Towards novel strategies to improve lipid homeostasis
van der Wulp, Mariëtte Ymkje Maria

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Chapter 2

Laxative treatment with polyethylene glycol does not affect lipid absorption in rats

Mariëtte Y.M. van der Wulp 1,2, Frans J.C. Cuperus 2, Frans Stellaard 2, Theo H. van Dijk 2, Jan Dekker 1, Edmond H.H.M. Rings 1,2, Albert K. Groen 1,2 and Henkjan J. Verkade 1,2

1 Top Institute Food and Nutrition, Wageningen, Gelderland, The Netherlands
2 Pediatric Gastroenterology and Hepatology, Department of Pediatrics, Beatrix Children’s Hospital, Groningen University Institute for Drug Exploration, Center for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Center Groningen, Groningen, Groningen, The Netherlands

Abstract

Objectives Polyethylene glycol (PEG) is a frequently used laxative agent. It is unknown, however, if PEG affects the absorptive capacity of the intestine. Reduced lipid (dietary fat and cholesterol) absorption induced by long-term PEG treatment could negatively affect growth in children. We tested whether PEG accelerates the gastrointestinal transit and alters lipid absorption and plasma lipid levels.

Methods Wistar rats were administered drinking water with or without PEG (7%) for two weeks. We studied whole gut transit time by recording the first appearance of red feces after intragastric carmine red administration. We measured plasma concentrations of cholesterol and triglycerides, dietary fat absorption by 48h fat balance and by plasma appearance of intragastrically administered stable-isotope labeled fats, and cholesterol absorption with a dual stable isotope technique.

Results PEG decreased whole gut transit time by 20% (p = 0.028) without causing diarrhea. PEG treatment did neither affect overall dietary fat balance, nor fat uptake kinetics, cholesterol absorption or plasma lipid concentrations.

Conclusion PEG does not affect lipid absorption, nor steady state plasma lipid levels in rats, although it accelerates the gastrointestinal transit.
Introduction

Polyethylene glycol (PEG) is an inert polymer that originally was used to prepare bowels for endoscopy.\(^1\) In the 1990's PEG became available as osmotic laxative for the treatment of chronic constipation.\(^2\) Currently it is one of the most widely prescribed laxative agents. Long-term treatment with PEG seems safe and effective in adults\(^3\) as well as children.\(^4,5\) Recently it was stated that PEG shows better outcomes than lactulose in terms of stool frequency per week, stool form, relief of abdominal pain and the need for additional products.\(^6\) However, it is not known whether PEG treatment affects intestinal absorptive functions, for example by its effect on gastrointestinal transit.

Little attention has been given to the effect of PEG on gastrointestinal transit. Coremans et al. reported acceleration of oro-cecal transit during PEG treatment as determined by breath tests in healthy volunteers.\(^7\) In constipated children, both high-dose (1.5 g. kg\(^{-1}\). d\(^{-1}\)) and low dose (0.3 g. kg\(^{-1}\). d\(^{-1}\)) PEG were shown to accelerate total and segmental colonic transit after six and fourteen days of treatment, respectively.\(^8,9\) Only one study, performed in healthy adults, determined fecal excretion of nutrients during PEG ingestion.\(^10\) In that study, designed to study effects of diarrhea, PEG was shown to increase fecal loss of fat and carbohydrates.\(^10\) However, it has not been studied in detail in an appropriate animal model whether long-term, non-diarrhea inducing, PEG dosages affect intestinal lipid (dietary fat and cholesterol) absorption.

Several steps are required for adequate absorption of dietary lipids in the intestine, which in theory could be affected by acceleration of intestinal transit, particularly of the small intestine. Triglycerides, the main components of dietary fat, are partly lipolyzed in the stomach.\(^11\) Emulsification in the stomach further enhances lipolysis by pancreatic lipase in the intestinal lumen.\(^12\) Lipolytic products and cholesterol are solubilized by bile salts, providing efficient translocation into enterocytes.\(^13\)

The aim of this study was to test whether PEG, by acceleration of whole gut transit time (WGTT), alters intestinal lipid absorption.

