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Regional hemodynamic effects of the N-(2-benzoylphenyl)-L-tyrosine peroxisome proliferator-activated receptor (PPAR) -γ-ligand, GI 262570, in conscious rats

Sheila M Gardiner[1], Derek J R Nunez[2], Philip G Baer[2], Kathleen K Brown[2] & Terence Bennett[1]

Centre for Integrated Systems Biology & Medicine, School of Biomedical Sciences, University of Nottingham Medical School, Queen`s Medical Centre, Nottingham NG7 2UH, UK.

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Running title Cardiovascular actions of PPAR ligands

Corresponding author Professor Sheila M Gardiner

School of Biomedical Sciences

University of Nottingham Medical School

Queen's Medical Centre

Nottingham NG7 2UH, UK

Tel 001 (0) 1159709476, Fax 001 (0) 115709259

e-mail **sheila.gardiner@nottingham.ac.uk**

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PPAR: peroxisome proliferator-activated receptor; L-NAME: N^G nitro-L-arginine methyl ester; AT1: angiotensin type 1 receptor; ETA/B: endothelin type A/B receptor

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ABSTRACT

This study provides novel data on the regional hemodynamic effects of the peroxisome proliferator-activated receptor (PPAR) -γ activator, GI 262570, in conscious, male, Sprague-Dawley rats. Administration of GI 262570 twice daily for 4 days caused a slowly-developing, modest, fall in mean arterial blood pressure, associated with a progressive, hyperaemic hindquarters vasodilatation, but with no consistent changes in renal or mesenteric hemodynamics. The hindquarters vasodilator effect of GI 262570 was not inhibited by the β_2-adrenoceptor antagonist, ICI 118551, and was still apparent in the presence of the α-adrenoceptor antagonist, phentolamine. Neither the latter, nor antagonism of angiotensin (AT_1) and endothelin (ET_A and ET_B) receptors unmasked vasodilator responses to GI 262570 in the renal or mesenteric vascular beds. In the presence of GI 262570, vasodilator responses to acetylcholine, and vasoconstrictor responses to methoxamine were normal. Furthermore, the cardiovascular responses to non-selective nitric oxide synthase inhibition were not influenced by GI 262570. Collectively, these results indicate that the vasodilator action of GI 262570 is specific to the hindquarters vascular bed (of those studied), does not involve α- or β_2-adrenoceptors, and is not associated with a change in basal or stimulated nitric oxide release.

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The thiazolidinediones were shown to enhance insulin sensitivity before it was known that they were activators of PPAR-γ (Speigelman, 1998; Willson et al., 2000; Willson et al., 2001; Rosen and Spiegelman, 2001). Although PPAR-γ is highly expressed in fat, it occurs in muscle and liver as well, and, to a lesser extent, in tissues of the cardiovascular system (Spiegelman, 1998; Bishop-Bailey, 2000). In the latter context, it is now apparent that the thiazolidinediones may influence cardiovascular function, particularly in patients with diabetes mellitus (Mudaliar and Henry, 2001; Martens et al., 2002), but it is not clear to what extent this involves PPAR-γ-dependent or -independent effects, changes in insulin sensitivity, and/or modulation of inflammatory processes (Spiegelman, 1998; Rosen and Spiegelman, 2001; Klappacher and Glass, 2002; Shiojiri et al., 2002; Rival et al., 2002). Moreover, the acute hemodynamic effects of PPAR-γ activation in the non-diabetic state have not been described in detail.

A novel series of N-(2-benzoylphenyl)-L-tyrosine compounds has been identified as potent PPAR-γ agonists (Henke et al., 1998; Collins et al., 1998; Cobb et al., 1998), and shown to be antidiabetic *in vivo* (Brown et al., 1999). Of this series, GI 262570, a non-thiazolidinedione, tyrosine-based insulin sensitizer, is the most potent *in vitro* (Willson et al., 2000), but there are few published data on the *in vivo* cardiovascular effects of this compound (Grillot et al., 2001; Callahan et al., 2002). Such data would be of value because of the potential link between muscle haemodynamics and glucose disposal resulting from PPARγ-mediated increases in insulin sensitivity (Baron, 1994). Given the dearth of information about the hemodynamic consequences of PPAR-γ activation in the non-diabetic state generally, and the relative lack of detail about the cardiovascular actions of GI 262570 in particular, the first objective of the present study was to delineate the regional hemodynamic responses to acute and chronic administration of GI 262570 in conscious rats. Since those initial experiments

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revealed a striking, selective, hindquarters hyperaemic vasodilator effect of GI 262570, further experiments were designed to explore mechanisms.

Firstly, we investigated the possibility that the hindquarters vasodilator effect of GI 262570 was mediated by β_2-adrenoceptors, since GI 262570-induced changes in insulin-mediated glucoregulation could influence adrenal medullary adrenaline release, and adrenaline-induced β_2-adrenoceptor activation is particularly prominent in the skeletal muscle vascular bed (Gardiner and Bennett, 1988; Gardiner et al., 1991a).

Secondly, we determined whether or not activation of compensatory vasoconstrictor systems (renin-angiotensin, endothelin, sympathetic), in the presence of GI 262570, might oppose any vasodilator influence in renal and mesenteric vascular beds. In addition, using this approach, we were able to determine if GI 262570-induced inhibition of such vasoconstrictor mechanisms in the hindquarters was responsible for the vasodilatation observed specifically in that region.

Thirdly, we explored the possibility that up-regulation of basal and/or stimulated nitric oxide release was involved in the cardiovascular actions of GI 262570.

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METHODS

Male, Sprague-Dawley rats (initial weights 300 - 325g) were obtained from Charles River (UK) at least 2 weeks before undergoing any procedure. During that period they were group-housed (4-5/cage), in a temperature-controlled environment (20-22°C), with a 12 h light-dark cycle (lights on at 06.00h), and free access to tap water and food (BeeKay Foods, Standard Rat Diet BK 001E). The procedures were approved by the University of Nottingham Ethical Review Committee, and were performed under Home Office Project Licence authority.

