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RESEARCH ARTICLE | *Vascular Biology and Microcirculation*

Persistent insulin signaling coupled with restricted PI3K activation causes insulin-induced vasoconstriction

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Olver TD, Grunewald ZI, Ghiarone T, Restaino RM, Sales AR, Park LK, Thorne PK, Ganga RR, Emter CA, Lemon PW, Shoemaker JK, Manrique-Acevedo C, Martinez-Lemus LA, Padilla J. Persistent insulin signaling coupled with restricted PI3K activation causes insulin-induced vasoconstriction. *Am J Physiol Heart Circ Physiol* 317: H1166–H1172, 2019. First published October 11, 2019; doi:10.1152/ajpheart.00464.2019.—Insulin modulates vasomotor tone through vasodilator and vasoconstrictor signaling pathways. The purpose of the present work was to determine whether insulin-stimulated vasoconstriction is a pathophysiological phenomenon that can result from a combination of persistent insulin signaling, suppressed phosphatidylinositol-3 kinase (PI3K) activation, and an ensuing relative increase in MAPK/endothelin-1 (ET-1) activity. First, we examined previously published work from our group where we assessed changes in lower-limb blood flow in response to an oral glucose tolerance test (endogenous insulin stimulation) in lean and obese subjects. The new analyses showed that the peak rise in vascular resistance during the postprandial state was greater in obese compared with lean subjects. We next extended on these findings by demonstrating that insulin-induced vasoconstriction in isolated resistance arteries from obese subjects was attenuated with ET-1 receptor antagonism, thus implicating ET-1 signaling in this constriction response. Last, we examined in isolated resistance arteries from pigs the dual roles of persistent insulin signaling and blunted PI3K activation in modulating vasomotor responses to insulin. We found that prolonged insulin stimulation did not alter vasomotor responses to insulin when insulin-signaling pathways remained unrestricted. However, prolonged insulinization along with pharmacological suppression of PI3K activity resulted in insulin-induced vasoconstriction, rather than vasodilation. Notably, such aberrant vascular response was rescued with either MAPK inhibition or ET-1 receptor antagonism. In summary, we demonstrate that insulin-induced vasoconstriction is a pathophysiological phenomenon that can be recapitulated when sustained insulin signaling is coupled with depressed PI3K activation and the concomitant relative increase in MAPK/ET-1 activity.

NEW & NOTEWORTHY This study reveals that insulin-induced vasoconstriction is a pathophysiological phenomenon. We also provide evidence that in the setting of persistent insulin signaling, impaired phosphatidylinositol-3 kinase activation appears to be a requisite feature precipitating MAPK/endothelin 1-dependent insulin-induced vasoconstriction.

diabetes; endothelin-1; MAPK; obesity; selective insulin resistance

INTRODUCTION

Insulin is a vasoactive hormone that stimulates vasodilation across mammalian species (1, 3, 5, 12, 19, 26, 33, 45, 48). The vasodilator actions of insulin contribute to insulin delivery, glucose disposal and glycemic control (4, 5, 18). At the endothelial cell level, insulin activates two primary signaling cascades, the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K)/protein kinase B (Akt) pathway and the Ras/mitogen-activated protein kinase (MAPK) pathway. Activation of the PI3K/Akt pathway stimulates production of the vasodilator nitric oxide (NO), whereas activation of the MAPK pathway stimulates production of the vasoconstrictor endothelin-1 (ET-1). The net effect of insulin on vascular tone is influenced by the activation of these two signaling cascades (22, 31).

Obesity and insulin resistance are associated with impaired insulin-stimulated vasodilation (10, 23, 24, 33, 34, 36, 38, 39, 41, 42) and limited delivery of insulin and glucose to the target organs (4–6, 18). Although it is less frequently observed, insulin-stimulated vasoconstriction has also been reported in obese rats and humans (13, 16). A primary mechanism implicated in this pathological adaptation is the discriminatory imbalance in endothelial insulin signaling, portrayed by a decrease in PI3K/Akt with either no change or heightened MAPK/ET-1 signaling (6, 12, 13, 21, 22, 29, 31, 36, 39, 41).