Previous studies on acceleration of intestinal transit were mainly executed with metabolized drugs.\(^14\) Therefore, it is not clear whether the effects observed were directly related to acceleration of gastrointestinal transit. PEG however, is minimally absorbed and not metabolized in the intestine.\(^15\) We attempted to accelerate WGTT with PEG without causing side-effects such as diarrhea and dehydration, similar to its optimal clinical effect in patients.

We determined absorption and plasma levels of dietary fats and cholesterol. We studied fat absorption with two independent methodologies, namely fat balance and stable isotope techniques. The quantitative fat balance involves comparing the amount of fat intake and fecal output per time unit. The fat balance technique can not discriminate between potential causes of fat malabsorption (e.g. intraluminal versus intracellular processes).\(^16\) We performed a qualitative analysis of fat absorption\(^16,17\) by administering stable isotope labeled triglycerides and measuring plasma appearance of triglyceride-derived fatty acids. This technique allows to assess differences in the rate of fat digestion and absorption. We simultaneously studied the absorption of a stable isotope labeled free fatty acid (FFA) and triglyceride.
By comparison of the plasma appearance of the two labels, the intestinal fatty acid absorption *per se* can be discriminated from the process of digestion. 16 Cholesterol absorption was determined by a dual stable isotope test. 18

**Materials and Methods**

**Materials**

Colofort® (polyethylene glycol + electrolytes) was obtained from Ipsen Farmaceutica B.V. (Hoofddorp, The Netherlands). Colofort® contained per sachet (74 g): 64 g PEG, molecular weight 4 kDa, 5.7 g sodium sulphate (anhydric), 1.68 g sodium bicarbonate, 1.46 g sodium chloride and 0.75 g potassium chloride. Carmine was obtained from Macro-imPulse Saveur Ltd. (Stadtoldendorf, Germany). Intralipid® (20%) was obtained from Fresenius Kabi, Den Bosch, The Netherlands. 2,2,4,4,6-Deuterium-cholesterol (D₅-cholesterol) was obtained from Medical Isotopes and 25,26,26,26,27,27,27-Deuterium-cholesterol (D₇-cholesterol) from Cambridge Isotope Laboratories Inc. 1-¹³C-stearate and tri-1-¹³C-palmitate were obtained from Sigma Aldrich (St. Louis, MO). All isotopes were of 98-99% isotopic purity.

**Animals**

Growing male Wistar Unilever rats (150-174 g) were obtained from Harlan (Horst, The Netherlands). Rats were housed individually in an environmentally controlled facility with diurnal (12/12h) light cycle. Food and water were available *ad libitum* during the entire study period. The experiments were performed in conformity with Public Health Service policy and in accordance with the national laws. The Ethics Committee for Animal Experiments of the University Medical Center of Groningen approved the experimental protocols.

**Cholesterol absorption study**

Rats were maintained on semisynthetic purified diet (supplementary table 1, code 4063.02, obtained from Arie Blok, Woerden, The Netherlands). Upon arrival rats were randomly given a number from 1 to 14. After a three week run-in period on semisynthetic diet, baseline parameters of all rats were obtained, including WGTT. Subsequently, rats with an even number received PEG treatment (n= 7), whereas rats with an uneven number were assigned to control group (no treatment; n= 7) for a total period of 16 days. PEG was continuously available to the rats in their drinking water at a concentration (71g.l⁻¹ PEG4000) previously found to significantly accelerate intestinal transit. 19 Steady state conditions are expected to be reached within five days of treatment. 15 Feces were collected during a period of 48h before the start of treatment and again after one week of PEG treatment. WGTT was measured after intragastric administration of carmine red inert dye 20 (1 ml 60 mg.ml⁻¹ drinking water 19) under brief isoflurane/ oxygen inhalation anesthesia (for handling) one day before, and during PEG treatment at day 14 at 9 A.M. during the light phase. WGTT was defined as the time to appearance of the first red feces after administration of carmine.
Food and fluids were available to the rats during the entire experiment. Food and fluid intake as well as body weight (BW) were measured daily.