Surgical preparation

Following the settling-in period, rats (now weighing 350 - 400g) were anesthetised (fentanyl and medetomidine, 300 μg kg^{-1} of each i.p., supplemented as required) and had pulsed Doppler probes implanted around the left renal and superior mesenteric arteries, and the distal abdominal aorta, through a midline laparotomy (Gardiner and Bennett, 1988; Gardiner et al., 1991a). Post-surgery, anesthesia was reversed and analgesia provided with atipamezole and nalbuphine, respectively (1 mg kg^{-1} s.c. of each). Thereafter, animals were housed individually for 7-14 days before implantation of intra-arterial (abdominal aorta via ventral caudal artery) and intravenous (right jugular vein) catheters. Procedures for anesthesia, reversal, and analgesia for catheterisation were as above. Following catheterisation, animals were housed in individual home cages for the whole of the experimental protocol with access to food and water *ad libitum*.

Cardiovascular recordings

Experiments began the day after catheter implantation. From previous studies using the approaches described here, we have seen no evidence of hang-over effects of the anesthetic regime (Wakefield et al., 2003; Woolard et al., 2004). Furthermore, in the present study hemodynamic responses to vasoconstrictor and vasodilator challenges were consistent across

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the 4 experimental days in vehicle-infused rats (see Results). Animals were connected (via a tether system) to a transducer amplifier (Gould) and Doppler flowmeter (Crystal Biotech) which were, in turn, connected to a custom-designed microprocessor system (Instrumentation Laboratories, University of Maastricht). The system was set to sample the signals (arterial blood pressure and renal, mesenteric and hindquarters Doppler shifts), every 2 ms, average them every cardiac cycle, and store them to disc at 5s intervals. Recordings were made as described under Data Analysis (below).

Experiment 1. Regional hemodynamic responses to GI 262570

GI 262570 is not soluble in saline; furthermore, polyethylene glycol, as used for oral dosing (e.g., Yang et al., 2003), cannot be used i.v. Therefore, GI 262570 (2 mg ml^{-1}) was solubilised in a vehicle containing glycine (225.2 mg), NaOH (0.1 M, 12.1 ml), NaCl (805.9 mg) and sterile water (to make up to 100 ml). GI 262570 or vehicle were infused (0.4 ml h^{-1} i.v.) continuously for 2 hours, twice daily (between 07 45h and 09 45h and between 13 30h and 15 30h) for 4 days. The continuous infusions of vehicle or GI 262570 were given to different rats (n = 9 in each group) through 0.22 μm filters. This infusion rate of GI 262570 produced a daily drug exposure in the rats which was equivalent to that achieved by oral dosing (8 mg kg^{-1} b.i.d.) of non-diabetic rats, and shown to have hemodynamic effects (Callahan et al., 2002). This dose has also been shown to be maximally efficacious for glucose lowering in diabetic rodents (Brown et al., 2000), although above what would be expected for efficacy in patients with type 2 diabetes mellitus (Fiedorek et al., 2000).

Experiment 2. Effects of ICI 118551 on regional hemodynamic responses to GI 262570

Rats were given GI 262570 together with the β_2-adrenoceptor antagonist, ICI 118551, (0.2 mg kg^{-1}; 0.1 mg kg^{-1} h^{-1}; n = 8), or its vehicle (saline, 0.4 ml h^{-1}; n = 8), over the same time course as the first experiment, i.e., ICI 118551 or its vehicle were administered for the 2 hour

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periods during which GI 262570 was given. The dose of ICI 118551 used has been shown to have little or no effects on baseline hemodynamics but to cause marked inhibition of the hindquarters vasodilator responses to exogenous and endogenous β_2-adrenoceptor agonists (Gardiner and Bennett, 1988; Gardiner et al., 1992; Gardiner et al., 2002).

Experiment 3. Effects of ZD 7155 and SB 209670 or phentolamine on regional hemodynamic responses to GI 262570

There were 6 experimental groups in this part of the study.

Groups 1 & 2 were given GI 262570 (2 mg ml^{-1} at 0.4 ml h^{-1}, n=8) or vehicle (as above, n=8) twice daily, at the same times as in Experiment 1, together with sterile saline (0.4 ml h^{-1}) which was the vehicle for the antagonists used (see below).

Groups 3 & 4 were given the AT_1-receptor antagonist, ZD 7155 (Jungrenn et al., 1996) as a bolus at a dose of 2 mg kg^{-1} (i.e., 0.8 mg in 0.1 ml). In pilot studies (n = 2) we confirmed this dose of ZD 7155 blocked responses to exogenous angiotensin II (50 pmol)). Simultaneously, the ET_A- ET_B-receptor antagonist, SB 209670 was administered as a primed infusion (600 µg kg^{-1} bolus, 600 µg kg^{-1} h^{-1} infusion; bolus in 0.1 ml, infusion at 0.4 ml h^{-1}) (Gardiner et al., 1996), and the administration of GI 262570 (n=8) or vehicle (n=8) was begun (as above), such that the infused drugs reached the animal together, 15 min later, and the 2h measurement period began from that time-point. We adopted this approach in order to optimise the chances of seeing any influence of the endogenous vasoconstrictor systems. Since no such effect was observed (see Results), we considered it unnecessary to examine the effects of inhibiting either the renin-angiotensin system or endothelin alone.

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Groups 5 & 6 were given phentolamine as a primed infusion (1 mg kg^{-1} bolus, 1 mg kg^{-1} h^{-1} infusion; bolus in 0.1 ml, infusion at 0.4 ml h^{-1}) (Janssen et al., 1991), and at the same time the infusion of GI 262570 (n=8) or vehicle (n=8) was begun (as above).

Experiment 4. Responses to acetylcholine, methoxamine and N^G nitro-L-arginine methyl ester (L-NAME) in the presence of GI 262570

After the last recording period following the afternoon infusion session on all four experimental days, all animals in Experiment 3 were challenged with randomised, 3 min infusions (0.15 ml min^{-1}) of acetylcholine (27 μg ml^{-1}) and methoxamine (200 μg ml^{-1}). Between each infusion, sufficient time (> 15 min) was left for all variables to return to their pre-infusion values. On the fourth experimental day only, and at least 15 min after the last infusion of either acetylcholine or methoxamine, a bolus injection of L-NAME was given (10 mg kg^{-1}) (Gardiner et al., 1990) and recordings continued for an additional 30 min.