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This imbalance has been termed "selective insulin resistance" (22, 52). Hyperinsulinemia develops as a compensatory mechanism to offset insulin resistance, but because activation of MAPK remains intact, chronic insulin signaling can have deleterious vascular consequences. It is possible that the functional interplay between basal hyperinsulinemia and selective insulin resistance underlies insulin-induced vasoconstriction in obesity. Thus, the purpose of the present work was to determine whether insulin-induced vasoconstriction is a pathophysiological phenomenon that can result from a combination of persistent insulin signaling, suppressed PI3K activation, and an ensuing relative increase in MAPK/ET-1 activity. First, we tested the hypothesis that peripheral vasoconstriction during endogenous insulin stimulation occurs in the setting of obesity. To extend on these findings, we next determined if ET-1 signaling is implicated in insulin-induced vasoconstriction in isolated arteries from obese subjects undergoing abdominal surgery. Lastly, to understand the dual roles of persistent insulin signaling and blunted PI3K activation in modulating vasomotor responses to insulin, we performed a series of functional experiments in isolated arteries from pigs. Specifically, we tested the hypothesis that prior insulinization coupled with diminished PI3K activity evokes insulin-induced vasoconstriction through a relative enhancement of the countercurrent MAPK/ET-1 pathway.

METHODS

Human Studies

All human study procedures conformed to the Declaration of Helsinki and were approved by the Institutional Review Board at The University of Western Ontario or University of Missouri. All subjects provided written, informed consent before participation in the study.

In vivo experiment. Hemodynamic data from a previously published study from our group were analyzed retrospectively for determination of peak increases in lower-limb vascular resistance during an oral glucose tolerance test (OGTT, endogenous insulin stimulation). Briefly, as previously described here (34), beat-by-beat noninvasive blood pressure was monitored from the left middle finger by photoplethysmographic methods, and brachial blood pressure was estimated using waveform reconstruction and regression equations (Finometer Model 1, Finapress Medical Systems). Additionally, superficial femoral artery blood flow was measured at the midpoint between the patella and iliac crest using Doppler ultrasound (4.7–10 MHz linear array probe; System 5; GE/Vingmed). Hemodynamics were studied at baseline and every 30 min for 2 h during the OGTT in lean ($n = 8$ women; age = 24 ± 1 yr, BMI = 21 ± 0 kg/m^2, fasting blood glucose = 4.9 ± 0.1 mmol/L) and obese ($n = 8$ women; age = 24 ± 1 yr, BMI = 30 ± 2 kg/m^2, fasting blood glucose = 5.1 ± 0.2 mmol/L) subjects. The Matsuda index of insulin sensitivity was calculated (28). In the original study, lower-limb blood flow and vascular conductance responses were reported at each time point, and in the present article hemodynamic variables are displayed at baseline and at peak vascular resistance. Vascular resistance was calculated as the quotient of mean arterial pressure and arterial blood flow (40). This analysis provided evidence that endogenous insulin stimulation can induce vasoconstriction in the setting of obesity.

Ex vivo experiments. To expand on the above observations, we examined whether the vasoconstrictor properties of insulin in obesity relate to ET-1 signaling. This was accomplished by assessing the effect of ET-1 receptor blockade in isolated omental adipose tissue resistance arteries from obese subjects displaying insulin-induced vasoconstriction. Arteries from six obese subjects that underwent abdominal surgery at the University of Missouri Hospital were included in this analysis. Determination of type 2 diabetes and hypertension was made by a physician, and this information was obtained from the medical history. Briefly, isolated resistance arteries were mounted on 40-μm stainless steel wires in oxygenated physiological saline solution (PSS, 95% O_2-5% CO_2) in a small vessel wire myograph for isometric tension recording (Danish Myo Technology, Aarhus, Denmark), as previously described (7, 30). After vessels were warmed to 37°C and equilibration, normalization was performed (43), and vessels were stretched to achieve an internal circumference corresponding to a transmural pressure of 70 mmHg. The viability of each artery was assessed by exposure to 60 mM KCl for 5 min. Thereafter, arteries remained untreated or were incubated with an ET-1 receptor antagonist (bosentan,10 μM; Product HY-A0013; MedChemExpress, Monmouth Junction, NJ) for 30 min, before being preconstricted with 60 mM KCl and undergoing a dose-response curve to insulin (whole log doses; $1e^{-9}$–$1e^{-6}$ M). Vasomotor responses were expressed as percent vasoconstriction relative to preconstriction. The area under the curve (net incremental; AUC) was calculated to approximate the net contraction response for each experimental condition.

