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Metformin treatment significantly enhances intestinal glucose uptake in patients with type 2 diabetes: Results from a randomized clinical trial

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ABSTRACT

Aims: Metformin therapy is associated with diffuse intestinal ¹⁸F-fluoro-deoxyglucose (FDG) accumulation in clinical diagnostics using routine FDG-PET imaging. We aimed to study whether metformin induced glucose uptake in intestine is associated with the improved glycaemic control in patients with type 2 diabetes. Therefore, we compared the effects of metformin and rosiglitazone on intestinal glucose metabolism in patients with type 2 diabetes in a randomized placebo controlled clinical trial, and further, to understand the underlying mechanism, evaluated the effect of metformin in rats.

Methods: Forty-one patients with newly diagnosed type 2 diabetes were randomized to metformin (1 g, b.i.d), rosiglitazone (4 mg, b.i.d), or placebo in a 26-week double-blind trial. Tissue specific intestinal glucose uptake was measured before and after the treatment period using FDG-PET during euglycemic hyperinsulinemia. In addition, rats were treated with metformin or vehicle for 12 weeks, and intestinal FDG uptake was measured *in vivo* and with autoradiography.

Results: Glucose uptake increased 2-fold in the small intestine and 3-fold in the colon for the metformin group and associated with improved glycaemic control. Rosiglitazone

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increased only slightly intestinal glucose uptake. In rodents, metformin treatment enhanced intestinal FDG retention ($P = 0.002$), which was localized in the mucosal enterocytes of the small intestine.

Conclusions: Metformin treatment significantly enhances intestinal glucose uptake from the circulation of patients with type 2 diabetes. This intestine-specific effect is associated with improved glycemic control and localized to mucosal layer. These human findings demonstrate direct effect of metformin on intestinal metabolism and elucidate the actions of metformin.

Clinical trial number [NCT02526615](#)

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1. Introduction

The intestine has numerous metabolic functions: nutrient absorption, hormone secretion, systemic immune response and glucose production. The intestine is gluconeogenic organ that expresses glucose-6-phosphatase and is capable of endogenous glucose production (EGP) [1–3]. EGP is elevated in impaired glucose tolerance and frank diabetes [4,5]. Furthermore, the intestine regulates nutrient absorption and controls energy balance mediated by incretin hormones. The function of these hormones is severely impaired in type 2 diabetes [6], which leads to beta cell dysfunction and insulin resistance. Metformin seems to interact with the incretin axis by increasing plasma glucagon-like peptide 1 (GLP-1) levels and expression of the genes encoding the receptors for GLP-1 and glucose-dependent insulinotropic polypeptide in mouse pancreatic islets [7].

Despite decades of use of metformin as a first line therapy for type 2 diabetes, its mechanisms of action are still poorly understood. Metformin has previously been demonstrated to control hepatic glucose production through mechanisms that involve adenosine monophosphate (AMP)-activated kinase [8], mitochondrial metabolism [9,10] and recently, glucagon receptor signalling and cyclic AMP production [11]. Experimental rodent models administered metformin orally have shown, that metformin accumulates in the mucosa of the small intestine [12] in concentrations up to 300 times higher than those in plasma [13].

In clinical diagnostics [^{18}F]-fluoro-2-deoxy-D-glucose (FDG) combination with positron emission tomography (PET), metformin treatment elicited increased FDG uptake can mimic pathologic uptake in the gut [14]. An increase in FDG bowel uptake takes place mainly in the colon and, to a lesser extent, in the small intestine [14]. Penicaud and co workers demonstrated almost two decades ago that metformin enhances glucose uptake in intestinal mucosa in obese rats [15]. We have recently showed that intestinal glucose uptake can be monitored using quantitative FDG-PET analysis methodology [16]. We demonstrated also, that insulin increases intestinal glucose uptake in healthy subjects, but not subjects with morbid obesity [17]. The present study was undertaken to assess specifically the intestinal effects of metformin and rosiglitazone monotherapy on insulin stimulated GU in a randomised clinical trial. The effects on glycemic control and insulin sensitivity have been published earlier [2,18]. Metformin treat-

ment improved glycemic control but did not change insulin sensitivity which was in line with previous publications [19]. In this study we wanted to assess in which extent metformin increases intestinal GU and does it correlate to glycemic control.

