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Enhanced intestinal 2-deoxy-2-[^{18}F]fluoro-D-glucose uptake under metformin is not fully suppressed by loperamide

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Objective. This study investigated whether the metformin (Met)-induced enhanced intestinal uptake of 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) is reduced by loperamide, a long-acting anti-diarrheal agent.

Methods. Mean ^{18}F-FDG uptake in the mouse small intestine and colon with Met exposure was compared with that in control mice. In the Met group, high-dose (1.0 mg/kg body weight) and low-dose (0.1 mg/kg body weight) loperamide were introduced, and ^{18}F-FDG uptake in the small intestine and colon was compared with that of control mice administered high-dose loperamide. The percent injected dose of ^{18}F-FDG per gram of tissue (%ID/g) in the extracted tissues was then determined.

Results. ^{18}F-FDG uptake increased significantly in the small intestine (0.64±0.06 vs. 1.01±0.15, p=0.040) and, especially, the colon (0.46±0.13 vs. 2.16±0.51, p<0.001) after Met exposure. Neither high-dose nor low-dose loperamide significantly reduced ^{18}F-FDG uptake in the small intestine (0.82±0.31 vs. 0.84±0.22, p=0.93 and 0.78±0.25 vs. 0.70±0.15, p=0.13, respectively) or colon (2.13±0.41 vs. 1.67±0.55, p=0.063 and 1.77±0.39 vs. 1.80±0.25, p=0.56, respectively). The colonic %ID/g was significantly higher in Met groups irrespective of loperamide introduction than in control group, whereas the significant difference in the small intestine was observed only between Met and control groups.

Conclusion. Metformin increased ^{18}F-FDG uptake in intestines especially in colon. Loperamide administration partially, but not sufficiently, suppresses the Met-induced increased colonic uptake of ^{18}F-FDG.

Key words: ^{18}F-FDG, PET/CT, metformin, loperamide, intestine, physiological

Positron emission tomography (PET) with 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-FDG) is frequently used in the detection and management of malignant diseases. However, ^{18}F-FDG accumulates physiologically in several organs, which can be misinterpreted as positive findings in image evaluation. In the gastrointestinal tract, physiological ^{18}F-FDG accumulation is augmented by metformin (Met), an oral antidiabetic drug commonly prescribed for patients with type 2 diabetes (Gontier et al. 2008). In these patients, the Met-induced accumulation of ^{18}F-FDG may obscure an unexpected tumor in the intestinal tract, leading to a delay in its identification. Previous studies have shown that the Met-induced increase in ^{18}F-FDG uptake can be avoided by stopping Met for a few days prior to the ^{18}F-FDG exam (Oh et al. 2010;

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Ozulker et al. 2010; Lee et al. 2016). However, the procedure may not be always accepted in routine clinical studies, and to cease the drug can cause hyperglycemia, which may adversely affect the diagnostic value.

Among the possible mechanisms resulting in enhanced intestinal ^{18}F-FDG uptake by Met are the increased expression of glucose transporters (GLUTs) and the increased availability of phosphorylated adenosine monophosphate (AMP)-activated protein kinase (pAMPK) (Massollo et al. 2013). However, the segmental rather than diffuse increase in intestinal ^{18}F-FDG uptake in Met-treated patients (Oh et al. 2010) suggests the involvement of other mechanisms. As one of the side effects of Met is diarrhea, increased intestinal motility may contribute to the elevated ^{18}F-FDG uptake. Therefore, we investigated whether loperamide (Lop), an anti-diarrheal drug that suppresses intestinal motility (Schiller et al. 1984), would prevent Met-induced enhanced ^{18}F-FDG uptake.

Materials and methods

Animal preparation. All animal experiments were approved by the responsible authorities of our institution. Male C57BL/6N mice (age, 10–13 weeks) were used in this study. All mice had free access to food and water. Nineteen mice were used in the Met group and nine in the control group.

