Journal of Animal and Feed Sciences, 34, 2, 2025, xxx–xxx, https://doi.org/10.22358jafs/197869/2025
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

ORIGINAL PAPER

ARTICLE IN PRESS

Effect of co-incubation of HPAd-derived adipocytes with enterocytes on inflammatory agents expression and bacterial adhesion in Caco-2-derived enterocytes: a preliminary study

H. Antushevich[1,*], M. Wójcik[1], M. Załęcki[2], K. Wojtulewicz[1], M. Matusiewicz[3] and J. Bochenek[1]

[1] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Department of Genetic Engineering, Instytucka 3, 05-110 Jabłonna, Poland
[2] University of Warmia and Mazury, Department of Animal Anatomy, Faculty of Veterinary Medicine, 10-719 Olsztyn, Poland
[3] Warsaw University of Life Sciences, Department of Nanobiotechnology, Institute of Biology, 02-786 Warsaw, Poland

KEY WORDS: adipocytes, bacterial adhesion, enterocytes, lipopolysaccharide, inflammation

Received: 12 December 2024
Revised: 17 December 2024
Accepted: 17 December 2024

* Corresponding author:
e-mail: a.antuszewicz@ifzz.pl

ABSTRACT. White adipose tissue is the largest endocrine organ in the body, which through endocrine or paracrine mechanisms, communicates with several tissues. Our preliminary study involved two independent experiments. The primary objective of Experiment 1 was to examine the effect of co-incubating Caco-2-derived enterocytes with human preadipocytes (HPAd)-derived adipocytes on cellular processes in the studied cells. Experiment 2 aimed to explore the impact of co-incubation of adipocytes with Caco-2-derived enterocytes on bacterial adhesion of conventional and pathogenic bacterial strains to Caco-2 cells and their viability after treatment with the studied bacteria. Caco-2 cells were seeded in inserts, and on the day of the experiment, these inserts with fully differentiated enterocytes were transferred to plates containing fully differentiated adipocytes. This approach created a model where metabolites from adipocytes contacted enterocytes from the opposite side of the intestinal lumen, simulating the conditions found in obese individuals, where visceral fat covers internal organs, including the intestines. Furthermore, due to the observed persistent inflammation in obese individuals resulting from the excess visceral fat, some groups of HPAd cells were stimulated with lipopolysaccharide (LPS) to induce the synthesis of inflammatory agents. Based on the data obtained in *in vitro* experiments, it can be concluded that the co-incubation of Caco-2 cells with adipocytes positively affects cellular processes occurring in Caco-2 cells by reducing the expression of proinflammatory cytokines and apoptosis markers. Moreover, pre-incubation of Caco-2 enterocytes with adipocytes protects these cells against *Salmonella enterica* by reducing bacterial adhesion and increasing the viability of Caco-2 cells. Incubation of Caco-2-derived enterocytes with LPS-pre-treated adipocytes did not cause significant changes compared to non-treated adipocytes.

Introduction

The manuscript is focused on an important topic for animals and human health – intestinal health in the context of obesity on the molecular level. Obesity is usually associated with morbidity related to cardiovascular diseases or diabetes mellitus, but also, it is a direct cause of many gastrointestinal diseases. There is a strong correlation between increased body mass index (BMI) and the development of intestinal inflammation, acute pancreatitis, and liver disease (Stabroth-Akil et al., 2015; Parekh et al., 2018; Wypych et al., 2024). Obese individuals are more susceptible to illnesses due to the

excess of adipose tissue in their organisms. This tissue covers organs in the body and on the paracrine level can affect multiple biological systems (Fain et al., 2004).

Adipose tissue is a heterogeneous endocrine organ that performs many vital functions in the body (Richard et al., 2000). The roles of adipose tissue are determined by the origin and composition of its cells (Richard et al., 2000). To date, various types of adipocytes have been identified and isolated, including yellow adipocytes – present in the marrow of long bones and spine, pink adipocytes – found in breast tissue, beige adipocytes – located in subcutaneous white adipose tissue, brown adipocytes – mainly forming small clusters in the upper chest, and white adipocytes – situated in subcutaneous and visceral depots, responsible for their expansion in obesity (Giordano et al., 2014). It should be noted that white adipocytes are the dominant fat cells in the body (Kershaw and Flier, 2004). The maturation of adipocytes occurs through the process of adipogenesis involving 2 phases: determination and terminal differentiation, wherein mesenchymal stem cells (preadipocytes) differentiate into mature adipocytes (Hutchings et al., 2020). During the determination phase, pluripotent stem cells transform into unipotent preadipocytes (Ambele et al., 2020), and in the terminal differentiation phase, preadipocytes acquire the phenotype and functional characteristics of mature adipocytes. Under both physiological conditions and in many diseases, mature adipocytes can dedifferentiate and reacquire the properties of preadipocytes or pluripotent stem cells. For example, through transdifferentiation, white subcutaneous adipocytes can transform into pink and beige adipocytes, and dermal adipocytes can undergo a transformation into myofibroblasts and participate in wound healing processes (Ambele et al., 2020).

White adipose tissue is the largest endocrine organ in the body that exerts its effects through endocrine, paracrine or autocrine mechanisms and communicates with the central nervous system, muscle tissue, blood vessels, kidney, pancreas and various other organs and tissues (Kershaw and Flier, 2004; Yi and Tschöp, 2012; Rome, 2022; Wypych et al., 2023). Visceral adipocytes play a crucial role by expressing and secreting several biologically active substances known as adipokines (Chait and den Hartigh, 2020). Among these, visceral adipocytes can produce enzymes involved in the metabolism of glucocorticoids and sex steroids, proteins of the renin-angiotensin system (RAS), resistin, acylation-stimulating protein (ASP), adipsin, adiponectin, plasminogen activator inhibitor (PAI)-1, macrophages and monocyte chemoattractant protein (MCP)-1, proinflammatory cytokines (interleukines: IL-1β, IL-6, and tumour necrosis factor α (TNFα), leptin, apelin and more (Ambele et al., 2020; Wypych et al., 2023; Szlis et al., 2024). Consequently, the bodies of obese individuals are subject to a chronic, low-grade proinflammatory state (Chait and den Hartigh, 2020; Elmas et al., 2022).

Metabolites from adipose tissue, transported through the bloodstream, reach cells in the gastrointestinal tract, including enterocytes, goblet cells, enteroendocrine cells, stem cells, and others. These metabolites affect the metabolism of these cells and influence physiological processes occurring in the intestines (Swierczynski and Sledzinski, 2012; Tsai et al., 2020).

It should also be noted that the gastrointestinal tract forms a complex ecosystem consisting of intestinal epithelial cells (enterocytes), immune cells and gut microbiota (McCracken and Lorenz, 2001; Shi et al., 2022; Wahid et al., 2022; Dunisławska et al., 2023). Enterocytes play a dominant role in the intestines, contributing significantly to digestion by ensuring the uptake of ions, water, nutrients, vitamins and absorption of unconjugated bile salts. Enterocytes are involved not only in the chemical processing of food, but also in the induction of immunological tolerance and bacterial adhesion to these cells (Miron and Cristea, 2012; Šikić Pogačar et al., 2020). Enterocytes possess specialized receptors belonging to the pathogen recognition receptors (PRR) family, including Toll-like receptors and nucleotide oligomerization domain (NOD)-like receptors. These receptors recognize highly conserved molecular structures in bacteria—microbe-associated molecular patterns (MAMPs)—triggering the activation of inflammatory mechanisms (Miron and Cristea, 2012). The key process for both commensal and pathogenic bacteria to survive and colonize the gastrointestinal tract is adhesion to gut epithelial cells (Ageorges et al., 2020; Šikić Pogačar et al., 2020; Zeng et al., 2021). It has been observed that the composition of the gut microbiota of obese individuals is different compared to those with normal body weight (Ettehad Marvasti et al., 2020; Li et al., 2021). It is known that intestinal flora can regulate energy absorption and nutrient metabolism, increasing energy accumulation from the diet, and it has been hypothesized that gut microbiota is one of the factors contributing to obesity (Xiao and Kang, 2020).

In the literature data, we can find a lot of information describing that obesity and adipokines promote the development of different diseases in the

organism, but there are no reports concerning the investigation of the direct *in vitro* effect of adipose tissue on the enterocyte cell condition. There is lack of data about mechanisms and signaling pathways explaining the direct paracrine action of adipocyte cells on bacterial adhesion. The proposed preliminary research is designed to verify the hypothesis that the adipose tissue metabolites by the paracrine way can influence the basic function of enterocytes and may act on the microbiota composition by influencing the bacterial adherence process. Therefore, the study aimed to determine in the *in vitro* model (using co-culture of adipocytes with enterocytes) how adipocytes in different conditions (normal and treated by pro-inflammatory agent lipopolysaccharide (LPS) influence the enterocyte cellular processes and the conventional and pathogenic bacterial strain's adhesion to the small intestine epithelium.