2. Materials and method

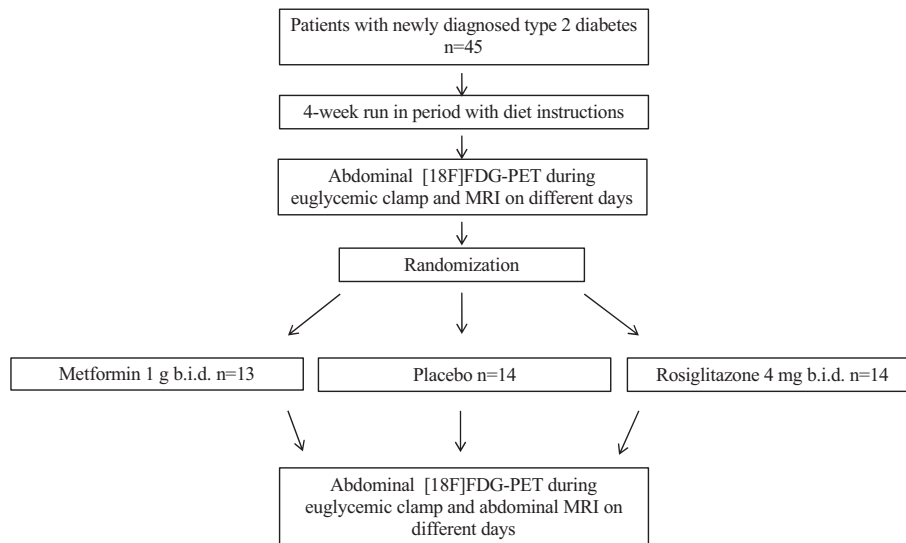
2.1. Human study

The study design was previously described [2]. A total of 45 patients with newly diagnosed uncomplicated type 2 diabetes with no prior antidiabetic medicine (Table 1) [20], were assigned to the protocol and randomized for sex and smoking (Clinical trial number NCT02526615). Two patients in the metformin group and one patient in the rosiglitazone group was excluded [2]. Follow-up data were not obtained from one patient in the rosiglitazone group and from patients the placebo group. The local ethics committee approved the study protocol. The study consisted of a 4-week run-in period after which patients were randomly assigned for treatment with rosiglitazone (2 mg b.i.d. for 2 weeks, thereafter 4 mg b.i.d.), metformin (500 mg b.i.d. for 2 weeks, thereafter 1 g b.i.d.), or placebo for a 26-week double-blinded trial (Fig. 1). PET studies were performed using the same protocol before treatment and in the 26th week of the trial [2]. The rates of whole-body, skeletal (quadriceps) muscle and intestinal GU were determined after an overnight fast by combining the euglycemic-hyperinsulinemic clamp for 140 min (with insulin infusion of $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and FDG PET scanning [2]. MRIs were done right after PET studies on the same day to avoid inconvenience for the study subjects. Whole-body GU (Mvalue) was calculated according to previous publication [21]. The FDG was injected intravenously 90 min after starting the clamp and a dynamic scan of 20 min was performed for skeletal muscle and consecutive 18-min dynamic scan of the abdominal area was obtained with arterial blood sampling [2,18]. Endogenous glucose production (EGP) calculations were based on the plasma clearance of FDG used to estimate the rate of appearance of glucose which was validated against deuterated glucose [3]. Since FDG is partly lost in urine (overestimating the metabolic clearance of glucose), we estimated the urinary loss from our previous data [3] and subtracted this factor in the calculation of EGP.

Table 1 – Anthropometric and metabolic characteristics of subjects before and after intervention.

