ARTICLE

Direct effect of GLP-1 infusion on endogenous glucose production in humans

M. Seghieri · E. Rebelos · A. Gastaldelli ·
B. D. Astiarraga · A. Casolaro · E. Barsotti · A. Pocai ·
M. Nauck · E. Muscelli · E. Ferrannini

Abstract

Aims/hypothesis Glucagon-like peptide-1 (GLP-1) lowers glucose levels by potentiating glucose-induced insulin secretion and inhibiting glucagon release. The question of whether GLP-1 exerts direct effects on the liver, independently of the hormonal changes, is controversial. We tested whether an exogenous GLP-1 infusion, designed to achieve physiological postprandial levels, directly affects endogenous glucose production (EGP) under conditions mimicking the fasting state in diabetes.

Methods In 14 healthy volunteers, we applied the pancreatic clamp technique, whereby plasma insulin and glucagon levels are clamped using somatostatin and hormone replacement. The clamp was applied in paired, 4 h experiments, during which saline (control) or GLP-1(7–37)amide (0.4 pmol min^{-1} kg^{-1}) was infused.

Results During the control study, plasma insulin and glucagon were maintained at basal levels and plasma C-peptide was suppressed, such that plasma glucose rose to a plateau of ~10.5 mmol/l and tracer-determined EGP increased by ~60%. During GLP-1 infusion at matched plasma glucose levels, the rise of EGP from baseline was fully prevented. Lipolysis (as indexed by NEFA concentrations and tracer-determined glycerol rate of appearance) and substrate utilisation (by indirect calorimetry) were similar between control and GLP-1 infusion.

Conclusions/interpretation GLP-1 inhibits EGP under conditions where plasma insulin and glucagon are not allowed to change and glucose concentrations are matched, indicating either a direct effect on hepatocytes or neurally mediated inhibition.

Keywords Endogenous glucose production · GLP-1 · Liver glucose output · Pancreatic clamp

Abbreviations

EGP	Endogenous glucose production
FFM	Fat-free mass
GLP-1	Glucagon-like peptide-1
Ra	Rate of appearance
Rd	Rate of disappearance
RQ	Respiratory quotient
SRIF	Somatotropin release-inhibiting factor
TTR	Tracer:tracer ratio

M. Seghieri · E. Rebelos · B. D. Astiarraga · A. Casolaro ·
E. Barsotti · E. Muscelli · E. Ferrannini (✉)
Department of Internal Medicine,
University of Pisa School of Medicine,
Via Roma, 67,
56100 Pisa, Italy
e-mail: ferranni@ifc.cnr.it

A. Gastaldelli · E. Ferrannini
C.N.R. Institute of Clinical Physiology,
Pisa, Italy

A. Pocai
Merck Sharp & Dohme,
Whitehouse Station, NJ, USA

M. Nauck
Diabeteszentrum Bad Lauterberg,
Bad Lauterberg im Harz, Germany

Introduction

The classical physiological actions of glucagon-like peptide-1 (GLP-1) include potentiation of glucose-induced insulin secretion, suppression of glucagon release, inhibition of gastric emptying and enhancement of satiety [1]. The opposing effects on insulin and glucagon secretion result in reductions of endogenous glucose production (EGP) and blood glucose levels. The question of whether the hormone exerts direct actions on insulin target tissues, i.e. liver, adipose and skeletal muscle tissue, is controversial.

GLP-1 receptors were originally not found in human liver [2]. However, the results of more recent in vitro studies are compatible with the presence of GLP-1 receptors in human hepatocytes [3, 4]. Additionally, GLP-1 has been reported to increase glucose transporter levels and insulin-mediated glucose uptake in 3T3-L1 adipocytes [5], and glucose transport in cultured human myocytes [6]. GLP-1 receptor mRNA has also been described in neurons in the hepatic portal region [7].

Studies in humans are scarce and inconsistent. Hvidberg et al [8] concluded that the decrease in EGP and increase in glucose rate of disappearance (Rd) during GLP-1 infusion in healthy volunteers could be entirely explained by the changes in insulin and glucagon concentrations. Likewise, others [9, 10] reported that the effects of GLP-1 on EGP and glucose disposal were abolished when co-infusing somatotropin release-inhibiting factor (SRIF), thereby blocking the insulin and glucagon response to GLP-1. The same conclusion was reached in experiments using somatostatin infusion during a high-dose hyperinsulinaemic–euglycaemic clamp [11]. In contrast, in uncontrolled studies in healthy volunteers, Prigeon et al used the pancreatic clamp technique to show that fasting EGP and plasma glucose concentrations declined ~20% upon adding a short-term (60 min), high-dose GLP-1 infusion [12].

