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Original article

Intestinal permeability parameters in obese patients are correlated with metabolic syndrome risk factors☆

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SUMMARY

Background & aims: Altered intestinal permeability has been shown to be associated with metabolic alterations in animal models of obesity, but not in humans. The aim of this study was to assess intestinal permeability in obese women and verify if there is any association with anthropometric measurements, body composition or biochemical variables.
Methods: Twenty lean and twenty obese females participated in the study. Anthropometric measurements, body composition and blood pressure were assessed and biochemical analyses were performed. Administration of lactulose and mannitol followed by their quantification in urine was used to assess the intestinal permeability of volunteers.
Results: The obese group showed lower HDL ($p < 0.05$), higher fasting glucose, insulin, HOMA index and lactulose excretion than the lean group ($p < 0.05$), suggesting increased paracellular permeability. Lactulose excretion showed positive correlation ($p < 0.05$) with waist and abdominal circumference. Blood insulin and the HOMA index also increased with the increase in mannitol and lactulose excretion and in the L/M ratio ($p < 0.05$). L/M ratio presented a negative correlation with HDL concentration ($p < 0.05$).
Conclusions: We demonstrated that intestinal permeability parameters in obese women are positively correlated with anthropometric measurements and metabolic variables. Therapeutic interventions focused on intestine health and the modulation of intestinal permeability should be explored in the context of obesity.

1. Introduction

Obesity is a worldwide epidemic problem[1] and metabolic syndrome is an increasingly common disorder as a result of the growing prevalence of obesity.[2] Insulin resistance, along with visceral adiposity, dyslipidemia and subclinical inflammatory state, are the main features of metabolic syndrome.[3]

Intestinal barrier function has been viewed as an interface between health and disease[4] and therapies aiming to correct abnormal intestinal permeability may play a role in treating or preventing some diseases.[5,6] Altered intestinal permeability has so far only been shown to be affected by obesity in animal models.[7,8] Its reduction, through the administration of prebiotics and changes in the microbiota, improve systemic and hepatic inflammation, modulate gut peptides and adiposity[8] indicating that therapeutic approaches to improve intestinal permeability could have a positive impact on variables of metabolic syndrome.

In humans, if there is an association between altered intestinal permeability, and adiposity and insulin resistance, therapies aimed

Abbreviations: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HOMA, Homeostasis model assessment index; HDL, high density lipoprotein; LDL, low density lipoprotein; % M, percentage of mannitol urinary excretion; %L, percentage of lactulose urinary excretion; L/M, lactulose/mannitol excretion ratio; LPS, lipopolysaccharide; TNF-α, Tumour necrosis factor alpha; LAMECC, Laboratory of Energetic Metabolism and Body Composition.

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at correcting abnormally increased intestinal permeability may play a role in the context of obesity. Therefore, the aim of this study was to assess intestinal permeability in obese women and verify if there was any association with anthropometric measurements, body composition and biochemical variables.

2. Subjects and methods

2.1. Ethical approval

The study was approved by the ethical committee of Universidade Federal de Viçosa (protocol number 001/2010) and the participants provided written informed consent.

2.2. Subjects

Healthy women volunteers were recruited through written advertisements. Exclusion criteria were: younger than 18 years of age, pregnant or breast-feeding, menopause, thyroid diseases, renal failure, cirrhosis, congestive heart failure, nephritic syndrome, diabetes, coeliac disease, Crohn's disease, irritable bowel syndrome, hepatitis, ulcers, use of vitamin/mineral supplements, use of non steroidal anti-inflammatory drugs and use of laxatives. According to a physical examination and a brief medical history, all subjects were in good health. Twenty lean females (BMI 19.0–24.99 kg/m^2) and twenty obese females (BMI >30 kg/m^2) of similar age (mean age of the lean and obese group 28.5 ± 7.6 *vs*. 30.7 ± 6.5, $p = 0.33$) participated in the study. None of the subjects were taking any form of medication except for contraceptives pills.

