

RESEARCH ARTICLE

## Novel effects of the gastrointestinal hormone secretin on cardiac metabolism and renal function

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### Abstract

The cardiac benefits of gastrointestinal hormones have been of interest in recent years. The aim of this study was to explore the myocardial and renal effects of the gastrointestinal hormone secretin in the GUTBAT trial (NCT03290846). A placebo-controlled crossover study was conducted on 15 healthy males in fasting conditions, where subjects were blinded to the intervention. Myocardial glucose uptake was measured with [<sup>18</sup>F]2-fluoro-2-deoxy-D-glucose ([<sup>18</sup>F]FDG) positron emission tomography. Kidney function was measured with [<sup>18</sup>F]FDG renal clearance and estimated glomerular filtration rate (eGFR). Secretin increased myocardial glucose uptake compared with placebo (secretin vs. placebo, means  $\pm$  SD,  $15.5 \pm 7.4$  vs.  $9.7 \pm 4.9$   $\mu\text{mol}/100$  g/min, 95% confidence interval (CI) [2.2, 9.4],  $P = 0.004$ ). Secretin also increased [<sup>18</sup>F]FDG renal clearance ( $44.5 \pm 5.4$  vs.  $39.5 \pm 8.5$  mL/min, 95%CI [1.9, 8.1],  $P = 0.004$ ), and eGFR was significantly increased from baseline after secretin, compared with placebo ( $17.8 \pm 9.8$  vs.  $6.0 \pm 5.2$   $\Delta\text{mL}/\text{min}/1.73$  m<sup>2</sup>, 95%CI [6.0, 17.6],  $P = 0.001$ ). Our results implicate that secretin increases heart work and renal filtration, making it an interesting drug candidate for future studies in heart and kidney failure.

**NEW & NOTEWORTHY** Secretin increases myocardial glucose uptake compared with placebo, supporting a previously proposed inotropic effect. Secretin also increased renal filtration rate.

*gastrointestinal hormone; kidney function; myocardial metabolism; secretin*

### INTRODUCTION

The cardiac benefits of gastrointestinal (GI) peptides have been of great interest in recent years. For the first time in the history of diabetes treatment, type 2 diabetes (T2D) medications have shown benefits in cardiovascular mortality (1). One such drug class is glucagon-like peptide-1 (GLP-1) analogs, but their precise mechanisms for cardiac benefits are still being uncovered (2). Secretin, which belongs in the same family of GI peptides as GLP-1, is the first hormone discovered and with it came the concept of endocrine regulation in the 1920s (3). It is secreted during feeding and its best-established effect is induction of pancreatic exocrine secretion (4). We recently showed that secretin is not only a digestive hormone but also controls appetite and activates meal-associated brown adipose tissue thermogenesis (5, 6).

Evidently, secretin has pleiotropic effects, as human secretin receptors are present in multiple organs and tissues, including the heart and kidney (7).

In the early 1980s, intravenous secretin infusion was shown to increase cardiac output and stroke volume in patients with heart failure (8) and patients with angina, but normal ventricular function (9). Since systemic resistance also fell, the secretin-induced effect was proposed to be mainly through a decrease in afterload. However, due to the substantial 20% increase in cardiac output, the authors speculated that there might also be an increase in myocardial contraction. Since then animal studies have provided support for an inotropic effect (10, 11), but it could not be proven in humans with the previously implemented method. Furthermore, several studies have also indicated that secretin has an effect on fluid homeostasis (12, 13), both centrally



and through aquaporin channels in the kidneys, independent of vasopressin (14). It also increases renal blood flow (15). In earlier clinical studies, secretin was shown to have diuretic effects in humans (16–18).

Secretin's cardiorenal effects have not been studied in humans with current, state-of-the-art methods. We recently reported the prespecified endpoints of the GUTBAT clinical trial, a randomized placebo-controlled crossover trial examining the effect of secretin on brown adipose tissue and appetite (5). In the present exploratory study, we aimed to investigate the cardiorenal effects of secretin in the participants of the GUTBAT trial (Clinical trial No.: NCT03290846).

## MATERIALS AND METHODS

### Study Subjects

Subjects ( $n = 16$ ) who filled the inclusion criteria [body mass index (BMI) 20–26 kg/m<sup>2</sup>, male, age 18–65 yr, no chronic disease that would affect the outcome] and who presented healthy at the screening visit [according to their medical history, as well as when assessed by their cardiovascular status, standard 2-h oral glucose tolerance test (OGTT), routine laboratory tests, electrocardiogram (ECG), and blood pressure] were enrolled in the PET/computed tomography (CT) study. After 1 subject dropped out before the completion of the study, 15 subjects were included in the analysis [means  $\pm$  standard deviation (SD) age 40.9  $\pm$  12.5 yr, median BMI 24.0  $\pm$  1.9 kg/m<sup>2</sup>]. Written informed consent was provided by all subjects. All participants were recruited between the years 2016 and 2017. The trial ended when all preplanned studies were completed (Clinical Trials Number: NCT03290846). No important harms or unintended effects were observed. The study protocol was reviewed and approved by the Ethics Committee of the Hospital District of Southwest Finland before starting the study. The study was performed according to the principles of the Declaration of Helsinki.