Studies in Pig-Isolated Arteries and Western Blot Analysis Experiments

The animal protocol conformed to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (2011) and was approved by the University of Missouri Animal Care and Use Committee. Female farm pigs ($n = 14$, age = 3 ± 0 mo, mass = 29 ± 1 kg) were housed under temperature-controlled conditions, with a 12-h: 12-h light-dark cycle and consumed a standard commercially available chow diet (5L80, Laboratory Diet; 2.98 kcal/g; 70% carbohydrate, 21% protein, and 9% fat). Pigs were anesthetized with telozol (5 mg/kg im)-xylazine (2.25 mg/kg im), followed by 5% inhaled isoflurane for 20 min, and then euthanized by removal of the heart or exsanguination. The brachial arteries and lateral head of the triceps were then harvested for ex vivo experiments. Experiments were designed to study the dual roles of persistent insulin stimulation and blunted PI3K activation in modulating vasomotor responses to insulin. We used wortmannin, a PI3K inhibitor, to recapitulate the phenotype of selective insulin resistance (i.e., to produce an imbalance between Akt and MAPK signaling). This imbalance in insulin signaling was verified by measuring activation of Akt and MAPK via Western blot analysis in whole artery segments (from the brachial artery), as well as in cultured endothelial cells, i.e., human umbilical vein endothelial cells (HUVECs; experimental details in figure legend). Resistance arteries from the triceps were used for vasomotor function experiments. Briefly, arteries were dissected from the muscle, transferred to a plexiglass chamber filled with PSS and cannulated with two glass micropipettes (60–75 μm) filled with PSS (with 10 g/L albumin added). The chambers were transferred to the stage of an inverted microscope (Nikon Diaphot 200) attached to a video camera (Javelin Electronics, Los Angeles, CA), video micrometer (Microcirculation Research Inst., Texas A&M University) and a Powerlab data acquisition system (ADInstruments, Colorado Springs, CO), as previously described (32, 33, 35, 39, 51). Fluid-filled reservoirs were used to set intraluminal pressure at 60 mmHg, and luminal diameter was monitored throughout the experiment. Arteries were allotted 30 min to stabilize, at which point maximal arterial vasoconstriction in response to 80 mM KCl was determined. Thirty minutes later, arteries were incubated for 3 h with versus without insulin (10 nM) in the presence or absence of the PI3K inhibitor wortmannin (100 nM, Cat. No.: 19545-26-7, Sigma-Aldrich; 2% DMSO used as vehicle control) (25). Thereafter, arteries were washed and wortmannin or vehicle control, but not insulin, were readded to the bath. Arteries were then preconstricted with a thromboxane A_2 analog (U-46619, $1e^{-7}$–$1e^{-4}$ M to achieve 20–40% tone) and underwent a dose-response curve for insulin (whole log doses; $1e^{-9}$–$1e^{-6}$ M). While plasma insulin during the OGTT was elevated by ~10-fold and almost reached the nanomolar range in

obese subjects, it should be acknowledged that the insulin concentration used for these ex vivo vasoreactivity studies ranged from high physiological (i.e., 1 nM) to supraphysiological levels (i.e., 1 μM). After the experiment, all vessels were washed twice with Ca^{2+} free PSS to determine maximal passive diameter. Vasomotor responses were expressed as percent vasodilation (i.e., the quotient of Δ diameter - baseline diameter and Δ maximal Ca^{2+} free diameter - baseline diameter, multiplied by 100) or vasoconstriction (quotient of Δdiameter - baseline diameter and baseline diameter). The area under (or over) the curve (net incremental; AUC) was calculated to approximate the net dilatory or constrictor response for each experimental condition (17, 38).

A subsequent experimental series was conducted to examine the role of MAPK and ET-1 in mediating insulin-induced constriction. To accomplish this, additional arteries were harvested from the pig triceps and underwent the same preparatory steps as described above. Briefly, arteries incubated with insulin and the PI3K inhibitor wortmannin for 3 h were cotreated with versus without a MAPK inhibitor (PD98059, 50 μM; Cat No.: 167869-21-8, Cell Signal) (14) or an ET-1 receptor inhibitor (tezosentan, 3 μM) (10). Thereafter, arteries were washed and all drugs except insulin were readded to the bath. Arteries were then preconstricted and underwent a dose-response curve for insulin, as described above. The sample size for each experiment is indicated in the figure legend. Vessel characteristics for all isolated pig forelimb arteries are as follows: average KCl-induced vasoconstriction = 72 ± 3%, average passive lumen diameter = 124 ± 4 μm, and average wall thickness = 23 ± 1 μm.