2.3. Study design

The subjects were evaluated at the Laboratory of Energetic Metabolism and Body Composition (LAMECC) on two occasions: the first to provide information about their health history and to receive all the recommendations prior to the next meeting. In the second meeting, subjects arrived in the morning at LAMECC after fasting for 10 h and were asked to eliminate residual urine. All participants were weighed wearing light clothes, their body composition was analysed by tetra polar bioimpedance (BodySystems®, Washington, USA), blood pressure was assessed and blood samples were collected for future analyses. After that, a solution (120 ml) containing 6.25 g lactulose (95%, Sigma–Aldrich®, Germany) and 3 g mannitol (>98%, Sigma–Aldrich®, Germany) was ingested, and urine was collected over a period of 5 h. Two hours after the solution had been ingested, the volunteers were allowed to have a snack. At the end of this period, the whole volume of urine was measured and an aliquot of 50 ml was taken, to which 0.01 g of thimerosal (Labsynth®, Brazil) was added to prevent bacterial growth. The urine samples were stored at −20 °C.

2.4. Intestinal permeability analysis

To quantify the sugars administered, urine samples were derivatised according to Farhadi et al.[9] Briefly, 200 μl of the urine sample was centrifuged for 20 min at 2250 rpm and 40 μl of an internal standard solution was added (myo-inositol 20 mg/ml (Fluka®, Switzerland) and phenyl-β-D-glucoside 20 mg/ml (Acrós Organics®, Belgium). The samples were then evaporated to dryness at 70 °C under a continuous flux of nitrogen gas and re-suspended in 400 μl of anhydrous pyridine (Sigma–Aldrich®, Germany) containing hydroxylamine (25 mg/ml, Sigma–Aldrich®, Germany). Next, the sample was heated to 70 °C for 1 h and centrifuged at 2250 rpm for 5 min. The supernatant (100 μl) was transferred to a vial and 200 μl of N-trimethylsilylimidazole (Acrós Organics®, Belgium) was added, and incubated for 30 min at 70 °C. From this derivative, a 100 μl aliquot was transferred to an insert, and 1 μl was injected into a gas chromatograph (Shimadzu®, Japan) equipped with an auto injector, flame ionisation detector and capillary column (DB-5, 30 m, 0.25 mm × 0.25 μm, J&W Scientific®, USA) for analysis. The parameters used for sugar separation on the gas chromatograph were adapted from Farhadi et al.,[10] due to difficulties in lactulose detection using their original conditions. Thus, the column temperature was set at 190 °C for 5 min and then increased at a rate of 5 °C/min for 12 min, until reaching a final temperature of 250 °C. This temperature was maintained for 15 min and the total run time was 32 min. The results were expressed as percentage of mannitol (%M) and lactulose (%L) excretion and as a Lactulose/Mannitol ratio (L/M).

2.5. Biochemical analysis

Blood samples were centrifuged at 3500 rpm for 15 min and the plasma was processed at the Laboratory of Clinical Analysis of the Health Division at Federal University of Viçosa. The biochemical assessments were: haemogram (Coulter T-890/Beckman Coulter®, USA), total cholesterol and lipoproteins (enzymatic colourimetric method), aspartate (AST) and alanine (ALT) aminotransferases (kinetic colourimetric method), fasting plasma glucose (enzymatic colourimetric method of glucose-oxidase) (all the kits used were from Bioclin/Quibasa, Brazil) and insulin through eletrochemiluminescence method using the Modular Analytics E170 e Elecsys 2010 (Roche Diagnostics®). The LDL concentration was estimated by the Friedwald formula.[11] Homeostasis model assessment (HOMA) index were calculated as follow: fasting glucose (mmol/l) × fasting insulin (mU/L)/22.5.[12]