### Study Design

The study was conducted at Turku PET Center and consisted of two separate [<sup>18</sup>F]2-fluoro-2-deoxy-D-glucose ([<sup>18</sup>F]FDG) PET scan days after  $\geq 12$  h of fasting. The scans were conducted within the interval of 2–28 days of each other (Fig. 1). Subjects were blinded to the intervention and randomized to receive placebo (saline) and secretin (secretin pentahydrochloride 1 IU/kg  $\times$  2) twice intravenously as a 2-min infusion on different days. Whole body energy expenditure (EE) was assessed with indirect calorimeter (Deltatrac II, Datex-Ohmeda) during the PET scans (19). Conventional 12-lead ECG was recorded at baseline, at 1 h, and at 2 h. Repeated arterialized venous samples were collected from the antecubital vein during the scanning days.

### Scanning Protocol

[<sup>18</sup>F]FDG was produced at the Turku PET Center, as described previously (20). The two PET/CT scans (GE Discovery ST System, General Electric Systems, Milwaukee, MI) were conducted according to identical scan acquisition protocols. A 2-min intravenous infusion of saline or secretin was given 20 min before the administration of 150 MBq of [<sup>18</sup>F]FDG. Subsequent to the radiotracer injection, a second 2-

min infusion of placebo or secretin was initiated. A dynamic PET scan was started simultaneously and acquired on the neck region for 40 min [results have been reported previously (5)] and the thoracic region for 15 min (frames: 5  $\times$  3 min) (Fig. 1). A low-dose CT was conducted before each dynamic PET scan for attenuation correction and anatomical localization.

### Image analysis.

Image analysis was conducted with Carimas 2.8 software (Turku PET Center, Turku, Finland). An automated cardiac analysis tool was used for myocardial time-activity curves (TAC). Regional TAC data were then analyzed, taking into account radioactivity in arterialized plasma, by using fractional uptake rate (FUR) (21). Arterialized plasma radioactivity was assessed with an automatic gamma counter (Wizard 1480, Wallac, Turku, Finland). Myocardial glucose uptake ( $\mu$ mol/100 g/min) was calculated by multiplying FUR with plasma glucose levels and dividing by tissue density of 1.0298 g/mL (22) and a lumped constant value of 1 (23). Brown adipose tissue (BAT) and skeletal muscle glucose uptake was calculated as previously reported (5). Glucose uptake rates of these tissues are also expressed in  $\mu$ mol/min, by multiplying for organ mass. For the heart, the reference value of adult males (332 g) was used (22). BAT mass was measured as previously described (24) and skeletal muscle mass was estimated from age, height, weight, and waist circumference as previously described (25).

### ECG assessments.

Twelve-lead ECGs were recorded before scanning, as well as 60 and 120 min after the first secretin or placebo dose (Fig. 1). ECG was recorded using GE Medical Systems MAC 5000 resting ECG analysis system. Heart rate, PR-, QRS, and QT intervals were automatically measured and assessed by a qualified cardiologist. Heart rate corrected QT interval (QTc) was calculated with Bazett's formula (26).

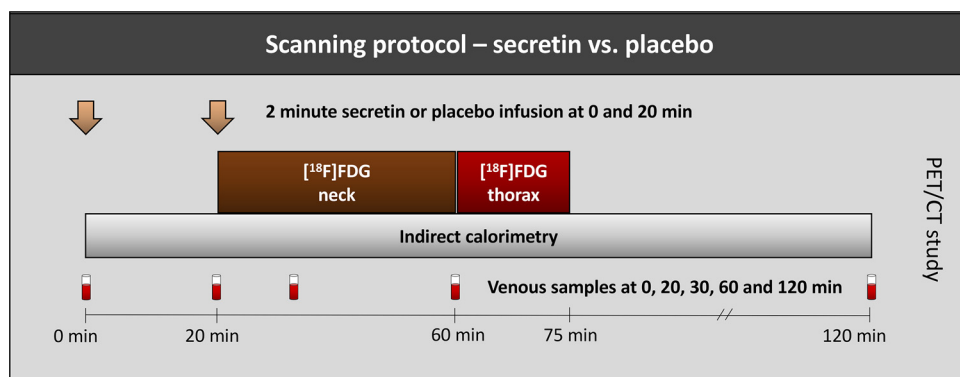
### Indirect calorimetry analysis.

Analysis was started 10 min after the first secretin dose. Whole body energy expenditure (EE) and the rate at which carbohydrates (CHO) and lipids (FO) were oxidized for EE were calculated with Matlab (Version: R2011a), using the Weir equation (27) and the manufacturer's equations (28). Whole body energy expenditure, carbohydrate oxidation, and fat oxidation by indirect calorimetry measurements are expressed in kcal/day. Protein metabolism was calculated by assuming urine nitrogen as 13 g/24 h (29).