Data Analysis

On each experimental day, data were recorded continuously for about 10 h. For measurement purposes, the experimental day was divided into five blocks, i.e., (i) the initial 30-45 min period, (ii) the first 2 h infusion period, (iii) the interval of about 3-4 h between the infusions, (iv) the second infusion period, and (v) the terminal recording period of about 1 h. The data collected in the first measurement period of the first day were taken as baseline, and all subsequent values were expressed as changes relative to these baseline values. Responses to acetylcholine or methoxamine were measured during the last minute of the 3 min infusion, when all variables were steady. Responses to L-NAME were measured 10 min after injection, when steady-state had been achieved.

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The measured variables were extracted into a custom-designed statistical package which performed all arithmetical calculations (means, standard errors, etc.) and allowed appropriate, statistical analysis. As described elsewhere (Gardiner and Bennett, 1988), under the conditions of our experiments, % changes in Doppler shift are directly related to % changes in blood flow and are used to calculate % changes in vascular conductance, where vascular conductance is Doppler shift / mean blood pressure.

Within a group, changes relative to baseline were assessed using Friedman's test (Theodorsson-Norheim, 1987); between-group analysis was by the Mann-Whitney U test, applied to integrated responses (areas under or over curves). The significance level was set at $P \leq 0.05$.

Drugs

GI 262570 was provided by GlaxoSmithKline as a sterile solution (2 mg ml^{-1}) in a glycine-based vehicle. SB 209670 ([(+)-1S,2R,3S)-3-(carboxymethoxy-4-methoxyphenyl)-1-13,4-methylenedioxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid]) was a gift from Dr E. Ohlstein (SKB, USA). ICI 118551 ((±)-1-[2,3-(Dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride), and ZD 7155 (5,7-Diethyl-3,4-dihydro-1-[[2'-(1*H*-tetrazol-5-yl)[1,1'-biphenyl]-4-yl]methyl]-1,6-naphthyridin-2(1*H*)-one hydrochloride) were purchased from Tocris UK. Phentolamine ((2-[N-(m-Hydroxyphenyl)-p-toluidinomethyl]-imidazoline) methanesulfonate), acetylcholine chloride, methoxamine hydrochloride and L-NAME (N^G nitro-L-arginine methyl ester) were purchased from Sigma UK.

Anesthetic, analgesic and reversing agents were as follows:- fentanyl citrate (Martindale), medetomidine hydrochloride (Domitor, Pfizer), nalbuphine hydrochloride (Nubain, DuPont), atipamezole hydrochloride (Antisedan, Pfizer).

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RESULTS

Experiment 1. Regional hemodynamic responses to GI 262570

Resting cardiovascular variables for these groups of animals before treatment on Day 1 are shown in Table 1, and Figure 1 illustrates all the data as changes relative to these baseline values, throughout the experiment.

Across the 4 days of the experiment, the group of animals receiving vehicle showed no significant changes in their arterial blood pressure, heart rate or renal flow or vascular conductance (Figure 1). However, hindquarters flow and vascular conductance tended to drift downwards, and some of the reductions on day 3 were significant (Figure 1). Mesenteric flow and vascular conductance showed clear circadian variation in the vehicle-infused rats, values being highest at the beginning of the daily recording period (about 0700 h) (Figure 1). We have noted this phenomenon elsewhere (Gardiner et al., 1995) and have attributed it to a waning of post-prandial hyperaemia during the day, with the rats sleeping during the light phase, having eaten during the dark phase (1800 - 0600 h).

In the animals given GI 262570, there was an initial rise and then a delayed, but progressive, fall in mean arterial blood pressure and variable increases in heart rate over the four experimental days. For both variables, the integrated changes (areas under or over curves), were significantly different from those in animals given vehicle (Figure 1). Although animals given GI 262570 showed a fall in renal flow, the integrated change was not different from that in the vehicle-treated group, and there was no difference in renal vascular conductance between the two groups (Figure 1). The pattern of change in mesenteric flow was not different between GI 262570- and vehicle-treated groups, but the former showed relatively

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less mesenteric vasoconstriction, specifically since they showed a more marked "postprandial" mesenteric vasodilatation. Hence, the integrated changes in mesenteric vascular conductance in the two groups were significantly different (Figure 1).

The most striking effect of GI 262570 was in the hindquarters, where it caused substantial increases in flow and vascular conductance which were significantly greater than the changes seen in vehicle-treated animals (Figures 1).

Experiment 2. Effects of ICI 118551 on regional hemodynamic responses to GI 262570

Resting cardiovascular variables for these groups of animals before treatment on Day 1 are shown in Table 1, and Figure 2 illustrates all the data as changes relative to these baseline values, throughout the experiment.

Animals receiving the vehicle for ICI 118551, together with GI 262570, showed hemodynamic changes qualitatively similar to those receiving GI 262570 in Experiment 1 (Figure 2). Moreover, in those animals given ICI 118551 together with GI 262570, there was no significant effect on any of the hemodynamic actions of the latter compound, and certainly not on its hyperaemic, vasodilator action in the hindquarters (Figure 2).

Experiment 3. Effects of ZD 7155 and SB 209670, or phentolamine, on regional hemodynamic responses to GI 262570

Resting cardiovascular variables for these groups of animals before treatment on Day 1 are shown in Table 1, and Figures 3-5 illustrate all the data as changes relative to these baseline values, throughout the experiments.

Effects of GI 262570 or vehicle

As in the previous study, GI 262570 caused clear hyperaemic vasodilatation in the hindquarters together with increases in heart rate, and both these effects were significantly

greater than seen with vehicle (Figure 3). However, compared to the previous study, the hypotensive effect of GI 262570 was only slight, and not different from that of vehicle and there was no post-prandial mesenteric hyperaemia (Figure 3).

Effects of ZD 7155 + SB 209670 on responses to GI 262570

Co-administration of ZD 7155 and SB 209670 caused significant hypotension and tachycardia, accompanied by increases in renal, mesenteric and hindquarters flows, although the times of onset of these effects differed (Figure 4). However, there was dilatation in all three vascular beds during the first infusion period on the first experimental day (Figure 4). A waning of the effects of ZD 7155 and SB 209670 between experimental days (i.e., when they were not being administered) was apparent (Figure 4).

The increases in renal flow and vascular conductance seen in the presence of ZD 7155 and SB 209670 were significantly less when GI 262570 was co-administered, but the latter had an additional dilator effect in the hindquarters vascular bed (Figure 4).