Western blot analysis. Triton X-100 cell and tissue lysates were prepared in Laemmli buffer. Protein samples (6 μg/lane) were separated via Criterion Tris-Glycine eXtended-PAGE precast gels (Bio-Rad). Proteins were next transferred onto polyvinylidene difluoride membranes and blocked with 5% nonfat dry milk or BSA. Membranes were probed for total p44/42 MAPK (1:500; Cat. No. 4695, Cell Signaling) and phosphorylated p44/42 $MAPK^{(Thr202/Tyr204)}$ (1:250, Cat. No. 4370, Cell Signaling) as well as total Akt (1:500; Cat. No.4691, Cell Signaling) and phosphorylated $Akt^{(Ser473)}$ (1:250; No. 4060, Cell Signaling). Molecular weights of protein bands were confirmed by comparison to a visual ladder. Intensity of individual protein bands was quantified via densitometry using the Bio-Rad ChemiDoc XRS+ System (Bio-Rad, Hercules, CA) and Image Laboratory Software (version 6.0.1) and expressed as the ratio of phosphorylated to total Akt and MAPK.

Statistical Analyses

For human in vivo data, hemodynamics and blood parameters were analyzed using a mixed model repeated measures ANOVA with a prior comparisons for baseline and peak vascular resistance. Peak rise in vascular resistance (relative to baseline) between groups was analyzed by unpaired two-tailed *t*-test. Experiments in isolated arteries from humans with obesity were analyzed using a paired *t*-test (untreated vs. ET-1 receptor antagonism conditions). For Western blot analysis data and functional experiments in pig isolated arteries, protein expression and AUC were analyzed using ANOVA. Pairwise comparisons were performed using a post hoc Student-Newman-Keul test. The significance level was set at $P < 0.05$. Data are presented as means ± SE.

RESULTS

Human Studies

For the original description of these data, see Olver and colleagues (34). New data from the current analyses show that during the OGTT, the peak increase in lower-limb vascular resistance was greater in obese versus lean subjects ($P < 0.05$; Fig. 1*A*). There were no differences in basal MAP or lower-limb blood flow ($P \geq 0.88$). Relative to baseline, at peak vascular resistance during the OGTT, increases in MAP approached significance (main effect of time point, $P = 0.09$) and reductions in lower-limb blood flow approached significance in the obese group only ($P = 0.09$). As also displayed in Fig. 1*A*, the OGTT elicited an increase in blood glucose and insulin concentrations in both groups ($P < 0.01$). Fasting and postprandial blood glucose at peak vascular resistance were similar between groups ($P \geq 0.15$). Fasting plasma insulin was similar between groups ($P = 0.76$), but postprandial plasma insulin at peak vascular resistance was greater in the obese group ($P = 0.02$). The Matsuda index of insulin sensitivity was not different between groups (lean = 8.6 ± 0.9 vs. obese = 6.2 ± 1.5; $P = 0.18$). In isolated omental arteries from obese subjects undergoing abdominal surgery, insulin-induced vasoconstriction was attenuated with ET-1 receptor antagonism ($P < 0.05$; Fig. 1*B*). Participant characteristics for the obese subjects undergoing abdominal surgery are presented in Table 1.

Studies in Pig-Isolated Arteries and Western Blot Analysis Experiments

As intended, in HUVECs and isolated pig arterial segments, PI3K inhibition using wortmannin attenuated Akt activation ($P < 0.05$) without altering MAPK activation ($P \geq 0.68$; Fig. 2, *B* and *F*). Accordingly, exposure of pig resistance arteries to PI3K inhibition for 3 h blunted insulin-induced vasodilation ($P < 0.05$; Fig. 2*C*). Similarly, in HUVECs as well as in