μPET/CT with FDG. The mice were injected with 37 MBq ^{18}F-FDG/kg via the tail vein and then after 60 min underwent a 5-min micro-PET/computed tomography (μPET/CT) scan using the Triumph small animal PET/single photon emission computed tomography/CT system (TriFoil Imaging, Chatsworth, CA USA). The mean standardized uptake value (SUVmean) in the intestine was calculated using the AMIDE software (SlashdotMedia, La Jolla, CA USA). The three-dimensional (3D) spherical volume of interest (VOI) (1.2×1.2×1.2 mm) and 3D cylindrical VOI (1.2×1.2×5 mm) were defined for the small intestine and colon, respectively, based on the area showing the highest ^{18}F-FDG uptake. Anal uptake was excluded from the analysis to avoid the influence of anal sphincter muscle contraction. The VOI was set carefully, avoiding any activity in the bladder or urinary tract.

Experimental procedures. In a first study (Study A), the effect of Met on the intestines was investigated in 12 mice, with nine allocated to the Met group and three to the control (Con) group. For mice in the Met group their normal drinking water was replaced for 4 weeks with drinking water containing 1.0 mg Met/ml. Mice in the control group continued to receive normal drinking water. The two groups underwent ^{18}F-FDG-PET/CT both at the beginning of the study (1st PET/CT) and 4 weeks after (2nd PET/CT) the administration of drinking water with or without Met.

In a second study (Study B), the effect of Lop was studied in 13 of the Met-treated mice, including three from Study A, and in six control mice. The mice underwent a 1st PET/CT scan without Lop (pre-Lop PET/CT) followed 3 days later by a 2nd PET/CT scan with Lop (post-Lop PET/CT). Met intake was continued in the Met group during this period. The Lop solution, prepared in saline, was injected intraperitoneally twice, 30 min before and after ^{18}F-FDG administration. For post-Lop PET/CT, the 13 Met-treated mice were divided into high-dose (1.0 mg/kg: M+high L; n=7) and low-dose (0.1 mg/kg: M+low L; n=6) Lop groups. The six control mice received 1.0 mg Lop/kg at the post-Lop PET/CT (C+high L). The doses of Lop, i.e. 1.0 mg/kg and 0.1mg/kg, were determined referring to a previous study of colonic transit which investigated the effects on opioid receptors (Krevsky et al. 1991).

Immediately after the post-Lop PET/CT, all of the mice were euthanized and their small intestine, colon, and blood were extracted for γ-counting. Several short segments from the small and large intestine were sliced open and the intestinal contents removed. The % injected dose of ^{18}F-FDG per gram of tissue (%ID/g) was calculated from the count data, tissue weight, and administered dose of FDG.

Statistical analysis. Numerical data are expressed as the mean±standard deviation. A Wilcoxon signed rank test was performed to compare the SUVmean values of the paired groups, and the Wilcoxon rank sum test to compare the data of non-paired groups. The Tukey-Kramer test was applied for γ-counting. All statistical analyses were performed using JMP pro version 12. A bilateral p value <0.05 was considered to indicate statistical significance.

Results

Figure 1 shows the procedure used in this study and includes representative images.

Study A. Mice in the Met group consumed 3.3–3.7 mg Met per day. The body weights of mice in the Met and Con groups did not differ significantly at either the 1st PET/CT (Met; 25.7±1.9 g, Con; 25.4±1.1 g, p=0.78) or the 2nd PET/CT (Met; 30.9±3.3 g, Con; 31.2±1.3 g, p=0.89) scans. Table 1 summarizes the results of Study A. In the Met group, FDG uptake in the small intestine was significantly higher in

seven of the nine mice, as determined in the 1st vs. 2nd PET/CT scan (0.64±0.06 vs. 1.01±0.15, p=0.040; Figure 2a). By contrast, ^{18}F-FDG uptake in small intestine of the Con group were not significantly different (0.55±0.10 vs. 0.65±0.25, p=0.73), nor were the 2nd PET/CT of the small intestines of mice in the Met and Con groups (p=0.23). In the colon, ^{18}F-FDG uptake was significantly increased in all mice in the Met group (0.46±0.13 vs. 2.16±0.51, p<0.001), whereas the differences in the Con group were not significant (0.39±0.05 vs. 0.45±0.06, p=0.38; Figure 2b). In a comparison of the 2nd PET/CT scans, ^{18}F-FDG uptake in the colon was significantly (p=0.013) higher in the Met than in the Con group.

