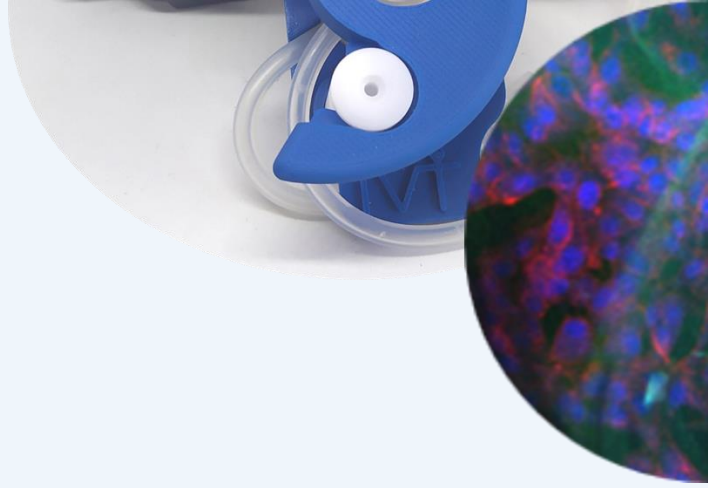


DIGESTION & METABOLISM: A NEW IN-VITRO PERSPECTIVE

May is the month dedicated to the awareness of the digestive diseases and disorders.
These conditions involve one or more organs of the gastrointestinal (GI) tract, and they range from mild to severe, such as gastrointestinal reflux, irritable bowel syndrome and cancers (e.g., liver, colon, kidney).[1]
Some risk factors, which lead to the occurrence of the gastrointestinal disorders, are represented by overweight and obesity.[2]
According to the data published by the World Health Organization (WHO)[3], the worldwide obesity has nearly tripled since 1975.
Moreover, this problem has started to occur also in the urban setting of low- and middle-income countries, where the co-existence of obesity and undernutrition is not so uncommon. *(Fig. 1)*

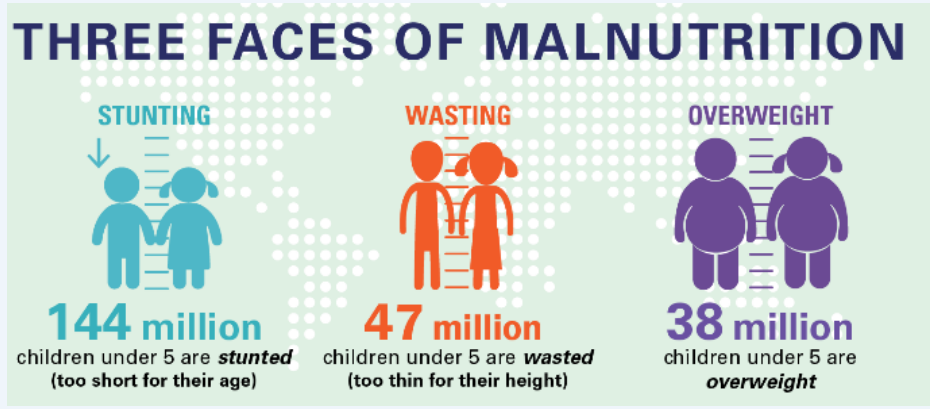
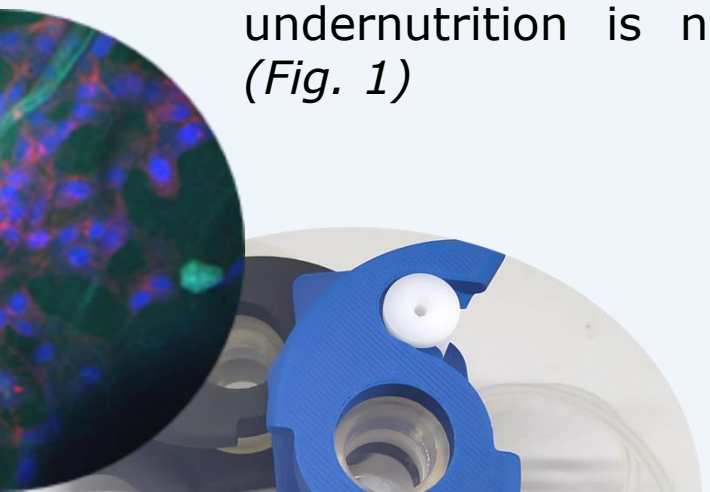