### Renal Function Measurements

Serum samples of the first  $n = 10$  scanned subjects were analyzed for a metabolic panel, including creatinine, the Nightingale Health laboratory (Helsinki, Finland), with nuclear magnetic resonance (NMR) spectroscopy (30). Glomerular filtration rate was measured with Cockcroft–Gault equation (31). All subjects voided before start of scan and after the scan. Times were recorded and urine volumes measured. Subjects received a slow saline infusion (NaCl 0.9) during the scan for sampling purposes and infusion volumes were not controlled. Urine radioactivity was assessed with an automatic gamma counter at the end of the scan (Wizard 1480, Wallac, Turku, Finland).

**Figure 1.** Overview of the scanning protocol. Two PET/CT scans were conducted in fasting conditions, in a single-blinded and randomized order, with a placebo (saline) and secretin (secretin pentahydrochloride 1 IU/kg \*2) intervention. Arrows indicate the timing of intravenous secretin/placebo infusions. [ $^{18}\text{F}$ ]FDG (150 MBq) was injected at 20 min, after which PET scanning of the neck, and then the thoracic region, was initiated. Indirect calorimetry was conducted for 2 h. Timing of arterialized samples is indicated with test tube figures. Twelve-lead ECG was collected at timepoints 0, 60 and 120 min. CT, computed tomography; [ $^{18}\text{F}$ ]FDG, [ $^{18}\text{F}$ ]2-fluoro-2-deoxy-D-glucose.



[ $^{18}\text{F}$ ]FDG renal clearance rate ( $n = 15$ ) was calculated as previously described, with urine activity ( $\text{FDG}_{\text{urine}}$ ) divided by area under the curve (AUC) arterialized plasma radioactivity from beginning of the scan to the end (32).

$$\text{Renal Clearance}_{\text{FDG}} = \frac{\text{FDG}_{\text{urine}}}{\text{AUC}_{0 \rightarrow \text{sampling time}}}$$

### Rate of Disappearance of [ $^{18}\text{F}$ ]FDG

The effect of secretin on whole body glucose metabolism was studied using the rate of disappearance (Rd) of glucose under placebo and secretin infusion. Rd is calculated as follows:

$$\text{Rd}_{\text{glucose}} = \frac{\text{FDG}_{\text{dose}} - \text{FDG}_{\text{urine}}}{\text{AUC}_{0 \rightarrow \text{sampling time}}} \times \text{glucose}_{0 \rightarrow \text{end of sampling}},$$

where  $\text{FDG}_{\text{dose}}$  is the injected [ $^{18}\text{F}$ ]FDG dose,  $\text{FDG}_{\text{urine}}$  is the decay-corrected quantity of [ $^{18}\text{F}$ ]FDG measured in the total volume of urine from [ $^{18}\text{F}$ ]FDG injection to the end of the PET scan, AUC is the area under the curve of [ $^{18}\text{F}$ ]FDG in plasma from [ $^{18}\text{F}$ ]FDG injection to the end of sampling, and glucose is the average glycemia from [ $^{18}\text{F}$ ]FDG injection to the end of sampling (33, 34).

### Statistical Analysis

Sample size calculations for the primary endpoint have been previously reported (5). Data are reported as means  $\pm$  standard deviation (SD). Statistical analysis was performed with IBM SPSS Statistics (version 27). The prespecified primary and secondary endpoints of the GUTBAT Trial have been reported previously and results reported here are exploratory (5). Myocardial glucose uptake is expressed as  $\mu\text{mol}/100 \text{ g}/\text{min}$ . Whole body carbohydrate oxidation by indirect calorimetry is expressed in kcal/day. Student's paired  $t$  test was used to compare PET/CT data. Correlation was analyzed with Pearson's correlation, unless otherwise stated. For serum creatinine analysis, R-studio was used for repeated-measures ANOVA.  $P$  values of  $<0.05$  were considered as statistically significant. Randomized allocation sequences for the order of placebo and secretin interventions were generated with the randomized blocks method, with block size of 6, using SAS (v. 9.4 for Windows). The allocation sequence was generated by the Turku University statistics department that was not otherwise involved in the study. Participants were assigned to the sequence in order of