	Placebo		Metformin		Rosiglitazone	
	Baseline	26 weeks	Baseline	26 weeks	Baseline	26 weeks
n	14		13		14	
Sex (M/F)	10/4		8/5		10/4	
Age	57.5(1.9)		57.8(2.2)		58.6(2.0)	
BMI (kg/m ²)	30.3(4.4)	30.3(4.4)	29.9(3.8)	29.2(3.9) [#]	29.1(3.8)	29.5(4.2)
Hb _A 1c (NGSP/IFCC units)	6.3(0.1)/45	6.1(0.1)/43	6.9(0.2)/52	6.2(0.2)/44 ^{**}	6.8(0.2)/51	6.5(0.2)/45 [#]
fS-Insulin	10.1(4.9)	9.6(3.4)	11.7(7.7)	8.8(4.1)	8.6(5.6)	6.6(1.6) [*]
Insulin clearance (ml/min)	1.10(0.22)	1.29(0.54)	1.09(0.29)	1.25(0.17) [#]	1.00(0.18)	1.26(0.23) [#]
EGP (μmol/min/kg)	19.4(6.4)	24.5(10.6)	21.2(8.1)	19.4(5.5)	20.7(12.0)	27.6(9.9) [#]
P-lactate (mmol/l)	1.0(0.1)	0.9(0.3)	1.0(0.1)	1.2(0.3) [*]	0.9(0.1)	0.7(0.4)

Data are means (SE).
^{*} P < 0.01.
^{**} P < 0.0001 vs. Placebo.
[#] P < 0.05 vs baseline.

**Fig. 1 – Study design. [¹⁸F]-fluoro-2-deoxy-D-glucose tracer (FDG) combined with positron emission tomography (PET) imaging (FDG-PET), magnetic resonance imaging (MRI).**

2.2. Analyses of PET images

Small intestine, colon and skeletal muscle GU values were measured by manually drawing regions of interest (ROIs) in the intestine [16] (Fig. 2. J and K) and in the quadriceps muscle using the Carimas 2.9 program. The intestinal ROIs were carefully shaped to contour the intestinal wall, avoiding the intestinal contents and also the external metabolically active organs. The localization of the intestine was done on fused PET and MR images then the final localization was confirmed visually on the PET images. Hepatic GU was analysed from the PET images as reported formerly [18].

The three-compartment model of FDG kinetics was used [22]. Plasma and tissue time-activity curves were analysed graphically [23] to quantify the fractional rate of tracer uptake (K_i) [24]. A lumped constant value of 1.15 for the intestine and 1.2 for skeletal muscle were used as previously described [16,25,26]. Measurement of whole-body GU was done as previously described using the euglycemic insulin clamp tech-