Material and methods

The experiments were carried out at the Department of Genetic Engineering of The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences. The Department has two state-of-the-art modern *in vitro* laboratories equipped for cell culture, holding the necessary permissions for working with cell lines. The study involved two independent experiments. The aim of Experiment 1 was to investigate the effect of co-incubation of differentiated human preadipocytes (HPAd) cells (human adipocytes) with differentiated Caco-2 cells (human enterocytes) on cellular processes in the latter cells. The objective of Experiment 2 was to examine the effect of co-incubation of human adipocytes with enterocytes on the adhesion of conventional and pathogenic bacterial strains to Caco-2-derived enterocytes and viability of these cells after treatment with the bacteria studied. Since excessive visceral adipose tissue in obese individuals leads to persistent inflammation in the body, certain groups of HPAd cells were subjected to stimulation with LPS to induce the synthesis of inflammatory agents.

Experiment 1

Prior to experiments, the cells were cultured separately until they acquired phenotypical features of enterocytes and adipocytes. HPAd cells (human preadipocytes 6 passage), were obtained from CELL Applications, Inc. (cat. no. 802S-05A; Sigma-Aldrich, St. Louis, MO, USA) were seeded in 24-well plates at a concentration of 9×10^4 adipocytes per well/ml. Initially, preadipocytes were cultured in Human Preadipocyte Growth Medium (Sigma-Aldrich, St. Louis, MO, USA), and subsequently differentiated into mature adipocytes using Human Adipocyte Differentiation Medium (Sigma-Aldrich, St. Louis, MO, USA). Following differentiation, HPAd cells were maintained in Preadipocyte Basal Medium (Sigma-Aldrich, St. Louis, MO, USA). The Caco-2 cell line (human enterocytes 59 passage) was obtained from European Collection of Authenticated Cell Cultures (ECCC) (cat. no. 86010202; Sigma-Aldrich, St. Louis, MO, USA) were seeded at 5×10^4 well/0.35 ml to 4.0-µm inserts (BD Bioscience, San Jose, CA, USA) and cultured in 24-well plates in 0.8 ml of Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA). Medium composition was as follows: 2 mM L-glutamine, 1% non-essential amino acids, 10% heat-inactivated foetal bovine serum, 10 IU/ml penicillin G, 100 µg/ml streptomycin sulphate and 250 ng/ml amphotericin B. HPAd and Caco-2 cells were cultured in an environment of 5% CO_2, 95% relative humidity at 37 °C, with the medium changed every other day. Before the beginning of the experiment, 0.1 µg/ml lipopolysaccharide (LPS) (Sigma-Aldrich, St. Louis, MO, USA) was added to the appropriate groups for 24 h to induce the synthesis of pro-inflammatory substances in adipocytes. Following this period, the medium was replaced with Dulbecco's modified Eagle's medium without LPS. On the day of the experiment, inserts with fully differentiated enterocytes were transferred to plates with adipocytes, and the cells were divided into the following groups (n = 4): Control Adipocytes (HPAd without Caco-2); Ad+LPS (HPAd stimulated with LPS); Ad+Caco-2 (HPAd+Caco-2); Ad+LPS+Caco-2 (LPS-pretreated HPAd+Caco-2); Control Caco-2 (Caco-2 without HPAd); Caco-2+Ad (Caco-2+HPAd); Caco-2+Ad+LPS (Caco-2+LPS-pretreated HPAd). Caco-2 cells were seeded in inserts and transferred to plates with fully differentiated adipocytes, creating a model where adipocyte metabolites contacted enterocytes from the opposite side of the intestinal lumen, resembling the conditions in an obese person where visceral fat covers all internal organs, including the intestines. The co-cultures were incubated for 24 h. After this period, adipocytes and enterocytes were examined for the expression of the following marker genes: proliferation (marker of proliferation Ki-67 (*MKI67*)), apoptosis (caspase 3 (*CAS3*)), DNA damage (8-oxoguanine DNA glycosylase (*OGG1*)), proinflammatory cytokines and receptors *TNF*, *IL6*; transient receptor potential

cation channel subfamily V member 1 (*TRPV1*), toll like receptor 2 (*TLR2*), toll like receptor 4 (*TLR4*)), and only in Caco-2 cells: adhesion receptors (integrin subunit alpha 1 (*ITGA1*), cadherin 1 (*CDH1*)) and proinflammatory cytokines *IL1B*. The analysis was conducted using real-time RT-PCR.

Experiment 2

The following bacterial strains were used in the experiment: *Lactobacillus paracasei* IBB2588 (IBB PAS, Warsaw, Poland) and *Salmonella enterica* subsp. *enterica* serovar Enteritidis KOS 1663 (The National *Salmonella* Centre, Gdansk, Poland). *L. paracasei* IBB2588 belongs to the commensal Gram-positive bacteria. This strain is added to the milk products, it is present in the human gastrointestinal tract and has probiotic properties. *S. enterica* subsp. *enterica* serovar Enteritidis KOS 1663 was isolated during *Salmonella* food infection outbreak (1988, Poland) from a commercial instant soup, bacateriophage type 1 (according to the Lalko phage collection). Before the experiment, the designated bacterial strains were cultured overnight in an appropriate liquid media. *S. enterica* was cultured in Luria-Bertani (LB) broth (Merck KGaA, Darmstadt, Germany) under aerobic conditions, while the *Lactobacillus* strain was cultured in MRS broth (Merck KGaA, Darmstadt, Germany) under anaerobic conditions in an anaerobic jar (OXOID Ltd, Basingstoke, UK) at 37 °C.

Caco-2 and HPAd cells were prepared as in Experiment 1, only using a medium without antibiotics and antimycotics. The co-cultured cells were grown for 24 h. On the day of the experiment, the co-culture plates from the '*In vitro* room' were transferred to the 'Microbiological room' where the inserts with Caco-2 cells had their medium changed to a medium with a 100-fold dilution of the studied bacterial strain pre-cultured overnight. The experiment included the following groups (n = 4): C (Caco-2 without bacteria), LD1 (Caco-2+*L. paracasei)*; LD2 (Caco-2+HPAd+*L. paracasei);* LD3 (Caco-2+LPS-pretreated HPAd+*L. paracasei*); SD1 (Caco-2+*S. enterica)*; SD2 (Caco-2+HPAd+*S. enterica);* SD3 (Caco-2+LPS-pretreated HPAd+*S. enterica*). The cells were incubated with bacteria for 3 h. Additionally, during the viability assay, a control group of Caco-2 cells without pre-incubation with adipocytes was added. After the incubation, viability was examined in both adipocytes and enterocytes, and bacterial adhesion was assessed in Caco-2 cells.

Cell viability assay

A mixture of 25 µl of trypan blue solution, 0.4% (Sigma-Aldrich, St. Louis, MO, USA) and 25 µl of cell suspension (1:1) was incubated for approximately 3 min at room temperature. Subsequently, the mixture was applied to a Bürker haematocytometer chamber (Merck KGaA, Darmstadt, Germany) and live and dead cells were counted in the squares under an inverted light microscope (40× objective; LSM Pascal, ZEISS, Oberkochen, Germany). Dead cells had a blue colour, while viable cells retained their original coloration. The obtained data were calculated using the following formula: total cells/ml = (total cells counted × dilution factor × 10000 cells/ml) / number of squares counted.

Bacterial adhesion assay

To analyse bacterial adhesion for Caco-2 cells, the experimental medium with bacteria was removed. The cells were then washed 3 times with sterile phosphate-buffered saline buffer (pH 7.4) and detached using trypsin-PBS solution (Sigma-Aldrich, St. Louis, MO, USA). Following trypsinisation, one portion of Caco-2 cells was used to calculate the total number of Caco-2 cells in a Bürker haematocytometer chamber (Merck KGaA, Darmstadt, Germany), whereas the second portion of Caco-2 cells with the adhered bacterial strain was diluted in physiological saline (*L. paracasei* 1×10^{-2}, *S. enterica* 1×10^{-4}). *L. paracasei* was plated on De Man–Rogosa–Sharpe agar plates (MRS broth supplemented with 1.5% agar, Merck KGaA, Darmstadt, Germany) and cultured under anaerobic conditions (in an anaerobic jar; OXOID Ltd, Basingstoke, UK) at 37 °C for 40–48 h. The pathogenic *S. enterica* was plated on lysogeny broth (LB) plates (LB supplemented with 1.5% agar, Merck KGaA, Darmstadt, Germany) and cultured at 37 °C for 18–20 h under aerobic conditions. After this time, colony-forming units (CFU) were calculated. The number of bacteria adhering to Caco-2 cells was expressed as CFU, and adhesion was expressed as the number of bacterial cells adhered to one Caco-2 cell.