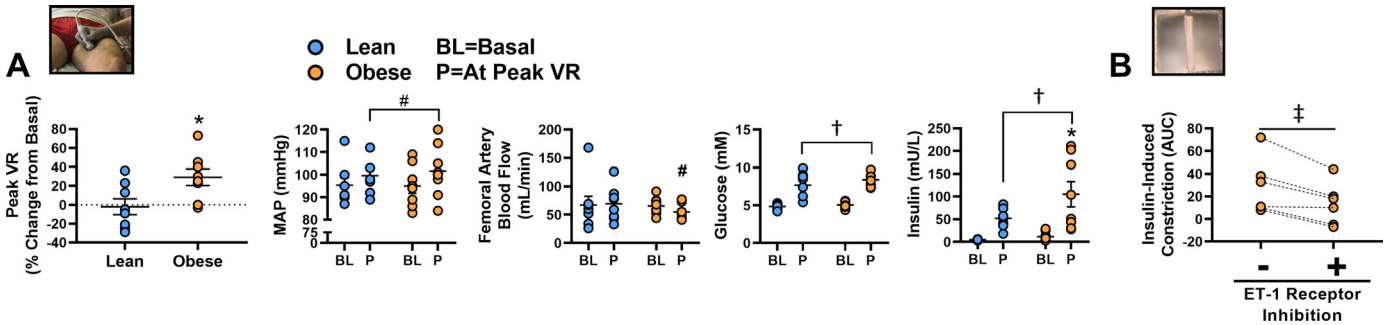

Fig. 1. Insulin-induced vasoconstriction in obesity and the role of endothelin-1 (ET-1) signaling. *A*: peak lower-limb vascular resistance (VR) during a 2-h oral glucose tolerance test (OGTT; 75 g glucose load) in lean ($n = 8$) and obese ($n = 8$) subjects. Plasma glucose and insulin, mean arterial pressure (MAP), and femoral artery blood flow at baseline and at peak VR are also presented; individual responses are displayed. *B*: ET-1 receptor inhibitor (bosentan, 10 μM) attenuates insulin-induced vasoconstriction in isolated omental adipose tissue resistance arteries from obese subjects ($n = 6$; individual responses presented). AUR, area under curve. *$P < 0.05$, difference between groups; #$P = 0.09$, trend for effect of time point; †$P < 0.05$, effect of time point; ‡$P < 0.05$, effect of ET-1 receptor inhibition. Data are expressed as means ± SE.

Table 1. *Patient characteristics*

Sex (women/men)	5/1
Age, yr	56 ± 8
Diabetes (yes/no)	4/2
Hypertension (yes/no)	5/1
Body mass index, kg/m^2	49 ± 3

Values of age and body mass index are means + SE.

isolated pig arterial segments that were insulin-treated, PI3K inhibition blunted Akt activation ($P < 0.05$) without disrupting MAPK activation ($P \geq 0.52$; Fig. 2, *D* and *F*). While insulin stimulation increased phosphorylation of Akt, it also attenuated total Akt in pig arterial segments ($P < 0.05$). Expression of the housekeeping protein vinculin was similar across conditions ($P = 0.89$). Notably, while 3 h of insulin stimulation alone did not alter vasomotor function in response to insulin ($P = 0.20$), the combined exposure of insulin and PI3K inhibition promoted insulin-stimulated vasoconstriction ($P < 0.05$; Fig. 2*E*). The vasoconstrictor effect of insulin stimulation combined with PI3K inhibition was abolished by MAPK inhibition as well as by ET-1 receptor antagonism ($P < 0.05$; Fig. 3). Collectively, these data indicate that when PI3K signaling is interrupted, prolonged insulin stimulation results in MAPK/ET-1-dependent insulin-induced vasoconstriction.

DISCUSSION

Findings from the present investigation support the hypothesis that insulin-induced vasoconstriction is a pathophysiological phenomenon that may result from simultaneous insulin stimulation and suppression of PI3K signaling (i.e., selective