Effects of phentolamine on responses to GI 262570

On initial administration, on the first experimental day, phentolamine caused a transient hypotension and a substantial tachycardia (Figure 5). These effects were accompanied by slight, transient reductions in renal and mesenteric flows and vascular conductances, but very marked increases in hindquarters flow and vascular conductance (Figure 5). The effects of phentolamine waned with repeated administration over the four experimental days (Figure 5).

In the presence of phentolamine, GI 262570 caused enhanced hypotension and tachycardia, and the integrated vasodilator changes in the hindquarters were significantly greater in the presence of phentolamine and GI 262570 than in the presence of phentolamine alone (Figure 5).

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Experiment 4. Responses to acetylcholine, methoxamine and L-NAME in the presence of GI 262570

Responses to acetylcholine

In animals given saline and the vehicle for GI 262570, there was a marked increase in renal vascular conductance at the end of a 3 min infusion of acetylcholine (+37±5, +35±4, +39±6, +48±5 % on Days 1, 2, 3 and 4, respectively); the corresponding changes in animals treated with GI 262570 were not significantly different (+44±13, +44±9, +43±8 +51±8 %, respectively).

Responses to methoxamine

On each experimental day, infusion of methoxamine had significant pressor and bradycardic effects, accompanied by renal, mesenteric and hindquarters vasoconstriction, and these effects were not influenced by GI 262570 (data not shown).

Responses to L-NAME

In animals given saline and vehicle, 10 min after injection of L-NAME, there was marked hypertension (+55±5 mmHg), bradycardia (-58±8 beats min^{-1}), and falls in renal, mesenteric, and hindquarters vascular conductances (-56±4, -72±7, -60±5 %, respectively); the corresponding changes in animals given saline and GI 262570 were not significantly different (hypertension (+51±2 mmHg), bradycardia (-87±9 beats min^{-1}), falls in renal, mesenteric and hindquarters vascular conductances (-52±5, -67±1, -52±3 %, respectively)).

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DISCUSSION

The degree to which the cardiovascular actions of thiazolidinediones involve changes in insulin sensitivity is unresolved, as is the possibility that some of the effects of these drugs are independent of PPAR-γ (see Introduction).

In the light of hepatic effects seen with troglitazone, a series of chiral, non-racemate, N-(2-benzoylphenyl)-L-tyrosine PPAR-γ agonists have been synthesized (Henke et al., 1998). They are more potent than troglitazone, and hence may show greater therapeutic effect in type 2 diabetes mellitus (Henke et al., 1998). However, nothing is known of the putative regional hemodynamic effects of these compounds in the non-diabetic state. Therefore, the first objective of the present work was to assess the regional hemodynamic changes occurring in conscious, chronically-instrumented rats treated over 4 days with the N-(2-benzoylphenyl)-L-tyrosine PPAR-γ ligand, GI 262570 (Henke et al., 1998), or its vehicle.

Our first experiment demonstrated clearly that GI 262570 selectively increased hindquarters flow (increased skeletal muscle blood flow) and vascular conductance progressively over a 4-day, intermittent infusion protocol. The magnitude of this effect, and its time course, were such as to fit with the proposition that the previously observed effects of GI 262570 on cardiac output and total peripheral conductance (Callahan et al., 2002) were attributable to the hindquarters hyperaemic action of the drug.

The selectivity of the vasodilator action of GI 262570 for the hindquarters vascular bed raised the possibility that β_2-adrenoceptor activation contributed to this effect since we have shown marked vasodilator responses to β_2-adrenoceptor agonists in the hindquarters vascular bed (Gardiner and Bennett, 1988; Gardiner et al., 1991a), and adrenomedullary adrenaline release would be expected to activate this process particularly effectively (Gardiner et al., 1992).

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For this reason, in the second experiment, we assessed the effects of the β_2-adrenoceptor antagonist, ICI 118551, on the hindquarters vasodilator effects of GI 262570. Those experiments showed very clearly that ICI 118551 was entirely without inhibitory effect on the hindquarters vasodilator action of GI 262570, consistent with no role of β_2-adrenoceptors in this phenomenon. Moreover, these observations indicated that a putative vasodilator effect of insulin, mediated through β-adrenoceptors (Creager et al., 1985), possibly as a result of insulin-mediated sensitization of adenylyl cyclase activation (e.g., Feldman, 1993) did not contribute to the hindquarters vasodilator action of GI 262570. Nonetheless, the apparent selectivity of vasodilator effect of GI 262570 in the hindquarters echoes the insulin-mediated glucose uptake which occurs principally in skeletal muscle, (see Baron (1994) for review). However, as Yki-Järvinen & Utriainen (1998) have pointed out, physiological levels of insulin which stimulate muscle glucose uptake substantially, have little effect on skeletal muscle blood flow, consistent with the former event being independent of the latter, although changes in microvascular hemodynamics might be important (Vincent et al., 2000).

The findings above raised the possibility that the apparent selectivity of the vasodilator action of GI 262570 for the hindquarters was due to the drug-induced changes in hemodynamics evoking compensatory activation of vasoconstrictor mechanisms (renin-angiotensin, sympathetic) which offset any direct GI 262570-induced vasodilatation in the renal and mesenteric vascular beds. In addition, it is feasible that endothelin could have contributed to such an effect, since GI 262570, which increases insulin sensitivity, could thereby stimulate endothelin release (Verma et al., 2001; Misurski et al., 2001), although there is also evidence that PPAR γ activators inhibit endothelin release from endothelial cells (Satoh et al., 1999; Delerive et al., 1999; Fukunaga et al., 2001).

For these reasons, the third experiment was designed to assess effects of GI 262570 in the absence and presence of antagonists of AT_1-, and ET_A- and ET_B-receptors (with ZD 7155 and

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SB 209670, respectively), or of antagonism of α_1- and α_2-adrenoceptors (with phentolamine), to detect any signs of unmasking of renal and/or mesenteric vasodilator actions of the drug and, at the same time, to determine if GI 262570 exerted greater or lesser hindquarters vasodilator effects in the presence of the antagonists.