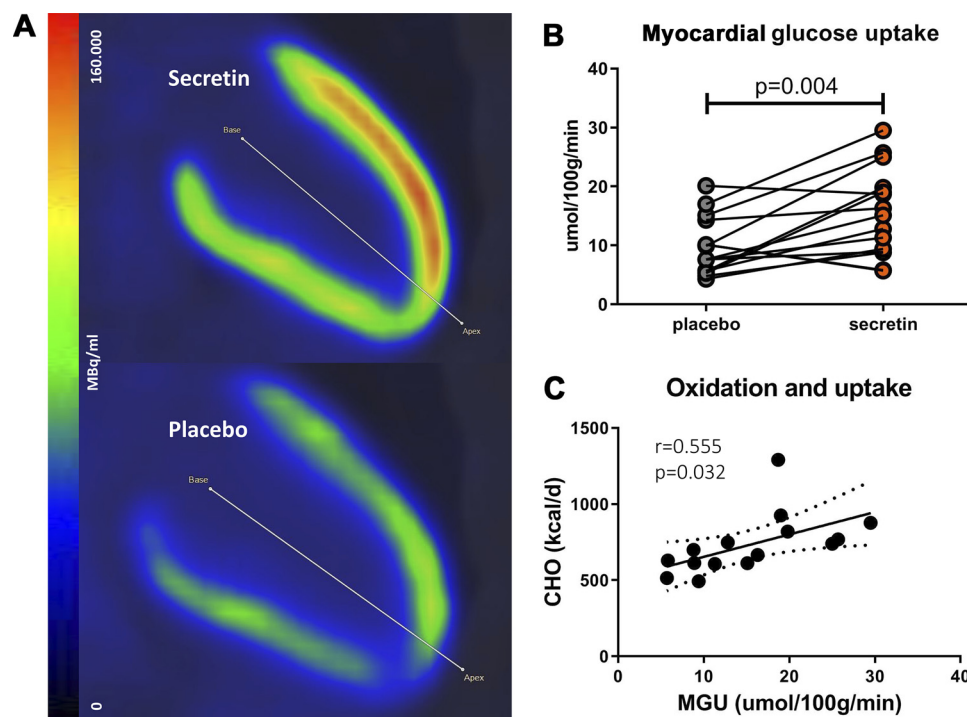
enrollment, by study personnel enrolling participants into the study.

## RESULTS

Myocardial glucose uptake (GU) was significantly higher after secretin compared with placebo ( $15.5 \pm 7.4$  vs.  $9.7 \pm 4.9 \mu\text{mol}/100 \text{ g}/\text{min}$ ,  $P = 0.004$ ; Fig. 2, A and B). Secretin-induced myocardial GU was not associated with previously reported insulin levels (5) (Spearman correlation between myocardial GU and plasma insulin at 0 min:  $r = 0.527$ ,  $P = 0.123$ ; 20 min:  $r = 0.275$ ,  $P = 0.441$ ; 60 min:  $r = 0.092$ ,  $P = 0.800$ ; 120 min:  $r = 0.080$ ,  $P = 0.827$ ). Previously reported serum insulin, glucose, and free fatty acid levels are shown in Supplemental Table S1 (see <https://doi.org/10.6084/m9.figshare.16912816>) (5). There was no significant difference in heart rate between the interventions at 1 h [ $57 \pm 8$  beats/min (bpm) vs.  $57 \pm 8$  bpm,  $P = 0.92$ ], which suggests that secretin does not have a chronotropic effect. This is supported by previous studies (8, 9). Interestingly, QTc was shortened after the two secretin infusions at 1 h compared with placebo ( $410.2 \pm 26.1$  vs.  $417.0 \pm 21.7$  ms,  $P = 0.045$ ). All ECG interval results are shown in Table 1.

To further investigate whether the increase in glucose uptake is due to increased heart work, we analyzed associations between myocardial GU and previously reported whole body energy expenditure. Whole body energy expenditure, which was 2% higher after secretin administration compared with placebo (5), was not associated with myocardial GU ( $r = -0.07$ ,  $P = 0.79$ ). This could indicate that the catabolic effect of secretin is not driven by heart work. However, whole body carbohydrate oxidation (CHO) was strongly associated with myocardial GU ( $r = 0.555$ ,  $P = 0.032$ ), which could indicate that glucose taken up by the myocardium is oxidized (Fig. 2C).

Rate of disappearance of glucose (Rd) was significantly higher after secretin administration compared with placebo ( $11.9 \pm 2.2$  vs.  $10.8 \pm 1.6 \mu\text{mol}/\text{kg}/\text{min}$ ,  $P = 0.045$ ), which is indicative of increased whole body GU during secretin infusion. We reported in our previous study that brown adipose tissue (BAT) and skeletal muscle glucose uptake are increased by secretin administration compared with placebo administration (5). Skeletal muscle has the largest influence on whole body glucose metabolism, due to its large mass compared with BAT and the myocardium (Supplemental Table S2; see <https://doi.org/10.6084/m9.figshare.16912810>).



**Figure 2.** Myocardial glucose uptake results. A: representative [ $^{18}\text{F}$ ]FDG PET/CT vertical long axis images showing Ki of the heart after secretin and placebo infusions ( $n = 1$ ). Short axis images are shown in Supplemental Fig. S1. (see <https://doi.org/10.6084/m9.figshare.16912807>). B: the effect of secretin infusion on myocardial glucose uptake compared with placebo ( $n = 15$ ). Data were analyzed by Student's paired  $t$  test. C: whole body carbohydrate oxidation (CHO) correlates with myocardial glucose uptake (MGU) after secretin administration ( $n = 15$ ). Data were analyzed by Pearson correlation. A line has been drawn on the data to indicate a significant association whereas dotted curves represent confidence interval. CT, computed tomography; [ $^{18}\text{F}$ ]FDG, [ $^{18}\text{F}$ ]2-fluoro-2-deoxy-D-glucose.

