TNFα regulates sugar transporters in the human intestinal epithelial cell line Caco-2

Jaione Barrenetxe[1], Olga Sánchez [1], Ana Barber [1], SoniaGascón[2,3], Mª Jesús Rodríguez-Yoldi [2,3], Maria Pilar Lostao [1].

[1]Department of Nutrition, Food Science and Physiology.University of Navarra, Pamplona 31008.Spain

[2]Department of Pharmacology and Physiology.University of Zaragoza, Zaragoza 50013. Spain

[3]CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn), Instituto de Salud Carlos III (ISCIII), Spain.

Address for correspondence: JaioneBarrenetxe, Department of Nutrition, Food Science and Physiology. Universityof Navarra, Pamplona 31008. Spain. E-mail: jaiobar@unav.es. Telephone: 34- 948 425600. Fax: 34- 948 425740

ABSTRACT

Purpose: During intestinal inflammation TNFα levels are increasedandas a consequencemalabsorptionof nutrients may occur. We have previously demonstrated that TNFα inhibits galactose, fructose and leucineintestinal absorption in animal models. In continuation with our work, thepurpose of the present study was to investigatein the human intestinal epithelial cell line Caco-2, the effect of TNFα on sugartransportand to identify the intracellular mechanisms involved.

Methods: Caco-2 cells were grown on culture plates and pre-incubated during different periods with various TNFα concentrationsbefore measuring the apical uptake of galactose, α-methyl-glucoside (MG) or fructose for 15 min. To elucidate the signalling pathway implicated, cells were pre-incubated for 30 min with the PKA inhibitor H-89 or the PKC inhibitor chelerythrine, before measuring the sugar uptake. The expression in the apical membrane of the transporters implicated in the sugars uptake process (SGLT1 and GLUT5) was determined by Western blot.

Results: TNFα inhibited 0.1 mM MG uptake after pre-incubation of the cells for 6-48 h with the cytokine and in the absence of cytokine pre-incubation. In contrast, 5 mM fructose uptake was stimulated by TNFα only after long pre-incubation times (24 and 48 h).These effects were mediated by the binding of the cytokine to its specific receptor TNFR1, present in the apical membrane of the Caco-2 cells. Analysis of the expression of the MG and fructose transporters at the brush border membrane of the cells, after 24 h pre-incubation with the cytokine, revealed decrease on the amount of SGLT1 and increase on the amount of GLUT5 proteins. Short-term inhibition of MG transport by TNFα was not modified by H-89 but was blocked by chelerythrine.

Conclusions: SGLT1 and GLUT5 expression in the plasma membrane is regulated by TNFα in the human epithelial cell lineCaco-2 cells, leading to alteration on sugars transport,

suggesting that TNFα could be considered as a physiological local regulator of nutrients absorption in response to an intestinal inflammatory status.

Keywords: Caco-2 cells, GLUT5, PKC, PKA, SGLT1,TNFα.

1. Introduction

The gastrointestinal mucosa constitutes a physical barrier between the external environment and the organism that is permanently exposed to potential antigenic substances.As a result, inflammatory reaction may occur and, if this reaction is not properly controlled by the immune system, chronic inflammation can appear leading to an excess of pro-inflammatory cytokines synthesis, as it happens in inflammatory bowel diseases (IBD),Crohn's disease (CD) and ulcerative colitis (UC) [1].Among the pro-inflammatory cytokines, TNFα plays a key role in the pathogenesis of IBD [1].Thus, it has been reported that TNFα concentrationcan be 400 fold higher in the blood [2] and in the intestine [3] of patients with IBD compared to thatofhealthy people. TNFα is released in the intestinal mucosa by activated T-cells and macrophages, and is able to act locally and systemically by binding to its receptors, TNFR1 and TNFR2 [4]. As a result, nuclear factor NF-kβ is activated and stimulates the production of other pro-inflammatory cytokines, including TNFα itself, which triggerthe immune response [1].TNFα activatesothersintracellular signalling pathwayswhich includes the MAPK and c-Jun N-terminal kinases pathways, PKA and PKC [5,6].

IBD patients usually presentlong malnutrition periods due to a diminished food intake and alterationsof the digestion process. In addition, in the intestinal inflammatory state, expression and activity of nutrient and electrolytes transporters may be altered leading to malabsorption and diarrhoea that typically appearin IBD patients [7]. Previous studies from our group performed in rabbithave shown that the i.v. administration of TNFα decreases galactose,

fructose and leucine intestinal transport and the expression of the corresponding transporters in the brush border membrane (BBM) of the enterocytes, with the implication of nitric oxide, PKC, PKA and several MAPKs [5,8,9]. Likewise, in rat everted intestinal rings, TNFα inhibitsgalactoseuptakeafter short time incubation [10]. Studies performed in Caco-2 cells have also shown that TNFα modifies the transporters implicated in the uptake of serotonin, taurine, iron and peptides [11-14].