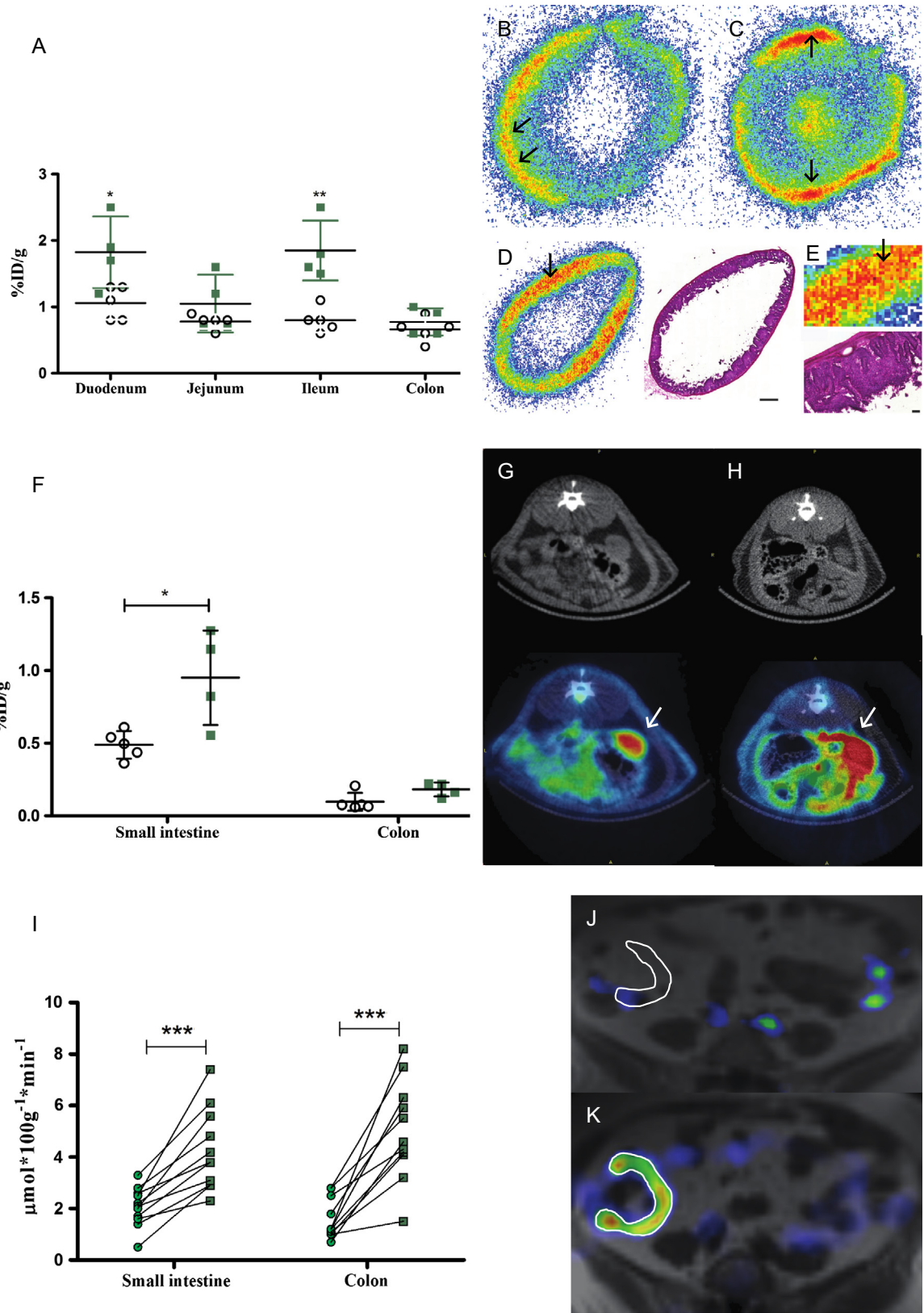
nique [2,21] (for additional details see [supplemental materials](#)).

2.3. Experimental animals

Nine male adult normoglycemic Bio-Breeding Diabetes Resistant rats [27], were treated by metformin or used as controls. Metformin was administered via osmotic pumps (50 mg/kg/24 h); placed subcutaneously between the scapulae of the rats for three months (for more details see the [supplemental materials](#)).

Each animal was examined after a three to five hours' fast by dynamic FDG-PET imaging and the tissue uptake was calculated as the percentage of the administered dose of tracer taken up per gram of tissue (%ID/g).

After the PET-scanning the rats were sacrificed and the biodistribution (BD) of the radioactivity in the different tissues were measured, and reported as a percentages of the injected dose per gram of tissue (%ID/g). Tracer uptake in tis-