**Plasma dual isotope ratio method**

At day 10 after the start of PEG treatment, rats received an intravenous injection (tail) of 1.5 mg D<sub>7</sub>-cholesterol dissolved in 500 μl intralipid and an oral dose of 3 mg D<sub>5</sub>-cholesterol dissolved in 1 ml medium chain triglyceride (MCT) oil. At time points 0, 3, 6, 9, 12, 24, 48, 72, 96, 120, 144 and 168h after administration, blood samples were obtained from the tail vein under isoflurane anesthesia. Blood was collected in sodium-heparinized micro-hematocrit tubes (75 μl). Plasma was separated by centrifugation (10 min, 2000 rpm, 4°C) and stored at -20°C until analyses. Feces were collected from day three until day six after label administration.

At day 17, rats were anesthetized one by one by intraperitoneal injection of a mixture of Hypnorm (fentanyl/ fluanisone 1 ml.kg<sup>-1</sup>) and diazepam (10 mg.kg<sup>-1</sup>). The common bile duct was cannulated for bile collection. To ensure that hepatic production was accurately measured, bile produced during the initial 5 min after cannulation was discarded, and bile was sampled for 30 min thereafter. During the bile collection period, body temperature was maintained by keeping animals in a humidified incubator. Blood was obtained by cardiac puncture and divided over lithium-heparin (for determination of renal parameters) and EDTA-containing tubes. Rats were terminated by cervical dislocation.

**Analytical procedures and calculations**

**Plasma parameters**

Plasma cholesterol and triglyceride concentrations were determined using commercially available kits (Roche Diagnostics, Mannheim, Germany). Renal functions (sodium, potassium, urea and creatinin) were determined in plasma by routine spectrophotometry on a P800 unit of a modular analytics serum work area from Roche Diagnostics Ltd. (Basel, Switzerland).

**Feces**

Fecal calcium and phosphate output were measured as described previously. We measured calcium concentration in 1.5 g aliquots of freeze-dried feces, and phosphate concentration in 1.0 g aliquots.

**Fat balance**

Fatty acid concentrations were determined in 50 mg aliquots of feces and of crushed food. Fatty acid methyl ester derivatives were measured by gas-chromatography (GC) on an HP-Ultra-1 column from Hewlett-Packard (Palo-Alto, CA), using 100 μl heptadecanoic acid (C17:0, 50 mg.100 ml<sup>-1</sup>) as internal standard. Fat absorption was calculated for individual and total fatty acids as (intake - output)/ intake *100%.
Chapter 2

**Cholesterol absorption**

Cholesterol was extracted from 10 μl plasma and isotope enrichments of sterols were determined in the cholesterol fraction by gas chromatography-mass spectrometry (GC-MS). Total fecal neutral sterols (cholesterol plus bacterial metabolites) were separately determined by GC. Biliary lipids were extracted according to the method of Bligh and Dyer. Total plasma and biliary cholesterol concentrations were determined as described previously. Fractional cholesterol absorption was determined as previously described by Van der Veen et al., modified for the influx of labeled cholesterol.

**Fat absorption kinetic study**

Theoretically, the composition of the diet could affect results. To evaluate this, we also performed experiments with rats fed a different diet, containing for example less sugars and fiber (supplementary table 1, AIN-93G, Research Diet Services BV, Wijk bij Duurstede, The Netherlands). Rats served as their own controls. We analyzed the effect of diet by repeating key measurements in rats before or during PEG treatment. Body weight, fluid and food intake were determined daily. Feces (48h) and food were collected. We administered stable isotope labeled saturated fat, in the form of FFA 1-13C-stearate and triglyceride tri-1-13C-palmitate.