2.6. Statistical analysis

Statistical analyses were performed with the use of the software Sigma Plot for Windows version 11.0 (Systat® Software, Chicago, USA). To compare if all variables assessed differed between obese and lean individuals, Student's *t* test (parametric) was used for those variables that passed the normality and equal variance test (Shapiro Wilk test), while the Mann–Whitney test (non-parametric) was used for the others. The same tests and criteria were used to compare all 40 volunteers distributed according to the reference variable values below and above the median of lactulose and L/M ratio and the mean of mannitol excretion, below and above the threshold value for insulin resistance (HOMA index > 2.71) proposed by Geloneze et al.[13] The significance level was 5%. Throughout the manuscript, the data are expressed as means ± SD and median. To measure the degree of correlation between intestinal permeability variables and other metric variables, Pearson's test was performed for mannitol excretion, while Spearman's test was applied for lactulose excretion and L/M.

3. Results

3.1. Anthropometric and body composition variables and blood pressure

As shown in Table 1, except for height, that was similar in both groups, anthropometric and body composition variables and blood pressure differed between obese and lean group as expected ($p < 0.05$).

3.2. Biochemical analysis

The collection of a blood sample from one volunteer of the lean group was not possible due to difficulties in venous access. All the

Table 1
Anthropometric, body composition and blood pressure variables of obese and lean women.

	Lean ($n = 20$)	Obese ($n = 20$)	p value
	Mean ± SD (median)	Mean ± SD (median)	
Systolic blood pressure (mmHg)	104 ± 8.2 (100)	113 ± 10.3 (120)	0.005[a]
Dyastolic blood pressure (mmHg)	64 ± 8.8 (60)	74 ± 9.4 (80)	0.002[a]
Weight (kg)	55.2 ± 5.2 (54.6)	88.06 ± 11.02 (88.02)	<0.001[a]
Height (cm)	159.9 ± 5.6 (159.5)	158.5 ± 4.2 (159)	NS
BMI (kg/m^2)	21.5 ± 1.39 (21.2)	35.04 ± 3.98 (34.4)	<0.001[a]
Waist circumference (cm)	69.57 ± 3.81 (68.5)	94.47 ± 8.16 (94.5)	<0.001[a]
Abdominal circumference (cm)	80.2 ± 4.33 (80.5)	110.02 ± 10.7 (108)	<0.001[a]
Hip (cm)	94.2 ± 3.5 (93.5)	117.17 ± 6.6 (117.2)	<0.001[b]
Waist/hip ratio	0.738 ± 0.026 (0.73)	0.807 ± 0.061 (0.79)	<0.001[b]
Body fat (%)	21.58 ± 3.52 (22.6)	37.48 ± 3.5 (37.4)	<0.001[b]

BMI = Body Mass Index.
[a] Mann–Whitney.
[b] Student t test.

variables relating to the haemogram did not differ among the groups (data not shown). The lipoprotein HDL was reduced in the obese group, while the ratios of total cholesterol/HDL and LDL/HDL were increased ($p < 0.05$). Fasting glucose, insulin and the HOMA index were also increased in the obese group ($p < 0.05$) (Table 2).

3.3. Intestinal permeability

The parameters, percentage of lactulose and mannitol excretion, and the ratio L/M are represented in Table 3. Mannitol excretion tended towards being higher in the obese group ($p = 0.06$). Lactulose excretion was higher in the obese group ($p = 0.04$). The L/M ratio was higher in the obese group, but not statistically significant (Table 3). Significant correlation between intestinal permeability parameters and variables that are related to metabolic risk factors are shown in Table 4.