Study B. In the pre-Lop PET/CT, ^{18}F-FDG uptake in small intestine tended to be higher in the Met group than in the Con group, without statistical significance (0.80±0.27 vs. 0.69±0.17, p=0.48). However, ^{18}F-FDG uptake in the colon was significantly higher in the Met group (1.96±0.43 vs. 0.46±0.06, p=0.0006), consistent with results of Study A.

The results of Study B are summarized in Table 2. As shown in Figure 3a, ^{18}F-FDG uptake in the small intestine did not differ significantly in the three groups (M+high L: 0.82±0.31 in the pre-Lop PET/CT vs. 0.84±0.22 in the post-Lop PET/CT, p=0.93; M+low L: 0.78±0.25 vs. 0.70±0.15, p=0.13; C+high L: 0.69±0.17 vs. 0.68±0.09, p=0.92). In the colon of five of the seven mice in the M+high L group, ^{18}F-FDG

Table 1
Effect of metformin on intestinal ^{18}F-FDG accumulation.

		SUVmean		p-value
		1st PET	2nd PET	
Small intestine	Met	0.64±0.06	1.01±0.15	0.040*
	Con	0.55±0.10	0.65±0.25	0.73
Colon	Met	0.46±0.13	2.16±0.51	<0.001*
	Con	0.39±0.05	0.45±0.06	0.38

Abbreviation: Met – Metformin group; Con – Control group.
*p<0.05.