The intestinal absorption of glucose and galactose is mainly mediated by the apical sodium-glucose co-transporter SGLT1/SLC5A1 located at the BBM of the enterocytes. Fructose transport occurs through the facilitative transporter GLUT5/SLC2A5 [15]. The three sugars leave the cells via the basolateral transporter GLUT2/SLC2A2, although expression of GLUT5 in the basolateral membrane of human enterocytes has also been documented [16].After a meal, sugarsreach high concentration in the intestinal lumen and in this particular situation, GLUT2 is also transiently expressed in the apical membrane allowing a rapidsugar transport without cell energetic cost [17].

In continuation with our previous studies performed in animal models, the aim of the present work was to studythe effect of TNFα on sugartransportin the human intestinal epithelial cell line Caco-2. We havealso investigated whether TNFα affects the transporters expression and analyzed the possible implication of PKA and PKC in this effect. The results suggest that TNFα could be considered as a physiological local regulator of sugarsabsorption in response to intestinal inflammation.

2. Materialand Methods

2.1 Chemicals

Human recombinant TNFα (Peprotech Inc. UK) was reconstituted in H_2O at a concentration of 100 mg/ml following the manufacturer's indications. Dubelcco´s Modified Eagles medium (DMEM) and supplements were purchased from Gibco BRL (Paisley, UK). [^{14}C]-α-

methylglucoside (313 mCi/mmol) was from GE Healthcare (LittleChaldfont, UK), [U-^{14}C]-D-galactose (309 mCi/mmol) and [U-^{14}C]-D-fructose (299 mCi/mmol) were obtained from GE Healthcare Life Sciences (Madrid, Spain). All unlabeled amino acids and α-methyl-glucoside (MG), chelerythrine chloride (PKC inhibitor) and H-89 (PKA inhibitor) were obtained from Sigma Chemicals Inc (St Louis, MO, USA) and Calbiochem (Nottingham,UK) respectively. Stock solutionsofprotein kinases inhibitors were prepared in DMSO and freshly diluted into cell culture medium before use.The TNFα receptor 1(TNFR1) antagonist H3736 was purchased from Bachem (Switzerland).

2.2 Cell culture

Caco-2 cells were maintained in a humidified atmosphere of 5% CO_2-95% at 37ºC. Cells (passages 50-70) were grown in DMEM (Gibco Invitrogen, Paisley, UK) supplemented with 10% foetal bovine serum (FBS), 1% non essential amino acids, 1% penicillin (1000 U/ml), 1% streptomycin (1000 μg/ml) and 1% amphoterycin (250 U/ml). Once the cells reached 80% confluence, they were dissociated with 0.05% trypsin-EDTA and sub-cultured on 25 or 75 cm^2 plastic flasks at a 25x10^4 cells/cm^2 density. For transport studies, the cells were seeded in 24 well culture platesat a densityof 6 x 10^4cells/cm^2. Culture medium was replaced every 2 days. Cell confluence was confirmed by microscopic observance. Uptake experiments were performed at 17-21 days post-seeding.

2.3 Uptake experiments

Galactose,α-methyl-glucoside (MG) and fructose uptake experiments were performed in the absence (control condition) or presence of different TNFα concentrations. In some experiments, cells were pre-incubated with TNFαin glucose and FBS-free DMEM for different incubation times. In this medium, used as uptake buffer, 0.1 mMgalactose, MG (SGLT1 specific substrate) or 5 mM Fructose (GLUT5 specific substrate) were diluted with traces of its corresponding radiolabeled substrate in the absence or presence of TNFα,

depending on the experiment. The uptake was stopped with ice cold free-substrate buffer followed by aspiration.Cells were again washed twice with ice-cold buffer to eliminate non-specific radioactivity fixation and were finally solubilized in 500 µl 1% Triton X-100 in 0.1 N NaOH. Samples (100 µl) were taken to measure radioactivity by liquid scintillation counting. Protein concentration was determined by theBradford method (Bio-Rad Protein Assay, Bio-Rad laboratories, Hercules, CA).In those experiments in which the TNFR1 antagonist was used, the cells were incubated for 24 h in the presence of the antagonist (250 ng/ml) or TNFα (10 or 25 ng/ml) before measuring 0.1 mM MG or 5 mMfructose uptake (15 min) in the presence or the absence of TNFα. For the analysis of the implication of PKC and PKA in the actions mediated by TNFα, the PKA inhibitor H-89 and the PKC inhibitor chelerythrinewere used. The cells were pre-incubated for 30 min in the presence of the inhibitor at a concentration of 1 µM or 2 µM respectively, before the addition of the substrate uptake solution.In both type of experiments, after the uptake period, the cells were processed as previously described.