The 40 women were also analysed by dividing them by the median or mean of the intestinal permeability parameters (Table 3). The comparison between women below and above the median of lactulose excretion showed that the group excreting higher quantities of lactulose also presented higher body weight (78.8 ± 19.9 kg *vs*. 64.4 ± 14.4 kg, $p = 0.01$), BMI (31.2 ± 7.8 kg/m^2 *vs*. 25.4 ± 5.7 kg/m^2, $p = 0.01$), waist (87.9 ± 15 cm *vs*. 76.1 ± 10.4 cm, $p = 0.007$) and abdominal circumference (101.7 ± 18.5 cm *vs*. 88.5 ± 12.9 cm, $p = 0.01$), body fat percentage (33.0 ± 8.2% *vs* 26.0 ± 7.9%, $p = 0.01$), fasting insulin (14.3 ± 7.8 mU/L *vs*. 8.6 ± 2.7 mU/L $p = 0.008$), HOMA index (3.15 ± 1.8 *vs*. 1.89 ± 0.66, $p = 0.008$), percentage of mannitol excretion and L/M ratio (Table 3). The use of the mean percentage of mannitol excretion as the criteria to divide all 40 women showed that those excreting a higher amount of mannitol presented higher lactulose excretion (Table 3), weight (77.6 ± 19.1 kg *vs*. 67.6 ± 17.6 kg, $p = 0.09$), BMI (30.8 ± 7.0 kg/m^2 *vs*. 26.6 ± 7.3 kg/m^2, $p = 0.08$) and waist circumference (87.6 ± 14.5 cm *vs*. 78.2 ± 12.7 cm; $p = 0.03$).

Conversely, when dividing all volunteers by the median of the L/M ratio, differences related to anthropometric and body composition variables were not observed, but women that showed L/M values above the median had lower HDL levels (43.8 ± 7.0 mg/dl *vs*. 54.6 ± 15.6 mg/dl, $p = 0.03$) and higher values of TC/HDL ratio (4.1 ± 1.1 *vs*. 3.3 ± 0.7, $p = 0.02$), LDL/HDL ratio (2.69 ± 1.0 *vs*. 2.0 ± 0.7, $p = 0.02$), insulin (12.6 ± 5.1 mU/L *vs*. 10.4 ± 7.6 mU/L, $p = 0.02$), HOMA index (2.76 ± 1.1 *vs*. 2.3 ± 1.8, $p = 0.01$) and percentage of lactulose excretion (Table 3).

The threshold value for the HOMA index (>2.71) to characterise insulin resistance proposed by Geloneze et al.[13] for a Brazilian population, was also used to compare all the variables. From this perspective, 25% of all volunteers ($n = 10$) presented insulin resistance, all being from the obese group, except for one from the lean group. Comparing women with an HOMA index above or below 2.71, in the insulin resistant volunteers all anthropometric and body composition variables analysed were higher ($p < 0.05$). Interestingly, of all the comparisons performed, this was the only one in which higher values for leukocytes were observed (7320 ± 1029 mm^3 *vs*. 5617 ± 1348 mm^3, $p < 0.001$), lymphocytes (2402 ± 569 mm^3 *vs*. 1957 ± 552 mm^3, $p = 0.03$) and platelets (268.4 ± 71.6 thousand/mm^3 *vs*. 205.5 ± 44.8 thousand/mm^3, $p = 0.008$) in the group above the cut-off point. They also showed higher mannitol and lactulose excretion percentages (Table 3).

The mean value + 2 SD in the lean group for each of the intestinal permeability variables were used to verify how many volunteers would be above this value. Considering all 40 women, 10% (three from the obese group and one from the lean group) were above the mannitol cut-off point (> 31.9% of excretion), 22.5% (nine from the obese group and none from the lean group) for lactulose (>0.421% of excretion) and 12.5% (four from the obese group and one from the lean) for the L/M ratio (>0.0264).

Table 2
Biochemical variables of obese and lean women.