In the presence of ZD 7155 and SB 209670 there was no unmasking of a renal or mesenteric vasodilator effect of GI 262570. Indeed, the latter appeared to suppress the increases in renal flow and vascular conductance caused by co-administration of ZD 7155 and SB 209670. It is feasible that this was due to GI 262570 causing renal 'steal' through further increase in hindquarters flow and vascular conductance (see below), although this phenomenon was not apparent on the first experimental day, when the suppression of the ZD 7155 and SB 209670-induced increases in renal flow and vascular conductance were present (Figure 4). Since co-administration of ZD 7155 and SB 209670 did not augment the vasodilator effect of GI 262570, we did not consider it necessary to examine the effects of either antagonist alone. However, we acknowledge that, with the use of selective antagonists of ET_A and ET_B receptors, one could gain more information regarding a possible vascular effect of endothelin.

While there is some evidence that glitazones can inhibit Ca^{2+} channels and thereby suppress the action of vasoconstrictors (e.g., Buchanan et al., 1995), the observation that additional hypotensive and hindquarters vasodilator effects of GI 262570 were seen in the presence of ZD 7155 and SB 209670 indicates that these effects normally do not depend upon inhibition of the constrictor actions of AII and/or endothelin.

We noted less marked recovery in blood pressure following co-administration of ZD 7155 and SB 209670 in the presence of GI 262570, compared to that in its absence (see Figures 3

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and 4 at the start of each experimental day). Considering the long duration of action of the AT_1-receptor antagonist used (Junggren et al., 1996), it is likely that the recovery seen in the vehicle-treated animals was due to a waning of the action of SB 209670. Therefore, these data could indicate a modulation by GI 262570 of either endothelin's action, or the pharmacokinetics of SB 209670.

The dramatic hemodynamic effect of phentolamine administration alone is consistent with previous studies from our laboratory, in which we have shown a substantial component of the hindquarters vasodilatation to be dependent on activation of β_2-adrenoceptors (Gardiner and Bennett, 1988; Woolard et al., 2004). It was interesting to see here, that with repeated phentolamine administration, the degree of hindquarters vasodilatation, but not the tachycardia, waned.

In the presence of phentolamine, GI 262570 still caused additional hindquarters vasodilatation; moreover, GI 262570 did not modify vasoconstrictor responses to methoxamine. These findings are consistent with the hindquarters vasodilator effects of GI 262570 being due to something other than inhibition of α-adrenoceptor-mediated tone. Furthermore, as in the case of the experiments performed in the presence of ZD 7155 and SB 209670, there was no renal or mesenteric vasodilator action of GI 262570 unmasked in the presence of phentolamine. Thus, collectively, the results indicate that the vasodilator effect of GI 262570 is specific for the hindquarters (of the vascular beds monitored) and is largely independent of angiotensin (AT_1) and endothelin (ET_A and ET_B)- receptors, and α_1- and α_2-adrenoceptors.

We know from previous studies that the renal vasodilator effect of acetylcholine infused over 3 min (as in the present study) is inhibited by the NO synthase inhibitor, L-NAME, and is therefore likely to be due to stimulated release of NO (Gardiner et al., 1991b). The lack of effect of GI 262570 on either acetylcholine-induced renal vasodilatation or L-NAME-induced

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hemodynamic changes thus suggests that the compound did not influence either stimulated or basal release of NO. This is somewhat surprising, considering that insulin increases vascular NO production, although such an effect would not be expected to have been confined to the hindquarters (Hilzenrat et al., 2001). But, while an NO-dependent component of the vasodilator action of insulin is widely acknowledged (see Hsueh and Law (1999) for review, Bursztyn et al., 2000; Molinari et al., 2001), there is evidence that the vasodilator action of thiazolidenediones on rat coronary vessels (Uchida et al., 2000), and the human forearm (Fujishima et al., 1998), are NO-independent. In fact, there is evidence that PPAR-γ ligands may suppress iNOS expression while increasing heme-oxygenase-1 expression in rat glial cells (Kitamura et al., 1999). Thus, one could hypothesise that carbon monoxide may contribute to the vasodilator action of compounds such as GI 262570. A very recent study (Yang et al., 2003) reported that 10 days treatment with GI 262570 produced significant elevation of plasma and skeletal muscle nitrate and nitrite levels, but these data are not inconsistent with our findings since no significant changes in the measured indices of NO activity were seen after 4 days of treatment (Yang et al., 2003).

In conclusion, although PPARγ has been localised to cardiovascular tissues, our data showing marked differences in regional hemodynamic effects of GI 262570, and the lack of effect thereupon of pharmacological interventions, perhaps make it unlikely that the compound exerts a primary vascular action. We acknowledge the fact that our study is limited to the normal state, and that effects in pathological conditions, such as diabetes and hypertension, may differ from those described here.

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Footnotes

This study was undertaken with financial support from GlaxoSmithKline

Some of the results reported herein have been presented to the British Pharmacological Society (*Br J Pharmacol* (2002) 137: 29P).

Reprint requests to:-

Professor Sheila M Gardiner

School of Biomedical Sciences

University of Nottingham Medical School

Queen's Medical Centre

Nottingham NG7 2UH, UK

[1] Centre for Integrated Systems Biology & Medicine, School of Biomedical Sciences,

University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH

[2] GlaxoSmithKline, Research Triangle Park, North Carolina, USA

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Figure 1:

Cardiovascular responses (mean ± SE) to vehicle (open circles, n=9) or GI 262570 (closed circles, n=9). Infusions of GI 262570 (2 mg ml^{-1}) or glycine-based vehicle were given twice daily for 2h on four experimental days. On each experimental day, data were recorded continuously for about 10 h. For measurement purposes, the experimental day was divided into five blocks, i.e., (i) the initial 30-45 min period, (ii) the first 2 h infusion period, (iii) the interval of about 3-4 h between the infusions, (iv) the second infusion period, and (v) the terminal recording period of about 1 h. The first measurement period on Day 1 was taken as the original baseline. *P < 0.05 versus original baseline (Friedman's test), # P < 0.05 for integrated responses (areas under or over curves) (Mann-Whitney U test).

Figure 2:

Cardiovascular effects (mean ± SE) of GI 262570 in the absence (closed circles, n=8) and presence (closed triangles, n=8) of β_2-adrenoceptor antagonism with ICI 118551. See Figure 1 for explanation of measurement periods. *P < 0.05 versus original baseline (Friedman's test).