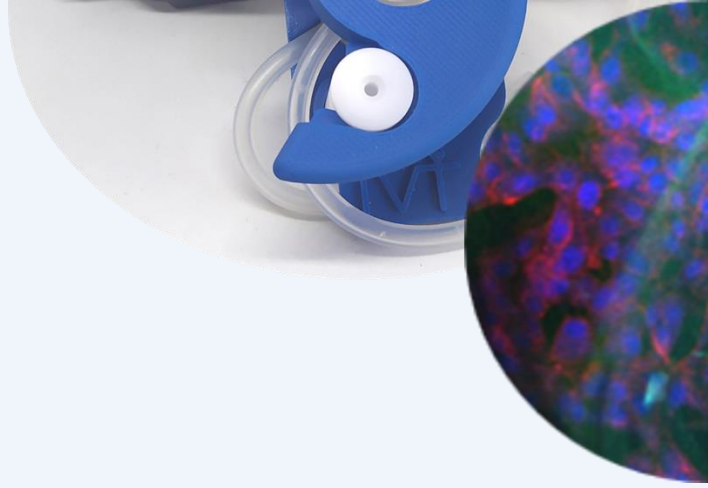
Fig. 1: The side effects of malnutrition in children in 2020 [3]

These data highlight the need to slow down this trend, considering that these conditions are correlated with the onset of some chronic diseases – *i.e., cardiovascular and respiratory diseases, diabetes or cancer* – known to be the leading causes of death worldwide.

In the next paragraphs, we deal with the use of advanced in-vitro models as a tool to evaluate several processes associated with the Human GI tract.



[1] https://www.hopkinsmedicine.org/
[2] Emerenziani S., *et al.*, *Role of Overweight and Obesity in Gastrointestinal Disease*, Nutrients vol 12,1 111. 31 Dec. 2019, doi:10.3390/nu12010111
[3] https://www.who.int/

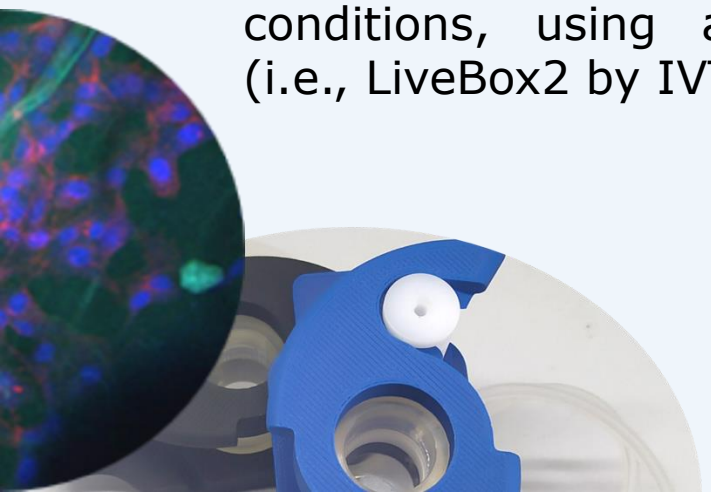


PERMEABILITY EVALUATION OF THE INTESTINAL BARRIER

The intestinal epithelium is one of the most investigated tissues of the GI tract, considering its role in the absorption of substances (i.e., foods, drugs). The Caco-2 cell culture is widely accepted as an efficient tool to assess the permeability of exogenous compounds through the barrier. However, since the in-vivo permeation is influenced by several factors, there is a need to increase the complexity of the model, introducing a dynamic stimulation.

Starting from these considerations, Costa *et al.,*[4] have evaluated the cellular uptake of a membrane protein (P-gp), measuring the efflux of its substrate Rhodamine-123.

The researchers have simulated the intestinal epithelium cultivating the Caco-2 cells both in static and dynamic conditions, using a fluidic chamber (i.e., LiveBox2 by IVTech srl).



