Electronic Supplementary Material (ESI) for Food & Function.
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2 **Supplementary Information**

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4 ZnO Nanoparticles Affect Intestinal Function in an *In Vitro* Model

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1 ***In vitro* digestion**

2 The NP were suspended in 20 mL of a 140mM NaCl and 5mM KCl. The pH was then adjusted to 3 2 using HCl and then 1 mL of 20 mg/mL pepsin solution was added. The suspensions were placed 4 on a rocker for 1 hour at 55 oscillations/minute in an incubator at 37 °C, 0.5% CO_2. The pH was 5 then adjusted 5.5-6.0 using 1.0 M $NaHCO_3$, and 5.25 mL of 1.4 mg/mL pancreatin and 8.6 mg/mL 6 bile in 0.1M $NaHCO_3$ solution were added and the pH was adjusted to 6.9-7. Next, the volume 7 was adjusted to 30 mL using a 140mM NaCl, 5mM KCl solution at a 6.7 pH and samples were 8 referred to as "digests." The digests were then warmed in the water bath to 37°C before being 9 added to the cell cultures.

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11 **Cell viability**

12 Cells were seeded into black, clear bottom 96 well plates (Corning). After growing for 2 weeks, 13 the cells were exposed to the NP suspensions for 4 hours and then carefully washed with 14 phosphate-buffered saline solution (PBS) 3 times before adding 100 µL of a 0.6 µM calcein AM/ 15 0.9 µM propodium iodide solution to each well. These concentrations were determined by testing 16 several concentrations of the dyes until finding the optimal fluorescence for these cell cultures. 17 After a 45 minutes incubation at 37 ºC, the fluorescence of the cells was read with a microplate 18 reader (Synergy H1, BioTek) at 494 nm excitation and 530 nm emission using Gen Five software. 19 For the analysis, the cells treated with control DMEM were set to be 100% viable, and the viability 20 of the rest of the treatments were determined by calculating the ratio of a specific treatment to the 21 DMEM control. The same analysis was done to determine the percentage of dead cells. In addition, 22 the cells were imaged with a fluorescent microscope using a 10X objective (Nikon Eclipse Ti, 23 Boston Industries) to confirm the viability results.

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25 **Transepithelial electrical resistance**

26 The Endohm chamber was sterilized by soaking it in 70% ethanol for 15 minutes, then 2 mL of 27 sterile 100 mM KCl solution was added to the chamber, and the chamber was then connected to 28 the EVOM2 and left to equilibrate for 2 hours before starting the experiments. After 2 hours, 29 600 µL of fresh KCl was added into chamber, along with a sterilized Calicell that was used to 30 calibrate the meter. After calibration, the Calicell was removed and the Endohm chamber was 31 rinsed with sterile 18MΩ deionized (DI) water. The Endohm chamber was equilibrated for 15

1 minutes with 2 mL serum free DMEM, during that 15 minute equilibration, the Transwells were
2 taken out of the incubator to stabilize to room temperature before starting the measurements. Next
3 the equilibration DMEM was replaced with 600 µL serum free DMEM and the TEER of every
4 sample was measured.
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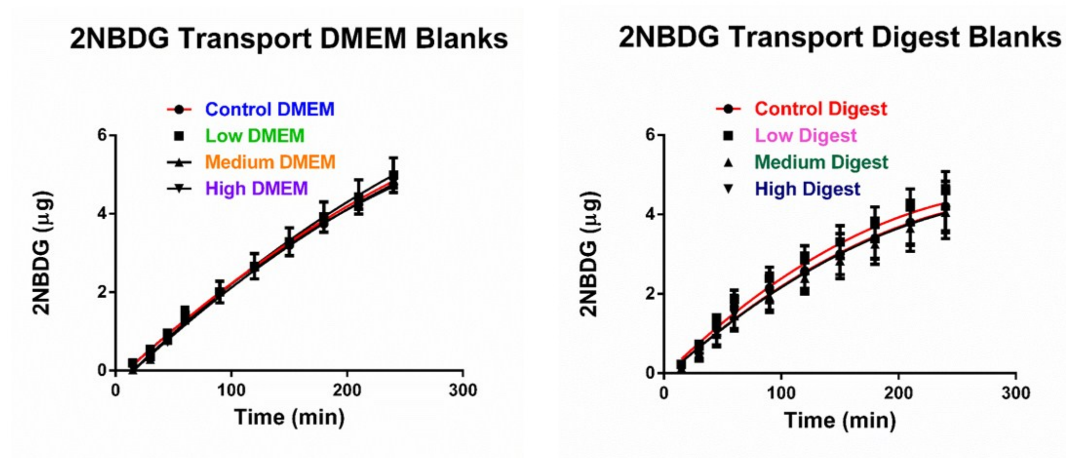
6 **Glucose Transport**