	Lean ($n = 19$)	Obese ($n = 20$)	p value
	Mean ± SD (median)	Mean ± SD (median)	
TC (mg/dl)	178.68 ± 32.9 (178)	169.5 ± 24.7 (166)	0.33[b]
HDL (mg/dl)	55.4 ± 13.6 (52)	43.1 ± 9.35 (42)	0.001[a]
LDL (mg/dl)	107.32 ± 28.26 (101.4)	108.69 ± 25.18 (100.9)	0.87[b]
TGL (mg/dl)	79.68 ± 31.12 (73)	88.75 ± 29.7 (85)	0.35[b]
VLDL (mg/dl)	15.93 ± 6.22 (15.9)	17.76 ± 5.96 (17)	0.35[b]
TC/HDL	3.37 ± 0.98 (3.09)	4.08 ± 1.07 (3.84)	0.016[a]
LDL/HDL	2.07 ± 0.87 (2.07)	2.65 ± 0.96 (2.51)	0.025[a]
Fasting glucose (mg/dl)	86.15 ± 5.49 (86)	89.8 ± 4.32 (89.5)	0.027[b]
Fasting insulin (mU/L)	8.17 ± 2.59 (8.1)	14.8 ± 7.49 (11.4)	<0.001[a]
HOMA	1.74 ± 0.59 (1.65)	3.29 ± 1.71 (2.55)	<0.001[a]
AST (U/l)	19.21 ± 5.0 (18)	17.95 ± 3.88 (17.5)	0.47[a]
ALT (U/l)	14.78 ± 5.88 (13)	15.2 ± 5.73 (14)	0.82[a]

TC = Total cholesterol; HDL = High density lipoprotein; LDL = Low density lipoprotein; VLDL = Very low density lipoprotein; TC/HDL = total cholesterol/high density lipoprotein; HOMA = Homeostasis Model Assessment; AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase.
[a] Mann–Whitney.
[b] Student t test.

4. Discussion

Obesity is the most critical factor in the emergence of metabolic diseases,[14–16] which are considered together with glucose intolerance, hypertension and dyslipidemia for metabolic syndrome diagnosis.[2] All of the screening variables (waist circumference, HDL levels, insulin and blood pressure) used to identify this syndrome,[17] were evaluated in this study, and differ ($p < 0.05$) between lean and obese volunteers. None of the obese women were diabetic, however 45% were insulin resistant. Insulin resistance and obesity have been seen to be induced by increased levels of circulating endotoxins (LPS) in animal models under a high fat diet.[18] Furthermore, it was demonstrated that this increase in endotoxins can be due to increased intestinal permeability in an animal model of obesity, whether genetical[7] or diet-induced.[19]

Studies on intestinal permeability and obesity in humans are scarce. The major finding of this study was the higher lactulose excretion observed in the obese group and its positive correlation with metabolic syndrome variables, such as HOMA index, waist and

Table 3
Intestinal permeability parameters of lean and obese women.

Comparisons	% Mannitol excretion mean ± SD (median)	% Lactulose excretion mean ± SD (median)	L/M ratio mean ± SD (median)	Weight
Lean ($n = 20$)	17.32 ± 7.31 (17.4)	0.247 ± 0.087 (0.23)	0.0144 ± 0.006 (0.013)	55.2 ± 5.2
Obese ($n = 20$)	21.86 ± 7.77 (21.6)	0.418 ± 0.267 (0.37)	0.018 ± 0.008 (0.015)	88.06 ± 11.02
p value	0.06[b]	0.041[a]	0.13[a]	<0.001[a]
Below median % L ($n = 20$)	15.4 ± 6.0 (16.6)	0.18 ± 0.06 (0.19)	0.11 ± 0.00 (0.012)	64.4 ± 14.4
Above median % L ($n = 20$)	23.7 ± 7.2 (22.5)	0.47 ± 0.21 (0.41)	0.020 ± 0.007 (0.018)	78.8 ± 19.9
p value	<0.001[b]	<0.001[a]	<0.001[a]	0.013[b]
Below % M mean ($n = 24$)	14.7 ± 4.7 (16.6)	0.24 ± 0.12 (0.22)	0.011 ± 0.006 (0.014)	67.6 ± 17.6
Above % M mean ($n = 16$)	26.8 ± 5.5 (25.5)	0.46 ± 0.25 (0.37)	0.017 ± 0.008 (0.015)	77.6 ± 19.1
p value	<0.001[b]	<0.001[a]	0.58[a]	0.09[b]
Below L/M median ($n = 20$)	19.2 ± 8.5 (18.1)	0.21 ± 0.11 (0.17)	0.019 ± 0.002 (0.012)	68.4 ± 19.6
Above L/M median ($n = 20$)	19.8 ± 7.2 (18.9)	0.44 ± 0.22 (0.39)	0.021 ± 0.006 (0.019)	74.5 ± 17.7
p value	0.79[b]	<0.001[a]	<0.001[a]	0.213[a]
Below HOMA 2.71 ($n = 30$)	18.03 ± 6.9 (17.5)	0.30 ± 0.22 (0.23)	0.015 ± 0.007 (0.014)	65.7 ± 15.5
Above HOMA 2.71 ($n = 10$)	24.2 ± 8.5 (21.2)	0.41 ± 0.16 (0.40)	0.017 ± 0.006 (0.015)	89.35 ± 16.4
p value	0.026[b]	0.026[a]	0.36[a]	0.001[a]