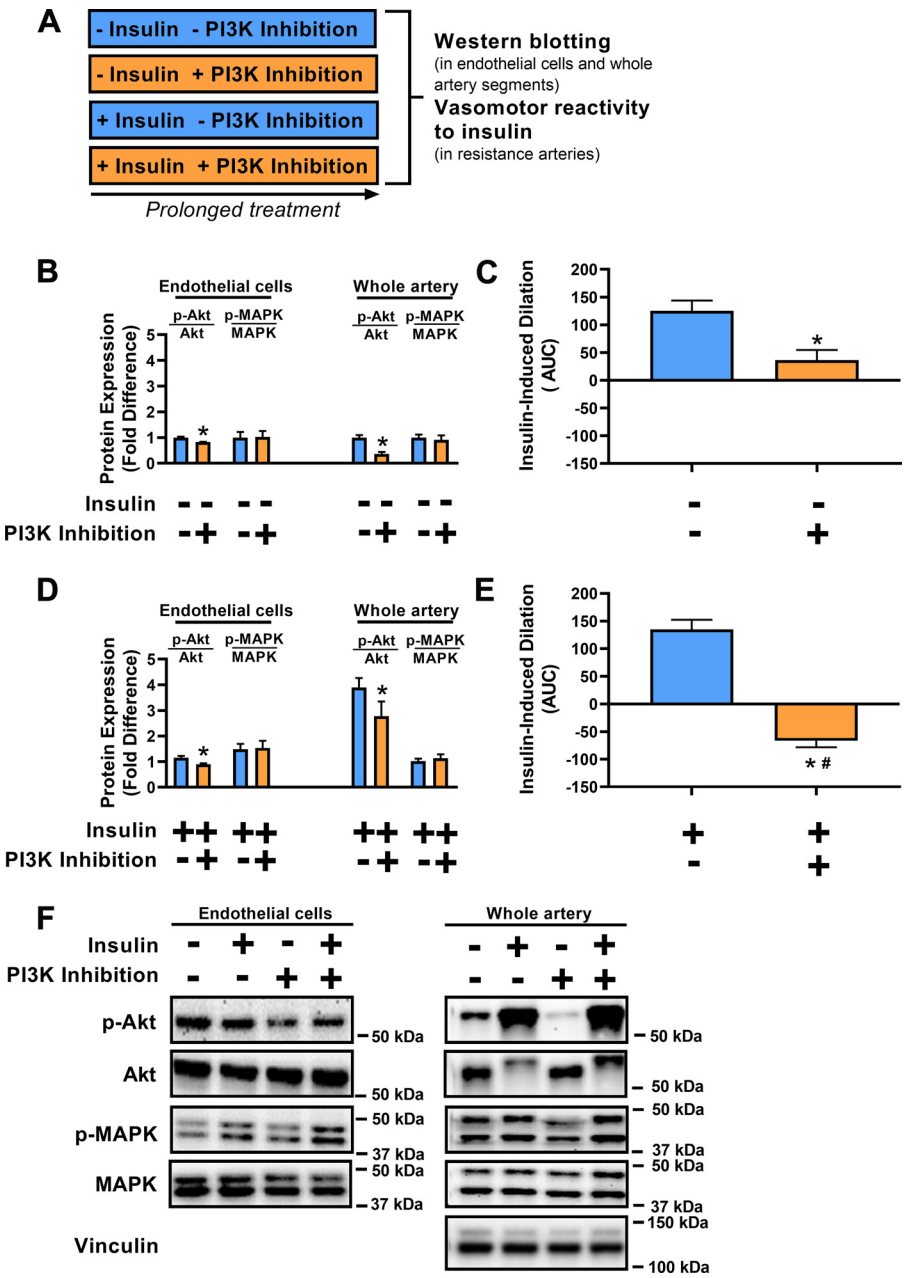

Fig. 2. Persistent insulin signaling coupled with restricted phosphatidylinositol-3 kinase (PI3K) activation causes insulin-induced vasoconstriction. *A*: illustration of 4 experimental conditions. *B*: Akt and MAPK 44/42 activation in human umbilical vein endothelial cells (HUVECs; $n = 6$/condition) and porcine brachial artery homogenates (whole artery; $n = 5$/condition). HUVECs (CC-2519, Lonza, *passages 3–7* in VascuLife EnGS medium, serum starved for 24 h with 0.5% FBS) and whole artery (~5-mm segments placed in DMEM + 0.1% FBS and allowed to acclimate for 1 h at 37°C on a rocking platform) were treated with vs. without the PI3K inhibitor wortmannin (100 nM) for 48 and 3 h, respectively. Data expressed as fold difference from the PI3K inhibitor-untreated condition. *C*: insulin-induced dilation in isolated porcine triceps resistance arteries following treatment with vs. without the PI3K inhibitor wortmannin (100 nM) for 3 h ($n = 8$/condition). AUC, area under curve. *D*: Akt and MAPK 44/42 activation in HUVECs ($n = 6$/condition) and porcine brachial artery homogenates (whole artery; $n = 5$/condition). HUVECs and whole artery segments were treated with vs. without the PI3K inhibitor wortmannin (100 nM) for 48 and 3 h, respectively, in the presence of insulin (100 nM for HUVECs and 10 nM for whole artery). Data expressed as fold difference from the insulin-untreated and PI3K inhibitor-untreated condition in *A*. *E*: insulin-induced dilation in isolated porcine triceps resistance arteries following treatment with vs. without the PI3K inhibitor wortmannin (100 nM) for 3 h in the presence of insulin (10 nM) ($n = 14$/condition). *F*: representative Western blot images of HUVECs and whole artery segments. *$P < 0.05$, statistical significance from the PI3K inhibitor-untreated condition. #$P < 0.05$, statistical significance from the insulin-untreated condition in *B*. Data are expressed as means ± SE.