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8 **Suppmelemtary Figure 1. 2-NBDG transport in blank wells.**
9 Analysis of 2-NBDG transport from wells that do not contain cells (blanks). The wells were treated
10 with serum-free, glucose-free, phenol red-free DMEM (Thermo Fisher Scientific) for one hour and
11 then exposed to low, medium and high concentrations of ZnO NP in 2-NBDG immediately before
12 starting the time points. Mean ± SEM is shown, n = 3. Curve fits (solid black lines) were compared
13 using the AICs from a quadratic model. Low, medium and high refer to the dose of ZnO
14 nanoparticles (NP), where low = 9.7 x 10^{-6} mg/mL, medium = 9.7 x 10^{-4} mg/mL, and high = 9.7 x
15 10^{-2} mg/mL. Digest refers to ZnO nanoparticles that have been subjected to a simulated gastric
16 and intestinal digestion. There were no significant differences in glucose analog transport with
17 differing doses of ZnO NP, indicating the differences in 2-NBDG transport in wells containing
18 cells was due to NP interaction with cells and not 2NBDG binding to NP.

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Supplemental Table 1. Primer sequences used for RT-PCR

Protein	Gene	Primer	Reference
Glyceraldehydes-3-phosphate dehydrogenase	***GADPH***	Forward: 5'-GACCACAGTCCATGACATCACT-3' Reverse: 5'-TCCCACCACCCTGTTGCTGTAG-3'	1
Duodenal cytochrome-B	***Dctyb***	Forward: 5’- TCATCCAGGGCATCGCCATC-3’ Reverse: 5’-CGGAGCCCATGGAAGCAGAA-3’	2
Divalent metal transporter-1	***DMT1***	Forward: 5’-TGTCACCGTCAGTATCCCAA-3’ Reverse: 5’- GTGCAATGCAGGATTCAATG-3’	1
Hephaestin	***HEPH***	Forward: 5’-TCTCTGCTGCAGATCCCATA-3’ Forward: 5’-CAGAAACCCATTATTGGCATGC-3’	2
Ferroportin1	***FPN1***	Forward: 5’-TGTTTCTGGTAGAGCTCTAT-3’ Reverse: 5’-GATATAGCAGGAAGTGAGAA-3’	2
Zinc transporter-1	***ZnT1***	Forward: 5'- CAATACCAGCAACTCCAACGG-3’ Reverse: 5'- GCAAGGACCAGCCTCATAAAC-3’	3
Zrt, Irt-like protein-1	***ZIP1***	Forward: 5'- CCACTTGTCTCCTGGACCTGC-3’ Reverse: 5'- AGCCACCACCTGTGCCCTAA-3’	3
Liver-type fatty acid-binding protein (FABP-1)	***L-FABP***	Forward: 5'- GCAAGTACCAACTGCAGAGCCAGGAAAAGT-3' Reverse: 5'-TTGCTGATTCTCTTGAAGACAATGTCACCC-3'	4
Intestinal fatty acid-binding protein (FABP-2)	***I-FABP***	Forward: 5'-GATAAACTAAAAGCATAGGCTGCATATG-3' Reverse: 5'-TCAAAATCAGAATGGCAATTATCTCT-3'	5
Interleukin 8	***IL-8***	Forward: 5'-TACTCCAAACCTTTCCACCC-3' Reverse: 5'-AACTTCTCCACAACCCTCTG-3'	6
Tumor necrosis factor-alpha	***TNF-α***	Forward: 5’-TCAACCTCCTCTCTGCCATC-3’ Reverse: 5’- CCAAAGTAGACCTGCCCAGA-3’	7
Nuclear factor kappa-light-chain-enhancer of activated B cells	***NFκB1***	Forward: 5’- ACTGTGAGGATGGGATCTGC -3’ Reverse: 5’-GCACCAAGAGTCCAGGATTA-3’	8
Sodium glucose transporter 1	***SGLT1***	Forward: 5’- GCCCTGGTTTTGGTGGTTG -3’ Reverse: 5’- CGAGATCTTGGTGAAAATGTAGAGC -3’	9
Glucose transporter 2	***GLUT2***	Forward: 5’-AGTTAGATGAGGAAGTCAAAGCAA-3’ Reverse: 5’-TAGGCTGTCGGTAGCTGG-3’	10