Moreover, once the tissues were mature, they have been incubated with a non-cytotoxic curcumin solution, to assess its ability to inhibit the activity of the P-gp. *(Fig. 2)*

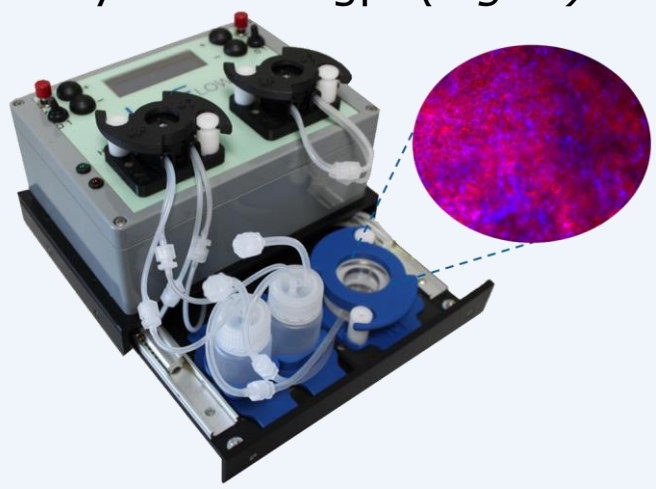
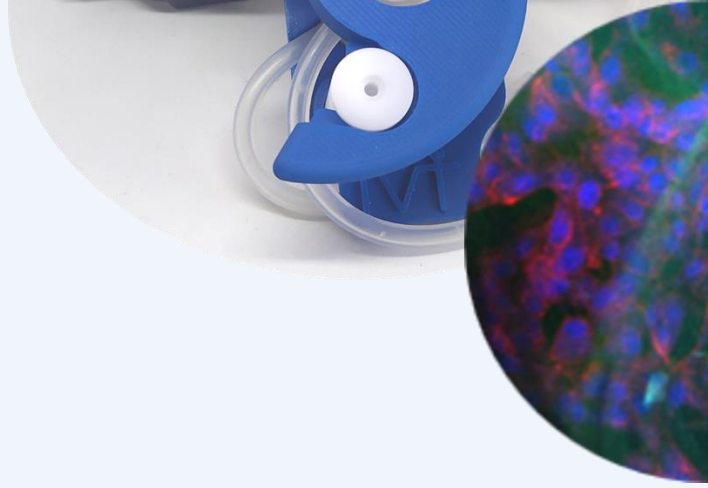


Fig. 2: Caco-2 cells cultivated in a LiveBox2 [4]
Day 20: Incubation with curcumin
Day 21: Efflux of Rhodamine-123

The obtained results have shown that the inhibitory effect of curcumin was more pronounced under dynamic conditions, due to the mechano-transduction effects, arising from the shear stress, associated with the flow of the culture medium.[4]

[4] Costa J., *et al.*, *A dynamic in-vitro model of the intestinal epithelium for the investigation of the P-glycoprotein activity*, Research Square, 07 Jul 2020, https://doi.org/10.21203/rs.3.rs-24178/v2



IN-VITRO SIMULATION OF THE DIGESTIVE PROCESS (1)

The digestive process begins in the mouth, with the primary digestion, to continue into the stomach.
The intestinal compartment is one of the most investigated, following a single-tissue approach. However, the digestive process is influenced by the cross-modulation between different tissues, highlighting the need to develop a multi-organ approach, where different organs communicate through an exchange of liquid (i.e., cell culture medium), imposing a mechanical stress on the pathway.
In the study carried out by Colombo *et al.,*[5] the development of an advanced in-vitro model to study the digestive process of methylglyoxal (MGO) is introduced. More in detail, the gastric cells (GIST-882) and the intestinal cells (Caco-2) have been independently cultivated using 2 fluidic chambers (i.e., LiveBox1 and LiveBox2 by IVTech srl). *(Fig. 3)*