2.4 Western blot analysis

Cells grown on 75 cm^2 plastic flasks were incubated for15 min with 0.1 mM MG in the presence or the absence of TNFα(50 ng/ml). In another set of experiments, the cells were pre-incubated for 24 hours in the presence of 10 or 25 ng/mlTNFα. Fifteen min before the end of the pre-incubation time, 0.1mM MG or 5 mMfructosewere respectively added to the pre-incubation medium containing 10 or 25 ng/mlTNFα, mimicking the conditions of the uptake experiments previously described.After the incubation period (15 min or 24 h), brush-border membrane vesicles (BBMV) were isolated [18]from each flask and from non-treated cells (incubated in DMEM) as control. The protein content of the vesicles was determined by the Bradford method (Bio-Rad Protein Assay, Bio-Rad laboratories, Hercules, CA).

Solubilized proteins (20 µg) were resolved by electrophoresis on 12% SDS-PAGE mini gels. The resolved proteins were transferred to a PVDF membrane (Hybond P, GE Healthcare),blockedin TBS buffer with 3% BSA for three hours at room temperature (RT), and incubated overnight at 4ºCwith the corresponding primary antibody: anti-rabbit SGLT-1 (1:3000) kindly donated by Ernest M. Wright (UCLA) or anti-goat GLUT-5 (1:500) from Santa Cruz Biotech (G-21; sc-14841). After the incubation, the membranes were washed three times in TBS-Tween 0.15% and incubated for one hour at RT with the corresponding peroxidase conjugated secondary antibody (Santa Cruz Biotech.).

Membranes were stripped and immunoblotted again with a monoclonal β-actin antibody (Santa Cruz Biotech.) used at 1:1000, in order to perform the loading control of the different wells. The immunoreactive bands were detected by enhanced chemiluminiscence (Super Signal West Dura, Thermo Scientific) and quantified by densitometric analysis (Gel Pro Analyzer 3.2). The results were expressed in % of the control value which was set to 100.

2.5 Statistical analysis

Transportexperimentaldata wereexpressed as nmol/mg of protein. All data are presented as % compared to controls. Statistical differences were evaluated by the two-way ANOVA test or the one-way ANOVA test followed by the *Dunnett* post-hoc test or Student *t*-test for parametric analysis and *U Mann-Whitney* test for non-parametric one. Differences were considered as statistically significant at $p<0.05$. The calculations were performed using the SPSS/WINDOWNS VERSION 15.0 statistical package (SPSS. Chicago, IL, USA).

3. Results

3.1 TNFα modulates sugar uptake

The effect of 10 and 25 ng/mlTNFα on 0.1mM galactoseuptake(15 min) was measured in Caco-2 cells pre-incubated with the cytokine for1,12, 24 or 48 h. The results in Fig. 1A show that both TNFαconcentrations inhibited galactose uptake. At each pre-incubation time, the

inhibition was higher with 25 ng/ml (40-60 %) than with 10 ng/ml (20-40 %)These results suggested that SGLT1 was regulated by TNFα since at the concentration of 0.1mM, below its $K_{0,5}$ [19],this is the main mechanism responsible for the sugar uptake. To confirm this hypothesis and to determine the minimum cytokine concentration needed to inhibit the sugar uptake,we studied TNFα effecton the uptake of MG, aspecific substrate of SGLT1, using lower concentrations of the cytokine.As shown in Fig. 1B, 10 ng/mlTNFα reduced 0.1mM MG uptake (~20 %) already after 1h pre-incubation, while at 1 ng/mlTNFα, the inhibitory effect of the cytokine was observed only after 24 h pre-incubation. Interestingly, at these conditionsTNFα produced the same degree of inhibition as 10ng/ml (~40 %).

In order to investigate whether TNFα was able to exert its effect without pre-incubation of the cells with the cytokine, uptake of MG for 15 and 60 min was determined in the presence of differentTNFα concentration. As shown in Fig.1C, 1ng/mlTNFα did not show significant effect on MG uptake after 15 min incubation, 10ng/mlinhibited the uptake by 20% and 25 and 50ng/mlinhibitedby ~40 %. In the experiments performed at 60 min incubation, both 1 and 10 ng/ml TNFα inhibited MG uptake by a 25%.

All these results demonstrated that TNFα inhibited, in a short and long-term manner, galactose and MG uptake by regulating SGLT1.

The effect of 25 ng/mlTNFαon 5 mMD-fructose uptake, a substrate of the facilitative transporterGLUT5, was also investigated. TNFα did not modify fructose transportwhen cells were not pre-incubated with the cytokine (data not shown) or after pre-incubation of the cells for 6 h (Fig. 2). However, when the cells were pre-incubated with TNFα for 24 or 48 h, the cytokine stimulated fructose uptake by a ~25 %.Therefore, contrary to SGLT1,TNFα increased GLUT5 activity andhigher cytokine concentrations and longer incubation times wereneeded to regulate GLUT5 activity compared with SGLT1.