Interestingly, glucose uptake of BAT is associated with myocardial GU after the secretin infusion ( $r = 0.592$ ,  $P = 0.020$ ) whereas muscle GU is not ( $r = 0.270$ ,  $P = 0.331$ ) (Fig. 3, A and C). This could indicate that both myocardial and brown adipose tissue glucose uptakes are increased by the direct effect of secretin through secretin receptors (6), although the effect is not as pronounced in skeletal muscle. In contrast, muscle GU and myocardial GU are associated in fasting conditions (Fig. 3D) whereas BAT GU and myocardial GU are not (Fig. 3B). Fatty acids are used as an energy source in fasting conditions instead of glucose and BAT is largely inactive.

Serum creatinine was measured as part of a metabolomics panel, taken at several timepoints during the scan (Fig. 1). Serum creatinine levels decreased from baseline after secretin administration, while no such decrease was observed subse-

quent to placebo administration (Fig. 4A). Accordingly, eGFR was increased from baseline after secretin administration at 30 min compared with placebo administration (Fig. 4B). This is also when serum secretin levels peaked, as reported by us previously (5). [ $^{18}\text{F}$ ]FDG renal clearance was significantly higher after secretin administration than placebo administration (secretin vs. placebo,  $44.5 \pm 5.4$  mL/min vs.  $39.5 \pm 8.5$  mL/min,  $P = 0.004$ ) (Fig. 4C). Urine volumes at the end of the study were not significantly different between interventions (secretin vs. placebo,  $380.9 \pm 138.1$  vs.  $338.1 \pm 199.5$ ,  $P = 0.391$ ).

## DISCUSSION

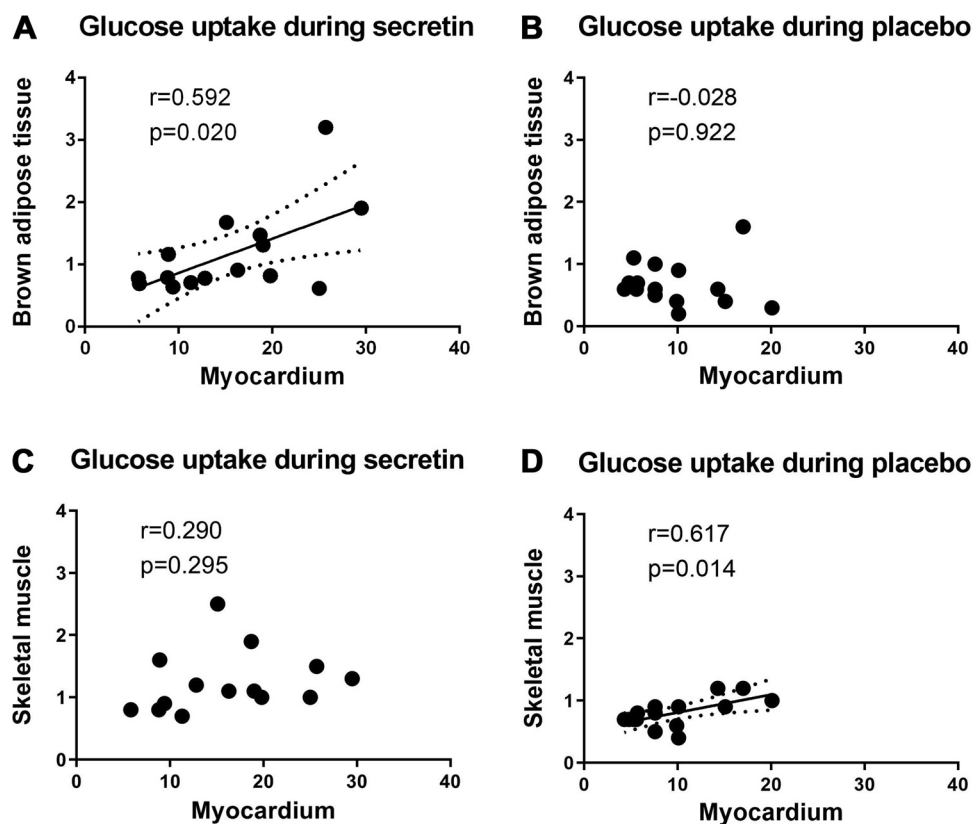
The main findings of this study are that secretin induces an increase in myocardial glucose uptake and increases renal

