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Hypothesis

Association between intestinal tight junction permeability and whole-body electrical resistance in healthy individuals: A hypothesis

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Abstract

Objective: Intestinal permeability describes non-carrier–mediated modes of transport through the intestinal epithelium. Wrist–ankle bioimpedance analysis (BIA) is a standard method to determine body composition based on the measurements of whole-body electrical resistance and reactance values. The present report deals with the coincidentally observed associations between permeability results and electrical raw values of BIA and their subsequent reproduction in a larger group of individuals.

Methods: Tetrapolar wrist–ankle BIA was performed on day 1 in the initial sample (12 women, 36 ± 11 y of age) and the validation sample (36 healthy subjects, 26 women and 10 men, 35 ± 14 y of age). Intestinal permeability tests (lactulose and mannitol) were implemented within 1 wk thereafter. Wrist–ankle electrical resistance plus electrical resistance between current-conducting electrodes and voltage-sensing electrodes (Rtotal) was measured at 5 kHz and 100 kHz.

Results: Permeability and bioimpedance raw values were normal, indicating normal tight junction permeability and normal hydration. Lactulose correlated to $R_{50total}$ in the initial sample ($\rho = 0.639$, $P = 0.025$) and in the validation sample ($\rho = 0.673$, $P < 0.001$). Weaker associations to $R_{50total}$ were observed with mannitol ($\rho = 0.381$, $P = 0.008$) and lactulose/mannitol ($\rho = 0.369$, $P = 0.010$) in the total group of individuals. Regression analyses demonstrated that $R_{50total}$ alone accounted for 41.3% of the variance in lactulose permeability.

Conclusion: The nature of the observed positive association between intestinal tight junction permeability and whole-body electrical resistance is unclear. We hypothesize that regulation involving submolecular mechanisms based on the principles of quantum physics might have caused the observed association. Such coherent mechanisms might possibly play a role in basal physiologic regulation in humans.

Keywords: Lactulose; Mannitol; Bioimpedance; Biofield; Coherence; Quantum physics

Introduction

The term "permeation" specifies non-carrier–mediated diffusion of water and solutes across epithelial or endothelial cell layers [1]. Permeation is generally considered an unregulated process that is largely dependent on the physicochemical characteristics of the processed molecule and

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osmotic pressure gradients [2,3]. So far research interests have mainly focused on clinical conditions associated with increased permeability of the intestine [4]. Increased intestinal permeability implicates the concept of compromised intestinal barrier function including bacterial translocation and thus increased susceptibility to systemic infections [5–7]. Normal intestinal permeability, however, has never really ignited intensive scientific research.

Intestinal permeability can be non-invasively determined by the urinary recovery of defined amounts of orally ingested probe sugars [1]. Chosen probe sugars must be neither metabolized nor synthesized in the human body. Thus their urinary recovery equals their intestinal uptake. Clinical in vivo tests most often use the ratio of urinary recovery of lactulose divided by the urinary recovery of mannitol. That ratio is called the permeability index (PI). The PI effectively minimizes the considerable interindividual difference of recovered probe sugars first observed in healthy individuals during development of the method [1]. That variation is usually explained by normal differences in non-mucosal factors such as gastric emptying time, small intestinal transit time, and renal clearance in healthy individuals [1,3,8].

Lactulose is a synthetic disaccharide that due to its size (diameter 1 nm, molecular weight 342 g/mol) exclusively permeates through the tight junctions (TJs) of epithelial crypts. Therefore, lactulose is considered a good TJ marker [1,8]. Permeation paths for mannitol (diameter 0.8 nm, molecular weight 182 g/mol) are less clear [8]. In vivo recovery of mannitol is approximately 50 times that of lactulose, suggesting that the smaller mannitol molecule additionally permeates transcellularly through water pores in the absorptive villi part of the epithelium [1].

The rationale of the present report underlies an incidental observation made in healthy controls in a previous study [9]. In that previous study we investigated if, in long-standing quiescent inflammatory bowel disease, decreased muscle mass [9,10] was triggered by increased intestinal permeability, which is often observed in those patients. The underlying hypothesis was that increased permeability might cause a systemic inflammatory response through intestinal translocation of endotoxins [11]. Thus we compared intestinal permeability values with body composition parameters derived from bioelectrical impedance (BIA) measurements. We found no correlations in patients. Surprisingly, however, we observed unexpected correlations in the control group with normal barrier function. That was neither previously reported not easily explicable. It suggested that possibly normal intestinal TJ permeation might be regulated by factors reflected in electrical whole-body resistance.

The aims of the present study were 1) to repeat the measurements of intestinal permeability and BIA in a larger group of healthy individuals using the same study conditions as in the previous study and 2) to integrate the previously unpublished results of the initial observation into the reproduced results. We now demonstrate that we were able to reproduce the correlations. However, we had to include skin resistances to receive significant results in both genders. We further discuss possible implications and hypotheses in regard to our findings.