3.2 TNFα regulation of sugar uptake is mediated by its specific receptor

In order to investigate whether TNFα effect was specific and, therefore, receptor-mediated, the effect of TNFα on0.1mMgalactose and 5mM fructose uptake (15 min) was measured after 24 h pre-incubation of the cells with a TNFR1 antagonist.As previously showed, TNFα reduced galactoseuptake (Fig. 3A) and increased fructose uptake (Fig. 3B).The TNFR1 antagonist alone did not modified galactose or fructose uptake but completely prevented the cytokine effect (Fig. 3A and B). The results demonstrated the involvement of TNFR1 in the TNFα regulation of SGLT1 and GLUT5 activities.

3.3 TNFα regulates SGLT1 and GLUT5 expression in the brush border membrane of the enterocytes

To investigate whether TNFα effect on MG and fructose uptake was due to modification on the amount of SGLT1 and GLUT5 proteins in the apical membrane of the enterocytes, the expression of these transporters in BBMV was analyzed by Western blot, after the cells were treated under the same experimental conditions than those ofthe functional studies. Expression of SGLT1 (~75 kDa) in the BBMV was not modified after 15 min incubation of the cells with 50 ng/mlTNFα (Fig. 4A). However, the incubation of the cells for 24hwith the cytokine (10 ng/ml),decreased the amount of SGLT1 in the BBM compared with the expression in control conditions (Fig. 4B)explaining the reduction of MG uptake found under these conditions. Likewise, in line with the functional results, GLUT5 (~48 kDa) expression in the BBMV was increased after 24h incubation of the cells with 25 ng/ml TNFα (Fig 4 C).

3.3 PKA and PKC are implicated in the effect of TNFα on sugar uptake

Since TNFα inhibited MG transport after 15 min incubation of the cells with both the sugar and the cytokine, but this effect was not accompanied by modification on the amount of

SGLT1 in the plasma membrane (Fig. 4A), we decided to investigate whether a PKA or PKC-dependent pathway could be implicated in the short-term inhibitory effect of SGLT1 by TNFα. For that, the cells were pre-exposed to thespecific inhibitor of each protein kinase for 30 min before measuring MG uptake for 15 min, as described in the Material and Methods section.

As shown in Fig5, chelerythrine, the PKC inhibitor, decreased MG uptake by ~15 %, but completely blocked the effect of TNFα (25 ng/ml). The PKA inhibitor H-89 alone also reduced 0.1mM MG uptake (~15%), but was not able to prevent the inhibitory effect of TNFα These results indicated that PKC was involved in the TNFα inhibitory effect on MG after 15 min incubation.

4. Discussion

The intestinal epithelium is responsible for the nutrients absorption from the lumen to the blood circulation. Enterocytes are polarized intestinal epithelial cellswhich are exposed to a microenvironmentthat is permanently changing,depending onthe physiological or pathological conditions of the organism. The brush border membrane of these cellsis a highly specialized membrane that can act as a nutrient digestive andabsorptive surface, and as a protective permeability barrier preventing the passage of luminal pathogens to the blood [20]. The enterocytes can thus detect specific changes in the intestinal lumen and activate different signalling pathways, in order to adapt nutrient absorption [21]. In addition, local and endocrine mediators as well as several neurotransmitters from the enteric nervous system are also able to regulate the intestinal absorption. Examples of these molecules are GLP-2[22], EGF [23], adrenomedullin-derived peptides [24] and leptin [25].Moreover,inflammatory and infectious diseases that affect the gastrointestinal tractalter the intestinal mucosa, triggering the secretion of diverse cytokines, including TNFαthat can lead to nutrient

malabsorption[5,8,9].TNFα is also expressed and secreted by human colon epithelial cells in response to bacterial infection or after TNFα or IL-1 stimulation [26]. During inflammatory processes, TNFα can induce intestinal barrier dysfunction [27], which would explain how cytokines can reach the apical membrane of the enterocytes and interact with its corresponding receptors, through autocrine and paracrine mechanisms.

In the present work, we showin the human intestinal cell line Caco-2 that TNFα, acting from the apical membrane through the TNFR1 receptor, inhibits the uptake of MG while stimulates the uptake of fructose, in a long-term dependent manner. This effect is accompanied by a decrease and an increase on the abundance of SGLT1 and GLUT5 transportersin the brush border membrane respectively. Uptake of MG is also inhibited by TNFα in a short-term manner being PKC implicated.

