of the bi-compartmental model enabled portions of intact whey proteins to

be introduced into the duodenum. This is believed to be a more realistic representation of digestion than batch models in which gastric emptying is unaccounted for and therefore duodenal digestion is routinely performed on fully digested samples. A similar observation was made in the corresponding elderly model (**Figure 1B**). Overall, these findings yielded more bio-relevant results, yet, this model focused solely on protein breakdown. Thus, it could be further improved, based on the additional information provided herein. Specifically, accounting for amylolytic and lipolytic activity in the stomach and duodenum of adults and the elderly could be applied based on the gathered information.

## Materials

Food grade whey protein isolate (WPI) (K-PRO 1050, 80% protein) was kindly donated by BA`EMEK Advanced Tecnologies Ltd. (Afula, Israel). All other materials and methods are described in the paper.

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## List of Tables and Figures

**Table I.** Definition of set points used to generate adult or elderly gastric pH profiles.

| Adult pH set points | | Elderly pH set points | |
|---|---|---|---|
| **Time [hr]** | **pH value** | **Time [hr]** | **pH value** |
| 0:00 | 4.5 | 0:00 | 6.2 |
| 0:05 | 3.9 | 0:30 | 5.0 |
| 0:09 | 3.4 | 1:00 | 4.5 |
| 0:10 | 3.2 | 1:30 | 4.0 |
| 0:15 | 3.0 | 2:00 | 3.6 |
| 0:20 | 2.8 | 2:30 | 3.2 |
| 0:40 | 1.8 | 2:40 | 3.0 |
| 1:00 | 1.7 | 2:45 | 3.0 |
| 2:00 | 1.5 | 2:50 | 3.0 |
| | | 2:55 | 2.0 |
| | | 3:00 | 2.0 |

**Figure 1.** SDS-PAGE analyses of WPI digesta collected during simulated digestion under (A) adult and (B) elderly conditions.