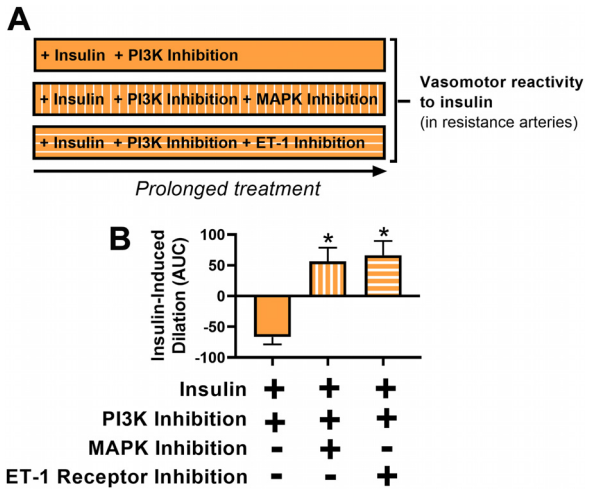

Fig. 3. MAPK and endothelin-1 (ET-1) receptor blockade rescue insulin-induced vasoconstriction caused by persistent insulin signaling in the setting of restricted phosphatidylinositol-3 kinase (PI3K) activation. *A*: illustration of the 3 experimental conditions. *B*: insulin-induced vasomotor responses of isolated porcine triceps resistance arteries following insulin stimulation (10 nM) coupled with PI3K inhibition (wortmannin, 100 nM) for 3 h with vs. without cotreatment of MAPK inhibitor (PD98059, 50 μM) or ET-1 receptor inhibitor (tezosentan, 3 μM); $n = 6$–14/condition. AUC, area under curve. *$P < 0.05$, statistical significance from the first bar. Data are expressed as means ± SE.

vascular insulin resistance), leading to an imbalance between Akt and MAPK/ET-1 activation in endothelial cells (Fig. 4; summary illustration). Indeed, examination of our existing human data revealed that vasoconstriction in the lower limb during an OGTT and attendant insulin stimulation is greater in the setting of obesity. In addition, the ex vivo arterial function experiments from obese humans indicated that this may be related, in part, to ET-1 signaling. Notably, findings in isolated arteries from pigs demonstrated that persistent insulin stimulation did not alter vasomotor responses to insulin when insulin signaling pathways remained preserved. However, prolonged insulinization coupled with restricted PI3K activity resulted in insulin-stimulated vasoconstriction, instead of vasodilation. This aberrant vascular response was rescued by either MAPK inhibition or ET-1 receptor antagonism, suggesting that MAPK/ET-1 signaling mediates insulin-induced vasoconstriction when PI3K/Akt signaling is dampened.

The retrospective analyses of human data provide evidence of the vasoconstrictor effects of endogenous insulin. Of note, typically both endogenous (4, 28, 34, 37) and exogenous (9, 26, 44, 48) insulin mediate a NO-dependent vasodilation. The dilatory response to insulin is blunted in insulin-resistant conditions, such as in obesity and T2D (10, 23, 24, 33, 34, 36, 38, 39, 41, 42). However, relative to the dilatory effects of insulin, the constrictor effects have not been well characterized. Previously, Gudbjörnsdottir and colleagues (16) reported that insulin stimulation increases forearm vascular resistance in obese, hypertensive subjects. The current analyses extend these initial observations and indicate that endogenous insulin-induced vasoconstriction can occur in the lower limb during an OGTT. Furthermore, given this response was observed in young obese subjects with no underlying cardiovascular disease, it may reflect an early pathophysiological alteration in the course of obesity-related vascular dysfunction. The mechanisms responsible for insulin-stimulated vasoconstriction in vivo may involve both neurohumoral inputs (i.e., increased sympathetic-mediated vasoconstriction) (2, 11, 26, 46, 47, 49, 50) and endothelial-derived signals (i.e., reduced NO bioavailability tied with increased MAPK/ET-1 signaling) (6, 8, 21, 22, 31, 33, 36, 41). Our finding that insulin-induced vasoconstriction was attenuated by ET-1 receptor antagonism in isolated resistance arteries from obese humans provides support for endothelial cell involvement in this vascular response.