We administered, through oral gavage, a bolus of 500 μl oil (olive oil: MCT oil 1:3) per 300 g BW, which represents 30% of daily fat intake. This bolus contained 10 mg of each labeled fat. Our rats consumed a diet containing 7.2% fat. Daily average food intake was 23 g, containing 1.7 g of fat. The daily consumed stearate and palmitate from food pellets was 4.6 mg (2.8 g. kg\(^{-1}\) in food) and 12.8 mg (7.7 g. kg\(^{-1}\) food) of stearate and palmitate, respectively. At time points 0, 2, 4, 6, 8 and 10 h after administration blood was drawn from the tail to determine label appearance. Baseline WGTT was determined two days later. One day after WGTT measurement, PEG treatment was started as described above and continued until termination. One week after the start of PEG treatment, all above measurements were repeated.

**Analytical procedures**

Plasma lipids were hydrolyzed with hydrogen chloride in acetonitrile. α-bromopentafluorotoluene derivatives of fatty acids were extracted with hexane and analyzed by GC-MS. Enrichment was defined as the increase in M\(_1\)/M\(_0\) fatty acid relative to baseline measurements. The ratio of plasma labeled fatty acid and administered label (μmol) was calculated to obtain the % enrichment of label in plasma. Palmitate % enrichment was corrected for molecular weight since we administered triglycerides, which are detected in plasma as FFA.

**Statistical Analyses**

Normal distribution was examined by normal probability plots and Shapiro-Wilk tests. Depending on normality of data, differences between two groups were determined by either Mann Whitney U or Student’s t-test. The effect of PEG on WGTT was evaluated by a one way ANOVA repeated measurements test, followed by post-hoc Wilcoxon Signed Rank tests to test within groups.
Results are presented as means ± SD. The level of significance for all statistical analyses was set at \( p < 0.05 \). Analyses were performed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL).

Results

Animal characteristics

PEG-treated rats ingested similar amounts of food and water and showed similar body weights and growth as control rats during the entire study period (table 1). PEG treatment did not affect biochemical plasma parameters of renal function. PEG increased fecal output (dry weight, +42%; \( p= 0.001 \)). No overt diarrhea was noticed during PEG treatment (fecal pellets were formed). Because increased intestinal calcium and phosphate availability can decrease fat absorption, we measured fecal concentrations and calculated their fecal excretion. Both calcium and phosphate excretion were decreased in PEG-treated vs. control rats (calcium -13% on average; \( p= 0.028 \); phosphate -19%; \( p= 0.022 \)).

### Table 1: Animal characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PEG treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>398 ± 32</td>
<td>391 ± 44</td>
</tr>
<tr>
<td>Growth (g)</td>
<td>31 ± 4</td>
<td>26 ± 16</td>
</tr>
<tr>
<td><strong>Intake and output</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Food intake (g·kg⁻¹·day⁻¹)</td>
<td>58.7 ± 2.3</td>
<td>58.4 ± 4.0</td>
</tr>
<tr>
<td>Fluid intake (g·kg⁻¹·day⁻¹)</td>
<td>66.9 ± 13.1</td>
<td>79.2 ± 12.0</td>
</tr>
<tr>
<td>PEG intake (g·kg⁻¹·day⁻¹)</td>
<td></td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td>Fecal dry weight (g·kg⁻¹·day⁻¹)</td>
<td>8.0 ± 0.8</td>
<td>11.4 ± 1.1*</td>
</tr>
<tr>
<td>Fecal calcium (mmol·kg⁻¹·day⁻¹)</td>
<td>4.8 ± 0.5</td>
<td>4.2 ± 0.4*</td>
</tr>
<tr>
<td>Fecal phosphate (mmol·kg⁻¹·day⁻¹)</td>
<td>3.8 ± 0.5</td>
<td>3.1 ± 0.5*</td>
</tr>
<tr>
<td><strong>Plasma parameters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.3 ± 0.5</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.0 ± 0.4</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>Creatinin (µmol/L)</td>
<td>21.7 ± 2.3</td>
<td>20.8 ± 2.4</td>
</tr>
<tr>
<td>Ureum (mmol/L)</td>
<td>8.5 ± 1.3</td>
<td>8.9 ± 1.0</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>142.2 ± 2.1</td>
<td>140.1 ± 4.7</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>5.4 ± 0.4</td>
<td>5.3 ± 0.9</td>
</tr>
</tbody>
</table>