With regard to the effects on whole-body glucose disposal, early studies [8, 11] found no direct effect of GLP-1, i.e. no effect that was independent of changes in insulin concentrations, on the potentiation of glucose disappearance. Subsequent work, however, reported an independent effect of GLP-1 on the promotion of glucose disposal in non-diabetic [13], obese [14] or diabetic participants [15].

Another potential extrapancreatic action of GLP-1 is on lipid metabolism. Although GLP-1 receptors are not produced in adipocytes, the peptide appeared to stimulate lipolysis in fat cells from obese participants [16]. In contrast, using in situ microdialysis and local GLP-1 perfusion, Bertin et al [17] detected no change in lipolysis or blood flow in adipose tissue or muscle. Finally, intracerebroventricular GLP-1 administration in mice [18] and peripheral GLP-1 infusions in man [19] increased sympathetic activity. It has not yet been determined whether this sympathoexcitatory action is mediated by insulin.

Here, we reassessed the in vivo direct effects of physiological GLP-1 elevations, created by exogenous administration of GLP-1(7-37)amide, on EGP, glucose disposal, lipolysis and indices of sympathetic activation in healthy volunteers.

Methods

Participants Healthy volunteers ($n=14$) aged 18 to 60 years and with a BMI <30 kg/m^2 participated in the study (Table 1). The nature and purpose of the study were carefully explained to all participants before they provided written consent to participate. The study procedures were approved by the Institutional Ethics Committee of Pisa University.

Study design and protocol Each participant underwent two studies within 7 to 14 days of each other. In each study, after an overnight (12 h) fast, catheters were inserted into an antecubital vein (for infusion of all test substances) and retrogradely into a vein on the dorsum of the hand for blood withdrawal. The hand was heated to 55°C to allow sampling of arterialised venous blood. At 09:00 hours primed continuous infusions of 6,6-[^{2}H$_2$]glucose (0.28 μmol min^{-1} kg^{-1}; prime 28.0 μmol/kg × [fasting plasma glucose/5]) and [^{2}H$_5$]glycerol (0.11 μmol min^{-1} kg^{-1}; prime 1.65 μmol/kg) were started and continued for the duration of the study (6 h). At time 0, constant infusions of SRIF (450 μg/h) and glucagon (1 ng min^{-1} kg^{-1}) were begun and continued for 4 h. At time 20 min, a primed continuous insulin (Humulin R; Eli Lilly, Indianapolis, IN, USA) infusion (12 pmol min^{-1} m^{-2}) was initiated, along with a saline drip. During the second study, from time 60 min onward, saline was replaced by a constant GLP-1(7–37)amide infusion (0.4 pmol min^{-1} kg^{-1}), while the plasma glucose profile of the first study was closely reproduced through a variable intravenous glucose infusion, using an algorithm developed ad hoc [20]. Plasma insulin, C-peptide, glycerol, glucagon and NEFA concentrations, as well as 6,6-[^{2}H$_2$]glucose and [^{2}H$_5$] glycerol enrichment were measured at pre-determined intervals.

In 13 of 14 participants, indirect calorimetry was used to measure the respiratory quotient (RQ) and substrate oxidation rates, using a continuous, open-circuit canopy system (Metabolic Measurement Cart Horizon; SensorMedics,

Table 1 Anthropometric and metabolic characteristics

Characteristic	Mean±SD	Range
n	14	–
Men (n)	11	–
Women (n)	3	–
Age (years)	26±2	21–30
Waist (cm)	84±5	75–91
Hip (cm)	105±7	96–114
BMI (kg/m^2)	25.5±3.7	19.0–30.0
Fasting glucose (mmol/l)	5.2±0.3	4.6–5.8
Total cholesterol (mmol/l)	4.1±0.5	3.5–5.5
HDL-cholesterol (mmol/l)	1.5±0.3	1.2–2.0
Triacylglycerol (mmol/l)	0.7±0.2	0.4–1.0
AST (μkat/l)	0.37±0.08	0.25–0.55
ALT (μkat/l)	0.28±0.07	0.15–0.43

AST, aspartate aminotransferase; ALT, alanine aminotransferase

Anaheim, CA, USA). These measurements were collected during the basal period (−40 to 0 min) and over the last 40 min of the study.