**Table 1.** ECG intervals

Measure	Minutes	Secretin Means $\pm$ SD	Placebo Means $\pm$ SD	P Value
Beats per minute, bpm	0	56.6 $\pm$ 7.5	55.4 $\pm$ 8.3	0.474
	60	56.8 $\pm$ 8.0	57.0 $\pm$ 7.8	0.917
	120	59.5 $\pm$ 7.8	57.8 $\pm$ 5.4	0.313
PR, mm	0	162.6 $\pm$ 24.5	158.8 $\pm$ 26.3	0.257
	60	157.4 $\pm$ 28.5	155.5 $\pm$ 24.7	0.584
	120	158.5 $\pm$ 24.7	156.8 $\pm$ 23.7	0.6
QRS, mm	0	100.5 $\pm$ 10.9	100.5 $\pm$ 10.1	1.0
	60	99.8 $\pm$ 6.6	99.8 $\pm$ 8.8	1.0
	120	100.9 $\pm$ 8.0	101.5 $\pm$ 8.5	0.524
QT, mm	0	428.0 $\pm$ 18.4	428.6 $\pm$ 18.0	0.899
	60	422.8 $\pm$ 13.2	429.5 $\pm$ 18.0	0.147
	120	421.9 $\pm$ 15.7	427.5 $\pm$ 13.9	0.122
QTc, mm	0	414.4 $\pm$ 24.1	410.2 $\pm$ 26.3	0.485
	60	410.2 $\pm$ 26.1	417.0 $\pm$ 21.7	0.045
	120	418.9 $\pm$ 24.6	418.9 $\pm$ 19.3	0.990

Values are means  $\pm$  SD. ECG intervals of  $n = 15$  subjects during secretin and placebo scans, measured before infusions, and 60 and 120 minutes after the first infusion. Data were analyzed by Student's paired  $t$  test for each timepoint. ECG tracings of  $n = 1$  subject are shown in Supplemental Fig. S2 (see <https://doi.org/10.6084/m9.figshare.16912813>).

**Figure 3.** Organ glucose uptake correlations ( $n = 15$ ). A: brown adipose tissue glucose uptake is strongly associated with myocardial glucose uptake after secretin infusion, while no association is seen during placebo (B). C: skeletal muscle glucose uptake is not associated with myocardial glucose uptake after secretin infusion, while an association exists during placebo (D). Data were analyzed by Pearson correlation. A line has been drawn on the data to indicate a significant association whereas dotted curves represent confidence interval. All units are  $\mu\text{mol}/100 \text{ g}/\text{min}$ .



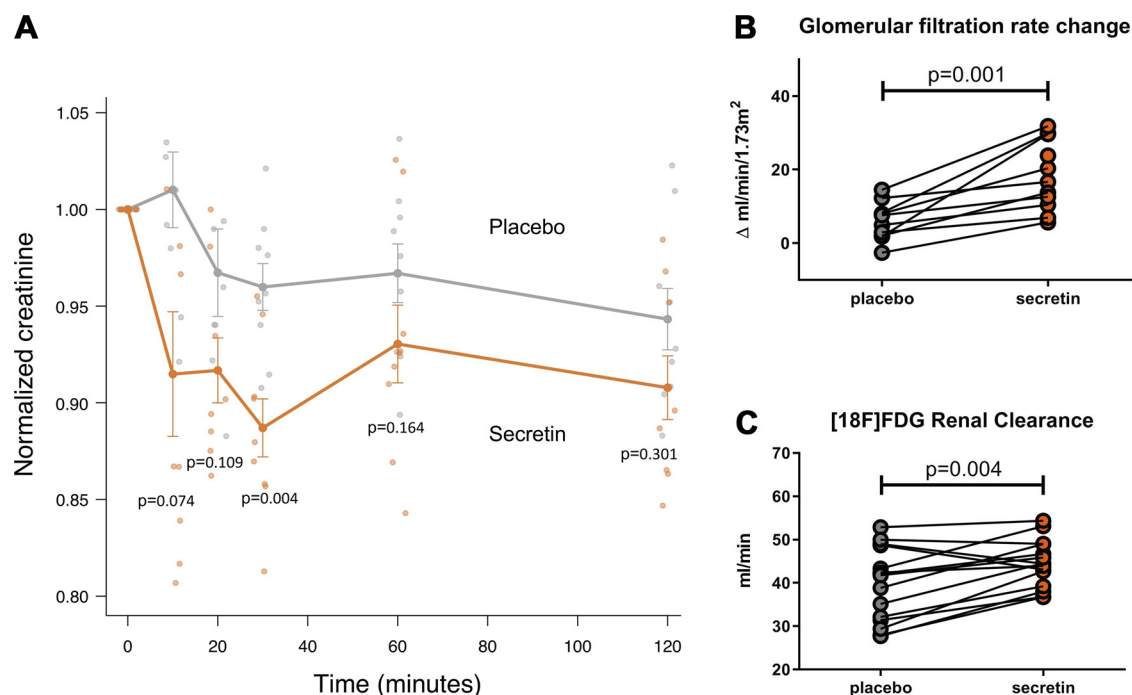
filtration, as indicated by the increased eGFR and clearance of [ $^{18}\text{F}$ ]FDG. Cardiac mortality benefits, shown by GLP-1 agonists, have sparked an interest in the cardiac effects of GI peptides, but the effects of secretin have not been previously studied in humans with modern imaging methods. Furthermore, the renal effects of any potential cardiovascular medications are of particular interest since renal failure exacerbates heart failure and vice versa. Our results in healthy, normal weight males highlight that studies on secretin in renocardiovascular pathologies are needed.