Body weight, growth, average daily intake and output, and plasma parameters in control rats and rats treated with 7% PEG in drinking water. Data are represented as mean ± SD, \( n=7 \) per group. Data of PEG treated rats were compared with those of control rats by unpaired two-sided Students’ t-tests. *\( p< 0.05 \)
PEG accelerates WGTT

PEG treatment decreased WGTT by 20% (from 10.1 ± 2.2h to 8.1 ± 2.7h; p= 0.028). Control rats showed similar WGTT in both episodes (11.2 ± 2.3h and 11.3 ± 3.3h, figure 1).

![Whole gut transit time](image1)

Figure 1. Effect of PEG on Whole gut transit time. WGTT was measured in rats fed a semisynthetic diet for 3 weeks. First a baseline measurement (0) was performed in all rats. Subsequently rats were randomly appointed to either control (white dots) or PEG treated (black dots) group and WGTT was measured in both groups (1). To test for differences in WGTT depending on PEG treatment, first a one way ANOVA repeated measurements test was executed. The error variance of the dependent variable (WGTT) was equal across groups (Levene’s test), indicating that it was correct to use this analysis. The repeated measurements test showed significant interaction (group*timepoint) (p<0.05). We used post-hoc Wilcoxon Signed rank tests to compare control WGTT at timepoints 0 and 1 (NS) and PEG treated WGTT (timepoint 1) compared to baseline WGTT in the same animals (timepoint 0; p=0.028). Data are represented as mean ± SD, n=7 per group. *P<0.05

Overall dietary fat balance is not affected by PEG treatment

We studied dietary fat absorption by subtracting fecal fat output from fat intake. Detailed analysis of fat absorption using GC demonstrated that the absorption of unsaturated fatty acids (oleic, linoleic and α-linolenic acid) was almost complete in control and PEG-treated animals (~93-100%). As described previously 27, control rats showed relative malabsorption of the long chain saturated fatty acids palmitic (~89%) and stearic acid (~66%) (figure 2).

PEG-treated animals absorbed significantly more saturated fatty acids than controls (palmitic acid 94 ± 2% vs. 90 ± 2%; p= 0.001; and stearic acid 76 ± 8 vs. 66 ± 7%; p= 0.03, respectively.) However, the absorption of unsaturated fatty acids did not differ between both groups.

![Fat balance of control and PEG treated rats](image2)

Figure 2. 48h Fat balance of control (white bars) and PEG treated rats (black bars). Molar absorption percentage of total and separate dietary fatty acids. Major dietary fatty acids: palmitic acid (C16:0), linoleic acid (C18:2n6) and oleic acid (C18:1n9). Minor dietary fatty acids: stearic acid (C18:0) and α-linolenic acid (C18:3n3). Data are presented as mean ± SD, n=7 per group. Data of PEG treated rats were compared with those of control rats by unpaired two-sided Students’t-tests. *P<0.05, **p<0.01
The overall percentage of lipid absorption did not differ between groups (96 ± 1 vs. 95 ± 2% in PEG-treated vs. control animals), attributable to the minor relative contribution of the long-chain saturated fatty acids to dietary fat.

In a separate experiment, we analyzed the effect of diet by repeating key measurements in rats on a different semisynthetic diet (AIN-93G), again with or without PEG treatment. All relevant effects of PEG treatment were virtually identical, including acceleration of WGTT by 27% and unchanged fat balance (data not shown). This indicated that the PEG effect does not (strongly) depend on the type of diet.