Fat-free mass (FFM) was evaluated using a body composition analyser (TB-300; Tanita, Tokyo, Japan); fat mass was then obtained as the difference between body weight and FFM.

Assays Plasma glucose was measured by the glucose oxidase technique (Beckman Glucose Analyzers; Beckman, Fullerton, CA, USA). Plasma insulin and C-peptide were measured by an electro-chemiluminescence assay on a COBAS e411 (both from Roche, Indianapolis, IN, USA). Glucagon was measured by radioimmunoassay (Millipore, Billerica, MA, USA). The tracer enrichment of $6,6-[^2H_2]$ glucose and $[^2H_5]$glycerol was measured by gas chromatography/mass spectrometry as previously described [21]. NEFA and glycerol were measured using an enzymatic colorimetric system (Syncron; Beckman).

Plasma samples were assayed for intact GLP-1 using a GLP-1 ELISA kit following the manufacturer's protocol (Millipore). The detection limit for this assay is 2 pmol/l in 100 μl plasma.

Calculations Glucose fluxes were expressed per kg of FFM. During the last 20 min of the basal tracer equilibration period, plasma glucose and glycerol concentrations, as well as $6,6-[^2H]$glucose and $[^2H_5]$glycerol enrichment (expressed as tracer:tracer ratio [TTR]) were stable in all participants. Therefore, EGP and the glycerol rate of appearance (Ra) were calculated as the ratio of tracer infusion rate to the plasma TTR (mean of three determinations). After starting SRIF infusion, the total glucose and glycerol Ra were calculated using Steele's equation, as previously described [22]. Before applying Steele's equation, plasma TTR data for $6,6-[^2H]$glucose were smoothed using a spline fitting approach to stabilise the calculation of the derivative of enrichment. The plasma glucose concentration resulting from EGP was obtained as the difference between total and exogenous glucose concentrations. The tracer-determined Rd provided a measure of insulin-mediated total-body glucose disposal.

Substrate oxidation rates were calculated from gas exchange measurements as described [23]. Areas under the time–concentration curve were calculated by the trapezium rule.

Statistical analysis Data are given as mean±SD. Differences between saline and GLP-1 infusion were analysed by Wilcoxon's signed-rank test. The time course of glucose fluxes was analysed by two-way, doubly repeated-measures ANOVA, modelling infusion (GLP-1 vs saline) and experimental time (and their interactions) as factors. A value of $p \leq 0.05$ was considered statistically significant.

Results

In the control study, glucose levels began to rise ~1 h into the SRIF infusion and levelled off at ~10.6 mmol/l during the last hour; this time course was reproduced in the GLP-1 study (Fig. 1a). Upon starting SRIF infusion, insulin concentrations initially dropped from baseline, then returned to the fasting value by ~60 min in the control and test study ($p=0.40$). During the last hour, however, plasma insulin levels were higher under GLP-1 infusion than under control conditions (38±18 vs 25±7 pmol/l, $p<0.002$), probably reflecting beta cell escape from SRIF blockade, as confirmed by the C-peptide time course (Fig. 1b, d). Plasma glucagon concentrations also decreased from baseline following the start of SRIF, then rose gradually and slightly until the end of the study, without significant ($p=0.18$) differences between saline and GLP-1 infusion (Fig. 1c).

The glucose Ra rose from baseline under saline and GLP-1 infusion, the time-pattern of the rise being similar in both (Fig. 2a). Exogenous glucose infusion rates, however, were higher with GLP-1 than saline infusion ($p<0.0001$); consequently, EGP was lower throughout the 3 h of GLP-1 infusion (Fig. 2b). Over the time-period when pancreatic hormones were closely superimposable between saline and GLP-1 (i.e. between 60 and 180 min), EGP was 27% lower (by 3.6 μmol kg^{-1}min^{-1}, 95% CI 2.4, 4.8, $p<0.0001$) with GLP-1 than with saline (Fig. 2c). The glucose Rd increased slightly only during the last hour of both studies ($p<0.01$ for saline and GLP-1), without differences between saline and GLP-1 (Fig. 2d).