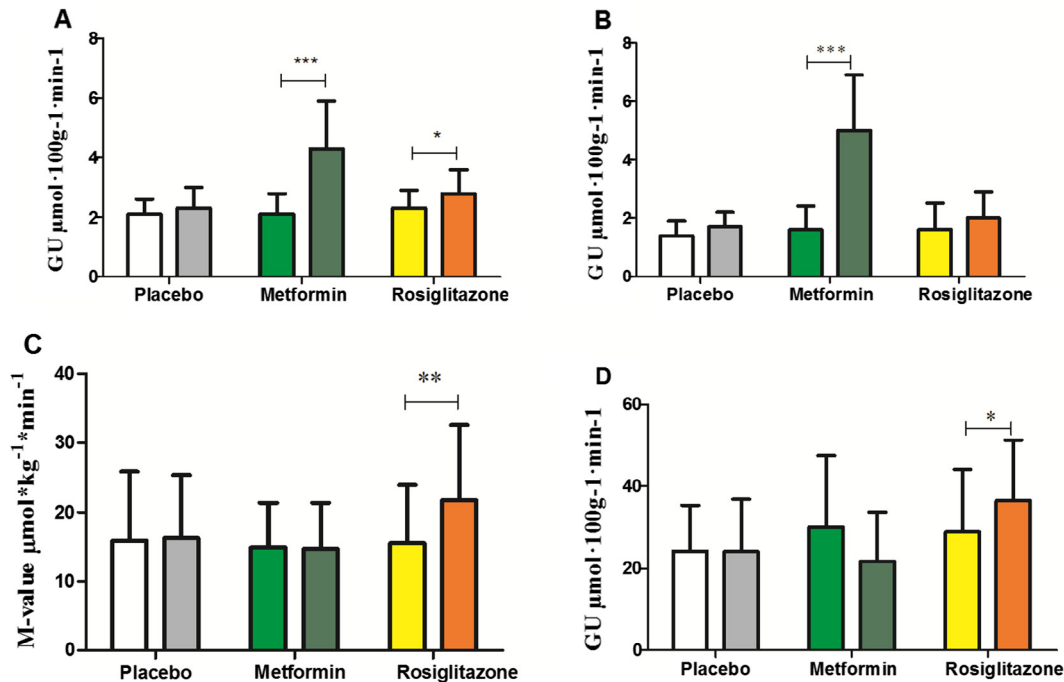


Fig. 3 – Tissue specific glucose uptake. Panel (A) showing small intestine and (B) colon GU (C) M-value (D) skeletal muscle GU in different intervention groups. Metformin significantly increased GU in the small intestine (2-fold) and in the colon (3-fold). Rosiglitazone treatment caused 22%, 44% and 25% incremental increases in the small intestine GU, M-value and skeletal muscle GU, respectively. * $P < 0.03$, ** $P < 0.05$ and *** $P < 0.001$ vs. baseline.

sues were corrected for blood glucose values. Cryosections were analysed using autoradiography (for more details see the [supplemental materials](#)).

2.4. Statistical methods

Statistical analyses were performed with SAS software for Windows version 9.2 (SAS Institute, Cary, NC). The data were expressed as means and standard deviations for variables with normal distributions. Differences between groups were compared using repeated measurements ANOVA and if a sig-

nificant interaction was found, by one-way ANOVA and Tukey's honestly significant difference post hoc test were performed to test changes between the groups. Differences between two groups of uneven size were evaluated using the Student's t-test for single repeated measurements. Pearson's or Spearman's correlation coefficients were calculated depending on the normality of the data. Values of $P < 0.05$ were considered statistically significant. Part comparisons of non-paired data between two uneven group sizes ($N = 4$ vs. $N = 5$) were made in the animal study using a Student's t test and a value of $P < 0.05$ was considered statistically significant.

Fig. 2 – Intestinal glucose uptake (GU) after metformin intervention. Panels A–H: experimental animals, I–K human data, green (metformin) and white (control) symbols indicate interventions. Metformin treated rats showed higher [^{18}F]-fluoro-2-deoxy-D-glucose (FDG) uptake in the small intestine in the biodistribution analysis (A). B–E: representative images of radiotracer accumulation in the mucosal layer (black arrows). Autoradiography confirmed radiotracer accumulation only in the mucosal layer of the intestinal sections (D and E, between white lines). In the metformin treated group (C) this accumulation was higher compared to control group (B) 239 (65.4) PSL/mm² vs. 185 (47.7), $P = 0.002$, respectively. The PET examinations (F) showed increased FDG uptake *in vivo* in small intestine in animals following metformin intervention compared to controls. There was also an increase in FDG uptake in the colon, although from a lower basal level. White arrows demonstrating increased FDG uptake in red areas. Small animal CT and PET images for control and metformin groups (G and H). Human data, show GU in the small bowel was increased by 2-fold and in the colon by 3-folds compared to baseline (I) after 26 weeks of metformin treatment. Fused PET/MRI images for same study subject in the baseline (J) and after metformin treatment (K). Region of interest drawn in fused PET-MRI (white line). Data are shown with median (A and F), * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ vs. control (for animal study) and baseline (for human study). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