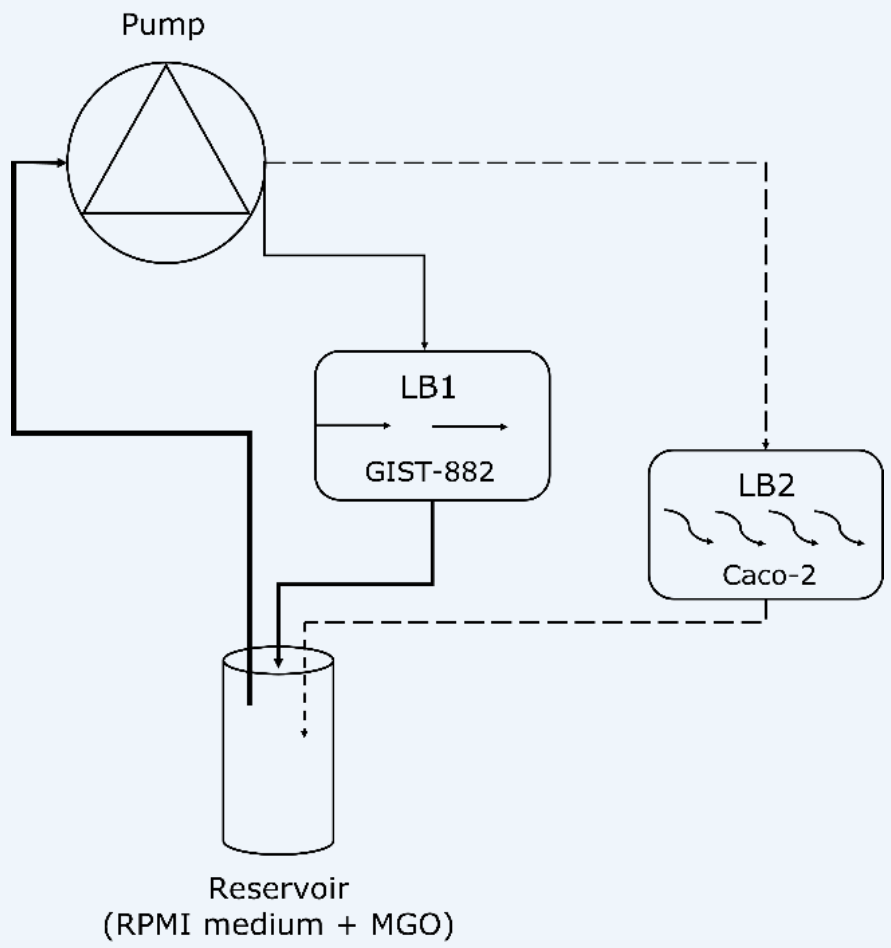
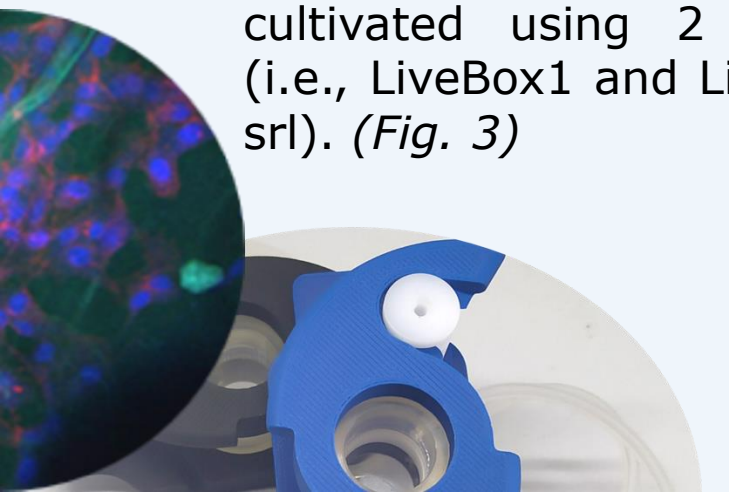
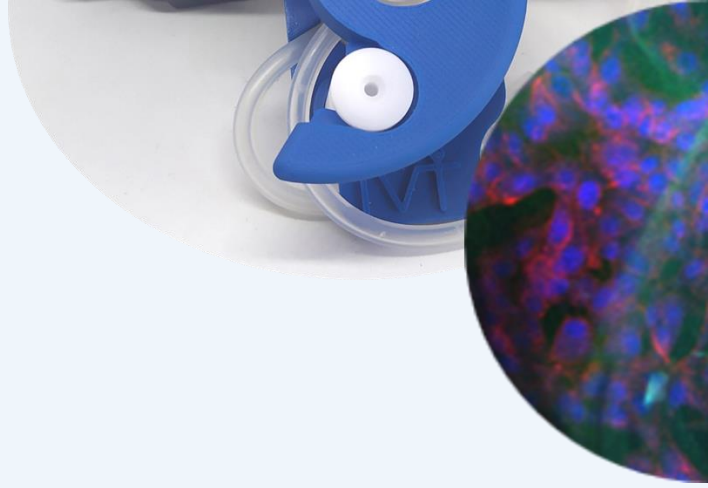