Plasma NEFA increased from baseline until 40 min (from 0.53±0.10 and 0.54±0.03 to 0.70±0.09 and 0.72±0.04 mEq/l, respectively, for saline and GLP-1), subsequently dropping below the basal levels, with no difference between the two studies (AUC$_{0–240 min}$ 115.5±13.2 vs 110.0±9.6 mEq/l×240 min, $p=0.65$) (Fig. 3a). The glycerol Ra averaged 2.72±0.24 and 3.11±0.22 μmol min^{-1}kg^{-1} during the baseline period of the saline and GLP-1 studies, respectively. During the infusion period, after an initial slight increase, the glycerol Ra declined slowly over time and to similar degrees under saline and GLP-1, to reach values somewhat lower with the latter (2.01±0.92) than the former (2.43±2.01) during the final hour of the study (Fig. 3b).

The RQ did not change between baseline (0.75±0.02 vs 0.76±0.02, saline vs GLP-1, $p=0.84$) through to the final hour of the study (0.76±0.03 vs 0.78±0.01, $p=0.33$). Accordingly, baseline rates of carbohydrate and lipid oxidation were similar between the two study days and did not change significantly with either saline or GLP-1 infusion (Fig. 4a, b).

During saline infusion, there was no change in intact GLP-1, whereas GLP-1 infusion raised the plasma levels of intact hormone threefold (AUC$_{60–180 min}$ 239±515 vs

Fig. 1 Time course of glucose (**a**), insulin (**b**), glucagon (**c**) and C-peptide (**d**) during the saline study (white circles) and GLP-1 infusion (black squares). In the final hour, plasma insulin levels were higher under GLP-1 infusion than control conditions ($p<0.002$)
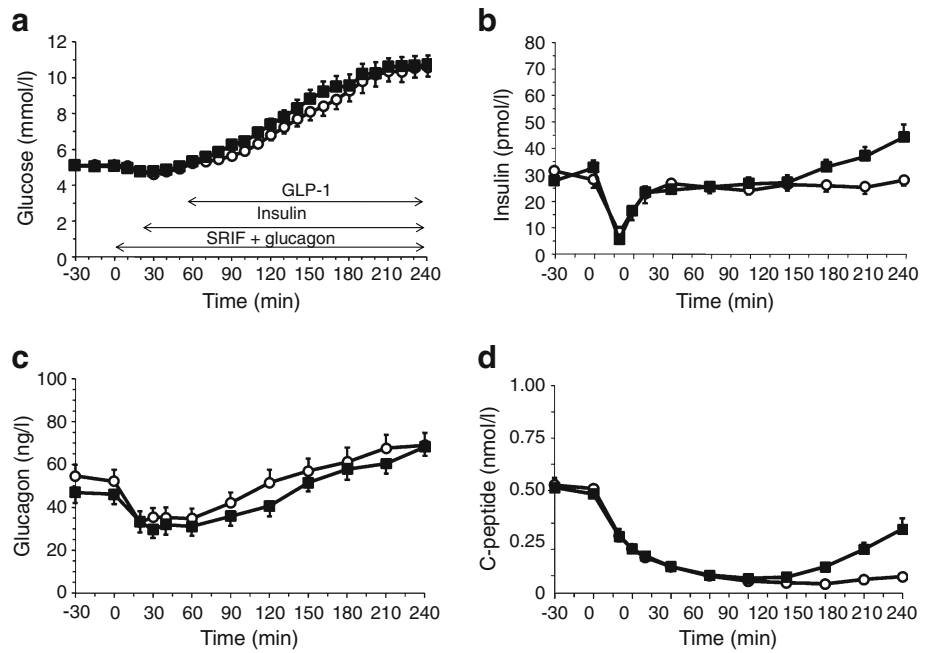

441±200 pmol/l×120 min, $p=0.001$). In the pooled saline and GLP-1 data, there was a significant, albeit weak, ($\rho=-0.49$, $p=0.01$) reciprocal relationship between EGP and intact GLP-1 concentrations measured over the 60–180 min time interval.