Because hyperinsulinemia and impaired PI3K activation are shared features of insulin resistance (15), here we interrogated the interplay between these two variables as a potential driver of insulin-induced vasoconstriction. As expected on the basis of previous work by others (12), we found that pharmacological inhibition of PI3K signaling alone impaired insulin-induced dilation. Notably, persistent insulin stimulation under control conditions (i.e., unrestricted insulin signaling) did not alter vasomotor responses to insulin; however, persistent insulin stimulation accompanied by inhibition of PI3K produced a vasoconstriction response. Collectively, these data indicate that restriction of the PI3K pathway may be a requisite to unmask the deleterious effects of sustained insulin signaling. Thus,

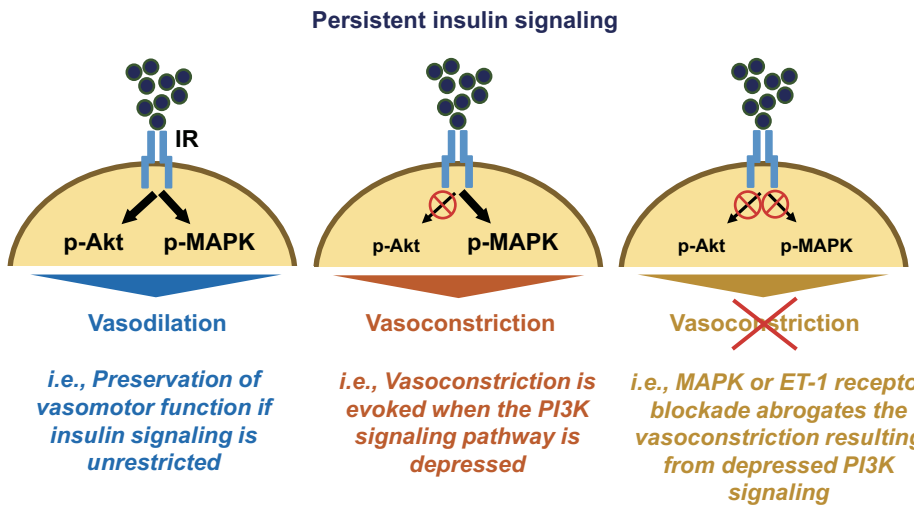

Fig. 4. Schematic summarizing the main findings of the study. Persistent (and unrestricted) insulin signaling does not alter vasomotor function in response to insulin (*left*); however, persistent insulin signaling in the setting where phosphatidylinositol-3 kinase (PI3K)/Akt signaling is depressed causes insulin-induced vasoconstriction (*middle*), thus recapitulating the pathophysiological phenomenon. This insulin-induced vasoconstriction is abolished with concurrent MAPK inhibition or endothelin-1 (ET-1) receptor antagonism (*right*). IR, insulin receptor.

diminished PI3K activation may be a critical step underpinning the increased insulin-induced vasoconstriction in the setting of obesity and compensatory hyperinsulinemia.

Recently, it was demonstrated that acute hyperinsulinemia increases endothelial NO synthase phosphorylation and ET-1 protein in human skeletal muscle homogenates (27). Perhaps the maintenance of both PI3K/Akt and MAPK/ET-1 signaling during prolonged insulin incubation may help explain why prior insulin stimulation alone did not impair subsequent insulin-induced vasodilation in isolated arteries. Importantly, as intended by design, PI3K inhibition reduced Akt phosphorylation without altering MAPK activity, thereby promoting an imbalance in favor of increased MAPK relative to Akt signaling. Notably, we found that insulin-induced vasoconstriction in our experimental ex vivo model of selective insulin resistance was rescued with MAPK inhibition (which targets one side of the imbalance directly) and with ET-1 receptor antagonism (which targets the net vasoconstrictor effect owing to the imbalance). These findings extend on previous work from our group (39, 51) as well as others (12, 13) and suggest that restoring the normal equilibrium between insulin-stimulated PI3K and MAPK activation may be a viable target for the treatment of vascular insulin resistance. In this regard, recent work demonstrates that selective activation of the insulin receptor PI3K branch, leaving the MAPK branch largely inactive, results in protection from atherosclerosis in a mouse model of metabolic syndrome (20).

In aggregate, this work provides evidence that insulin-induced vasoconstriction is a pathophysiological phenomenon. Furthermore, we report that, in the setting of persistent insulin signaling, impaired PI3K activation appears to be a requisite feature precipitating MAPK/ET-1-dependent insulin-induced vasoconstriction.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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

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