Fig. 3: Representation of the fluidic circuit. The black line is referred to the gastric circuit, the dashed line to the intestinal circuit and the marked line to the common medium input [5]



[5] Colombo R., *et al., A new millifluidic-based gastrointestinal platform to evaluate the effect of simulated dietary methylglyoxal intakes*, Food Funct., 2019, doi:10.1039/C9FO00332K



IN-VITRO SIMULATION OF THE DIGESTIVE PROCESS (2)

The GIST-882 cells have been exposed to a medium flow, added with MGO at different concentrations for 4h (e.g., time required for a complete digestion).
Then, the conditioned medium has been used to treat the Caco-2 cells, to mimic the physiological digestive process, avoiding the recirculation of the same medium through the gastric compartment.[5]
Further analyses have shown that the MGO metabolization start in the gastric compartment, as demonstrated by its reduction in all the concentrations tested.
Moreover, the exposition to Caco-2 cells has gradually reduced its concentration until its complete metabolization after 24h for all the tested concentrations. *(Fig. 4)*

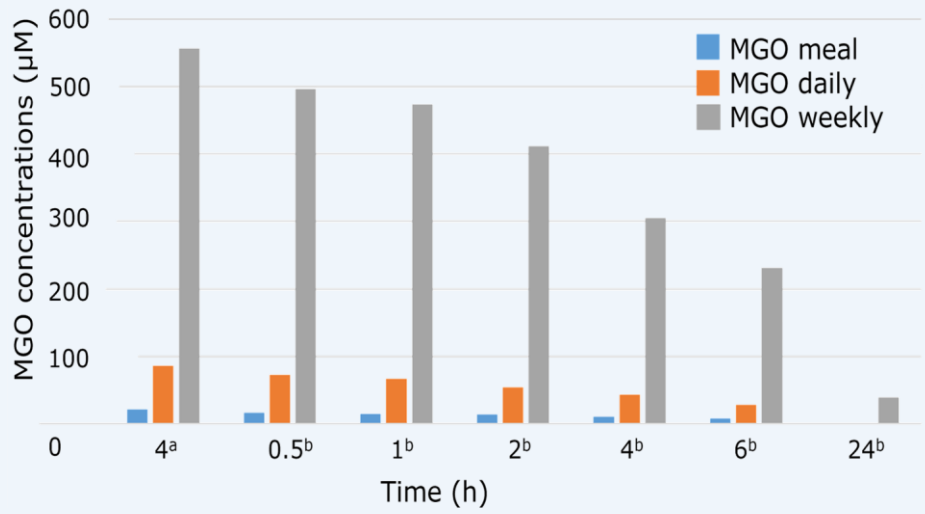
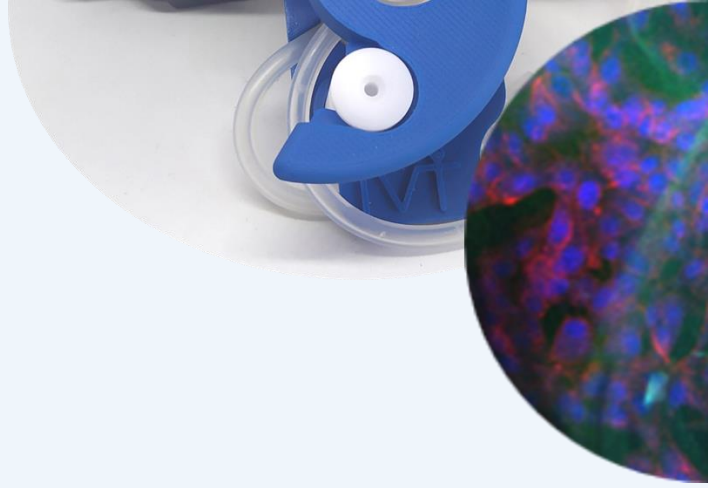