Materials and methods

Healthy subjects

In the initial group and in the validation study, health was defined as good nutritional status according to the Subjective Global Assessment [12], absence of acute or chronic disease, and no intake of acute or long-term medication within 3 wk before assessment except contraceptives. Exclusion criteria consisted of not achieving at least one health criterion, pregnancy, and cigarette smoking.

The study protocols were approved by the ethics committee of the Charité Universitätsmedizin Berlin and all subjects gave their informed consent before entering the study.

Initial results

For a nutritional study in patients with gastrointestinal problems [9], 47 healthy women were recruited as controls from the normal population from May to November 2005. Fourteen women of this group agreed to perform an intestinal permeability test in addition to the obligatory study program. One woman provided incomplete urine collection and another had increased intestinal permeability values. The remaining 12 women were included in the analysis.

Validation study

From June to August 2007 a total of 37 healthy individuals were recruited from among medical students and in-house staff personnel. In addition, historic results from 10 healthy individuals were included who were enrolled by another study group at our center (M.P.) as controls for an investigation performed in 2006.

A posteriori we had to exclude 11 individuals from the analysis due to increased gastroduodenal permeability [2], increased intestinal permeability [5], diarrhea developed during the permeability test [1], development of flu between the recruitment and performance of the permeability test [1], and incomplete urinary sampling [2].

The final sample consisted of 26 women and 10 men.

BIA for body composition

Measurement

All BIA measurements (Nutriguard M, Data Input GmbH, Darmstadt, Germany) were performed at our department on an appointed date after inclusion using the tetrapolar wrist–ankle technique and the same standardized protocol [13]. Subjects were instructed to refrain from alcohol for 24 h and from intense physical exercise for 48 h before the test.

The subjects were measured in the morning after an over-

night fast, voiding of urine from the bladder, and accurate measurements of height and weight. Subjects lay in the supine position for at least 15 min before BIA was conducted. The skin at the electrode sites was rubbed with ethanol/isopropanol (Softasept N, B. Braun, Melsungen, Germany) for 5 s and then allowed to dry. Current-conducting and voltage-sensing electrodes (Ag/AgCl, Bianostic Classic Electrodes, Data Input GmbH) were placed on the dorsum of the hand and foot at the right side of the body [14]. In BIA for body composition analyses, the tetrapolar (four-electrode) method is indispensable to reduce the contribution from the current constriction zones near the electrodes and to select the preferred volume to be measured [15]. Current-conducting electrodes were placed in the middle of the dorsal surface of the right hand and foot just below the metacarpal–phalangeal and metatarsal–phalangeal joints. Wrist–ankle resistance was determined by positioning two voltage-sensing electrodes on the pisiform prominence of the right wrist and between the medial and lateral malleoli of the ankle [14].

An alternating electric current of 800 μA was applied at 5, 50, and 100 kHz and the voltage drop of the real component of impedance (resistance [R]) and the imaginary component of impedance (capacitative reactance [Xc]) were directly measured. We routinely documented skin resistances at the source electrodes on the dorsum of the hand and foot for each measurement. Total body water was calculated as 0.396 (body height squared/$R_{50w\text{-}a}$) + 0.143 kg of body weight + 8399 in men and as 0.382 (body height squared/$R_{50w\text{-}a}$) + 0.105 kg of body weight + 8315 in women [16], where $R_{50w\text{-}a}$ represents wrist–ankle resistance at 50 kHz. Fat-free mass was calculated as total body water × 0.732 and body cell mass as fat-free mass × 0.29 × ln (α_{50}) [17].

We further calculated skeletal muscle mass using the formula of Janssen et al. [18] as ([height squared/R × 0.401] + [gender × 3.825] + [age × −0.071]) + 5.102. Skeletal muscle mass was normalized for height to receive the skeletal muscle index.

The measurement precision of the BIA device was regularly checked against a calibration resistor. The accuracies of measurements according to the manufacturer are ±0.5% for the R value and ±2.0% for the Xc value. The coefficient of variance of 10 repeated measurements of R and Xc at 50 kHz was assessed in five individuals; the coefficients of variation were <1.5% for R and <2.6% for Xc.

Theory

The measuring principle of bioimpedance for body composition analyses is based on the electrical conductivity of the human body. It largely relies on the 50-kHz frequency of the applied current.

According to theory using a constant signal frequency and a relatively constant conductor configuration, the body's impedance to current flow can be related to its electroconductive volume, because conductor volume equals the cross-sectional area multiplied by the length or height of the conductor [19]. To determine body composition using any method, volume or total body water must be known first. Electrically determined total body water equals body height squared/R_{50}, multiplied by a coefficient and constant (*y*-intercept) [20]. That coefficient is the relative volume resistivity per cubic liter of ionic water distributed in organized tissues. Fat-free mass is normally calculated from total body water by assuming a constant average hydration of 73% and fat mass is calculated by simply subtracting fat-free mass from body weight.