Secretin is a prandial hormone, secreted by S-cells in the duodenal epithelium. Its secretion is stimulated by the acidification of the duodenal lumen upon gastric emptying (4) and it binds to the G protein-coupled human secretin receptor (35). Secretin has an important role in the initiation of digestion, as it stimulates pancreatic exocrine secretion (4). Recently, we showed that it also has a role in postprandial thermogenesis and the termination of feeding through a gut-BAT-brain axis (6). Since cardiac output also increases postprandially (36), possibly to supply the splanchnic vasculature and facilitate nutrient distribution and digestion, we propose that secretin has a role in inducing this increase.

The cardiovascular effects of secretin were studied already 30 years ago, but possibly due to enalapril showing mortality benefits in heart failure and thus catching focus (37), and the practical pharmacological challenges of secretin being an intravenous drug with a short half-life (38), the potential of secretin as a treatment for heart failure was not further pursued. In the studies conducted by Gunnes et al, measurements were made with pulmonary artery catheterization and

thermodilution technique, while ECG and femoral artery pressure were also monitored (8, 9). Secretin induced an increase in cardiac output and a drop in systemic resistance. A reduction in systemic resistance increases output, but since the increase was considerable ( $\sim 20\%$ ), the authors suggested an inotropic effect as well. Animal studies have further confirmed this. In rat cardiomyocytes, secretin receptors stimulated contraction due to accumulation of cyclic adenosine monophosphate (cAMP) in cells (10). In pigs, intracoronary secretin increased cardiac function and perfusion through nitric oxide release and  $\beta$ -adrenoceptors (11). In coronary endothelial cells, this was also mediated through cAMP signaling and the effects were abolished by a secretin receptor antagonist (11). cAMP production is induced by  $G_s$  coupled secretin receptor activation (35). Interestingly,  $G_q$  coupling of the receptor is also recognized and it induces intracellular calcium mobilization (35). This could also contribute to increased cardiomyocyte contraction, but to our knowledge, the mechanism has never been investigated.

In addition to the shown increase in cardiac output (8, 9), one study also showed that secretin levels are associated with normal cardiac function. Circulating gastric peptide levels were measured in patients with chronic heart failure (39). Out of the gastric peptides studied, secretin and gastrin-releasing peptide were significantly lower in patients with chronic heart failure than in controls (39). This was not the case with vasoactive intestinal peptide (VIP), gastric inhibitory peptide (GIP), insulin, or glucagon (39). This could indicate a disturbance of secretin secretion in patients with chronic heart failure. One possible explanation could be the



**Figure 4.** Renal function results. **A:** secretin decreases serum creatinine compared with placebo ( $n = 10$ ). Values were normalized, dividing by the value of the first time point. Mean values and standard error are shown on graph. Each timepoint was analyzed by paired Wilcoxon signed-rank test. The secretin intervention is shown in orange whereas the placebo intervention is shown in gray. **B:** eGFR, calculated by Cockcroft–Gault equation, was increased by secretin from baseline at 30 minutes, compared with placebo ( $n = 10$ ). **C:**  $[^{18}\text{F}]\text{FDG}$  renal clearance was increased after secretin ( $n = 15$ ). **B** and **C:** data were analyzed by Student's paired  $t$  test.  $[^{18}\text{F}]\text{FDG}$ ,  $[^{18}\text{F}]\text{2-fluoro-2-deoxy-D-glucose}$ ; eGFR, estimated glomerular filtration rate.

sympathetic activation and increase in B-type natriuretic peptide (BNP) levels in patients with heart failure, which lead to increased white adipose tissue lipolysis (40). Since secretin is a powerful lipolytic agent (41), it could be downregulated between meals due to increased circulating FAs. There have not been studies addressing this question.

The heart is an omnivore, using glucose, FAs, lactate, and ketones for its metabolism, but long-chain fatty acids are the main substrate for energy metabolism (42). The rate of cardiac FA uptake is mostly determined by arterial FA concentration (43), whereas glucose uptake is regulated mainly through the recruitment of insulin-sensitive GLUT4 transporters (44). Glucose becomes the main substrate postprandially when glucose and insulin levels are high (45). It is notable, that secretin increased myocardial glucose uptake in our study, despite the concomitant increase in circulating FAs (5). Infusing FAs during a hyperinsulinemic euglycemic clamp is known to decrease myocardial glucose uptake in humans (46), confirming *in vivo* the Randle cycle. Furthermore, fasting myocardial  $[^{18}\text{F}]\text{FDG}$  uptake has been shown to correlate with increased heart work (47, 48). Taken together, our results support an inotropic effect of secretin, which was suggested in previous studies that showed an increase in cardiac output and stroke volume (8, 9).

Another interesting finding of this study was that QTc was shortened after secretin administration compared with placebo administration whereas there was no difference in heart rate. QTc interval shortens postprandially (49) and previous studies indicate that the change is not associated with insulin or glucose levels (50). In contrast, high carbohydrate

uptake and high insulin levels have been tied to a postprandial increase in heart rate (51). It has been suggested that postprandial QTc shortening is associated with the signaling pathways of  $\text{Ca}^{2+}$  cycling (52). This would be in line with a known postprandial increase in inotropy, also involving  $\text{Ca}^{2+}$  cycling (52). Taken together, our results suggest that secretin could influence postprandial QTc shortening through inotropy. This warrants further studies.