Relative gene expression assay

Total RNA from cells was isolated using the NucleoSpin RNA kit (Macherey-Nagel, Duren, Germany), according to the manufacturer's instruction. The quantity and quality of RNA was assessed through spectrophotometric measurements of optical density using a NanoDrop instrument, at wavelengths of 260 nm, 280 nm and 230 nm,

and electrophoresis in 1% agarose gel. Subsequently, 500 ng of RNA from Caco-2 cells or 800 ng of RNA from adipocytes were transcribed into cDNA using the Maxima™ First Strand cDNA Synthesis Kit for RT-qPCR (ThermoFisher Scientific, Waltham, MA, USA). The resulting template was used in the real-time PCR reaction, which was performed using FIREPol® HOT EvaGreen qPCR Mix® Plus solution (Solis Biodyne, Tartu, Estonia) based on EvaGreen, a fluorescent DNA intercalating agent. The temperature profile for each gene was selected after optimization of the standard protocol: 15 min at 95 °C for HOT FIREPol® DNA Polymerase activation, followed by 40 denaturation cycles at 95 °C for 6 s, annealing at 60 °C for 20 s, and extension at 72 °C for 5 s. After the reaction, a final melting curve analysis under continuous fluorescence measurements was performed to evaluate amplification specificity, which was confirmed by product sequencing (Genomed, Warsaw, Poland). PCR reactions were carried out using a RotorGene Q thermocycler (Qiagen, Hilden, Germany). Gene-specific primers were designed using the freely available Primer-BLAST program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). With respect to relative expression, the results obtained for the genes tested (Table 1) were normalized to the reference housekeeping gene. The reference gene was selected from among actin beta (*ACTB*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and heterogeneous nuclear ribonucleoprotein L (*HNRNPL*) using the NormFinder program (http://www.mdl.dk/publicationsnormfinder.htm), which identifies the gene with the most stable expression in the experimental system.

Statistical analysis

All data were expressed as means ± SEM. Differences between groups were determined using one-way analysis of variance (ANOVA), followed by Fisher's post hoc test (Statistica v.10.0, Stat Soft. Inc., Tulsa, OK, USA). A significance level of $P < 0.05$ was assumed for all statistical analyses.

Table 1. Genes analysed using real-time RT-PCR with full names, abbreviations, amplicon sizes and primer characteristics

GenBank acc. no.	Gene	Amplicon size, bp	Forward/ Reverse	Sequence 5'→3'
NM_001101.5	*ACTB (*actin beta)	116	Forward	CTCTTCCAGCCTTCCTTCC
			Reverse	AGCACTGTGTTGGCGTACAG
NM_001289745.3	*GAPDH (*glyceraldehyde-3-phosphate dehydrogenase)	131	Forward	GCCAAAAGGGTCATCATCTC
			Reverse	GGTGCTAAGCAGTTGGTGGT
XM_047438740.1	*HNRNPL (*heterogeneous nuclear ribonucleoprotein L)	82	Forward	CCAAGGCCTCTCTCAATGGG
			Reverse	TTCAAGCGTGTAGGCTTTGC
NM_001318796.2	*TLR2 (*toll like receptor 2)	133	Forward	CTGTGCTCTGTTCCTGCTGA
			Reverse	GATGTTCCTGCTGGGAGCTT
NM_003266.4	*TLR4 (*toll like receptor 4)	145	Forward	TGCTTCTTGCTGGCTGCATA
			Reverse	AGGCAGAGCTGAAATGGAGG
NM_000594.4	*TNF (*tumour necrosis factor)	146	Forward	TGCACTTTGGAGTGATCGGC
			Reverse	CTCAGCTTGAGGGTTTGCTAC
NM_000576.3	*IL1B* (interleukin 1 beta)	97	Forward	GCTCGCCAGTGAAATGATGG
			Reverse	GTCCTGGAAGGAGCACTTCAT
NM_001371096.1	*IL6* (interleukin 6)	139	Forward	CAGTTCCTGCAGAAAAAGGCAA
			Reverse	AGCTGCGCAGAATGAGATGA
NM_018727.5	*TRPV1* (transient receptor potential cation channel subfamily V member 1)	118	Forward	TGGGACAGATTGGTCAAGCG
			Reverse	TCTTAAAGGGAGGCAAGCCA
NM_001317185.2	*CDH1* (cadherin 1)	189	Forward	GCATTGCCACATACACTCTCTTCTC
			Reverse	TGGCTGTGACCTCCATCAC
NM_004346	*CASP3* (caspase 3)	112	Forward	TGCTATTGTGAGGCGGTTGT
			Reverse	TCACGGCCTGGGATTTCAAG
NM_002417	*MKI67* (marker of proliferation Ki-67)	154	Forward	AGCACGTCGTGTCTCAAGAT
			Reverse	TGAGTCATCTGCGGTACTGTC
NM_002542.6	*OGG1* (8-oxoguanine DNA glycosylase)	145	Forward	ACATTGCCCAACGTGACTACA
			Reverse	GCACTGAACAGCACCGCTT
NM_181501.2	*ITGA1* (integrin subunit alpha 1)	180	Forward	CCGAGATGTGGCCGTAGTTA
			Reverse	ACACGGTACTGCAAATCAGC

Results

Gene expression

Effect of co-incubation of HPAd cells with Caco-2 cells on *CASP3*, *MKI67*, *OGG1* gene expression in HPAd cells (adipocytes)

Co-incubation of Caco-2 cells with mature adipocytes decreased the expression of *CASP3* in adipocytes (Figure 1), whereas stimulation of adipocytes with LPS increased its expression in adipocytes compared to the control group. In the Ad+LPS+Caco-2 group, where adipocytes were pretreated with LPS and co-incubated with Caco-2, the expression of *CASP3* was lower compared to the LPS stimulated without enterocytes and control group, indicating a protective effect of substances synthesised by Caco-2 enterocytes against apoptosis in adipocytes.

Co-incubation of Caco-2 cells with mature adipocytes had no effect on *MKI67* gene expression in adipose cells (Figure 1). Only stimulation of adipocytes with LPS led to increased *MKI67* gene expression in adipocytes. These data indicated that co-incubation of Caco-2 cells with mature adipocytes did not affect adipocyte proliferation.

A decrease in *OGG1* gene expression was observed only in adipose cells from the Ad+LPS+Caco-2 group, where adipocytes were previously treated with LPS and co-incubated with Caco-2 cells compared to the control and other adipocyte groups (Figure 1). Co-incubation of Caco-2 cells with mature adipocytes did not affect the expression of *OGG1* in adipocytes, consequently not causing DNA damage or activation of the expression of the DNA repair enzyme OGG1. LPS treatment and the presence of Caco-2 cells appeared to suppress *OGG1* expression.

Effect of co-incubation of HPAd cells with Caco-2 cells on *TLR2*, *TLR4*, *TRPV1* gene expression in HPAd cells (adipocytes)

The results presented in Figure 2 show that co-incubation of Caco-2 cells with mature adipocytes increased *TLR2* gene expression in adipocytes in comparison to the control group. The highest level of *TLR2* expression was observed in the Ad+LPS+Caco-2 group. Stimulation of adipocytes with LPS also increased *TLR2* expression in adipocytes compared to the control group indicating an appropriate response to pathogen-associated molecular patterns expressed on infectious agents. The increased expression of *TLR2* in adipocytes co-incubated with Caco-2 cells suggested that Caco-2 cells synthesised some substances that influenced *TLR2* gene expression in adipose cells.

The data presented in Figure 2 also showed that co-incubation of adipocytes with Caco-2 cells decreased *TLR4* gene expression in adipose cells in comparison to the control and LPS-stimulated groups. LPS preincubation did not stimulate *TLR4*

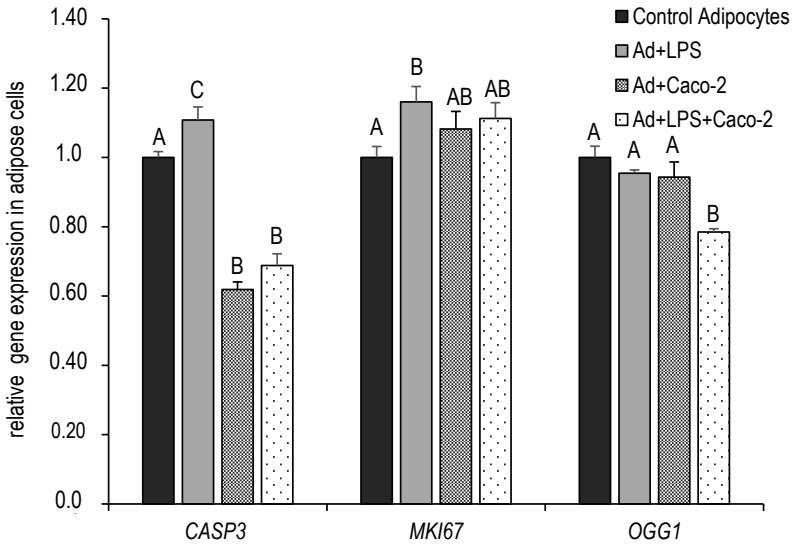

Figure 1. Effect of co-incubation of HPAd cells with Caco-2 cells on *CASP3*, *MKI67*, *OGG1* genes expression in HPAd cells (adipocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide, *CASP3* – caspase 3, *MKI67* – marker of proliferation Ki-67, *OGG1* – 8-oxoguanine DNA glycosylase; groups: Control adipocytes – HPAd without Caco-2, Ad+LPS – LPS-stimulated HPAd, Ad+Caco-2 – HPAd+Caco-2, Ad+LPS+Caco-2 – LPS-pretreated HPAd+Caco-2