Skin resistance/impedance

In body composition analysis, the effects of the zones near the current-carrying electrodes are to be reduced to focus on the volume measurement necessary for estimating body composition. This can be achieved by the four-electrode (tetrapolar) method [15,21]. The tetrapolar method makes it possible to subtract electrical resistance generated between the current-carrying and voltage-sensing electrodes on the hand and foot from the total whole-body resistance value. Those subtracted resistance values are commonly termed "skin resistances," although skin only partly contributes to that resistance value [15]. By reintegrating skin resistance to the resistance value used in body composition analysis (i.e., resistance between the two voltage-sensing electrodes, $R_{w\text{-}a}$), we increased subcutaneous contributions from the lower limbs.

Intestinal permeability

After an overnight fast, each subject collected a pretest urine sample and then drank 100 mL of water containing 10 g of lactulose and 5 g of mannitol. Urine was collected over 5 h into bottles containing sodium azide as a preservative. Subjects went without food during the test but were requested to drink 250 to 1000 mL of water after 2 h and to refrain from further drinking in the remaining 3 h of urine collection. Total urine volume was recorded on completion of the test and a 10-mL aliquot was stored at −20°C until analysis. Alcohol intake was not allowed in the 24 h before the test.

A volume of 50 μL of an internal standard (250 mmol/L of meso-erythritol, 15 mmol/L of turanose) was added to 0.5 mL of urine before the samples were desalted with Amberlite MB-3 resin in the acetate form, separated, analyzed, and quantified by high-performance liquid chromatography with pulsed electrochemical detection (Dionex, Idstein, Germany; chromatography module 250 × 40 mm Carbopac PA-1 column [Dionex], eluent 150 mmol of NaOH, flow 1 mL/min) [22]. Calibration with an external standard at multiple concentrations was performed in each run, with dilution of standards matching sample concentrations. Interassay relative standard deviations (and correlation coefficients) for lactulose and mannitol were 3.2% (99.9%) and 6.69% (99.8%), respectively.

Results are expressed as the percentage of urinary recovery of the ingested dose of the respective sugar. The PI is defined as percentage of recovery of lactulose divided by

the percentage of recovery of mannitol. Normal values for lactulose, mannitol, and PI are <0.44%, <29%, and <0.033, respectively.

Blood parameters

Blood was drawn from the cubital vein while subjects lay in the supine position waiting for BIA. Sodium, potassium, chloride, glucose, and osmolality were determined using routine methods. Blood parameters were not available from the 10 historic controls obtained from the other study group at our center.

Study design

In all groups BIA was performed first at our department. After the BIA measurement subjects received detailed instructions to perform the permeability test at home. All subjects implemented the permeability test within 1 to 7 d after BIA.

Statistics

Statistical analysis was carried out using the software package SPSS 14 (SPSS Inc., Chicago, IL, USA). Data showed skewness for some parameters; therefore, we consistently used non-parametric tests. Descriptive data are reported as medians and ranges. Correlations were calculated using Spearman's rank-order correlation coefficient (ρ). To identify independent influencing factors on permeability parameters, multiple logistic regression analyses were used. An acceptable level of statistical significance was established a priori at $P < 0.05$.

Results

Descriptive analysis of study populations

Table 1 lists the population characteristics of the initial observation and of the validation study. Permeability values were normal in all subjects except for two marginally increased lactulose values in the initial group. Resistance was normal in all individuals, indicating normal hydration status. Serum values for sodium (141 mmol/L, range 137–144, reference 134–145), potassium (3.8 mmol/L, range 3.1–4.7, reference 3.4–5.2), chloride (103 mmol/L, range 98–108, reference 95–112), osmolality (292 mosmol/L, range 288–302, reference 275–301), and glucose (81 mg/dL, range 46–96, reference <110) were also normal in all individuals.

Initial results

The coincidental observation raising our initial interest was the close association between lactulose and $R_{50w\text{-}a}$ ($\rho = 0.890$, $P < 0.001$) in 12 healthy women. $R_{50w\text{-}a}$ also corresponded to mannitol ($\rho = 0.643$, $P < 0.024$) and to a lesser degree to PI ($\rho = 0.448$, $P = 0.145$) but Xc at 50 kHz did not correlate to any permeability value.