Figure 3:

Cardiovascular responses (mean ±SE) to vehicle (open circles, n=8) or GI 262570 (closed circles, n=8) given in the presence of saline. See Figure 1 for explanation of measurement periods. *P < 0.05 versus original baseline (Friedman's test), # P < 0.05 for integrated responses (areas under or over curves) (Mann-Whitney U test).

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Figure 4:

Cardiovascular responses (mean ± SE) to vehicle (open circles, n=8) or GI 262570 (closed circles, n=8) given in the presence of angiotensin (AT_1) receptor antagonism (with ZD 7155) and endothelin (ET_A and ET_B) receptor antagonism (with SB 209670). See Figure 1 for explanation of measurement periods. *P < 0.05 versus original baseline (Friedman's test), # P < 0.05 for integrated responses (areas under or over curves) (Mann-Whitney U test).

Figure 5:

Cardiovascular responses (mean ±SE) to vehicle (open circles, n=8) or GI 262570 (closed circles, n=8) given in the presence of α-adrenoceptor antagonism (with phentolamine). See Figure 1 for explanation of measurement periods. *P < 0.05 versus original baseline (Friedman's test), # P < 0.05 for integrated responses (areas under or over curves) (Mann-Whitney U test).

Table 1. Resting cardiovascular variables (mean ± SEM) on Day 1 before any intervention. Units for vascular conductance (VC) are [kHz mm Hg^{-1}]10^3. DS=Doppler shift.

	A	B	C	D	E	F	G	H	J	K
Heart rate (beats min^{-1})	336±6	338±7	342±6	328±9	344±9	355±16	343±8	358±9	346±5	355±12
Mean blood pressure (mmHg)	100±2	103±2	110±4	108±4	104±3	101±3	103±2	100±2	102±3	101±2
Renal DS (kHz)	11.5±1.0	8.9±0.8	9.8±0.8	10.6±1.1	11.2±1.2	8.7±0.9	8.9±0.5	10.4±0.9	9.6±0.7	8.9±0.7
Mesenteric DS (kHz)	13.1±0.6	10.0±0.8	9.6±1.3	10.1±0.8	13.4±0.6	11.9±1.0	11.6±0.7	11.4±0.9	10.5±0.7	11.2±0.9
Hindquarters DS (kHz)	4.9±0.5	4.1±0.3	3.9±0.4	4.2±0.3	4.7±0.8	4.0±0.3	4.5±0.5	4.5±0.4	4.5±0.3	4.0±0.2
Renal VC (units)	115±10	86±7	89±8	98±8	107±8	86±9	87±4	105±8	95±7	88±5
Mesenteric VC (units)	133±7	98±9	90±14	94±8	131±8	118±9	113±6	115±10	105±9	112±9
Hindquarters VC (units)	49±5	40±3	36±4	40±4	46±9	41±4	44±6	45±5	44±3	40±3

Experiment 1. Vehicle (Group A), GI 262570 (Group B)
Experiment 2. GI 262570 + vehicle (Group C), GI 262570 + ICI 118551 (Group D)
Experiment 3. Vehicle + saline (Group E), GI 262570 + saline (Group F)
Vehicle + ZD 7155 + SB 209670 (Group G), GI 262570 + ZD 7155 + SB 209670 (Group H)
Vehicle + phentolamine (Group J), GI 262570 + phentolamine (Group K)

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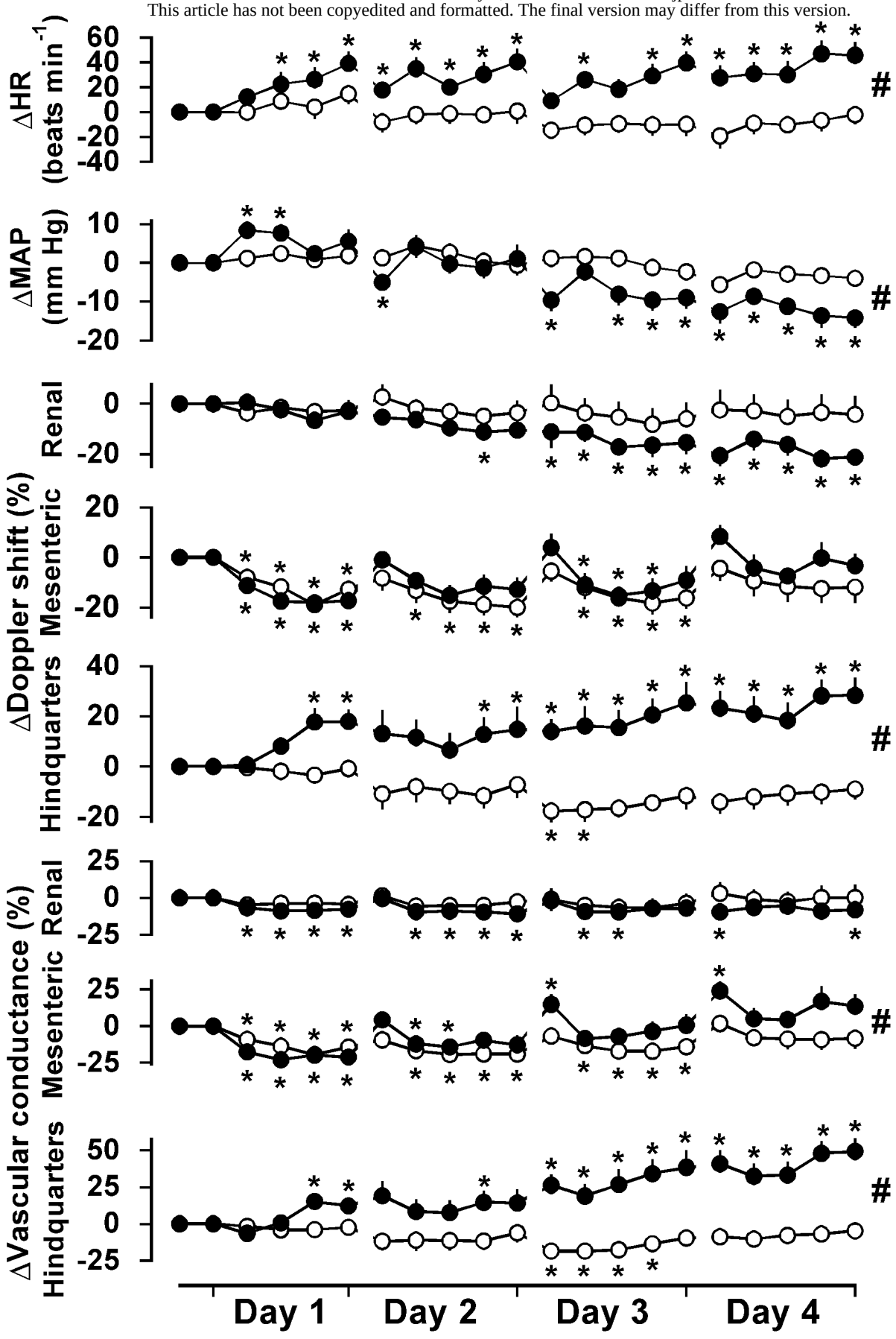