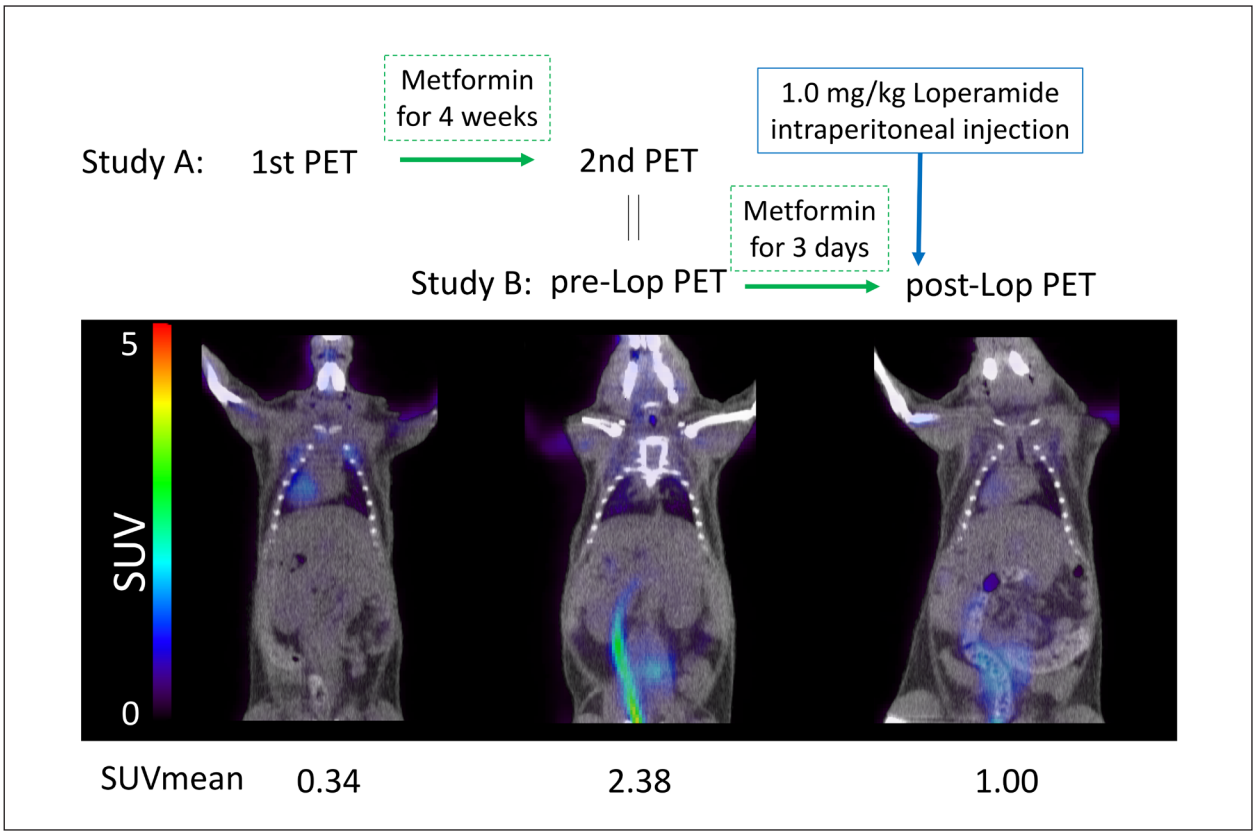

Figure 1. Representative coronal ^{18}F-FDG-PET/CT images of mice included in both study A and study B. Before metformin (Met) administration, there is no apparent ^{18}F-FDG uptake in the colon (SUVmean = 0.34); after Met exposure, ^{18}F-FDG accumulation in the colon is markedly increased (SUVmean = 2.38). A high dose of loperamide decreases ^{18}F-FDG uptake but it is still higher than that seen in the 1st PET/CT scan (SUVmean = 1.00).