Table 1
Population characteristics*

	Normal values	Initial study	Validation study		All
		Women (n = 12)	Women (n = 26)	Men (n = 10)	Women + men (n = 48)
General					
Age (y)	—	34 (21–55)	30 (19–64)	32 (21–56)	31 (19–64)
Weight (kg)	—	65 (50–77)	65 (50–89)	78‡ (67–103)	68 (50–103)
Height (cm)	—	168 (156–179)	167 (153–186)	180‡ (169–200)	170 (153–200)
BMI (kg/m^2)	—	21.8 (19.2–25.8)	22.8 (19.0–32.8)	24.5 (19.6–32.7)	23.9 (19.0–32.8)
Intestinal permeability					
Lactulose (%)	<0.44	0.30 (0.20–0.51)	0.27 (0.15–0.42)	0.27 (0.14–0.38)	0.27 (0.14–0.51)
Mannitol (%)	<29	16.9 (9.7–19.5)	18.0 (9.8–28.7)	15.6 (10.9–23.2)	17.2 (9.7–28.7)
PI (lactulose/mannitol)	<0.033	0.017 (0.013–0.030)	0.014 (0.010–0.032)	0.017 (0.009–0.024)	0.016 (0.009–0.032)
Bioimpedance analysis					
$R_{50w\text{-}a}$ (Ω)	F: 529–796†, M: 441–581	581 (492–669)	568 (511–724)	522‡ (421–573)	558 (421–724)
R_{50skin} (Ω)	NA	440 (296–543)	401 (190–551)	349 (246–457)	398 (190–551)
$R_{50total}$ (Ω)	NA	992 (846–1166)	961 (822–1174)	879‡ (747–972)	956 (747–1174)
Xc_{50} (Ω)	F: 53–75†, M: 49–71	58 (49–71)	64 (50–78)	62 (44–71)	63 (44–78)

BMI, body mass index; F, female; M, male; NA, not available; PI, permeability index; R_{50skin}, resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50total}$, wrist–ankle resistance at 50 kHz + resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50w\text{-}a}$, wrist–ankle resistance at 50 kHz; Xc_{50}, reactance at 50 kHz

* Permeability values were normal in all individuals except for marginally increased lactulose values in two women in the initial group (1 × 0.51%, 1 × 0.49%) who nevertheless had normal mannitol and PI values. Resistance was normal in all individuals, indicating normal hydration status. Data are reported as median (range).

† Reference values are based on the 10th and 90th percentiles of 29 409 healthy women and of 2224 healthy men 18–59 y of age from Germany [33].

‡ $P < 0.05$, male versus female subjects in the validation study.

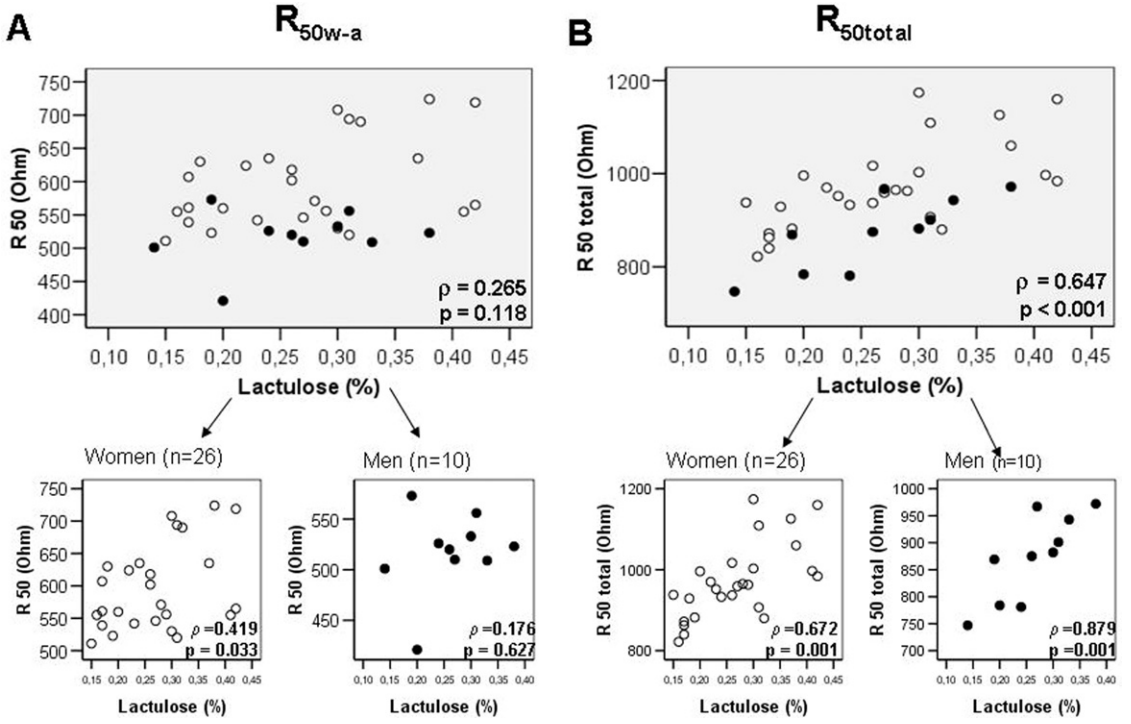

Fig. 1. Results of the validation study. (A) $R_{50w\text{-}a}$ did not correlate in the total validation group. Although an association could be confirmed in women, lactulose was not associated to $R_{50w\text{-}a}$ in men. (B) We then tentatively added skin resistances to the $R_{50w\text{-}a}$ value ($R_{50total}$) and were astonished to find improved and evident associations in all groups. R_{50}, resistance at 50 kHz; $R_{50total}$, wrist–ankle resistance at 50 kHz + resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50w\text{-}a}$, wrist–ankle resistance at 50 kHz.