Discussion

The present studies demonstrate that exogenous GLP-1 inhibits EGP by mechanisms that are largely independent

of changes in plasma glucose, insulin and glucagon levels. Our experimental settings mimicked a diabetic state, i.e. raised glucose concentrations and glucagon:insulin ratios. Under these conditions, EGP was increased by ~70% from baseline, with plasma glucose rising to a plateau of ~11.1 mmol/l. Replacing the saline with a GLP-1 infusion, at a rate producing steady-state plasma levels approximately in the postprandial range, caused a marked reduction of EGP, which remained close to the starting levels. Interestingly, insulin secretion during the 3rd hour of GLP-1 infusion tended to rise, reflecting an escape of the beta cells

Fig. 2 Time course of glucose Ra (**a**), glucose infusion rate (GIR) (**b**), glucose Rd (**d**) and EGP (**c**) during the saline study (white circles) and GLP-1 infusion (black squares)
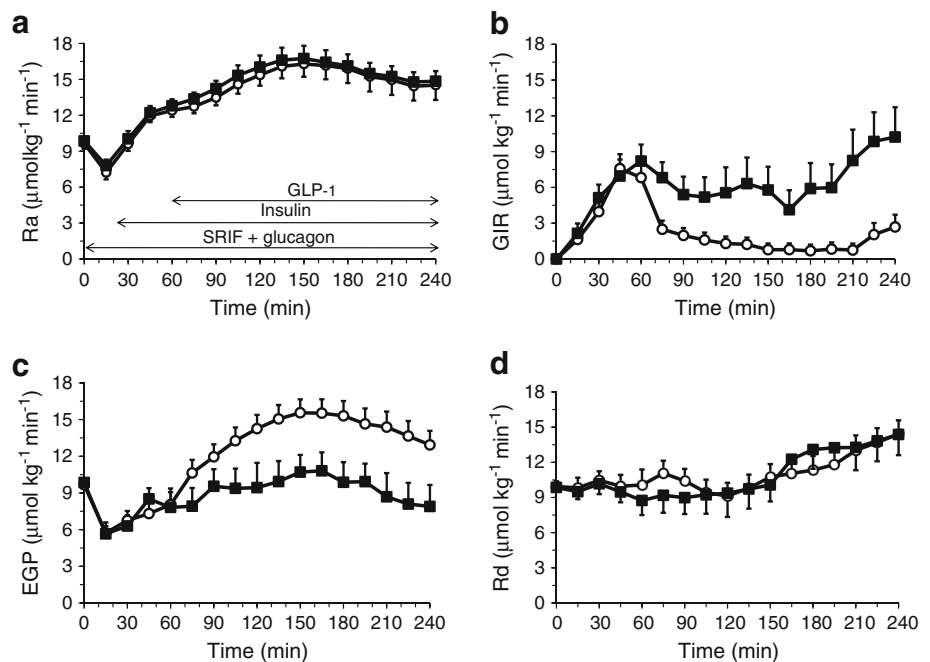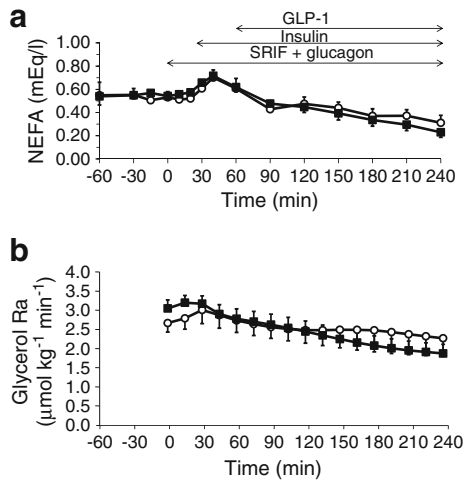
Fig. 3 Time course of plasma NEFA concentrations (a) and glycerol Ra (b) during saline (white circles) or GLP-1 infusion (black squares) in healthy volunteers

from SRIF blockade. Therefore, quantification of the GLP-1 effect was restricted to the 2 h during which plasma insulin and C-peptide levels were stable and superimposable between the two studies (Fig. 1).

Previous reports [11, 24] that failed to observe a direct inhibitory effect of GLP-1 on EGP under pancreatic clamp conditions can probably be explained by their use of high insulin replacement doses, which suppressed EGP completely [11] or by greater than 75% [24] in the control studies, thereby leaving little room for a further inhibitory action of GLP-1. In addition, we did not detect any effect on whole-body glucose disposal, in accordance with previous

Fig. 4 Rates of carbohydrate (a) and lipid (b) oxidation before starting SRIF (basal) and at the end of the 4 h infusion period (final). Light grey, saline; dark grey, GLP-1 infusion

findings in healthy volunteers [9, 10]. However, we cannot rule out the possibility that pharmacological doses of GLP-1 such as those used in previous studies [14–25] may promote whole-body glucose uptake.