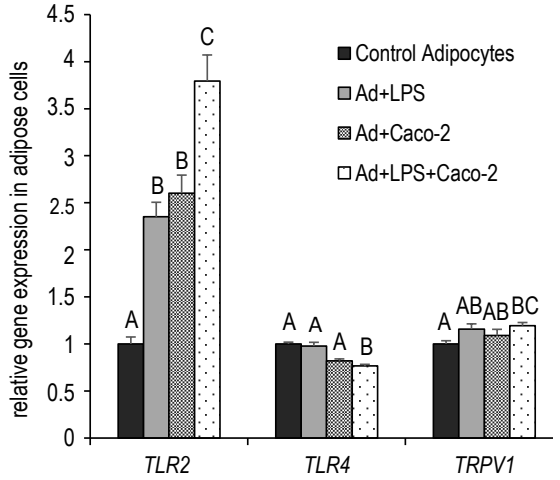

Figure 2. Effect of co-incubation of HPAd cells with Caco-2 cells on *TLR2*, *TLR4*, *TRPV1* genes expression in HPAd cells (adipocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide, *TLR2* – toll like receptor 2, *TLR4* – toll like receptor 4, *TRPV1* – transient receptor potential cation channel subfamily V member 1; groups: Control adipocytes – HPAd without Caco-2, Ad+LPS – LPS-stimulated HPAd, Ad+Caco-2 – HPAd+Caco-2, Ad+LPS+Caco-2 – LPS-pretreated HPAd+Caco-2

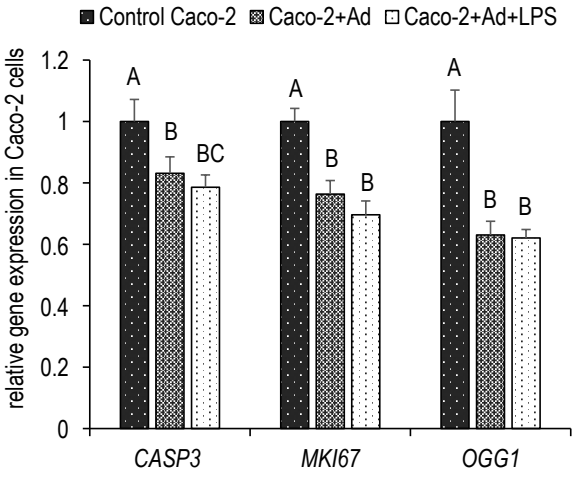

Figure 3. Effect of co-incubation of HPAd cells with Caco-2 cells on *CASP3, MKI67, OGG1* genes expression in Caco-2 cells (enterocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide, *CASP3* – caspase 3, *MKI67* – marker of proliferation Ki-67, OGG1 – 8-oxoguanine DNA glycosylase; groups: Control Caco-2 – Caco-2 without HPAd, Caco-2+Ad – Caco-2+HPAd, Caco-2+Ad+LPS – Caco-2+LPS-pretreated HPAd

gene expression in adipocytes. In addition, the presence of Caco-2 cells acted as an inhibitor of *TLR4* gene expression in adipocytes.

Only in the group where adipocytes were pretreated with LPS and co-incubated with Caco-2 cells (Figure 2) an increase of *TRPV1* gene expression in adipose cells in comparison to the control was observed. Rest of studied parametres did not affect on *TRPV1* gene expression in adipose cells. This data suggests that Caco-2 metabolites stimulated *TRPV1* gene expression in LPS-treated adipocytes and did not affect *TRPV1* expression in non-treated LPS adipocytes.

Effect of co-incubation of HPAd cells with Caco-2 cells on *CASP3*, *MKI67*, *OGG1* gene expression in Caco-2 cells (enterocytes)

Co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased the expression of *CASP3* in Caco-2 cells in comparison to the control group (Figure 3). No difference in co-incubation between Caco-2+Ad and Caco-2+Ad+LPS groups was observed. These data demonstrated that adipocytes may influence on apoptosis in enterocytes reducing *CASP3* expression in Caco-2 cells.

Co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased *MKI67* gene expression in Caco-2 cells (Figure 3) in comparison to the control group of enterocytes. However, when comparing the Caco-2+Ad group with the Caco-2+Ad+LPS group, it could be observed that stimulation of adipocytes with LPS did not influence *MKI67* gene expression in Caco-2 cells. These data showed that co-incubation of HPAd cells with Caco-2 cells suppressed enterocyte proliferation.

Co-incubation of Caco-2 cells with mature adipocytes visibly decreased the expression of *OGG1* in Caco-2 cells in comparison to the control group (Figure 3). However, data from the Caco-2+Ad and Caco-2+Ad+LPS groups showed that LPS-stimulated adipocytes did not affect *OGG1* gene expression in Caco-2 cells. These data suggested that co-incubation of Caco-2 cells with either LPS-stimulated or non-stimulated adipocytes neither caused DNA damage nor activation of the expression of the DNA repair *OGG1* enzyme in enterocytes.

Effect of co-incubation of HPAd cells with Caco-2 cells on *TLR2*, *TLR4*, *TRPV1* gene expression in Caco-2 cells (enterocytes)

Co-incubation of Caco-2 cells with mature adipocytes decreased the expression of *TLR2* in Caco-2 cells in comparison with the control group (Figure 4). No difference in co-incubation was observed in the Caco-2+Ad and Caco-2+Ad+LPS

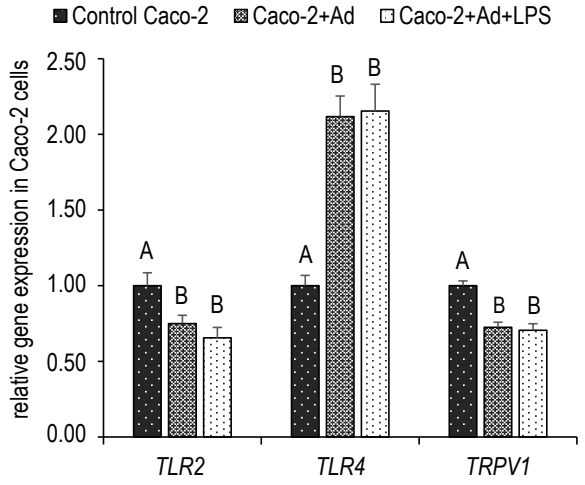

Figure 4. Effect of co-incubation of HPAd cells with Caco-2 cells on *TLR2,TLR4, TRPV1* genes expression in Caco-2 cells (enterocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide, *TLR2* – toll like receptor 2, *TLR4* – toll like receptor 4, *TRPV1* – transient receptor potential cation channel subfamily V member 1; groups: Control Caco-2 – Caco-2 without HPAd, Caco-2+Ad – Caco-2+HPAd, Caco-2+Ad+LPS – Caco-2+LPS-pretreated HPAd

groups. This data showed that substances synthesised by adipocytes could suppress *TLR2* expression in Caco-2 cells, thereby affecting the recognition of pathogens by enterocytes and activation of signalling pathways connected with this receptor.

Analysing the effect of co-incubation of adipose cells with Caco-2 cells on *TLR4* gene expression in Caco-2 cells (Figure 4), it can be observed that co-incubation drastically increased the expression of the *TLR4* gene in Caco-2 cells compared to the control group. No effect of co-incubation was observed for the Caco-2+Ad and Caco-2+Ad+LPS groups. The reason for these results can be connected with the fact that fatty acids and lipoproteins are ligands of the TLR4 receptor, and adipose cells activated the expression of this receptor by releasing these substances into the culture medium.

Figure 4 also shows that the relative expression of the *TRPV1* gene in Caco-2 cells after co-incubation was lower in comparison to the control group. No differences were observed between the Caco-2+Ad and Caco-2+Ad+LPS groups, indicating that LPS did not affect the tested traits. This data showed that adipocyte metabolites could reduce *TRPV1* expression in Caco-2 cells, thus influencing *TRPV1* activity in enterocytes.

Effect of co-incubation of HPAd cells with Caco-2 cells on *IL1B*, *ITGA1*, *CDH1* gene expression in Caco-2 cells (enterocytes)

Co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased the relative expression of the *IL1B* gene in Caco-2 cells compared to the control group (Figure 5). No effect of co-incubation and differences between the Caco-2+Ad and Caco-2+Ad+LPS groups was observed. In addition, the relative expression of *IL6* and *TNF* genes in Caco-2 cells were analysed, but PCR analysis did not detect any *IL6* and *TNF* expression. This data indicated that adipocyte metabolites could reduce the expression of proinflammatory cytokines in Caco-2 cells, thereby decreasing the synthesis of proinflammatory agents by enterocytes, and consequently, reducing inflammation in the intestines.