Validation results

Results could be reproduced in women but not in men.

Fig. 1A demonstrates that the observation was reproducible in women but not in men, leading to non-significant associations between $R_{50w\text{-}a}$ and lactulose (and any other permeability value) in the total group of individuals.

Adding resistances between current-conducting and voltage-sensing electrodes (R_{50skin}) to $R_{50w\text{-}a}$ results in significant associations in men and women

We tentatively added the skin resistances at the electrode sites to $R_{50w\text{-}a}$ and termed the new value $R_{50total}$. Figure 1B shows that $R_{50total}$ significantly correlated with lactulose in the total validation group and in the gender-separated groups. Furthermore, mannitol ($\rho = 0.413$, $P = 0.014$) and PI ($\rho = 0.333$, $P = 0.047$) correlated with $R_{50total}$ in the total validation group.

Final group

Initial results fitted well into validation results

We recalculated the initial results for the new parameter $R_{50total}$ and found significant correlations to lactulose ($\rho = 0.639$, $P = 0.025$) and weaker associations for mannitol ($\rho = 0.488$, $P = 0.107$) and PI ($\rho = 0.330$, $P = 0.295$). Figure 2 demonstrates that the initial results fitted well in the results of the validation group. Thus we combined the two groups for the final analysis.

Correlation analyses

Table 2 demonstrates that, similar to the initial results, lactulose revealed stronger associations to electrical values than did mannitol or PI. Among the electrical values $R_{50total}$ corresponded best to the permeability values. In the final sample also, Xc at 50 kHz correlated to both single-probe sugars but not to PI. Regarding body composition we found no associations of permeability parameters to total body water, fat-free mass, body cell mass (not shown), extracellular mass (not shown), or fat mass. However, the skeletal muscle index significantly correlated with both single probe sugars but not to PI. Age, anthropometric parameters, serum osmolality, and serum glucose were not associated to any permeability value.

We further investigated possible associations of serum electrolytes, serum glucose, or serum osmolality on BIA raw values. Table 3 demonstrates that only the reactance value but not the resistance values were associated to electrolytes in the final group. Gender-specific analysis, however, revealed that $R_{50w\text{-}a}$ was associated to sodium ($\rho = -0.401$, $P = 0.028$) and chloride ($\rho = -0.435$, $P = 0.018$) in women and to chloride values in men ($\rho = -0.766$, $P = 0.027$). R_{50skin} or $R_{50total}$ remained non-correlational to blood values also in the gender-separated groups.

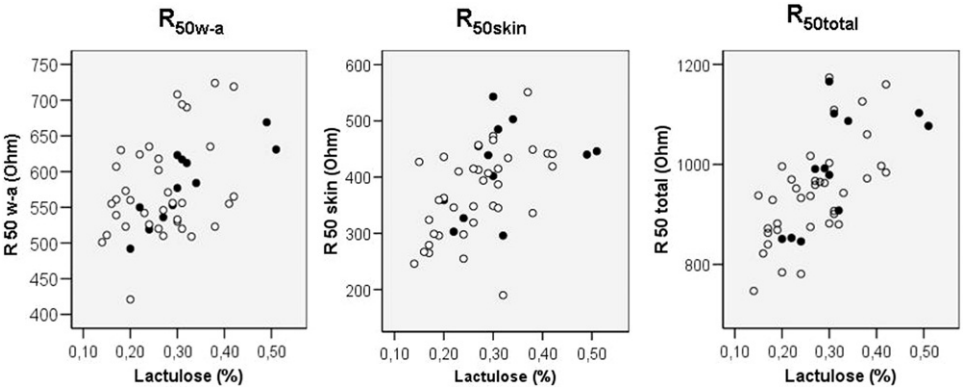

Fig. 2. Initial results (filled circles) fitted well into the results of the validation study (open circles). The scatter plots make also demonstrate that the association of lactulose to $R_{50total}$ is indeed superior to $R_{50w\text{-}a}$ or R_{50skin} alone. The total group consisted of 48 healthy individuals. Corresponding correlation coefficients are listed in Table 2. R_{50}, resistance at 50 kHz; R_{50skin}, resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50total}$, wrist–ankle resistance at 50 kHz + resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50w\text{-}a}$, wrist–ankle resistance at 50 kHz.