Our results are in agreement with those from other authors who have reported that both TNFR1 and TNFR2 are expressed in Caco-2 cells but only TNFR1 is implicated in the apical regulation by TNFαof the taurine transporter TAUT [28].

Interestingly,TNFαshowsopposite effect ontheactivityofSGLT1andGLUT5. One explanation could be that in metabolic stress situations such as inflammation, TNFα would regulate differently the energy-requiring transporters such as SGLT1 and the passive transporters such as GLUT5.In this sense,Sakar and colleagues have demonstrated thatleptin, a cytokine implicated in the regulation of intestinal nutrients absorption[25, 29, 30],also down regulates SGLT1 but up regulates GLUT 5 [31].

After 24 h treatment of the Caco-2 cells with TNFα, we observed a ~50 % reduction in MG uptake accompanied by a ~20 % decrease on the expression of SGLT1 in the BBM. The absence of correspondence between transporter expression and function could be related with an impaired Na^+ gradient across the intestinal barrier, due to modification of the

Na^+/K^+ATPase activity or alteration of the paracellular ion permeability by TNFα [32-35]. Inhibition of sugar uptake by TNFα could also be related with tight junction dysfunction, which would allow sugar passage through the paracellular pathway. Nevertheless, although increase in transepithelial permeability has been described in Caco-2 cells after 24 h incubation with TNFα, this leaded to alteration in the charge selectivity of the paracellular conductive pathway but was not associated with a significant increase of the transepithelial mannitol flux [36, 37].

In the case of GLUT5, since it is a Na^+ independent transporter, the absence of correspondence between transporter expression levels and function due to TNFα, could be explained by TNFα-mediated cytoskeleton disruption [39] that would impair the correct insertion of the transporter in the BBM leading to a reduced activity [40] even though its expression was increased.

Long-term pre-incubationof Caco-2 cells with TNFαpresent in the apical or basal medium increases taurine and peptidesuptake respectively, by regulatingprotein expression of the active co-transporters TAUT [12] and PepT1[14]. On the contrary, as we show for SGLT1, serotonine and iron transport is decreased in Caco-2 cells pre-incubated for 72 h with TNFα, acting from the apical and basolateral membrane respectively, due to a decrease in the expression of the secondary active transportersSERT and DMT1[41, 42].Therefore, it is clear that the expression of several intestinal transporters is regulated byTNFα, which may be important in intestinal inflammation.

Similar to the studies in Caco-2 cells, it has been shown in rabbit treated with i.v. TNFα to induce sepsis, thatgalactose absorption is inhibited by reduction on the amount of SGLT1in the apical membrane of the enterocytes but, in contrast to the Caco-2 results, fructose uptake in the jejunum was decreased in partdue to a reduction in the number of transporters present

in the brush border membrane [5, 8]. In this regard, it has been demonstrated that the post-transcriptional regulation of GLUT5 can be different between *in vitro* and *in vivo* models [43].

After short-term treatment of the Caco-2 cells with TNFα, the inhibition of SGLT1 activityinvolves PKC activation, without modification on the amount of SGLT1 in the apical membrane. It is known that TNFα induces activation of PKA and PKC[6], and thatthese protein kinases induce short-term regulation of SGLT1 through mechanisms that include trafficking from intracellular pools to the plasma membrane and activation or inactivation of the transporters function [44-47].In line with our results, in COS-7 cells, activation of PKC decreases sugar transport by SGLT1 without any effect on the number of transporters at the cell surface, suggesting that PKC may decrease the turnover rate of the transporter [46], which may also occur in the present work.

In summary, TNFα can modify SGLT1 and GLUT5 activity by mechanisms that include regulation of the expression of the transporters in the brush border membrane and activation of protein kinases, suggesting that this cytokine should be considered as a physiological local regulator of sugar absorption in response to an inflammatory status of the intestine.

Acknowledgments

We thank Asunción Redín for her unconditional technical assistance.

This work was supported by PIUNA (University of Navarra), “Departamento deEducación y Cultura” from the Navarra Government, Spain, and “FundaciónMarcelinoBotín”, and grants from FIS and ISCIII CB06/03/1012,” Departamento de Ciencia, Tecnología y Universidad” from the Aragón Government, Spain, and FSE: (A-32 and PI017/09).The groupsare members of the Network for Cooperative Research on Membrane Transport Proteins (REIT), co-funded

by the “Ministerio de Educación y Ciencia”, Spain and the European Regional Development Fund (ERDF) (Grant BFU2007-30688-E/BFI).

References

[1] Viscido A, Aratari A, Maccioni F, Signore A, Caprilli R. Inflammatory bowel diseases: clinical update of practical guidelines. Nucl Med Commun2005; 26: 649-655.