Figure 3. Fat uptake kinetics at baseline (white dots) and during PEG treatment (black dots). Panel A: Palmitate originating from tripalmitate was adequately absorbed during PEG-treatment, suggesting preserved digestion and absorption of saturated fat. Panel B: Absorption of free stearate was likewise preserved during PEG-treatment (Area Under Curve). Plasma kinetics were studied during ten hours after administration of stable isotope labeled stearate and tripalmitate by oral gavage. Rats were used as their own control. Data are presented as mean + SD, n= 7 per group. *P(Wilcoxon Signed Rank test) <0.05
PEG treatment does not affect the rate of fat digestion and absorption

**Plasma $^{13}$C-fatty acid concentrations**

Fat balance provides a quantitative analysis of fat absorption. It can not discriminate between potential causes of differences, such as intraluminal factors (lipolysis of triglycerides, solubilization) or intracellular factors (chylomicron formation). We used a different approach than fat balance to separately assess whether PEG affected the rate of triglyceride lipolysis or the absorption of FFA. We administered stable isotope labeled triglyceride (tri-$^{1-13}$C-palmitate) and FFA ($^{1}$-$^{13}$C-stearate) intragastrically and determined plasma appearance of $^{1-13}$C-palmitate and $^{1}$-$^{13}$C-stearate, respectively (figure 3). Plasma $^{1-13}$C-stearate was higher 2h after administration during PEG treatment ($p=0.028$). A similar (non-significant) effect was observed for $^{1-13}$C-palmitate ($p=0.063$).

Maximum enrichment values of $^{1-13}$C-palmitate were reached after 2h in PEG-treated vs. 6h at baseline conditions. Maximum values of $^{1-13}$C-stearate were reached after 6h in both conditions. The area under the curves for both $^{1-13}$C-stearate and $^{1-13}$C-palmitate ($0.81 \pm 0.16$ vs. $0.64 \pm 0.11$ and $0.91 \pm 0.11$ vs. $0.78 \pm 0.08$ % of administered dose.ml plasma$^{-1}$ under PEG treatment versus baseline conditions respectively (not shown) did not differ, indicating that PEG did neither affect lipolysis nor FFA absorption.

PEG treatment does not affect cholesterol absorption

Similarly to the absorption of long-chain saturated fatty acids, cholesterol absorption is strongly bile salt dependent. We studied cholesterol absorption by means of a dual stable isotope technique. With this technique we determine levels of stable isotope labeled cholesterols in plasma after prior i.v. and oral administration of differentially labeled cholesterols. This well-known methodology allows us to calculate the fractional absorption of cholesterol from the intestine. Similarly to overall fat absorption, fractional cholesterol absorption did not differ between PEG-treated and control rats (~54 and ~58%, respectively, figure 4). PEG neither affected biliary cholesterol secretion, nor fecal sterol excretion (data not shown).

![Figure 4. Fractional cholesterol absorption in control rats (white bars) and in rats treated with PEG in drinking water (black bars). Absorption kinetics were calculated from labeled cholesterol in plasma collected after administration of i.v. D$_{7}$- and intragastric D$_{5}$-cholesterol. Data are presented as mean + SD. Data were analyzed by unpaired two-sided Student’s t-test, n=7 per group.](image-url)
Discussion

In the present study we determined in a rat model whether a frequently applied laxative, PEG, influences intestinal lipid absorption via acceleration of the WGTT. Our data convincingly show that PEG treatment does accelerate WGTT, but that it neither affects absorption of triglycerides, fatty acids or cholesterol, nor plasma lipid levels.