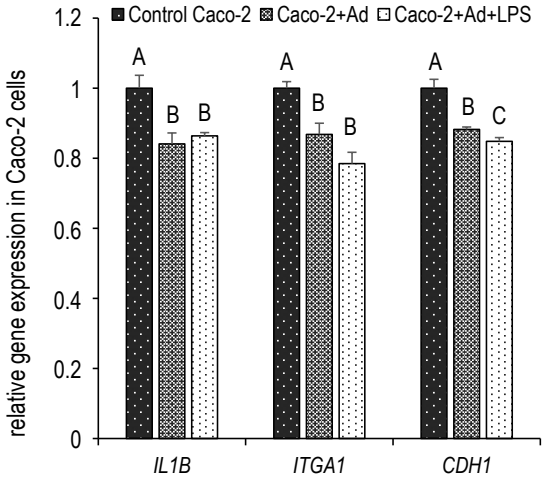

Figure 5. Effect of co-incubation of HPAd cells with Caco-2 cells on *IL1B, ITGA1, CDH1* genes expression in Caco-2 cells (enterocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide, *IL1B* – interleukin 1 beta, *ITGA1* – integrin subunit alpha 1, *CDH1* – cadherin 1; groups: Control Caco-2 – Caco-2 without HPAd, Caco-2+Ad – Caco-2+HPAd, Caco-2+Ad+LPS – Caco-2+LPS-pretreated HPAd

Co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased *ITGA1* gene expression in Caco-2 cells in comparison to the control group (Figure 5). No effect of co-incubation and differences between the Caco-2+Ad and Caco-2+Ad+LPS groups were observed. This data showed that adipocyte metabolites reduced *ITGA1* expression in Caco-2 cells, thus affecting, i.a., bacterial adhesion to enterocytes.

Co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased *CDH1* gene expression in Caco-2 cells in comparison to the control group (Figure 5). Moreover, it was found that *CDH1* expression in the Caco-2+Ad group was slightly higher compared to the Caco-2+Ad+LPS group. Based on this data, it can be assumed that adipocytes are able to mediate bacterial adhesion in the gut.

Caco-2 cell viability and bacterial adhesion

Effect of co-incubation of *L. paracasei* with Caco-2 cells on Caco-2 cell (enterocytes) viability

The data from Figure 6 showed that the number of viable Caco-2 cells co-incubated with *L. paracasei* and with adipocytes (stimulated and non-stimulated with LPS) was radically higher compared to the control group. There was no effect of co-incubation with the test agents on the number of dead cells in the LD1 and LD2 groups in comparison to the control group; however, in the LD3 group, where Caco-2 cells were incubated with LPS-pretreated adipocytes and then with *L. paracasei*, a decrease in the number of viable cells and an increase in the count of dead Caco-2 cells was observed. These data showed that adipocytes and *L. paracasei* did not negatively affect enterocyte viability, while LPS-stimulated adipocytes did have an adverse effect on the viability of these cells.

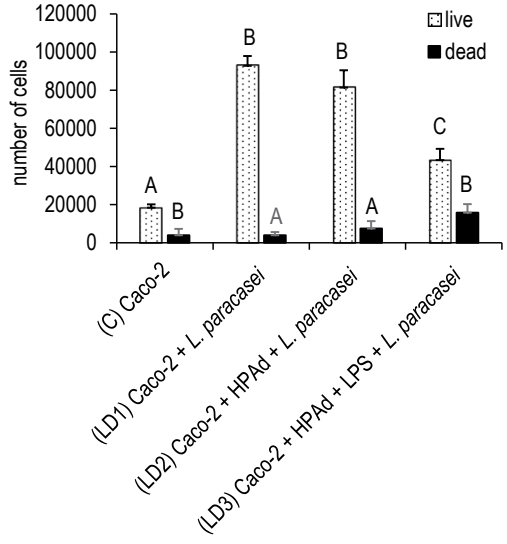

Figure 6. Effect of co-incubation of *Lactobacillus paracasei* with Caco-2 cells on Caco-2 cell (enterocytes) viability. All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide; groups: C – Caco-2 without bacteria, LD1 – Caco-2+*L. paracasei*, LD2 – Caco-2+HPAd+*L. paracasei*, LD3 – Caco-2+LPS-pretreated HPAd+*L. paracasei*

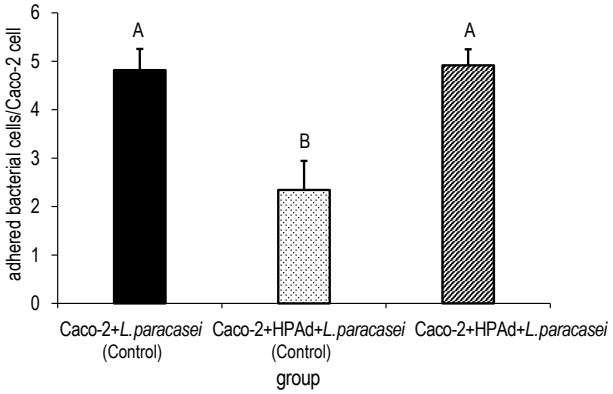

Figure 7. Effect of co-incubation of HPAd cells with Caco-2 cells on *Lactobacillus paracasei* adhesion to Caco-2 cells (enterocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide; groups: Caco-2+*L. paracasei* – Control, Caco-2+HPAd+*L. paracasei* – Caco-2 preincubated with adipocytes and incubated with *L. paracasei*, Caco-2+LPS-pretreated+*L. paracasei* – Caco-2 preincubated with LPS-pretreated adipocytes and incubated with *L. paracasei*

Effect of co-incubation of HPAd cells with Caco-2 cells on *L. paracasei* adhesion to Caco-2 cells (enterocytes)

Figure 7 demonstrates that co-incubation of Caco-2 cells with mature adipocytes reduced the number of adhered *L. paracasei* bacterial cells to Caco-2 cells compared to the control and Caco-2+LPS-pretreated HPAd+*L. paracasei* groups. This data shows that co-incubation of Caco-2 cells with non-LPS-stimulated adipocytes inhibited *L. paracasei* adhesion to Caco-2 cells, whereas LPS-stimulated adipocytes had no effect on this process.

Effect of co-incubation of *S. enterica* with Caco-2 cells on Caco-2 cell (enterocytes) viability

Co-incubation of Caco-2 cells with *S. enterica* and adipocytes increased the number of dead Caco-2 cells compared to the control group. An increase in the number of viable Caco-2 cells was observed only in the SD2 group, where Caco-2 cells were preincubated with adipocytes (Figure 8). These data suggests that *S. enterica* had a detrimental effect on enterocytes, leading to their death. However, metabolites produced by adipocytes appeared to exert a protective effect, mitigating the harmful actions of *S. enterica* on enterocytes.

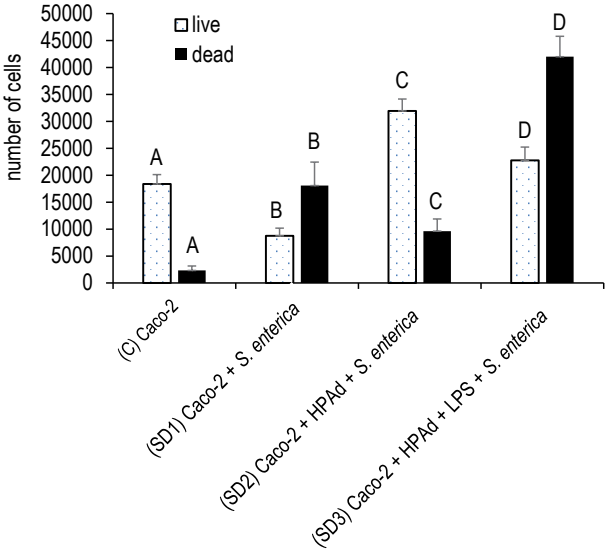

Figure 8. Effect of co-incubation of *Salmonella enterica* with Caco-2 cells on Caco-2 cell (enterocytes) viability. All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide; groups: C – Caco-2 without bacteria, SD1 – Caco-2+*S. enterica*, SD2 – Caco-2+HPAd+*S. enterica*, SD3 – Caco-2+LPS-pretreated HPAd+*S. enterica*

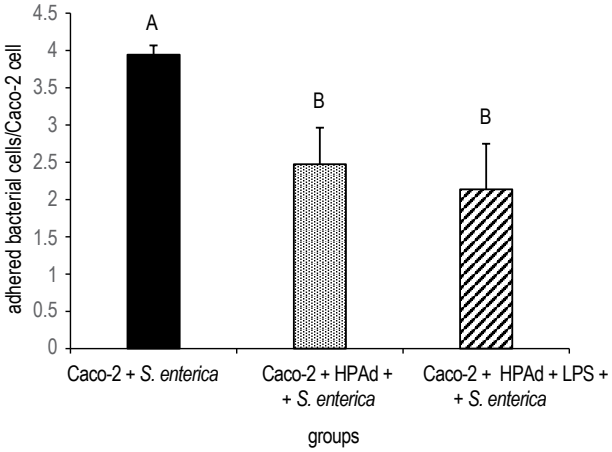

Figure 9. Effect of co-incubation of HPAd cells with Caco-2 cells on *Salmonella enterica* adhesion to Caco-2 cells (enterocytes). All data are presented as means (± SEM). Different capital letters indicate significant ($P < 0.05$) differences according to one-way ANOVA followed by Fisher's post-hoc test