Similar associations were observed using 5- and 100-kHz frequencies

We further evaluated R_{total} for all three measured frequencies (5, 50, and 100 kHz). Lactulose correlated similarly to $R_{50total}$ ($\rho = 0.673$, $P < 0.001$), R_{5total} ($\rho = 0.651$, $P < 0.001$), and $R_{100total}$ ($\rho = 0.588$, $P < 0.001$). The same was true in regard to mannitol and PI.

Multivariate regression analyses

Univariate analyses revealed that permeability values were also associated to some serum electrolytes (Table 2). Thus we controlled for possible confounding factors by performing a multivariate analysis. We entered lactulose as a dependent variable and $R_{50total}$, electrolytes, sex, age, height, and body weight as independent variables (Table 4).

Table 2
Correlation analysis ($n = 48$): Permeability values

	Lactulose (% urine recovery)		Mannitol (% urine recovery)		PI (lactose/mannitol ratio)	
	ρ	P	ρ	P	ρ	P
BIA and body composition ($n = 48$)						
$R_{50w\text{-}a}$ (Ω)	**0.402**	0.005	**0.327**	<0.020	0.096	0.515
R_{50skin} (Ω)	**0.550**	<0.001	0.151	0.312	**0.444**	0.002
$R_{50total}$ (Ω)	**0.673**	<0.001	**0.381**	0.008	**0.369**	0.010
Xc_{50} (Ω)	**0.296**	0.041	**0.337**	0.020	0.005	0.974
Imp index ($cm^2/Ω$)	**−0.313**	0.030	−0.100	0.502	−0.142	0.337
Total body water (kg)	−0.148	0.332	−0.111	0.474	0.010	0.950
Fat-free mass (kg)	−0.148	0.333	−0.110	0.477	0.009	0.951
Fat mass (kg)	0.278	0.064	0.264	0.084	0.066	0.666
Skeletal muscle index (kg/ht^2)*	**−0.455**	0.001	**−0.333**	0.022	−0.140	0.342
General ($n = 48$)						
Age (y)	0.083	0.575	0.097	0.518	0.018	0.904
Body height (cm)	−0.048	0.745	0.132	0.378	−0.066	0.655
Body weight (kg)	0.023	0.874	0.042	0.778	0.059	0.692
Body mass index (kg/m^2)	0.020	0.874	−0.013	0.932	0.062	0.673
Blood values ($n = 38$†)						
Sodium (mmol/L)	−0.079	0.637	−0.145	0.393	0.037	0.823
Potassium (mmol/L)	0.313	0.056	−0.113	0.505	**0.428**	0.007
Chloride (mmol/L)	**−0.409**	0.012	−0.130	0.450	**−0.381**	0.020
Osmolality (mosm/kg)	−0.073	0.670	0.016	0.927	−0.102	0.550
Glucose (mg/dL)	0.025	0.884	−0.317	0.056	0.279	0.090

BIA, bioimpedance analysis; Imp index, impedance index (body height squared/wrist–ankle resistance at 50 kHz); PI, permeability index; R_{50skin}, resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50total}$, wrist–ankle resistance at 50 kHz + resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50w\text{-}a}$, wrist–ankle resistance at 50 kHz; ρ, Spearman's rank-order coefficients; Xc_{50}, reactance at 50 kHz

* According to Janssen et al. [18].

† Blood values were not available from historic controls.

Table 3
Correlation analysis (n = 48): Bioimpedance analysis raw values

All (n = 38*)	$R_{50w\text{-}a}$ (Ω)		R_{50skin} (Ω)		$R_{50total}$ (Ω)		Xc_{50} (Ω)	
	ρ	P	ρ	P	ρ	P	ρ	P
Sodium (mmol/L)	−0.253	0.126	−0.071	0.672	0.205	0.217	**−0.337**	0.038
Potassium (mmol/L)	0.017	0.921	−0.331	0.043	0.274	0.097	−0.100	0.552
Chloride (mmol/L)	−0.278	0.095	−0.033	0.848	−0.137	0.419	**−0.407**	0.013
Osmolality (mosm/kg)	−0.099	0.561	0.098	0.564	0.061	0.720	−0.111	0.515
Glucose (mg/dL)	−0.152	0.364	0.097	0.564	−0.013	0.938	−0.170	0.308

R_{50skin}, resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50total}$, wrist–ankle resistance at 50 kHz + resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz; $R_{50w\text{-}a}$, wrist–ankle resistance at 50 kHz; ρ, Spearman's rank-order coefficients; Xc_{50}, reactance at 50 kHz

* Blood values were not available from historic controls (n = 10).