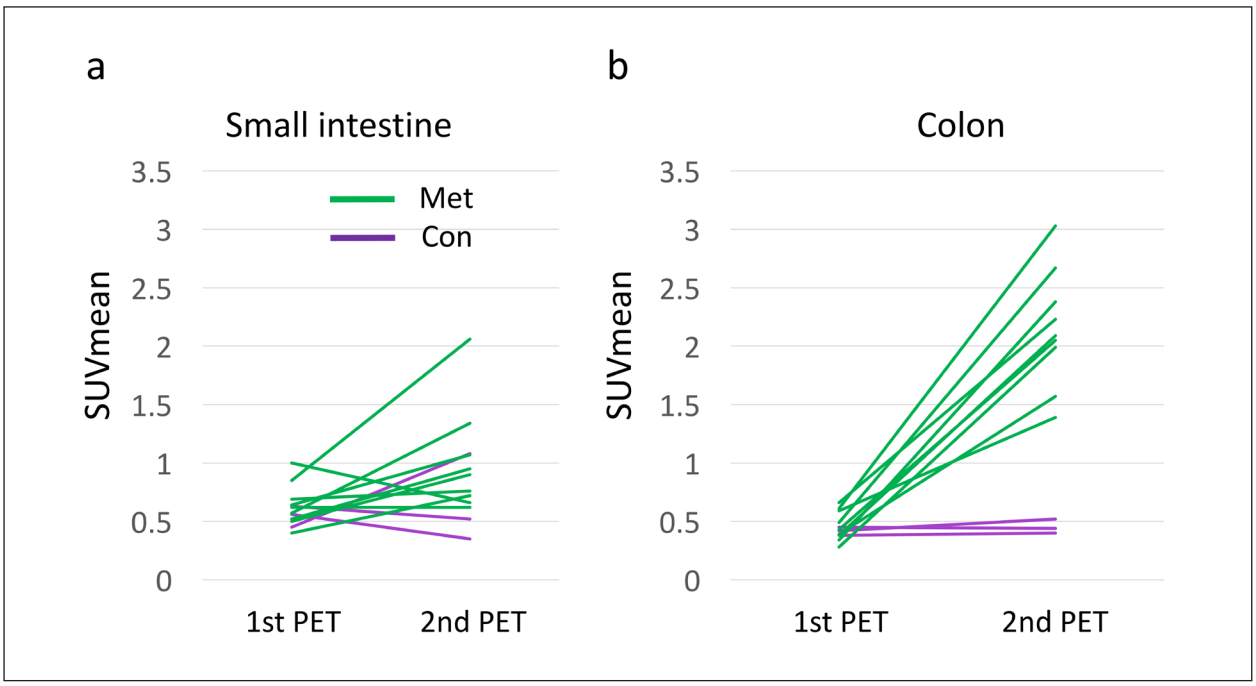

Figure 2. SUVmean of the 1st and 2nd PET/CT scans of the small intestine (**a**) and colon (**b**). Met; metformin, Con; control. On the 2nd PET/CT scan, ^{18}F-FDG uptake, especially in the colon, is increased in the Met group but stable in the Con group.

Table 2
Change in the SUVmean of the small intestine and colon before and after loperamide administration.

Group	n	Small intestine			Colon		
		Pre Lop	Post Lop	p-value	Pre Lop	Post Lop	p-value
M+high L	7	0.82±0.31	0.84±0.22	0.93	2.13±0.41	1.67±0.55	0.063
M+low L	6	0.78±0.25	0.70±0.15	0.13	1.77±0.39	1.80±0.25	0.56
C+high L	6	0.69±0.17	0.68±0.09	0.92	0.46±0.06	0.54±0.08	0.22

Abbreviation: SUVmean – mean standardized uptake value; M+high L – Metformin plus 1.0 mg loperamide/kg; M+low L – Metformin plus 0.1 mg loperamide/kg; C+high L – Controls plus 1.0 mg loperamide/kg.

uptake decreased substantially. However, as shown in Figure 3b, the changes among the mice in this group were variable such that the reduction in colon uptake was not significant (2.13±0.41 in the pre-Lop PET/CT vs. 1.67±0.55 in the post-Lop PET/CT, p=0.063). Furthermore, colonic uptake was still much higher in the scans of post-Lop PET/CT than of Con mice. The differences between the pre-Lop and post-Lop scans of the other two groups were not significant (M+low L: 1.77±0.39 vs. 1.80±0.25, p=0.56; C+high L: 0.46±0.06 vs. 0.54±0.08, p=0.22) (Figure 3b).

There was a significant difference in the average %ID/g of the small intestine between the C+high L, and M (2.30±0.89 vs. 4.14±1.22), but no significant difference with M+low L (2.69±1.22), and M+high L (2.47±0.92) groups, as determined by ex vivo γ-counting (Figure 4a). In the colon, the %ID/g of the M, the M+low L, the M+high L groups (13.02±7.38, 13.69±4.49, 16.79±5.32; respectively) was significantly higher than that of the C+high L group (4.29±0.73) (p=0.031, 0.019, 0.0012; respectively), whereas the difference between the other groups was not significant (Figure 4b). The differences among the blood samples of these groups were also not significant (data not shown).

Discussion

Metformin modifies the intestinal uptake of ^{18}F-FDG but its specific effects in the small intestine are as yet unknown. According to one report, Met increases ^{18}F-FDG uptake in the ileum but to a lesser