Fig. 4: Monitoring of MGO extracellular concentration in the dynamic model [5]
[a] passage through the gastric chamber
[b] passage through the intestinal chamber

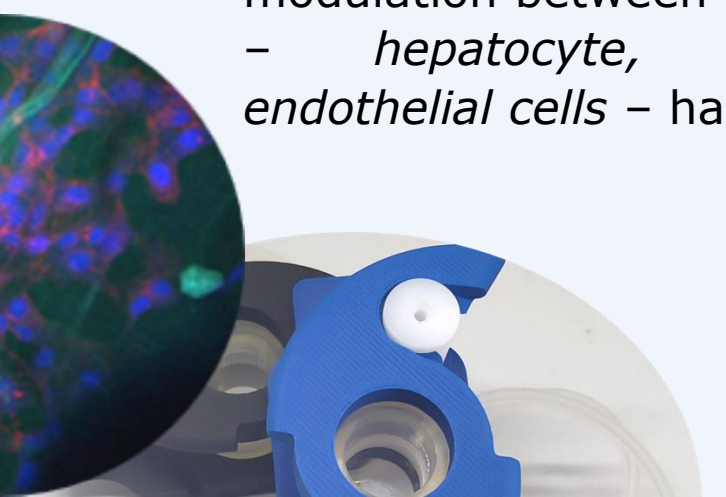
This trend has been confirmed by the data obtained from the static model. However, the evidence arising from the dynamic model have highlighted the role of the gastric cells in the first phase of MGO metabolization.
In conclusion, this model can be considered as a tool to study a physiological process as well as to monitor in real-time the effects of several compounds.[5]

[5] Colombo R., *et al., A new millifluidic-based gastrointestinal platform to evaluate the effect of simulated dietary methylglyoxal intakes*, Food Funct., 2019, doi:10.1039/C9FO00332K



IN-VITRO MODEL OF CENTRAL OBESITY (1)

Overweight and obesity are defined as an abnormal or excessive fat accumulation.[3]
These pathologies lead to an alteration of the metabolism of the tissues involved in the maintenance of energy homeostasis in the human body.
The side effects arising from these conditions can be assessed in-vitro developing a multi-organ approach (MOA), which allows to study the cross-modulation between different tissues, evaluating how they contribute to generate a synergic response.
In the study carried out by Ahluwalia *et al.,*[6] the development of a modular in-vitro model to evaluate the glucose and lipid metabolism has been introduced. Moreover, the cross-modulation between different cells type – *hepatocyte, adipocyte and endothelial cells* – has been analysed.



The researchers have gradually increased the complexity of the system, both involving more tissues in the metabolic pathway as well as varying the percentage of the adipose tissue (AT), simulating three different scenarios: normo-weight (12% AT), overweight (25% AT) and obese (35% AT). Each tissue has been independently cultivated in a fluidic chamber (i.e., LiveBox by IVTech srl and µSlide by Ibidi GmbH). Then, once they were mature, they have been interconnected, as shown in *Fig. 5.*