F-test disclosed a highly significant association (0.001) with the adjusted coefficient of determination (R^2 adjusted), indicating that 51.1% of the variance in lactulose values was explained by the model. $R_{50total}$ had by far the highest β-weight (0.672) of all entered variables and only chloride (−0.264), but not the remaining variables, had some additional impact. $R_{50total}$ alone accounted for 41.3% of the variations in lactulose. Replacing $R_{50total}$ by R_{50} or R_{50skin} decreased the fit of the model, with R^2 adjusted values of 0.324 and 0.374, respectively.

Discussion

We reported in healthy individuals a number of significant associations between BIA-derived electrical whole-body resistance and intestinal permeability values assessed in vivo. Our main finding was the close association of R_{total} (wrist–ankle resistance including skin resistances) with the urinary recovery of lactulose, a non-metabolizable sugar generally acknowledged as an intestinal TJ marker [1]. According to our calculation R_{total} alone accounts for 41% of variation in lactulose values.

Table 4
Multiple regression analyses for predicting lactulose from $R_{50total}$

	R	R^2 adjusted	b	β-weight	P
Model summary	**0.787**	**0.511**			<0.001
$R_{50total}$ (Ω)			**0.001**	**0.672**	<0.001
Chloride (mmol/L)			−0.010	−0.264	0.052
Potassium (mmol/L			0.040	0.193	0.155
Sodium (mmol/L)			0.005	0.134	0.298
Gender*			0.027	0.133	0.424
Weight (kg)			0.001	0.118	0.544
Height (cm)			0.001	0.096	0.656
Age			0.000	−0.014	0.915

b, regression coefficient; β-weight, standardized regression coefficient; R, multiple correlation coefficient; R^2, adjusted coefficient of determination; $R_{50total}$, wrist–ankle resistance at 50 kHz + resistance between current-conducting electrodes and voltage-sensing electrodes at 50 kHz

* Dummy-coded gender term (female = 0, male = 1).

The associations were unexpected for several reasons.

First, the starting point for relating intestinal permeability to BIA parameters was in a previous study [9] to test a disease-oriented hypothesis in patients with inflammatory bowel disease: An impaired intestinal barrier through translocation of endotoxins might trigger a systemic inflammatory response, which is involved in the pathogenesis of cachexia syndrome [11]. We therefore were astonished to find associations not in the patients but in the healthy controls with completely normal barrier function. Normal intestinal permeability seemed to be necessary to observe the association. That was further confirmed in the validation sample where we had to exclude 15% (n = 7) healthy individuals with increased permeability values, a prevalence rate in line with a recently reported 19% prevalence of increased permeability in healthy individuals [23]. Increased permeability of unknown origin in healthy individuals most probably hints at latent, subclinical intestinal inflammation. It is tempting to speculate that local intestinal aberrations, clinically evident or not, might superimpose or obscure the association.

Second, wrist–ankle BIA, a standard bedside method for body composition analysis, is well known for its inefficiency in measuring anything in the trunk region directly [13,24]. That very fact actually is often cited as one major flaw of the method [13,15,24]. The reason is simple: Electrical resistance relates to the diameter and length of the conductor. Limbs, because of a smaller circumference and greater length, contribute most to wrist–ankle resistance, usually about 95% [13]. Trunk resistance, however, is approximately only 5% of wrist–ankle resistance and hardly influences the results.

Furthermore, the most proximate explanation for our results did not work: Higher intestinal permeability (higher in the normal range) could have led to higher influxes of water from the intestine into the body. In BIA total body water is calculated by using $R_{50w\text{-}a}$ as the sole electrophysiologic parameter. That could have possibly explained the association. We discarded that hypothesis because Bijlsma et al. [3,25] showed that permeation of lactulose is completely independent of intestinal water absorption. Perme-

ation of mannitol, however, is to some extent affected by intestinal solvent drags in the villus tip [3,25], but with mannitol we observed relations much weaker than those seen for lactulose. In addition, in body composition analysis $R_{w\text{-}a}$ relates negatively to total body water [19] and we found positive relations of resistance values to probe sugar recovery. That meant that higher TJ permeability was associated to lower total water content in our findings. Our results also contrast the actual electrophysiologic characteristics of the intestinal epithelia ex vivo. Reims et al. [26] found an inverse relation of TJ permeability and resistance values in duodenal biopsies. Thus, our electrophysiologic results from whole-body measurements definitely did not reflect the electrophysiologic characteristics of intestinal TJs ex vivo and speak against any direct measurement of intestinal fluid, substance, or ion movement in the present experiment.