[2] Komatsu M, Kobayashi D, Saito K, Furuya D, Yagihashi A, Araake H, Tsuji N, Sakamaki S, Niitsu Y, Watanabe N. Tumor necrosis factor-alpha in serum of patients with inflammatory bowel disease as measured by a highly sensitive immuno-PCR. ClinChem2001; 47: 1297-1301.

[3] BreeseE, Michie C, Nicholls S, Murch S, Williams C, Domizio P, Walker-Smith J, MacDonald T. Tumour necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology 1994; 106: 1455-1466.

[4] Rossol M, Meusch U, Pierer M, Kaltenhäuser S, Häntzschel H, Hauschildt S, Wagner U. Interaction between transmembrane TNF and TNFR1/2 mediates the activation of monocytes by contact with T cells. J Immmunol2007; 179: 4239-4248.

[5] Amador P, García-Herrera, Marca M.C, De La Osada J, Acín S, Navarro, MA, Salvador MT, Lostao MP, Rodríguez-Yoldi, MJ. Inhibitory effect of TNF-α on the intestinal absorption of galactose. J CellBiochem 2007; 101: 99-111.

[6] Wajant H, Pfizenmaier K, Scheurich P. Tumor necrosis factor signaling.Cell Death Differ. 2003;10(1):45-65.

[7] McKay DM, Baird, AW. Cytokine regulation of epithelial permeability and ion transport. Gut 1999; 44: 283-289.

[8] García-Herrera J, Navarro MA., Marc, MC, de la Osada, J, Rodríguez-Yoldi MJ. The effect of tumour necrosis factor-alpha on D-fructose intestinal transport in rabbits. Cytokine 2004; 25: 21-30.

[9] Abad B, Mesonero JE, Salvador MT, Garcia-Herrera J, Rodriguez-Yoldi M.J. Tumor Necrosis Factor-α mediates inhibitory effect of Lipopolysaccharide on L-Leucine intestinal uptake. Dig Dis Sci 2002; 47: 1316-1322.

[10] Bértolo C, Barrenetxe J, Barber A, Lostao MP. Cytokine effect on intestinal galactose absorption. J Physiol Biochem 2002; 58, 61-62.

[11] Foley KF, Pantano C, Ciolino A, Mawe GM. IFN-γ and TNF-α decrease serotonin transporter function and expression in Caco-2 cells. Am J Physiol Gastrointest Liver Physiol 2007; 292: G779-784.

[12] Mochizuki T, Satsu H, Shimizu M. Tumor necrosis factor αstimulates taurine uptake and transporter gene expression in human intestinal Caco-2 cells. FEBS Lett 2002; 517: 92-96.

[13] Sharma N, Laftah A, Brookes M, Cooper B, Iqbal T, Tselepis C. A role for Tumour necrosis factor alpha in human small bowel iron transport. Biochem J 2005;390: 437-446.

[14] Vavricka SR, Musch MW, Fujiya M, Kles K, Chang L, Eloranta JJ, Kullak-Ublick G A, Drabik K, Merlin D, Chang EB. Tumor necrosis factor-α and Interferon-γ increase PEPT1 expression and activity in the human colon carcinoma cell line Caco-2/BBE and in mouse intestine. Pflugers Arch 2006; 452: 71-80.

[15] Wright EM, Loo DF, Hirayama BA& Turk E. Sugar absorption. In: L.R. Johnson ed. Physiology of the gastrointestinal tract 2006; pp 1653-1665. Elsevier, San Diego.

[16] Blakemore SJ, Aledo JC, James J, Campbell FC, Lucocq JM, Hundal HS. The GLUT5 hexose transporter is also localized to the basolateral membrane of the human jejunum. Biochem J 1995; 309: 7-12.

[17] Kellett GL, Brot-Laroche E, MaceOJ, Leturque A. Sugar Absorption in the intestine: the role of GLUT2. Annu Rev Nutr 2008; 28: 35-54.

[18] Garriga C, Rovira N, Moretó M, Planas JM.Expression of Na^+-D-glucose co-transporter in brush-border membrane of the chicken intestine. Am J Physiol 1999;276: R627-31.

[19] Hirayama BA, Lostao MP, Panayotova-Heiermann M, Loo DD, Turk E, Wright, EM. Kinetic and specificity differences between rat, human, and rabbit Na^+-Glucose co-transporters (SGLT-1). Am J Physiol 1996; 270: G919-926.

[20] Danielsen EM,Hansen GH. Lipid raft organization and function in the small intestinal brush border. J Physiol Biochem 2008; 64: 377-382.

[21] Raybould HE. Nutrient sensing in the gastrointestinal tract: possible role for nutrient transporters. J Physiol Biochem 2008; 64: 349-356.