HPAd – human preadipocytes, Caco-2 – human epithelial cell line (enterocytes), LPS – lipopolysaccharide; groups: Caco-2+*S. enterica* – Control, Caco-2+HPAd+*S. enterica* – Caco-2 preincubated with adipocytes and incubated with *S. enterica*, Caco-2+LPS-pretreated HPAd+*S. enterica* – Caco-2 preincubated LPS-pretreated adipocytes and incubated with *S. enterica*

Effect of co-incubation of HPAd cells with Caco-2 cells on *S. enterica* adhesion to Caco-2 cells (enterocytes)

Co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased the number of adhered *S. enterica* cells to Caco-2 enterocytes (Figure 9) in comparison to the control group. However, when comparing the Caco-2 + HPAd+*S. enterica* group with the Caco-2+LPS-pretreated HPA+*S. enterica* group, it was observed that stimulation of adipocytes with LPS did not influence *S. enterica* adhesion to Caco-2 cells. These data demonstrated that co-incubation of adipocytes with enterocytes suppressed *S. enterica* adhesion to enterocytes.

Discussion

Effect of co-incubation of HPAd cells with Caco-2 on cellular processes in these cells

Adipose tissue plays a crucial role in the body, and it is well established that both its deficiency and excess can disturb the appropriate functioning of the organism. While body fat and adipose tissue are often associated with negative impacts on health, such as cardiovascular diseases, diabetes mellitus or cancer, it is important to recognize their beneficial effects. Body fat performs many significant functions in the system, including energy storage and release, maintenance of energy balance, regulation of glucose and cholesterol levels; preservation of insulin sensitivity, cushioning surrounding soft organs, insulation from cold and heat, regulation of hunger and satiety, generation of thermogenic heat and contribution to immunity and sex hormones metabolism (Choe et al., 2016).

In the first part of the experiment, we investigated the impact of mature adipocytes on various processes in enterocytes, including proliferation (*CASP3*, *MKI67*), expression of the DNA repair enzyme (*OGG1*), factors responsible for inflammatory process (*TLR2*, *TLR4*, *IL1B*, *IL6*, *TNF*), transient receptor potential cation channel subfamily V member 1 (*TRPV1*) and receptors necessary for bacterial adhesion (*ITGA1*, *CDH1*). The results of this experiment demonstrated that co-incubation of Caco-2 cells with mature adipocytes (stimulated and non-stimulated with LPS) decreased the expression of *CASP3, MKI67* and the DNA repair enzyme *OGG1* in Caco-2 cells compared to the control group, suggesting that adipocytes could reduce apoptosis and affect proliferation in enterocytes.

Similarly, a decreased expression of the *TRPV1* receptor was observed, which is involved in the transmission and modulation of pain and integration of diverse painful stimuli. Moreover, a reduced expression of inflammatory factors in Caco-2 cells, such as the *TLR2* receptor and *IL1B*, *IL6*, *TNF* cytokines, was recorded. Taken together, these data indicated that adipocytes differentiated from HPAd cells prolonged the lifespan of Caco-2 cells and might possess anti-analgetic and anti-inflammatory properties. We believe that this effect may be attributed to the origin of HPAd cells. These cells were isolated from human subcutaneous adipose tissue and frozen as the primary culture, thus potentially containing all cell types present in adipose tissue. Fat tissue is a loose connective tissue derived from preadipocytes and, in addition to adipose cells, contains mesenchymal stem cells, vascular endothelial cells, preadipocytes, fibroblasts, and a variety of immune cells such as mature B and T cells, macrophages or myeloid cells. It has been established that adipose-derived cells (mesenchymal stem cells/stromal cells) show great potential in treating such diseases as diabetes, liver cirrhosis, cardiovascular disease, Crohn's disease, ulcerative colitis, wound healing, rheumatoid arthritis (Bunnell, 2021), multiple sclerosis (Riordan et al., 2009) or bone tissue restoration (Mesimäki et al., 2009).

In our experiment, we noticed that co-incubating Caco-2 cells with mature adipocytes derived from HPAd cells positively affected Caco-2 cell proliferation by reducing apoptosis and downregulating the DNA repair enzyme *OGG1*. Our findings are consistent with other studies that have investigated the influence of adipose cells on proliferation, particularly in the context of wound healing and colitis. For instance, several works evaluating the role of adipose-derived stem cells in wound healing showed that adipose tissue-derived stem cells regulated keratinocyte and dermal fibroblast proliferation and improved diabetic wound healing (Seo et al., 2017; Venter and Kelly, 2021). In addition, Sémont et al. (Sémont et al., 2013) described the regenerative properties of adipose tissue-derived mesenchymal stromal cells MSCs. Their results demonstrated that MSCs stimulated the proliferation of colonic epithelial cells, leading to reduced mucosal damage.

In the present study, we also observed that co-incubation of Caco-2 cells with mature adipocytes differentiated from HPAd cells decreased the expression of inflammatory agents such as the *TLR2* receptor and *IL1B*, *IL6*, *TNF* cytokines. These findings align with the results reported by other scientists, indicating that adipose-derived cells exhibit anti-inflammatory properties. For instance, Dirk Van de Putte (Van de putte et al., 2017) investigated the efficiency of using mesenchymal stromal cells from adipose tissue (Ad-MSCs) as a therapeutic agent during colonic anastomosis following high-dose irradiation. The results of this experiment demonstrated that Ad-MSC treatment suppressed inflammation, reduced ulcer size, increased mucosal vascular density and limited haemorrhage in the colon. Similar immunomodulatory properties of adipose-derived MSCs in the treatment of chronic colitis have been described in the work of Park et al. (Park et al., 2018). The latter authors investigated the anti-inflammatory mechanism and therapeutic effects of adipose tissue-derived MSCs using THP-1 macrophages in mice with dextran sodium sulphate (DSS)-induced chronic colitis. The findings from their experiment showed that adipose-derived MSCs inhibited the secretion of IL-1β and IL-18 cytokines in THP-1 macrophages and suppressed the inflammatory response, ameliorating colitis by decreasing the total number of macrophages in mice. The anti-inflammatory capacity of adipose-derived mesenchymal stem cells was also demonstrated by Liang et al. (Liang et al., 2019), who investigated the role of adipose-derived MSCs in acute liver injury in rats suffering from sepsis induced by ceacal ligation and puncture (CLP). The result of the experiment showed that adipose-derived MSCs decreased the levels of pro-inflammatory cytokines, including TNF-α, IL-6, AP-1 c-jun and NF-κB p56 after CLP administration, suggesting that injecting adipose-derived MSCs reduced systemic inflammation and increased the survival rate of septic rats.

Further, our experiments showed that co-incubation of adipose cells with Caco-2 cells drastically enhanced the expression of the *TLR4* gene in Caco-2 cells without causing increased expression of pro-inflammatory cytokines. The reason for these results may be connected with the fact that fatty acids and lipoproteins are ligands of the TLR4 receptor (Lu et al., 2014), and adipose cells, by releasing these substances into the culture medium, activated *TLR4* gene expression.

Furthermore, we analysed how enterocytes influenced cellular processes occurring in adipocytes; however, this was a secondary objective of our study, as the present model did not accurately replicate the conditions observed in the body. The aim of these analyses was to assess the health status of adipocytes. The data obtained from this experiment showed that co-incubation of Caco-2 cells with mature adipocytes

decreased *CASP3* and *TLR4* gene expression, increased *TLR2* and *TRPV1* gene expression and did not affect the expression of *MKI67* and *OGG1* genes in adipocytes. In contrast, stimulation of adipocytes with LPS elevated the expression of *CASP3*, *MKI67*, *TLR2* and *TRPV1*, reduced the expression of *OGG1* and did not affect *TLR4* gene expression in adipose cells. These findings imply that direct exposure to fluids secreted by both cell types induced a response in the two cell populations.

Effect of co-incubation of HPAd cells with Caco-2 cells on bacterial adhesion to Caco-2 cells (enterocytes)

In the second part of the experiment, we investigated the effect of co-incubation of HPAd cells with Caco-2 cells on the adhesion of both conventional and pathogenic bacterial strains to Caco-2 cells, as well as on enterocyte viability after treatment with the tested bacteria.