%L: percentage of lactulose excretion; %M: percentage of mannitol excretion; L/M: lactulose/mannitol ratio.
[a] Mann–Whitney test.
[b] Student test.

abdominal circumference. It was also shown that higher body weight, BMI, waist and abdominal circumference, body fat percentage and the HOMA index were found in women with higher lactulose excretion.

Higher lactulose permeation and excretion indicate a damaged functioning of tight junctions and hence a leaky gut, allowing a higher flux of molecules through the paracellular route.[20] Higher endotoxin uptake could occur due to reduced expression of proteins of the epithelial tight junctions, such as zonulin and occludin, contributing to deregulation of paracellular transport and increased intestinal permeability.[19] Lipopolysaccharides (LPS) can activate the local or systemic immune cells.[21,22] The activated immune system has been demonstrated to play a role in the pathogenesis of type two diabetes.[22] Our results indicate that in obese women, intestinal barrier dysfunction might play a possible role in insulin secretion and immune system activation, as higher lactulose excretion was positively correlated to higher HOMA index values, which in turn was related to a high number of blood immune cells. Although LPS or associated parameters (lipopolysaccharide binding protein, sCD14) were not assessed in this study, there is evidence that the development of obese phenotype, induced by a high fat diet, involves the action of LPS and intestinal permeability,[23] and that there is a positive correlation between endotoxins and insulin.[18]

It has been suggested that changes in the gut microbiota can influence the intestinal permeability and LPS levels.[8,19,23] One of the proposed mechanisms is that the gut microbiota modulates the intestinal endocannabinoid system tone, which in turn regulates gut permeability and plasma LPS levels. An increased endocannabinoid tone and LPS contribute to the deregulation of adipogenesis,[24] and the level of endotoxins is directly related to the systemic inflammatory status.[7] Obesity and type two diabetes have been characterised by a chronic inflammatory state, and upon the interference of the activation of inflammatory pathways, an improvement in insulin resistance has been observed, demonstrating the biological relevance of modulating inflammation for metabolic control.[25] Thus, it can be suggested that the interactions between gut microbiota, immune system, adipose tissue and hormones are the main framework underlying possible altered intestinal permeability in obesity.

Table 4
Correlation between intestinal permeability measurements and anthropometric, body composition and biochemical variables.

Variables	% Mannitol excretion[a] (r)	p	% Lactulose excretion[b] (r)	p	L/M ratio[b] (r)	p
Weight (kg)	0.18	0.26	0.30	0.05	0.28	0.07
BMI (kg/m^2)	0.18	0.25	0.24	0.12	0.25	0.10
Body fat (%)	0.19	0.24	0.27	0.08	0.24	0.13
Body fat (kg)	0.18	0.25	0.29	0.06	0.28	0.07
Waist (cm)	0.22	0.16	0.32	0.04	0.28	0.07
Abdomen (cm)	0.18	0.24	0.33	0.03	0.30	0.05
HDL (mg/dl)	0.005	0.97	−0.27	0.08	−0.39	0.01
HOMA	0.32	0.04	0.47	0.002	0.39	0.014

%M = percentage of mannitol excretion; %L = percentage of lactulose excretion; L/M = Lactulose/mannitol ratio.
[a] Pearson correlation test.
[b] Spearman correlation test.