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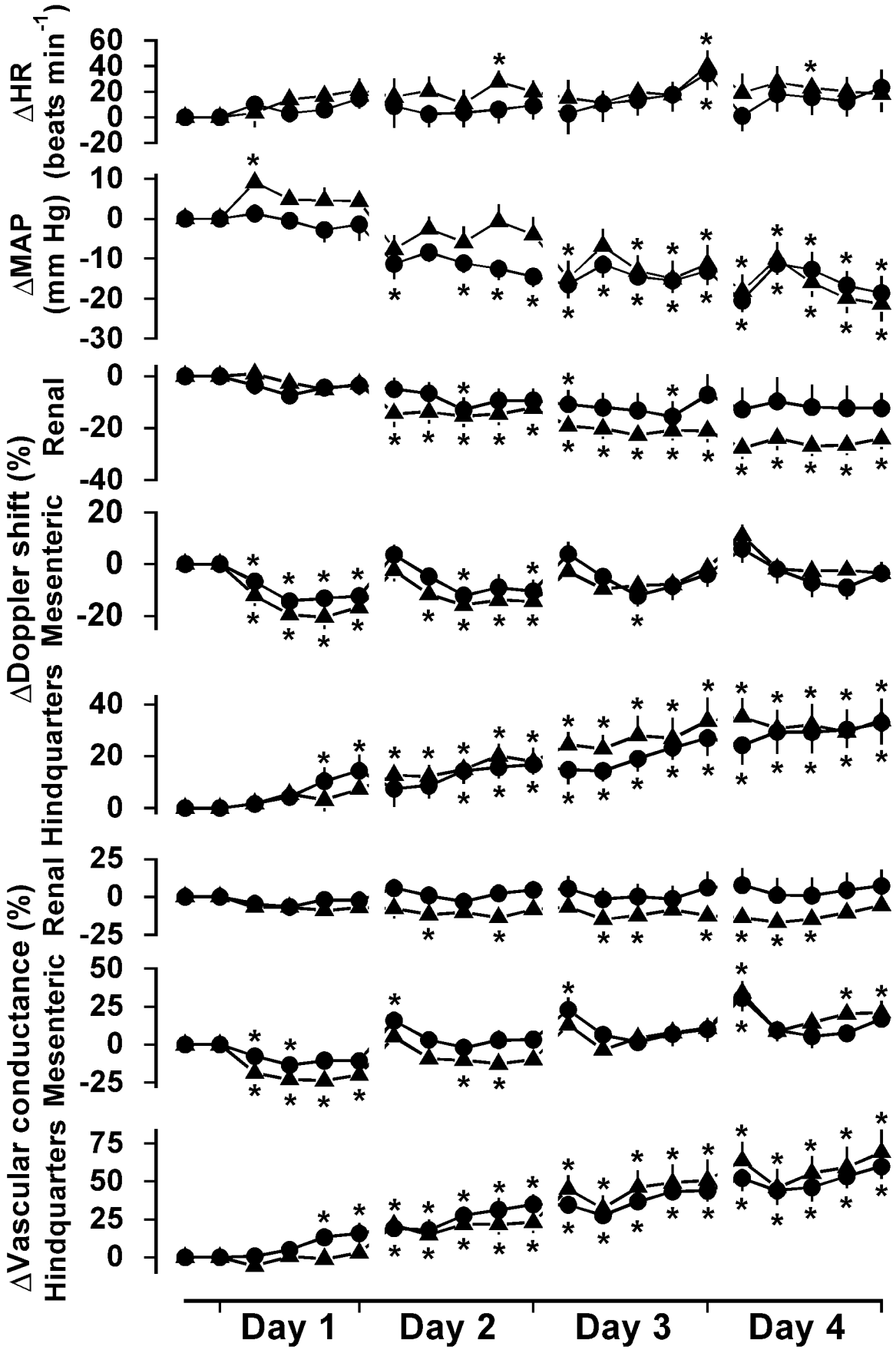