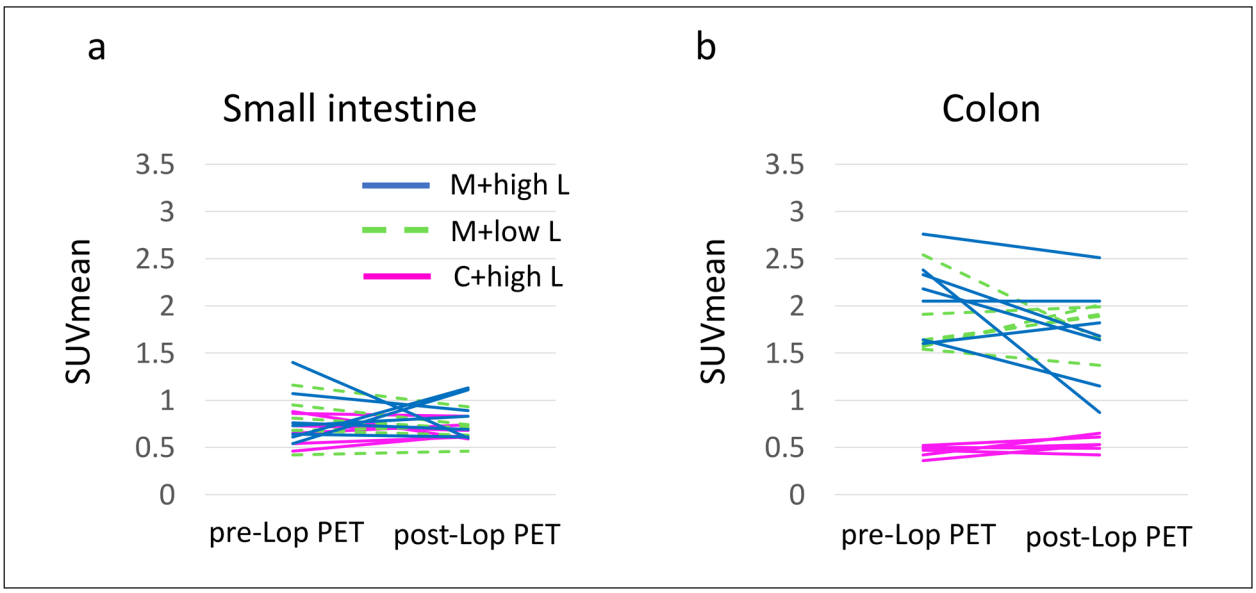

Figure 3. SUVmean as seen on the pre-Lop and post-Lop PET/CT scans of the small intestine (**a**) and colon (**b**). M+high L; Met plus 1.0 mg Lop/kg, M+low L; Met plus 0.1 mg Lop/kg, C+high L; controls plus 1.0 mg Lop/kg. The reduction in ^{18}F-FDG uptake in the small intestine and colon of Lop-treated mice is not significant, although colonic uptake after Lop administration is substantially decreased in 5 of the 7 mice in the M+high L group.