[22] Au A, Gupta A, Schembri P, Cheeseman C. Rapid insertion of GLUT2 into the rat jejunal brush-border membrane promoted by glucagon-like peptide 2. Biochem J 2002; 367: 247-254.

[23] Chung B, Wallace L, Hardin J, Gall D. The effect of epidermal growth factor on the distribution of SGLT-1 in rabbit jejunum. Can J Physiol Pharmacol 2002; 80: 872-878.

[24] Fernández De Arcaya I, Lostao MP, Martínez A, Berjón A, Barber A. Effect of adrenomedullin and proadrenomedullin N-terminal 20 peptide on sugar transport in the rat intestine. Regul Peptides 2005; 129: 147-154.

[25] Iñigo C, Patel N, Kellett G, Barber A, Lostao MP. Luminal Leptin inhibits intestinal sugar absorption in vivo. Acta Physiol 2007; 190: 303-310.

[26] Jung H C, Eckmann L, Yang SK, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J Clin Invest 1995; 95: 55-65.

[27] Wang F, Schwarz BT, Graham WV, Wang Y, Su L, Clayburg DR, Abraham C & Turner JR. IFN-γ-induced TNFR2 expression is required for TNF-dependent intestinal epithelial barrier dysfunction. Gastroenterol 2006; 131: 1153-1163.

[28] Mochizuki T, Satsu H, Shimizu M. Signalling pathways involved in tumor necrosis factor α–induced upregulation of the taurine transporter in Caco-2 cells. FEBS Letters 2005; 579: 3069–3074

[29] Ducroc, R, Sakar, Y, Fanjul, C, Barber, A, Bado, A, Lostao, MP. Luminal leptin inhibits L-glutamine transport in rat small intestine: involvement of ASCT2 and B0AT1. Am J Physiol Gastrointest Liver Physiol 2010; 299, G179-85.

[30] Fanjul C, Barrenetxe J, Iñigo C, Sakar Y, Ducroc R, Barber ALostao MP. Leptin regulates sugar and amino acids transport in the human intestinal cell line Caco-2 Acta Physiol (Oxf). 2012; 205(1), 82-91.

[31] Sakar, Y, Nazaret, C, Letteron, P, Ait Omar, A, Avenati, M, Viollet, B, Ducroc, R, Bado, A. Positive regulatory control loop between gut leptin and intestinal GLUT2/GLUT5 transporters links to hepatic metabolic functions in rodents. Plos One 2009; 4:e7935.

[32] Markossian S, Kreydiyyeh SI. TNF-αdown -regulates the Na^+-K^+ ATPase and the Na^+-K^+-2Cl- cotransporter in therat colon via PGE2. Cytokine 2005; 30;319-327.

[33] Moran GW, O´Neill C, McLaughlin. GLP-2 enhances barrier formation and attenuates TNFα-induced changes in a Caco-2 cell model of the intestinal barrier. Reg Peptides 2012; 178:95-101.

[34] Tamura A, Hayashi H, Imasato M, Yamazaki Y, Hagiwara A, Wada M, Noda T, Watanabe M, Suzuki Y, Tsukita S. Loss of Claudin-15, but not Claudin-2, causes Na^+ deficiency and glucose malabsorption in mouse small intestine. Gastroenterol 2011; 140: 913-23.

[35] Cui W, Li LX, Sun CM, Wen Y, Zhou Y, Dong YL, Liu P. Tumor necrosis factor alpha increases epithelial barrier permeability by disrupting tight junctions in Caco-2 cells. Brazilian J Med Bio Res 2010; 43:330-37.

[36] Marano CW, Lewis SA, Garulacan L A, Peralta Soler A, Mullin JM. Tumor necrosis factor alpha increases sodium and chloride conductance across the tight junction of

Caco-2 BBE, a human intestinal epithelial cell line. J Membrane Biol 1998; 161: 263-274.

[37] He F, Peng J, Deng XL, Yang LF, Camara AD, Omram A, Wang GL, Wu LW, Zhang CL, Yin F. Mechanism of tumor necrosis factor alpha induced leaks in intestine epithelial barrier. Cytokine 2012; 59: 264-272.

[38] Sundaram U, Wisel S, Rajendren VM, West AB. Unique mechanism of inhibition of Na^+-glucose cotransport in the chronically inflamed rabbit ileum. Am J Physiol Gastrointest Liver Physiol 1997; 273: G913-G919.

[39] Turner JR. Molecular basis of epithelial barrier regulation. Am J Pathol 2006; 169:1901-09.

[40] Zhen Y and Sarr MG. Translocation of transfected GLUT2 to the apical membrane in rat intetsinal IEC-6 cells. Dig Dis Sci 2012; 57:1203-12.

[41] Foley KF, Pantano C, Ciolino A, Mawe GM. IFN-gamma and TNF-alpha decrease serotonin transporter function and expression in Caco2 cells.Am J Physiol Gastrointest Liver Physiol. 2007;292(3):G779-84.