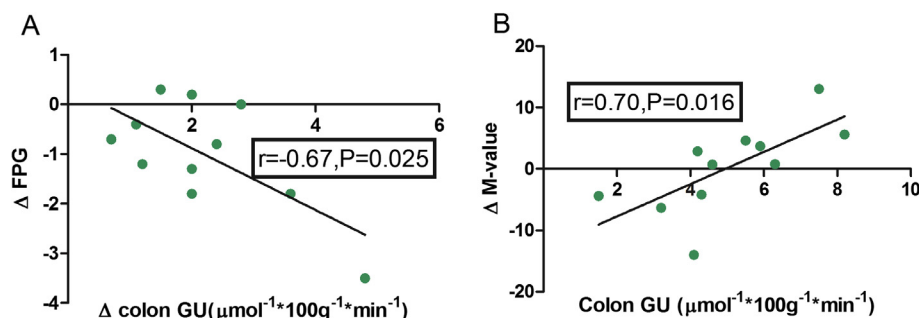


Fig. 4 – Relationship between intestinal metabolism and glycemic control. Increased glucose uptake (GU) in colon correlated with decrease in fasting plasma glucose in metformin group (A). Increased whole body GU correlated with postintervention intestinal GU in metformin (B) group.

3. Results

3.1. Intestinal effects of metformin and rosiglitazone in the clinical intervention

Metformin and rosiglitazone treatments improved Hb_{A1c} (Table 1) but only rosiglitazone improved whole body insulin sensitivity (M-value) ($P < 0.001$) and skeletal muscle insulin sensitivity ($P = 0.047$, Fig. 3).

Intestinal glucose uptake in the small intestine and in the colon were similar between the groups before the intervention (Fig. 3). After 26 weeks of treatment with metformin, GU in the small bowel increased 2-fold, ($P < 0.001$) and in the colon 3-fold, ($P < 0.001$) compared to baseline (Fig. 3). The GU in the small intestine increased only slightly for the rosiglitazone group but significantly compared to baseline ($P = 0.029$, Fig. 3). Changes in HbA_{1c} in the pooled dataset were associated with changes in GU in the small intestine ($rP = -0.40$, $P = 0.016$ and) and in the colon ($P = 0.005$, $rP = -0.46$), but not for groups separately. In addition, enhanced intestinal GU in colon correlated with changes in M-values and with fasting plasma glucose (Fig. 4). Changes in HOMA-IR and M-values were associated with differences in the small intestine GU in the rosiglitazone group ($r = 0.56$, $P = 0.056$ and $r = 0.67$, $P = 0.017$; respectively). Insulin clearance increased after metformin and rosiglitazone interventions (from 1.02 ± 0.27 ml/min to 1.23 ± 0.25 and from 0.96 ± 0.32 to 1.19 ± 0.29 ; $P = 0.01$ and $P = 0.001$; respectively). EGP/kg increased only in the rosiglitazone group from 20.7 ± 11.9 $\mu\text{mol}/\text{min} \cdot \text{kg}$ to 27.6 ± 9.9 ($P = 0.012$). Baseline vs. intervention lactate levels elevated in the metformin group from 1.0 ± 0.2 to 1.18 ± 0.3 mmol/l ($P = 0.01$) but no correlations between EGP were found.

3.2. Intestinal effect of metformin in rats

Biodistributional data showed that the metformin treated group had significantly higher FDG uptake in the small intestine compared to the controls in the duodenum (1.8 ± 0.5 ID%/g vs. 1.1 ± 0.3 ID%/g, $P = 0.026$) and ileum (1.8 ± 0.4 ID%/g vs. 0.8 ± 0.2 ID%/g, $P = 0.002$), (Fig. 2 A) but no difference in the colon. Metformin treatment increased FGD uptake in the mucosal layer of the small intestine as determined by photostimulated luminescence ($P = 0.002$ between metformin

and control, Fig. 2B–E). In concert with this, imaging derived FDG uptake was higher in the small intestine of rats given the metformin intervention compared to controls (10.0%ID/g vs. 4.4, $P = 0.002$, Fig. 2F). There was also an increase in FDG uptake by the colon albeit from a lower baseline level ($P < 0.026$).