The gastrointestinal tract is a complex ecosystem consisting of intestinal epithelial cells, immune cells and gut microbiota (Yoo et al., 2020). Numerous literature reports indicate that the composition of the microbiota in the gastrointestinal tract of obese individuals differs from that in individuals with normal body weight (Al-Assal et al., 2018). Additionally, it is known that intestinal flora can regulate energy absorption and nutrient metabolism, increasing energy accumulation from the diet (Krajmalnik-Brown et al., 2012). Disturbances in the gut flora can lead to the production of excessive lipopolysaccharides, which, upon absorption into the bloodstream, may contribute to the high-fat diet-associated obesity and metabolic syndrome (de La Serre et al., 2010; Lokapirnasari et al., 2022). Epithelial cells of the gastrointestinal tract are protected against pathogenic bacteria by many mechanisms, one involving a reducing pathogenic infections through competition of the microbiota for sites of adhesion (Alp and Kuleaşan, 2020). Several studies have indicated that lactic acid bacteria can prevent the attachment of pathogens, thereby reducing colonization and averting infections (Alp and Kuleaşan, 2020). Adhesion to mammalian epithelial cells is a crucial process for both commensal and pathogenic bacteria to survive and colonize the gastrointestinal tract. In our experiment, we aimed to investigate how co-incubation of adipocytes with enterocytes would affect the adhesion of conventional and pathogenic bacterial strains to enterocytes. Initially, we analysed how the addition of the studied bacterial strain to the insert with Caco-2 cells influenced the viability of Caco-2 cells. The data from our experiment showed that co-incubation of Caco-2 cells with *L. paracasei* and adipocytes (stimulated and non-stimulated with LPS) increased the number of viable Caco-2 cells compared to the control group. However, incubating Caco-2 cells with LPS-pretreated adipocytes and subsequently with *L. paracasei* reduced the number of viable cells and elevated the count of dead Caco-2 cells. These findings were consistent with the observations of other scientists. The protective capacity of *L. paracasei* was confirmed in the work of Chen et al. (2016), who reported that incubation of Caco-2 cells with *L. paracasei* enhanced Caco-2 growth, decreased Caco-2 cell death induced by LPS/TNF-α)/interferon-γ (IFN-γ), as well as lowered chemokine CCL-20 production. Additionally, it is known that LPS exerts harmful effects to enterocytes, and probiotic strains were shown to prevent pro-inflammatory cytokine production by the gut epithelial cells (Singh et al., 2018; Szczepkowska et al., 2022). Moreover, we found that co-incubation of Caco-2 cells with *S. enterica* and adipocytes increased the rate of Caco-2 cell death compared to the control group, and preincubation of Caco-2 with adipocytes reduced the number of dead cells. These data suggested that *S. enterica* could induce enterocyte death, but adipocyte metabolites were able to protect gut epithelial cells against the harmful action of *S. enterica*. Although there is a lack of literature data regarding the role of adipocytes in the aforementioned process, our findings indicated that adipocytes had a positive impact on enterocyte viability, while *S. enterica* and adipocytes stimulated with LPS negatively affected this parameter.

Subsequently, by analysing the adhesion of *L. paracasei* and *S. enterica* to Caco-2 cells, we found that simultaneous culture of Caco-2 cells with non-LPS-stimulated adipocytes reduced the number of adhered *L. paracasei* bacterial cells to Caco-2 cells. In contrast, LPS-stimulated adipocytes did not affect this process. On the other hand, the addition of *S. enterica* to Caco-2 culture co-incubated with mature adipocytes (stimulated and non-stimulated with LPS) reduced *S. enterica* adhesion to Caco-2 cells in comparison to the control group. These data demonstrate that co-incubation of adipocytes with enterocytes suppresses bacterial adhesion to gut epithelial cells. This effect may be associated with reduced expression of *ITGA1* and *CDH1* receptors, which are necessary for bacterial adhesion to cells.

Conclusions

Based on the outcomes of *in vitro* experiments, we can conclude that co-incubation of Caco-2 cells with adipocytes positively affects cellular processes occurring in Caco-2 cells, reducing the expression of proinflammatory cytokines and apoptosis markers. Moreover, preincubation of Caco-2 with adipocytes protects Caco-2 against deleterious effects of *Salmonella enterica*, reducing bacterial adhesion and increasing the viability of Caco-2-derived enterocytes. Incubation of Caco-2 with adipocytes pretreated with lipopolysaccharide did not result in significant changes in comparison to non-lipopolysaccharide-treated adipocytes.

Funding

This work was supported by the statutory funding of The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Ageorges V., Monteiro R., Leroy S., Burgess C.M., Pizza M., Chaucheyras-durand F., Desvaux M., 2020. Molecular determinants of surface colonisation in diarrhoeagenic *Escherichia coli* (DEC): from bacterial adhesion to biofilm formation. FEMS Microbiol. Rev. 44, 314–350, https://doi.org/10.1093/femsre/fuaa008

Al-Assal K., Martinez A.C., Torrinhas R.S., Cardinelli C., Waitzberg D., 2018. Gut microbiota and obesity. Clin. Nutr. Exp. 20, 60–64, https://doi.org/10.1016/j.yclnex.2018.03.001

Alp D., Kuleaşan H., 2020. Determination of competition and adhesion abilities of lactic acid bacteria against gut pathogens in a whole-tissue model. Biosci. Microbiota Food Health 39, 250–258, https://doi.org/10.12938/bmfh.2020-033

Ambele M.A., Dhanraj P., Giles R., Pepper M.S., 2020. Adipogenesis: a complex interplay of multiple molecular determinants and pathways. Int. J. Mol. Sci. 21, 4283, https://doi.org/10.3390/ijms21124283

Bunnell B.A., 2021. Adipose tissue-derived mesenchymal stem cells. Cells 10, 3433, https://doi.org/10.3390/cells10123433

Chait A., den Hartigh L.J., 2020. Adipose tissue distribution, inflammation and its metabolic consequences, including diabetes and cardiovascular disease. Front. Cardiovasc. Med. 7, 22, https://doi.org/10.3389/fcvm.2020.00022

Chen Y., Hsu C., Hung W., Chen M., 2016. Effects of *Lactobacillus paracasei* 01 fermented milk beverage on protection of intestinal epithelial cell in vitro. J. Sci. Food Agric. 96, 2154–2160, https://doi.org/10.1002/jsfa.7331

Choe S.S., Huh J.Y., Hwang I.J., Kim J.I., Kim J.B., 2016. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. Front. Endocrinol. 7, https://doi.org/10.3389/fendo.2016.00030

de La Serre C.B., Ellis C.L., Lee J., Hartman A.L., Rutledge J.C., Raybould H.E., 2010. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am. J. Physiol. Gastrointest. Liver Physiol. 299, G440–G448, https://doi.org/10.1152/ajpgi.00098.2010

Dunisławska A., Pietrzak E., Bełdowska A., Sławińska A., Siwek M., 2023. Response in liver gene expression and DNA methylationto changes in the intestinal microbial profileafter in ovo stimulation of chickens. J. Anim. Feed Sci. 32, 152–163, https://doi.org/10.22358/jafs/156098/2023

Elmas O., Cenik P., Sirinyildiz F., Elmas S., Sirin F., Cesur G., 2022. Relationship between cognitive functions, levels of NR2Aand NR2B subunits of hippocampal NMDA receptors, serumTGF-β1 level, and oxidative stress in rats fed a high-fat diet. J. Anim. Feed Sci. 31, 318–327, https://doi.org/10.22358/jafs/152027/2022

Ettehad Marvasti F., Moshiri A., Taghavi M.S. et al., 2020. The first report of differences in gut microbiota composition between obese and normal weight Iranian subjects. Iran Biomed. J. 24, 148–154, https://doi.org/10.29252/ibj.24.3.148

Fain J.N., Madan A.K., Hiler M.L., Cheema P., Bahouth S.W., 2004. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 145, 2273–2282, https://doi.org/10.1210/en.2003-1336

Giordano A., Smorlesi A., Frontini A., Barbatelli G., Cinti S., 2014. White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ. Eur. J. Endocrinol. 170, R159–R171, https://doi.org/10.1530/EJE-13-0945

Hutchings G., Janowicz K., Moncrieff L. et al., 2020. The proliferation and differentiation of adipose-derived stem cells in neovascularization and angiogenesis. Int. J. Mol. Sci. 21, 3790, https://doi.org/10.3390/ijms21113790

Kershaw E.E., Flier J.S., 2004. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. 89, 2548-2556, https://doi.org/10.1210/jc.2004-0395

Krajmalnik-Brown R., Ilhan Z., Kang D., DiBaise J.K., 2012. Effects of gut microbes on nutrient absorption and energy regulation. Nutr. Clin. Pract. 27, 201–214, https://doi.org/10.1177/0884533611436116

Li B., Ren S., Zhao W., Wang X., Tu F., 2021. Comparative analysis of the rectaland caecal microbial community composition and functionin adult Erhualian and Sushan pigs. J. Anim. Feed Sci. 30, 248–259, https://doi.org/10.22358/jafs/138777/2021

Liang H., Ding X., Yu Y., Zhang H., Wang L., Kan Q., Ma S., Guan F., Sun T., 2019. Adipose-derived mesenchymal stem cells ameliorate acute liver injury in rat model of CLP induced-sepsis via sTNFR1. Exp. Cell Res. 383, 111465, https://doi.org/10.1016/j.yexcr.2019.06.010