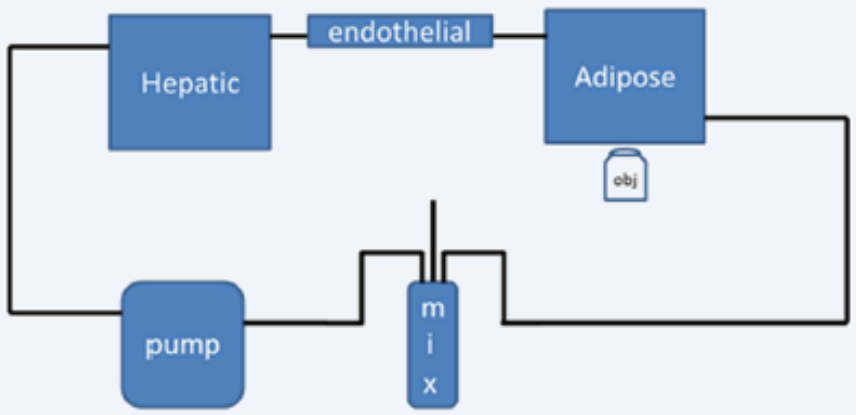
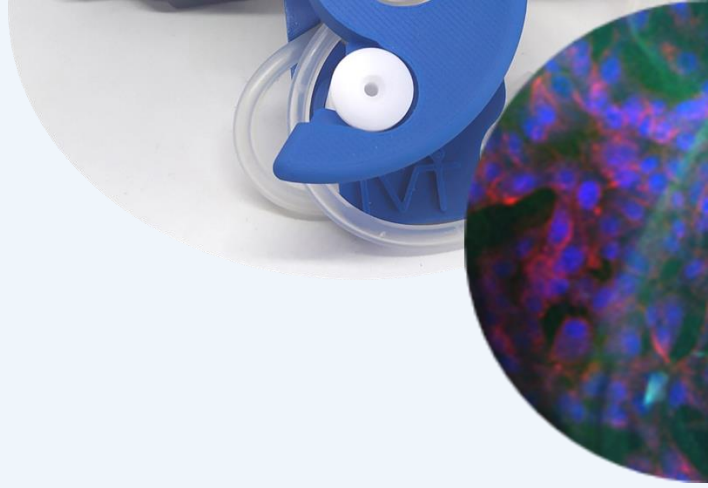


Fig. 5: Representation of the 3-way circuit [6]

[3] https://www.who.int/
[6] Ahluwalia A., *et al.,* (2018), *Systemic and vascular inflammation in an in-vitro model of central obesity*, PLoS ONE 13(2): e0192824, doi.org/10.1371/journal.pone.0192824



IN-VITRO MODEL OF CENTRAL OBESITY (2)

After 24 hours under dynamic stimulation, the production of lipid metabolites and pro-inflammatory markers has been analysed, to determine their correlation with the amount of adipose tissue and/or with the crosstalk between different tissues. More in detail, it has emerged that the release of glycerol (GLY) and triglycerides (TRG) was dependent on the percentage of AT and on the complexity of the system. *(Fig. 6)*

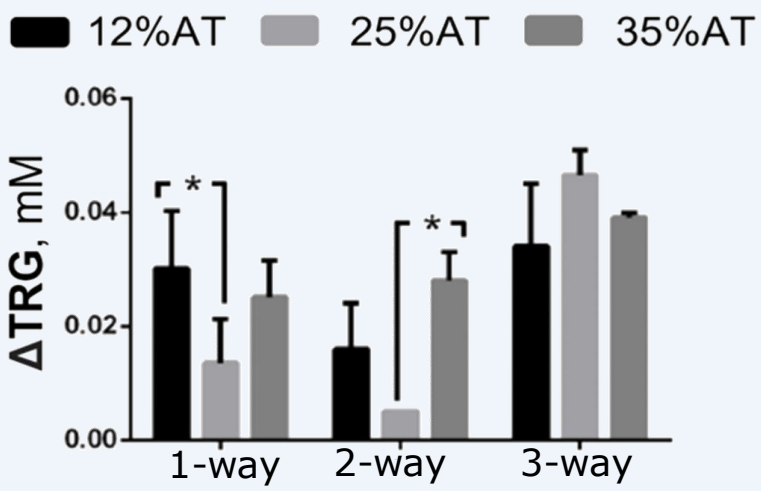
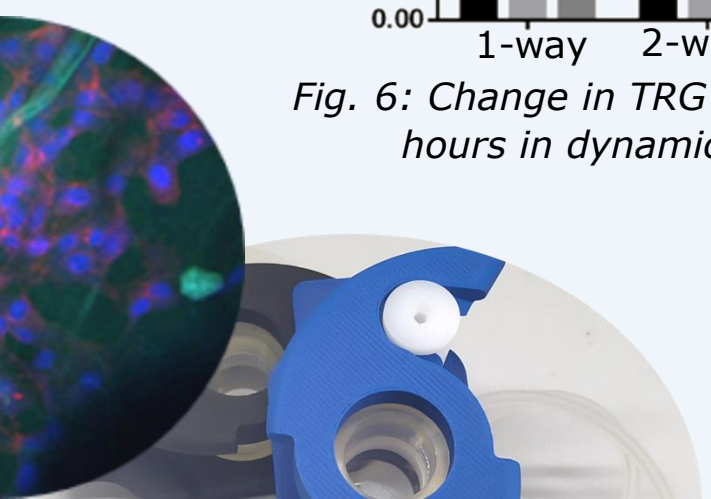