Radioactivity measurements of faecal samples showed a movement of FDG from the blood circulation to the intestinal lumen but no significant differences were seen in faecal activity between the metformin group and the control group (5.6 vs. 7.8 1 ID% $\text{g}^{-1} \cdot \text{Mm}$, $P = 0.096$).

4. Discussion

Our study highlights intestinal metabolic effects of metformin in patients with newly diagnosed type 2 diabetes. It shows that metformin treatment elevated GU several fold from the circulation into the enterocytes in the small intestine and the colon, indirectly suggesting the improved capacity of mucosal cells to function as an intestinal barrier. Although case reports on ‘intestinal hot spots’ in diagnostic patients on metformin are published [14,28], to the best of our knowledge the effects of metformin on intestinal metabolism have not been clarified in a prospective setting.

Compared to healthy subjects’ intestinal GU during euglycemic hyperinsulinemic clamp in our recent study [17], intestinal GU was lower in the diabetic patients involved in this trial and similar to the previously reported values in morbidly obese subjects [17]. The present study shows that 26 weeks of metformin intervention restored intestinal GU in human patients with newly diagnosed type 2 diabetes. In the experimental part, metformin treatment led to doubled intestinal GU in diabetic rats. This is in line with those of previous study of Mitheaux et al. [29], which showed that intestinal GU is almost totally suppressed in high fat fed rats and restored by metformin treatment.

Importantly, GU in the small intestine was inversely associated with fasting plasma glucose levels only in the metformin treated group (Fig. 4.) suggesting that intestinal uptake of glucose from the circulation is important for metformin action. Elevated lactate levels in metformin group implies indirectly increased anaerobic glucose metabolism in the intestine. While GU in the colon was associated with changes in M-values only in the metformin treated group

(Fig. 4.) metformin did not change whole-body or muscle insulin sensitivity in these newly diagnosed type 2 diabetic as shown previously [19]. The twofold increment in intestinal GU in the metformin group was not associated with improved insulin sensitivity in any other organs evaluated either [30].

Insulin clearance increased after metformin and rosiglitazone interventions, which might explain lower insulin levels during clamp and the lower EGP suppression. The data supports the finding that rosiglitazone had a greater effect than metformin on whole body glucose uptake. Despite the increment in postintervention lactatemia in metformin group no changes in the EGP/kg were found suggesting that the lactate does not affect EGP during hyperinsulinemia.

Our metformin data is in-line with several recent animal experiments. Previous studies in insulin resistant mice, fed a high-fat, low-carbohydrate diet, and treated with metformin increased the amount of GLUT2 transporter in apical enterocyte membranes in the small intestine [31]. Moreover, the same study found improved glucose homeostasis, while tripling glucose release into the intestinal lumen [31]. If glucose is actively transported into the enterocytes from the circulation, then the intestine can be assumed to act as a sink for the cleared glucose and thus plays a role in preventing hyperglycemia. Metformin intervention in our animal study increased mucosal GU but did not induce significant differences in luminal FDG concentration in the intervention groups. However, FDG transport from the circulation into the lumen did occur in both groups. Of note, unlike humans the GU uptake in the rats' colon was not increased after metformin intervention. The reason why metformin did not enhance GU in rat's colon remains obscure but might be related to small number of studied animals or *p.o* vs. *s.c* administration.