Lokapirnasari W., Al Arif M., Maslachah L., Kirana A., Suryandari A., Yulianto A., Sherasiya A., 2022. The potency of *Lactobacillus acidophillus* and *L. lactis* probioticsand *Guazuma ulmifolia* Lam. extract as feed additiveswith different application times to improve nutrient intakeand feed efficiency in *Coturnix coturnix japonica* females. J. Anim. Feed Sci. 32, 59–67, https://doi.org/10.22358/jafs/156018/2022

Lu P., Sodhi C.P., Hackam D.J., 2014. Toll-like receptor regulation of intestinal development and inflammation in the pathogenesis of necrotizing enterocolitis. Pathophysiol. 21, 81–93, https://doi.org/10.1016/j.pathophys.2013.11.007

McCracken V.J., Lorenz R.G., 2001. The gastrointestinal ecosystem: a precarious alliance among epithelium, immunity and microbiota. Cell Microbiol. 3, 1–11, https://doi.org/10.1046/j.1462-5822.2001.00090.x

Mesimäki K., Lindroos B., Törnwall J., Mauno J., Lindqvist C., Kontio R., Miettinen S., Suuronen R., 2009. Novel maxillary reconstruction with ectopic bone formation by GMP adipose stem cells. Int. J. Oral Maxillof. Surg. 38, 201–209, https://doi.org/10.1016/j.ijom.2009.01.001

Miron N., Cristea V., 2012. Enterocytes: active cells in tolerance to food and microbial antigens in the gut. Clin. Exp. Immunol. 167, 405–412, https://doi.org/10.1111/j.1365-2249.2011.04523.x

Parekh R., Abdulhamid A., Trudeau S., Kaur N., 2018. Tumor necrosis factor alpha inhibition for inflammatory bowel disease after liver transplant for primary sclerosing cholangitis. Case Rep. Gastrointest. Med. 2018, 1015408, https://doi.org/10.1155/2018/1015408

Park H.J., Kim J., Saima F.T., Rhee K.-J., Hwang S., Kim M.Y., Baik S.K., Eom Y.W., Kim H.-S., 2018. Adipose-derived stem cells ameliorate colitis by suppression of inflammasome formation and regulation of M1-macrophage population through prostaglandin E2. Biochem. Biophys. Res. Commun. 498, 988–995, https://doi.org/10.1016/j.bbrc.2018.03.096

Richard A.J., White U., Elks C.M., Stephens J.M., 2000. Adipose Tissue: Physiology to Metabolic Dysfunction. In: K.R. Feingold (Editor). Endotext. MDText.com, Inc. South Dartmouth, MA (USA)

Riordan N.H., Ichim T.E., Min W.-P. et al., 2009. Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. J. Transl. Med. 7, 29, https://doi.org/10.1186/1479-5876-7–29

Rome S., 2022. Muscle and adipose tissue communicate with extracellular vesicles. Int. J. Mol. Sci. 23, 7052, https://doi.org/10.3390/ijms23137052

Sémont A., Demarquay C., Bessout R., Durand C., Benderitter M., Mathieu N., 2013. Mesenchymal stem cell therapy stimulates endogenous host progenitor cells to improve colonic epithelial regeneration. PLoS ONE 8, e70170, https://doi.org/10.1371/journal.pone.0070170

Seo E., Lim J.S., Jun J.-B., Choi W., Hong I.-S., Jun H.-S., 2017. Exendin-4 in combination with adipose-derived stem cells promotes angiogenesis and improves diabetic wound healing. J. Transl. Med. 15, 35, https://doi.org/10.1186/s12967-017-1145-4

Shi M., Chang Y., Cao M., Zhang J., Zhang L., Xie H., Miao Z., 2022. Effects of dietary yam polysaccharide on growth performance andintestinal microflora in growing Huoyan geese. J. Anim. Feed Sci. 31, 328–334, https://doi.org/10.22358/jafs/151561/2022

Šikić Pogačar M., Langerholc T., Mičetić-Turk D., Možina S.S., Klančnik A., 2020. Effect of *Lactobacillus* spp. on adhesion, invasion, and translocation of *Campylobacter* jejuni in chicken and pig small-intestinal epithelial cell lines. BMC Vet. Res. 16, 34, https://doi.org/10.1186/s12917-020-2238-5

Singh S., Bhatia R., Singh A. et al., 2018. Probiotic attributes and prevention of LPS-induced pro-inflammatory stress in RAW264.7 macrophages and human intestinal epithelial cell line (Caco-2) by newly isolated Weissella cibaria strains. Food Funct. 9, 1254–1264, https://doi.org/10.1039/C7FO00469A

Stabroth-Akil D., Leifeld L., Pfützer R., Morgenstern J., Kruis W., 2015. The effect of body weight on the severity and clinical course of ulcerative colitis. Int. J. Colorectal Dis. 30, 237–242, https://doi.org/10.1007/s00384-014-2051-3

Swierczynski J., Sledzinski T., 2012. The role of adipokines and gastrointestinal tract hormones in obesity. In: W.K. Karcz, O. Thomusch (Editors). Principles of Metabolic Surgery. Springer Berlin Heidelberg Berlin, Heidelberg (Germany), pp. 53–79, https://doi.org/10.1007/978-3-642-02411-5_6

Szczepkowska A., Bochenek J., Wójcik M., Tomaszewska-Zaremba D., Antushevich H., Tomczyk M., Skipor J., Herman A., 2022. Effect of caffeine on adenosine and ryanodine receptorgene expression in the hypothalamus, pituitary, and choroidplexus in ewes under basal and LPS challenge conditions. J. Anim. Feed Sci. 32, 17–25, https://doi.org/10.22358/jafs/156762/2022

Szlis M., Przybył B.J., Pałatyńska K., Wójcik-Gładysz A., 2024. QRFP43 modulates the activity of the hypothalamic appetite regulatory centre in sheep. J. Anim. Feed Sci. 33, 281–286, https://doi.org/10.22358/jafs/186262/2024

Tsai Y.-W., Fu S.-H., Dong J.-L., Chien M.-W., Liu Y.-W., Hsu C.-Y., Sytwu H.-K., 2020. Adipokine-modulated immunological homeostasis shapes the pathophysiology of inflammatory bowel disease. Int. J. Mol. Sci. 21, 9564, https://doi.org/10.3390/ijms21249564

Van de putte D., Demarquay C., Van Daele E., Moussa L., Vanhove C., Benderitter M., Ceelen W., Pattyn P., Mathieu N., 2017. Adipose-derived mesenchymal stromal cells improve the healing of colonic anastomoses following high dose of irradiation through anti-inflammatory and angiogenic processes. Cell Transplant. 26, 1919–1930, https://doi.org/10.1177/0963689717721515

Venter M., Kelly A., 2021. The use of adipose tissue and its derivates to stimulate and support wound healing processes - An updated review. Interdiscip. Neurosurg. 26, 101304, https://doi.org/10.1016/j.inat.2021.101304

Wahid S., Lee B., Kim I., 2022. Effect of purified docosahexaenoic acid supplementationon production performance, meat quality,and intestinal microbiome of finishing pigs. J. Anim. Feed Sci. 31, 224–231, https://doi.org/10.22358/jafs/150033/2022

Wypych A., Dunisławska A., Grabowska M., Michałek K., Ożgo M., Liput K., Herosimczyk A., Poławska E., Pierzchała M., Lepczyński A., 2023. Effects of three-month feeding high-fat diets with differentfatty acid composition on kidney histology and expressionof genes related to cellular stress and water-electrolytehomeostasis in mice. J. Anim. Feed Sci. 32, 372–384, https://doi.org/10.22358/jafs/163179/2023

Wypych A., Ożgo M., Bernaciak M. et. al., 2024. Effect of feeding high fat diets differing in fatty acid composition on oxidative stress markers and protein expression in mouse kidney. J. Anim. Feed Sci. 33, 170–184, https://doi.org/10.22358/jafs/175920/2024

Xiao H., Kang S., 2020. The role of the gut microbiome in energy balance with a focus on the gut-adipose tissue axis. Front. Genet. 11, 297, https://doi.org/10.3389/fgene.2020.00297

Yi C.-X., Tschöp M.H., 2012. Brain-gut-adipose-tissue communication pathways at a glance. Dis. Models Mech. 5, 583–587, https://doi.org/10.1242/dmm.009902

Yoo J.Y., Groer M., Dutra S.V.O., Sarkar A., McSkimming D.I., 2020. Gut Microbiota and immune system interactions. Microorganisms 8, 1587, https://doi.org/10.3390/microorganisms8101587

Zeng Z., Tang L., Zhou Y., Wang B., Wang Q., Zou P., Zhan X., Fu L., Li W., 2021. Effect of pidotimod on growth performance, immune function,intestinal epithelial barriers and microbiota of piglets. J. Anim. Feed Sci. 30, 64–75, https://doi.org/10.22358/jafs/134117/2021