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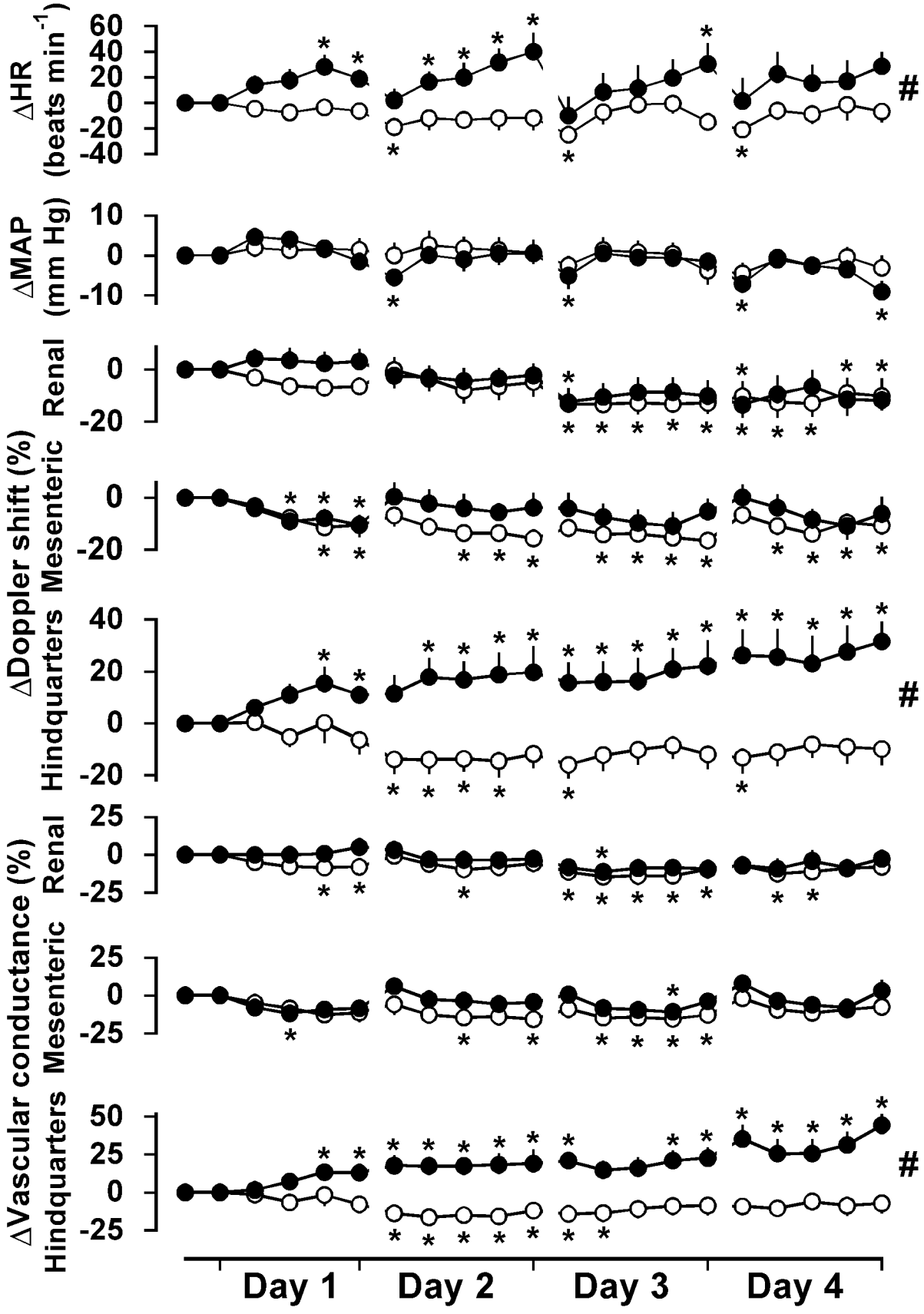

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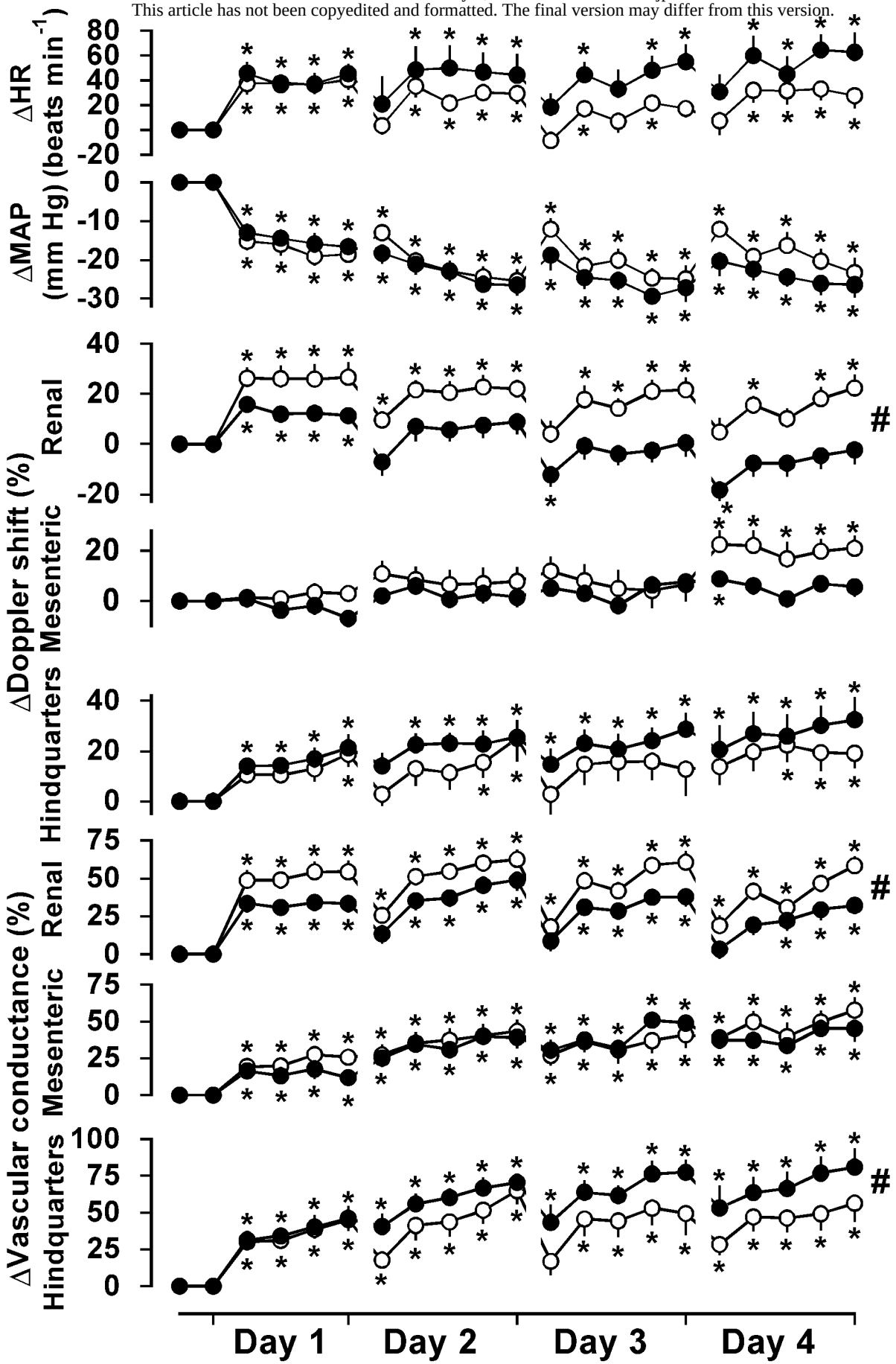

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