We used PEG with added electrolytes because of its broad applicability with no net exchange of electrolytes in the intestine. We considered this as a safe method during continuous administration of PEG in drinking water. To our knowledge, there are no studies that directly compare effects of PEG with or without added electrolytes in children. However, clinical experience and experimental data of children taking PEG with or without added electrolytes show that both methods are highly effective and safe. 29,30 PEG increased dry fecal output, which is mostly, if not entirely, attributable to appearance of PEG itself in feces. 15 Unexpectedly, we found decreased fecal calcium and phosphate excretion. We cannot exclude loss of these water soluble ions with fecal water in the bedding of PEG-treated rats. Although probably underestimated due to evaporation and loss in bedding, we found increased water content in PEG feces (up to 3-fold, data not shown) as determined by weight of fresh feces and that after freeze-drying. We did not use metabolic cages to collect feces for our study, since the extra stress would alter WGTT 31 and influence the effect of PEG. In addition, rats are able to consume feces directly from their anus, which cannot be prevented in metabolic cages. It is known that coprophagia could theoretically influence the absolute amounts of fat absorption. However, we do not have any indication that PEG treatment selectively affects coprophagia and thereby relative fat absorption. We analyzed the effects of PEG on dietary fat absorption with different, independent methodologies. 16,17 First, we studied fat balance comparing the amounts of fat ingested over 48h via the food and the amount excreted via feces. 16 PEG treatment did not affect the overall dietary fat absorption. PEG treatment did slightly increase saturated fatty acid absorption, compared with control rats. This could be due to increased intestinal bile salt availability as indicated by decreased fecal bile salt excretion during PEG treatment. 19 Fat balance cannot discriminate between potential causes of differences in fat absorption, such as intraluminal factors and intracellular factors. To further investigate fat uptake, we studied plasma appearance of $^{13}$C-labeled fats, derived from intragastrically administered $^{13}$C-labeled FFA or triglycerides. 16 We found absorption kinetics to be comparable in PEG-treated versus baseline conditions. The bolus of stable isotope labeled fat we administered contained 100-200% of the daily consumed saturated fats by diet. Apparently, the difference in absorption during PEG treatment of specifically the saturated fats (fat balance), when intake of those fats is minor, is abolished when we administer a single high dose. Together, these results indicate that there are no relevant changes in fat absorption during PEG treatment.

We cannot completely exclude that the amount of dietary fat may influence extrapolation of our results towards the human situation, where a Western high-fat diet is often consumed. However, only one study by Hammer et al. 10 in humans showed malabsorption of nutrients during PEG intake.
This particular study was designed to study effects of osmotic diarrhea induced by high dose PEG. PEG is nowadays most frequently used in lower dosages in humans to offer a long-term laxative treatment by softening of stools and not to induce diarrhea. We mimicked this scenario in our study and did not observe fat malabsorption.

We did not specifically measure small intestinal transit in this study, since it requires termination of the rats. However, we previously showed that PEG induces a significant acceleration of small intestinal transit in rats (+17%), comparable to the acceleration in total WGTT we found. 19

Whereas fat absorption percentage in healthy states is nearly complete 27, cholesterol absorption is substantially lower. At least in part depending on the method applied 32, cholesterol absorption percentage has been estimated between 30-70% in humans 33, in rodents 18,34,35 and in our present study in rats. The substantially lower basal cholesterol absorption may provide increased sensitivity to detect differences in cholesterol absorption as compared to fat absorption during an intervention. Our finding that not only fat but also cholesterol absorption was not changed during PEG treatment supports the concept that PEG treatment does not affect the absorptive capacity of the small intestine.

Together our data indicate that PEG treatment does not lead to relevant changes in lipid absorption or plasma lipid levels in rats. These data suggest that in terms of lipid absorption, PEG can be safely administered to children during growth and development.

Acknowledgements

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Conflicts of interest and source of funding

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References


Supplementary table 1. Composition of semisynthetic diets

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<th>4063.02</th>
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<tr>
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<tr>
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<td>7.2</td>
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<td>g kg⁻¹</td>
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<td>Casein</td>
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<td>200</td>
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<tr>
<td>Corn-starch</td>
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<tr>
<td>Maltodextrin</td>
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<td>Sucrose</td>
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<tr>
<td>Phosphate</td>
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</table>

During the cholesterol absorption study, rats were maintained on semisynthetic purified diet code 4063.02. The fat absorption experiment was performed with rats fed AIN-93G diet.