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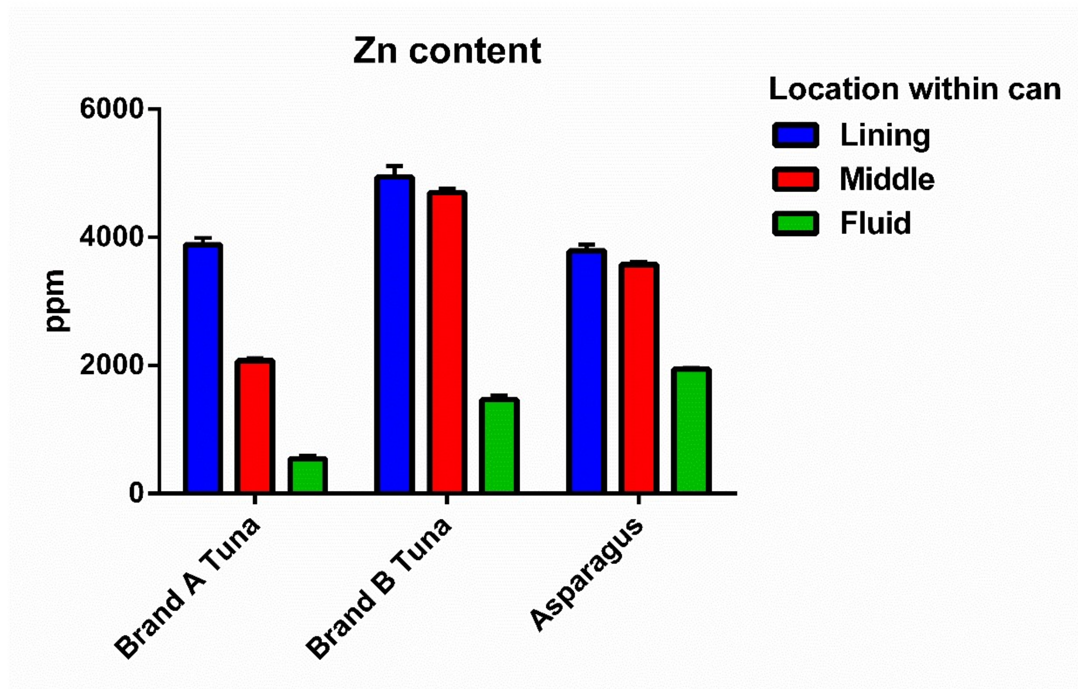

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3 **Supplementary Figure 2. Zn contents of tuna based on location within the can.**

4 Three different brands of canned food were purchased in the supermarket and the food samples
5 were removed from three different areas of the can: touching the lining, the center, and the fluid
6 that accumulates at the bottom. These samples were weighed before and after freeze-drying
7 (Labconco Freezone, Marshall Scientific) for 72 hours and then ground into powder. A 50 mg
8 aliquot of the powder was placed in 1.5 mL centrifuge tubes in triplicate and then digested in a
9 heat block with 70% HNO_3 until the acid looked clear and no food residue was left. Zn
10 concentration was measured using inductively coupled plasma mass spectrometry (ICP-MS,
11 NexION 300, Perkin Elmer) using Ytrium as internal standard. Measurements were made in
12 triplicate. Mean ± SEM is shown.

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