Fig. 6: Change in TRG metabolites after 24 hours in dynamic conditions [6]



The same trend has been observed analysing the production of the pro-inflammatory markers, such as Interleukin-6 and E-selectin, which is an indicator of endothelial damage. Indeed, endothelial dysfunctions are highly related with the cardiovascular complications, known to be a consequence of obesity. *(Fig 7)*

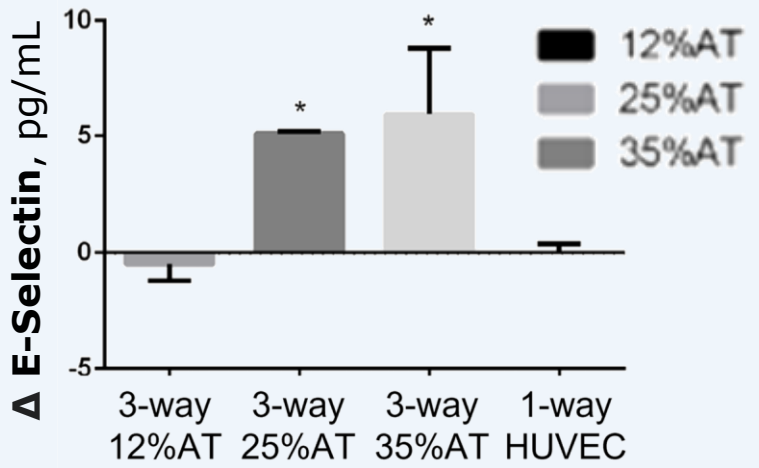
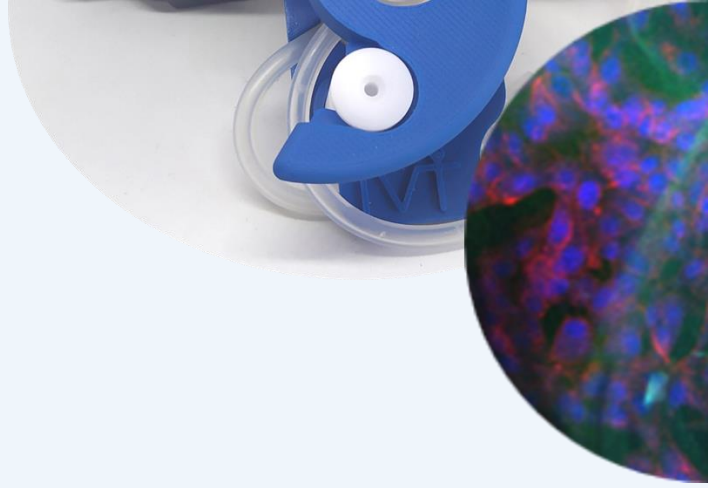


Fig. 7: Evaluation of E-selectin production [6]

These data have confirmed the link between the increase in the level of AT and the occurrence of a pro-inflammatory state and endothelial stress.[6]

[6] Ahluwalia A. *et al.,* (2018), *Systemic and vascular inflammation in an in-vitro model of central obesity*, PLoS ONE 13(2): e0192824, doi.org/10.1371/journal.pone.0192824



TAKE-HOME MESSAGES

This overview is about the use of advanced in-vitro models to study the GI tract and the related digestive process/diseases. More in detail, we have introduced three in-vitro models, developed to evaluate both physiological as well as pathological scenarios.
We want to provide some food for thoughts about:

- The advantages related with the presence of a **dynamic environment**, where the medium flows as blood in human circulation. Indeed, the flow of the medium improves the cells viability and metabolism, inducing a mechano-transduction effect on the surface of the tissues

- The complexity of the model can be increased step by step, following a **modular approach**. This strategy allows to evaluate complex scenarios, characterized by the interconnection between different tissues, in cross-modulation through an exchange of liquid

The advanced in-vitro models provide an approximation of the human reality. However, we consider the commercially available **fluidic platforms** as a valid tool to increase the predictivity of these models, leading to obtain results more predictive of the human reality.