The higher blood insulin and HOMA index observed among obese volunteers, which are associated with higher abdominal fat accumulation, indicates the presence of insulin resistance.[14] Insulin resistance theory provides a mechanistic explanation to the observed tendency to higher blood pressure,[26] higher plasma glucose[27] and lower HDL-cholesterol concentrations,[28] observed in the obese group. Our study is the first to suggest that increased lactulose excretion reflect an increased paracellular permeability in obese women, and can contribute to increased insulin levels.

The comparison of intestinal permeability among different groups is usually based on the ratio of two excretion probes.[21] The observation of the isolated behaviour of specific probes might disclose different mechanisms through which L/M ratio may be affected. An increase in the L/M ratio is commonly observed in the presence of organic diseases, and could be due to increased lactulose excretion, which suggests the presence of inflammation of the intestinal mucosa and/or reduced mannitol excretion, that could reflect abnormal villous morphology such as atrophy.[29]

In the present study, L/M ratio was slightly increased in the obese group but was not statistically significant, even though lactulose excretion was increased in obese women. It should be further investigated as to whether obese women have increased intestinal mucosa surface area, which could favour higher mannitol excretion, as shown in an animal model of genetic obesity.[30] In the majority of the comparisons shown in Table 3, the groups with higher weight excreted higher amounts of mannitol. If we assume that obese individuals might be absorbing proportionally higher quantities of both probes (mannitol and lactulose), the increase in L/M ratio might not be significantly different from lean, although

the permeability (i.e. the ease in which the membrane allows molecules to pass through by non-mediated diffusion[31]), of individual probes would be increased. This was clearly demonstrated when all 40 women were divided by the mean of mannitol excretion and by the HOMA cut-off point, where it was observed that mannitol and lactulose excretion were significantly higher in the group above the criteria considered, but having a similar L/M ratio.

An increase in mannitol excretion could also be related to the increased circulatory levels of insulin observed in the obese group. Insulin receptors are found at the basolateral membrane of intestinal cells, and the addition of insulin to the medium of an intestinal cell culture at the basolateral membrane induced a decline in transepithelial resistance and increased mannitol flux.[32] Our results suggest that some degree of intestinal mucosa inflammation might be present in obesity, favouring a higher proportion of lactulose excretion. Intestinal inflammation in mice was demonstrated to be an early consequence of the interaction between high fat diet and microbiota, which may contribute to obesity and insulin resistance.[33]

In a previous pilot study, differences were not found in the variables related to intestinal permeability when obese subjects were compared to the lean ones.[34] Although differences existed on the quantities of the administered sugar probes, on the quantification method, and in the form of expressing the results, the present investigation has the advantage of a larger sample of female volunteers. Thus, more studies are necessary to address the question of whether altered intestinal permeability contributes to obesity, and also if lactulose excretion could be considered a better marker than L/M ratio for the detection of altered intestinal permeability in obesity. This implies a higher paracellular absorption of substances, including bacterial material. Our data also suggest that lactulose excretion and L/M ratio could be considered as possible indicators to be included on the list of criteria for metabolic syndrome diagnosis or management.

As there is strong evidence that the detection, prevention and treatment of the underlying risk factors of metabolic syndrome would be of importance to reduce cardiovascular disease incidence and mortality, as well as all-cause mortality,[35] our data suggest that therapeutic interventions focused on intestine health and modulation of intestinal permeability, should be explored in the context of obesity, based on the findings that a positive correlation was found between higher lactulose excretion and alterations in anthropometric and metabolic measurements.

Statement of authorship

TFST, JB and MCGP participated in the conception and design of experiments. TFST also carried out the studies and drafted the manuscript; NCSS and PGC provided essential reagents and carried out urine samples analyses; SCCF performed statistical analysis; CLLFF helped to draft the manuscript.

Conflict of interest statement

None declared.

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