The only previous study that is indicative of a direct effect of GLP-1 on EGP [12] was carried out in eight healthy volunteers, who did not receive a saline infusion control study. Moreover, exogenous GLP-1 was infused, for a short time (60 min), at rates achieving total plasma GLP-1 concentrations that were twice as high as the steady-state levels created by us. More importantly, the insulin replacement (36 pmol min^{-1} kg^{-1}) was at least twice as high as ours (12 pmol min^{-1} kg^{-1}), achieving two to three times higher steady-state plasma insulin concentrations (thus raising plasma glucose clearance by ~50%). Thus, in Prigeon's protocol [12], the effect of short-lived, supraphysiological GLP-1 concentrations was tested under conditions of euglycaemia and hyperinsulinaemia. With the present protocol, we demonstrated that physiological GLP-1 increments prevent EGP from increasing under conditions simulating the fasting state in diabetes.

With regard to the mechanisms underlying the direct action of GLP-1 on EGP, we measured lipolysis, as indexed by glycerol Ra and plasma NEFA levels, and the pattern of substrate utilisation (using indirect calorimetry). As no differences, not even in trend, were observed between saline and GLP-1 infusion, we can rule out the possibility that the GLP-1-induced inhibition of EGP may have been due to a reduction of NEFA delivery to the liver, which would stimulate EGP via gluconeogenesis, or to an increase in sympathetic drive, which would stimulate lipolysis and shift the substrate oxidation pattern toward lipid oxidation.

In the present studies, the threefold elevated intact GLP-1 levels could have engaged hepatic GLP-1 receptors similar to those on beta cells [4]. Alternatively, the GLP-1(28–36)amide nonapeptide, which enters hepatocytes independently of the GLP-1 receptor, may have suppressed glucose production, as shown in mouse hepatocytes [26].

Experimental evidence for the possibility that GLP-1 may act on the liver by engaging sensors in the portal circulation or nerve endings in the intestinal wall comes from different animal species, but is convergent. Thus, in insulin clamp experiments in GLP-1 receptor knockout mice, insulin suppression of EGP was impaired and animals became hyperglycaemic during exercise [27]. Nakabayashi et al [28] measured changes in the impulse discharge rate of the hepatic afferent vagus, following a bolus intraportal GLP-1 injection in the rat. They found that the hormone dose-dependently increased the firing rate and that this effect could be cancelled by vagotomy. In catheterised dogs, Johnson et al [29] found that direct infusion of GLP-1 into the portal vein at matched plasma glucose, insulin and glucagon concentrations resulted in a more positive net
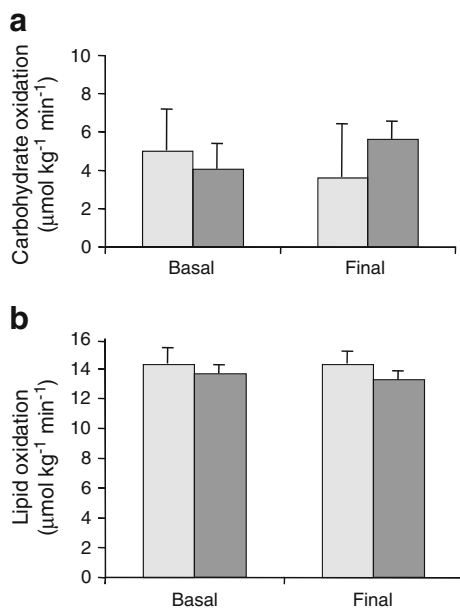
hepatic glucose balance, which is the net sum of EGP and hepatic glucose uptake.

In summary, the present studies provide conclusive evidence that a physiological action of GLP-1 inhibits glucose production under conditions where its major controlling signals, namely plasma insulin, glucagon and glucose concentrations, are not allowed to change. The effect is quantitatively significant and does not appear to be mediated by changes in substrate availability or sympathetic drive.

Contribution statement MS, ER, BDA, AG, AP, AC, EB and EM acquired and analysed the data and drafted the article. EM, EF and MN reviewed the article and were responsible for the conception of the study. All authors approved the final version.

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