SGLT2 inhibitors have sparked a new interest in the cardiorenal axis, as the drug class has shown benefits in both kidney and cardiovascular endpoints (53). Renal and heart failure exacerbate each other, and prescribed medications should ideally aim to treat both conditions simultaneously. In line with previous studies that have shown a diuretic effect of secretin (54, 55), our study also suggests a mild diuretic effect of secretin, as shown by the enhanced  $[^{18}\text{F}]\text{FDG}$  renal clearance.  $[^{18}\text{F}]\text{FDG}$  is filtrated into the urine, and in contrast to glucose, it is not reabsorbed by SGLT2 (56). Thus, it could reflect urine excretion. It is also of note that the increase in  $[^{18}\text{F}]\text{FDG}$  clearance occurred despite higher myocardial GU during the secretin experiment. This suggests that our  $[^{18}\text{F}]\text{FDG}$  clearance and glucose uptake results are, if any, under rather than overestimated.

Glomerular filtration rate was estimated using serum creatinine levels according to the Cockcroft–Gault equation (31). eGFR values are dependent on not only the rate of creatinine filtration but also the rate of creatinine production by the muscle. It is thus possible that the results reflect a decrease in creatinine production. However, there is no previous literature to support this in relation to secretin, and our  $[^{18}\text{F}]\text{FDG}$  renal clearance rate results support an increase in glomeru-

lar filtration. Interestingly, creatinine clearance rate has previously been shown to associate with brown adipose tissue activation in humans (57). The authors proposed that this could reflect an increase in creatine phosphate turnover, as eGFR also increases after muscular exercise (58). Beige adipose tissue thermogenesis is enhanced by a creatine-driven substrate cycle (59), one that has also been shown to have a role in diet-induced thermogenesis in mice (60). The study showed that the genetic depletion of adipose creatine metabolism drives diet-induced obesity (60). Since we previously showed that secretin activates brown adipose tissue (6), the increase in eGFR could also reflect BAT activation. Furthermore, brown adipose tissue glucose uptake was associated with myocardial glucose uptake after secretin, which indicates that the effects are linked. This is an interesting finding since brown adipose tissue activity has recently been shown to associate with cardiovascular health, especially in patients with obesity (61).

Strengths of the present study are the use of state-of-the-art techniques to measure myocardial substrate uptake rates in vivo and the randomized placebo-controlled crossover protocol used. Our study also has limitations. First, the sample size, even though sufficient for the primary endpoints of this clinical trial, was relatively small. Infusion volumes of saline were not controlled since urine volume was not a pre-specified endpoint in this study. These factors may have precluded us from finding a significant change in urine volume excretion induced by secretin. Our subjects were all healthy males and further studies are needed to determine whether our results are applicable to a wider population.

In conclusion, our study supports the previous hypothesis that secretin has an inotropic effect in humans. This is the first study to demonstrate that secretin may directly impact myocardial glucose metabolism, despite concomitant increase in circulating FA levels. We also showed for the first time that secretin induces QTc shortening, which is known to occur postprandially. Our results also indicate that secretin increases renal filtration in humans, suggesting that the hormone has an influence on the cardiorenal axis. Based on the present findings, we believe that larger studies are warranted to investigate whether secretin may have a place in the future treatment of heart failure.

## SUPPLEMENTAL DATA

Supplemental Table S1: <https://doi.org/10.6084/m9.figshare.16912816>

Supplemental Table S2: <https://doi.org/10.6084/m9.figshare.16912810>

Supplemental Fig. S1: <https://doi.org/10.6084/m9.figshare.16912807>

Supplemental Fig. S2: <https://doi.org/10.6084/m9.figshare.16912813>

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## DISCLOSURES

M.K. is an inventor on a patent application from the Technical University of Munich (Publication No. WO/2017/20285; International Application No. PCT/EP2017/062420) addressing the role of secretin receptor agonists and modulators in the regulation of energy homeostasis. This patent is based on the initial discovery that meal-induced secretin inhibits food intake, and this anorexigenic action of secretin depends on the activation of brown fat (6). None of the other authors has any conflicts of interest, financial or otherwise, to disclose.

## AUTHOR CONTRIBUTIONS

S.L., K.S., L.N., M.K., K.A.V., and P.N., conceived and designed research; S.L., M.L., and L.S. performed experiments; S.L., E.R., T.-M.P., R.K., M.U.-D., and M.-J.H. analyzed data; S.L. and E.R. interpreted results of experiments; S.L. and R.K. prepared figures; S.L. and E.R. drafted manuscript; S.L., E.R., K.S., R.K., and M.K., edited and revised manuscript; S.L., E.R., M.L., L.S., K.S., T.-M.P., R.K., M.U.-D. M.-J.H., O.E., A.K.K., L.N., M.K., K.A.V., and P.N. approved final version of manuscript.

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