[42] Johnson D, Bayele H, Johnston K, Tennant J, Srai SK, Sharp P.Tumour necrosis factor alpha regulates iron transport and transporter expression in human intestinal epithelial cells.FEBS Lett. 2004;573(1-3):195-201.

[43] Douard V, Ferraris RP. Regulation of the fructose transporter GLUT5 in health and disease. Am J Physiol Endocrinol Metab2008; 295(2): E227-37.

[44] Wright EM, Hirsch JR, Loo DD & Zampighi GA. Regulation of Na^+/Glucose cotransporters. J Exp Biol 1997; 200: 287-293.

[45] Barrenetxe J, Sainz N, Barber A, Lostao MP. Involvement of PKC and PKA in the inhibitory effect of leptin on intestinal galactose absorption. Biochem Biophys Res Commun2004;317: 717-721.

[46] Vayro S. Silverman M. PKC regulates turnover rate of rabbit intestinal Na^+-glucose transporter expressed in COS-7 cells. Am J Physiol1999; 276, C1053-1060.

[47] Castaneda-Sceppa C, Subramanian S, Castaneda F. Protein Kinase C mediated intracellular signalling pathways are involved in the regulation of Sodium-dependent glucose co-transporter SGLT1 activity. J Cell Biochem 2010; 109:1109-1117.

LEGENDS TO FIGURES

Figure 1: Effect of TNF-α on sugar uptake by Caco-2 cells.(A)Cells were pre-incubated with TNFα (10 or 25 ng/ml) for 1, 12, 24 or 48 h before measuring the uptake of 0.1 mMgalactose (Gal) for 15 min in the presence of the cytokine(control values ranged from 1.9 to 2.8 nmol/mgof protein)(B)Cells were pre-incubated with TNFα (1 or 10 ng/ml) for 1, 6 or 24 hours before measuring the uptake of 0.1 mMMG for 15 min in the presence of the cytokine.(Control values ranged from 0.2 to 0.35 nmol/mg of protein). (C) MG uptake was measured in Caco-2 cells for 15 and 60 min in the presence of TNFα (1, 10 or 25 ng/ml)(control values ranged from 0.5 to 1 nmol mg^{-1} of protein). Data (n=8-44) are expressed as % (mean ± SEM) of control values. *p< 0.05; **p< 0.01; ***p <0.001*vs* control.

Figure 2: Effect of TNFα on fructose uptake by Caco-2 cells. Cells were pre-incubated with TNFα (25 ng/ml) for 6, 24 or 48 h before measuring the uptake of 5mMfructose for 15 min in the presence of the cytokine. Data (n=26-29) are expressed as % (mean ± SEM) of control values*p<0.05; **p <0.01*vs.*control. (Control values ranged from 16 to 29 nmol/mg of protein)

Figure 3: TNF-α effect on sugar uptake is mediated by its specific receptor TNFR1. Cells were pre-incubated for 24 h with or without TNFα (25 ng/ml) in the presence and in the absence of TNFR1 antagonist (250ng/ml). Uptake of 1 mMgalactose(A) or 5 mM fructose (B) was measured for 15 min in the presence of the cytokine. Data (n=45-55) are expressed as % (mean ± SEM) of control values. *p< 0.05 *vs.* control.

Figure 4: Effect of TNFα on SGLT1and GLUT5 protein expression in BBMV of Caco-2 cells.BBMV were obtained after 15 min incubation of the cells with TNFα (50 ng/ml) and 0.1 mM MG(A) or after 24 h pre-incubation with TNFα(10 ng/ml) followed by 15 min incubation with the sugar(B).The intensity of the ~78 kDaimmunoreactive band (transporter/β-actin) is expressed as % of control (MG; n=4). A representative Western blot image is also represented. *p< 0.05 *vs*MG. In **(**C**)**BBMV were obtained after 24h pre-incubation of the cells with TNFα(25 ng/ml) followed by 15 min incubation with fructose (F). The intensity of the ~48 kDaimmunoreactive band (transporter/β-actin) is expressed as % of control (F; n=4). A representative Western blot image is also represented. ***p< 0.001*vs.* F.

Figure 5: Effect of TNFαon MG uptake in the presence of the PKA inhibitor (H-89) or the PKC inhibitor (chelerythrine).Cells were pre-incubated with 1 μM H-89 or 2 μM chelerythrine for 30 minutes before measuring the uptake of 0.1 mM MG for 15 min, in the absence or the presence of TNFα (25 ng/ml). Data (n=42-18) are expressed as % (mean ± SEM) of control values*p<0.05,***p< 0.001 *vs.* control (MG); ### p< 0.001 *vs.* TNFα.