Third, as mentioned in the INTRODUCTION, differences in probe sugar recoveries in healthy individuals are commonly attributed to individual differences in non-mucosal factors, such as gastric emptying, intestinal transit time, and kidney function [1]. That reasoning is well established and had never been questioned by us or other clinical researchers before. With the present results in hand, however, we started to search the literature thoroughly and were astonished to find no studies on the influences of gastric emptying and kidney functions on sugar recoveries in health. In addition, Van Nieuwenhoven et al. [27] demonstrated that moderate acceleration of small intestinal transit time from 60 to 90 min does not affect permeability results. Thus the effectual magnitude of pre- and postabsorptive factors on the recovery of probe sugars under normal conditions is still unclear and it might well be that additional factors contribute to normal variations observed in healthy individuals.

The correlations we found in healthy humans are unusual and we are aware that they do not necessarily imply causality. When permitting ourselves to assume causality, then, from the teleologic point of view, it is more reasonable to suggest that a factor reflected by resistance affects intestinal TJs than vice versa. That factor reflected by resistance is derived from the limbs to 95% and is thus not a local intestinal phenomenon. It can be described as a long-range correlation, as previously proposed for quantum physics based biological system dynamics [28]. When we further assume that the factor reflected by resistance is of endogenous electrophysiologic origin and underlies the laws of quantum physics, then the effects are coherent and simultaneous. That factor reflected by resistance might thereby regulate not only intestinal TJs but also TJs in other endothelial [29] or epithelial tissues. Considering the immense complexity of physiologic processes in living beings, such coherent regulation would be reasonable to maintain basic homeostasis, in addition to linear regulatory systems such as neural pathways or circulatory systems. We are aware of the purely speculative character of these thoughts.

Unfortunately the "factor reflected by resistance" is still unknown in terms of biological parameters and the concrete current paths in humans are also still unidentified [15]. Further limiting to the interpretation of our results is that the theoretical model for body composition analyses by BIA as presented in the methodology sections has never been fully transferred to the regression formulae used in body composition analysis. For example, regression formulae do not use constants for tissue resistivity because, from the electrical point of view, it is impossible to specify a fixed resistivity value valid for a group of humans [15]. Furthermore, the basic requirement of a constant conductor configuration is not provided in humans [30,31]. In fact, up to now, there is no fully satisfactory theory that adequately accounts for the success of the BIA technique [24].

We found clearly improved associations to permeability values with the combined $R_{50w\text{-}a}$ and R_{50skin} value in men and women and in the total group of individuals. In BIA skin resistances are mainly used to assess the quality of electrodes and thus the validity of the measurement [13]. To calculate body composition, however, it is fundamental to eliminate skin resistances from the total resistance value. That can be achieved by using the tetrapolar method [21]. It should be mentioned that the common term "skin resistance" only imprecisely expresses the character of this resistance value, which also includes major contributions from small-circumference subdermal tissue zones between the current-conducting electrode and the voltage-sensing electrode [15,32]. By adding so-called skin resistances from the hand and foot to $R_{w\text{-}a}$, the domination of lower arm and wrist contributions was enforced. The more surprising is that exactly that addition, which we termed R_{total}, produced our main results.

Reactance is the capacitive part of impedance and originates from the dielectric properties of cell membranes, which act as an insulator [13]. Xc was only marginally involved in the correlation with permeability values. We observed similar correlations between permeability values and R_{total} at 5, 50, and 100 kHz. According to theory electrical current is not able to penetrate cells at 5 kHz but penetrates cells fully at 100 kHz [13]. Provided that the relation is causal, the factor responsible for the association was seemingly present extracellularly and intracellularly to the same extent.

Limitations of the study

The finding is based on a coincidental observation. The methods used are simple standard clinical bedside methods. The standardized procedure in wrist–ankle BIA calls for defined electrode placement; still, it would be interesting to place voltage-sensing electrodes farther away from the ankle and wrist in further studies to limit domination by wrist or ankle volume. The sample is still small, especially for men. Therefore, we cannot say for sure if the different results between man and women in regard to $R_{w\text{-}a}$ are relevant or just due to the low statistical power in the male group. Serum ionic concentrations do not adequately reflect total or tissue ionic concen-

trations and thus results from multivariate analyses do not exclude electrolytes being confounding factors. Furthermore, data on normal between-day inter- and intraindividual variations for intestinal permeability assessed with probe sugars in healthy individuals are lacking. Thus we cannot prove near-to-constant permeability values in the same healthy individuals over a period of at least 5 d, which would be necessary in regard to our hypothesis. We nevertheless decided to open our results to professional discussion at the current stage to encourage scientific thought and further research.

Conclusion

Consistent with our initial hypothesis, the results suggest that intestinal TJ permeation might underlie regulative processes reflected by whole-body electrical resistance. If so, the observation might hint at a yet unknown regulatory mechanism concerning non-carrier–mediated transport as part of basal metabolism. We hypothesized that such regulation might underlie the principles of quantum physics implying coherence, which could explain long-distance regulation in complex systems like the human body. Further studies are required to test our hypothesis.

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