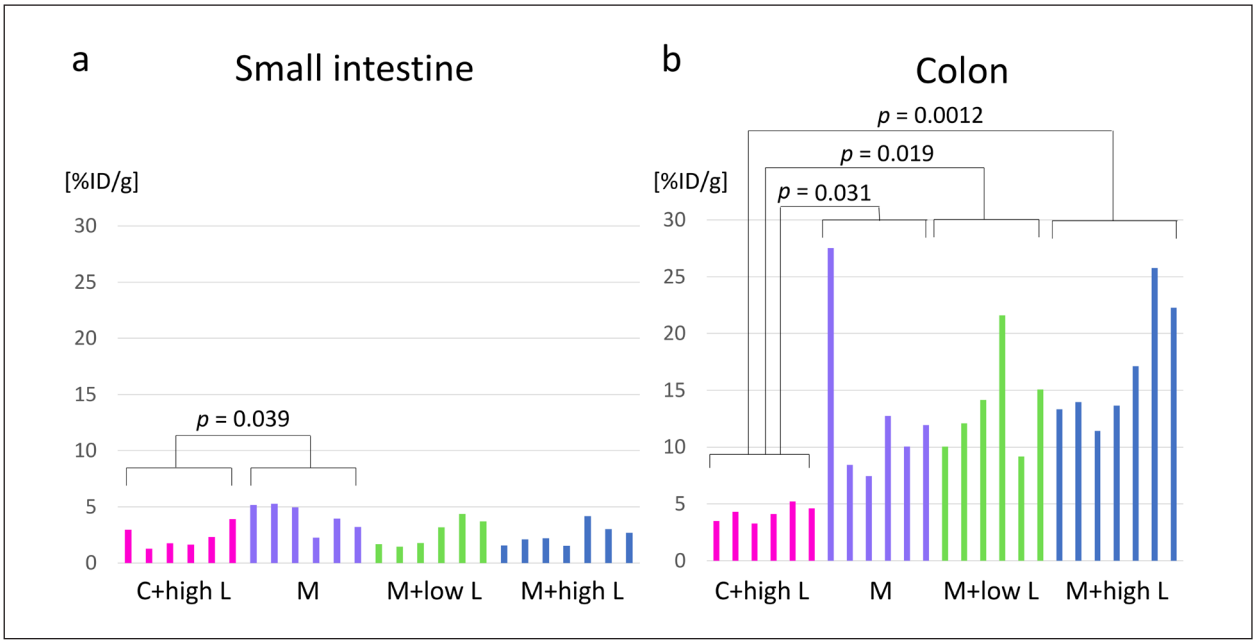

Figure 4. γ-counting results in specimens from the small intestine (**a**) and colon (**b**). C+high L; controls plus 1.0 mg Lop/kg, M; Met only, M+low L; Met plus 0.1 mg Lop/kg, M+high L; Met plus 1.0 mg Lop/kg. The average %ID/g of the small intestine was significantly higher in the M than the C+high L group (p=0.0039), whereas in the colon, the %ID/g was significantly higher in the M, and the M+low L, and the M+high L groups than in the C+high L group (p=0.031, 0.019, and 0.0012; respectively).

extent than in the colon (Gontier et al. 2008). The effect of Met on the jejunum is unclear (Oh et al. 2010; Ozulker et al. 2010). However, it is established that Met uniformly and significantly increases ^{18}F-FDG uptake in the colon (Gontier et al. 2008; McCreight et al. 2016). Our results on intestinal uptake were consistent with those of previous reports, as ^{18}F-FDG was significantly increased in mice orally administered

Met for 4 weeks. And ^{18}F-FDG uptake in the small intestine was much less than that in the colon, indicating that Met preferentially affects ^{18}F-FDG uptake in the colon.

Since the most common side effect of Met is diarrhea, we hypothesized that the Met-induced elevation of ^{18}F-FDG uptake is mediated by increased intestinal motility. We tested this hypothesis by evaluating whether Lop administration suppressed Met-induced ^{18}F-FDG uptake in the mouse intestine.

Loperamide is a popularly prescribed to reduce the bowel motility that causes diarrhea. It induces intestinal relaxation by activating opioid μ2 receptors via the cAMP-PKA pathway, which in turn prevents the exocytosis of acetylcholine from the parasympathetic nerves of the ileum, as demonstrated in mice (Chen et al. 2012). In the large intestine, Lop suppresses the release of 5-hydroxytryptamine (5-HT), an enhancer of colonic motility, from the colonic mucosa, in a process triggered by the NK3 receptor, probably by activating κ- and δ-opioid receptors, as shown in guinea pigs (Kojima et al. 2005). Based on these previous reports, we expected that Lop would reduce the motility of both the small and the large intestine. However, our results showed that even high-dose Lop only partially suppressed the Met-induced increase in ^{18}F-FDG uptake in the colon, and failed to eradicate completely the effect of Met on the small intestine. These results suggest that, while Met may increase intestinal motility, especially that of the colon, this effect does not appear to be the major contributor to the increased ^{18}F-FDG uptake.

In γ-counting, samples were randomly excised from several segments of the intestine, while the areas showing high ^{18}F-FDG uptake were selected in PET measurement. Nevertheless, the γ-counting data supported the post-Lop PET results, showing that, compared with the controls, colonic ^{18}F-FDG uptake was significantly elevated in Met mice treated with either high-dose or low-dose Lop. Since the intestinal contents were removed prior to γ-counting, it can be concluded that Met increases ^{18}F-FDG uptake in the colonic wall, not intraluminally.

In conclusion, metformin increases intestinal ^{18}F-FDG uptake in intestines, especially in the colon. Loperamide, an antidiarrheal drug, only partially suppressed the increased colonic uptake, suggesting that accelerated intestinal motility is not the major mechanism of action of Met that leads to increased ^{18}F-FDG accumulation. Further studies are required to elucidate the functional mechanism by which metformin enhances intestinal ^{18}F-FDG uptake for optimal ^{18}F-FDG-PET/CT studies in clinical.

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