Peroxisome proliferator-activated receptor γ (PPAR γ) activators such as rosiglitazone regulate free fatty acid (FFA) metabolism, intestinal cell proliferation and gut homeostasis [32]. Rosiglitazone slightly but significantly enhanced intestinal GU in the small bowel and this change was positively correlated with the change in the M-value and HOMA-IR. Unexpectedly rosiglitazone treatment had no effect on fasting plasma glucose contrary to findings in more insulin resistant patients in our previous reports [33]. This was likely due to small sample size and moderate hyperglycemia in studied patients. Rosiglitazone increased hepatic and peripheral insulin sensitivity [34] and our study shows that intestinal insulin sensitivity was also improved. No correlation was found between hepatic and intestinal GU, which implies that the drug effect is gut-specific. We have previously reported data from the same study cohort whereby PPAR γ -antagonism increased insulin sensitivity by 25% in muscle, 38% in heart and 34% in liver [2,18]. Therefore, the 20% increment found here in the intestine is very well in line with improved intestinal insulin sensitivity.

Several studies have shown that gut microbiota is altered in obesity and type 2 diabetes [35]. Shin et al. showed in rodents that metformin treatment increased appearance of the Akkermansia genus in the gut flora and the levels of goblet cells in the mucosa and intraluminal mucin levels in line with improved glycemic tolerance [36]. However, this action had not been shown in humans. The FDG tracer was also found in the faecal samples of rats taken from different parts

of the small intestine and colon in our study, which raises the question as to whether FDG is transported into goblet cells and secreted by the mucin into the gut lumen.

There are some limitations in this study. First, we injected glucose tracer to circulation and measured intestinal (enterocyte) GU using FDG-PET during insulin stimulation. Therefore we were not able to assess effects of metformin on glucose absorption from the intestinal lumen. Our study was done during normoglycemia, but it is likely that the GU by intestinal mucosa depends on plasma glucose concentrations [16]. Fluctuations in circulating glucose levels are frequent throughout the day in patients with diabetes. If so, this mechanism could be important in attenuating hyperglycemic peak values. It is noteworthy that in clinical routine FDG-PET scanning, intestinal 'hot spots' are found in patients on metformin treatment after over-night fast [14], which suggests that glucose uptake from the circulation is also significant during fasting.

Second, the PET methodology used in the data collection of the present study failed to provide detailed information on the colon mucosa FDG uptake, due to the limited spatial resolution of PET imaging, thickness of the colon mucosa and leakage of FDG into the lumen. Therefore, it is not possible to differentiate luminal and mucosal FDG activity in the colon with dynamic PET imaging. When biodistribution of FDG in luminal content and intestinal segments was compared in the rat model, no difference was found between control and metformin treated animals. Third, excessive motion artefacts because of peristalsis, cardiovascular pulsation, and respiration elicits challenge when the scanning time is more than few second. ROIs were drawn to quite fixed locations in the small intestine and colon and the anatomical correspondence was confirmed from the PET image using renals, spine and surrounding muscles as landmarks. Fourth, patients were randomized for sex and smoking which led to difference in the baseline levels of fasting plasma glucose (8.0 mmol/L in metformin group vs. 7.2 mmol/L in the other two groups, NS). This might have influenced on drug-induced lowering of FPG in metformin group but not the GU during clamp studies.

In conclusion, this study showed different responses to rosiglitazone and metformin treatments in early stage type 2 diabetes. Metformin remarkably increased GU by the enterocytes without changing skeletal muscle or whole body insulin sensitivity. The increment of intestinal GU was smaller with PPAR γ -agonist and associated with changes in insulin sensitivity in other tissues. These data suggest that intestinal insulin resistance is an early feature of type 2 diabetes and can be relieved with metformin therapy.

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Conflict of interest

No potential conflicts of interest relevant to this article were reported.

Author contributions

J.K. contributed to the design of the study, acquired and researched data, and wrote the manuscript. K.M., A.S. and O.E. contributed to the design of the animal study and discussion, researched data, and edited the manuscript. K.H. contributes to skeletal muscle GU analysis and acquired data. K.V. contributed to the discussion of the manuscript and acquired data. P.I. contributed to HGU analysis and discussion/revision of the manuscript. L.G. calculated EGP and contributed to the discussion. A.A., A.D., M.H., S.E., S.K., M.G., R.P., T.P., J.V., J.S. offered technical support and contributed to the discussion. P.N. was the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of these data and the accuracy of the data analysis. All 19 authors approved the final version of the manuscript.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.diabres.2017.07.015>.

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