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Effects of obesity on insulin: insulin-like growth factor 1 hybrid receptor expression and Akt phosphorylation in conduit and resistance arteries

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Abstract

Insulin and insulin-like growth factor-1 (IGF-1) stimulate specific responses in arteries, which may be disrupted by diet induced obesity. We examined: 1) Temporal effects of high-fat diet (HF) compared to low-fat diet (LF) in mice on insulin receptor (IR), IGF-1 receptor (IGF-1R), IR/IGF-1R hybrid receptor expression and insulin/IGF-1 mediated Akt phosphorylation in aorta. 2) Effects of HF on insulin and IGF-1 mediated Akt phosphorylation and vascular tone in resistance arteries.

Medium term HF (5-weeks), decreased IGF-1R expression and increased hybrid expression (~30%) only. After longer term (16 weeks) HF, IR expression was reduced by ~30%, IGF-1R expression decreased a further ~40% and hybrid expression increased a further ~60%. Independent correlates of hybrid receptor expression were HF, duration of HF and plasma IGF-1 (all P<0.05). In aorta, insulin was a more potent activator of Akt than IGF-1 whereas in resistance arteries, IGF-1 was more potent than insulin. HF blunted insulin-mediated vasorelaxation (P<0.01), but had no effect on IGF-1-mediated vasorelaxation in resistance arteries. Our findings support the possibility that hybrid receptor level is influenced by nutritional and metabolic cues. Moreover, vessel dependent effects of insulin and IGF-1 on vascular tone and Akt activation may have implications in treating obesity related vascular disease.
Introduction

Acting via their cognate receptors insulin and IGF-1 respond to environmental cues and nutrient availability to coordinate metabolism and growth (1). To do this, insulin and IGF-1 may act on multiple tissues including the vascular endothelium where they activate endothelial nitric oxide (NO) synthase via activation of the upstream kinase Akt (2). In aorta we have shown that insulin and IGF-1 stimulated vasorelaxation and activation of eNOS is blunted in obesity (2).

The insulin receptor (IR) and IGF-1 receptor (IGF-1R) are heterodimers consisting of two extracellular α-subunits and two transmembrane spanning β-subunits held together by disulphide bonds (3). Homology between IR and IGF-1R is high and as a result they can heterodimerize to form hybrid receptors composed of one IGF-1Rαβ complex and one IRαβ subunit complex (4). The proportion of hybrid dimerization is thought to be a function of the molar fraction of each receptor in the ER (5). According to this model, a marked increase in IR leads hybrids to form in preference to IGF-IR homodimers (6). Hybrid receptors are thought to have a binding affinity similar to the IGF-1R i.e. binding IGF-1, but not insulin, with high affinity (7). By reducing IR availability the formation of hybrid receptors has been suggested to have a negative regulatory effect on insulin signalling (8, 9).

In cross sectional studies in humans with insulin resistance of relatively short duration, increased hybrid receptor expression is not seen (10), whereas patients with type 2 diabetes have down-regulation of IR and increased expression of hybrids (11). The temporal relationship between expression of IR, IGF-1R and hybrids in obesity and their pathological correlates in the vasculature in vivo remains unclear. Moreover, whether IGF-1 has vasorelaxant effects in resistance arteries and the effect of obesity on these responses is also unclear. To answer these questions we examined: 1) The temporal changes in expression of IGF-1R, IR and hybrids in aorta and their correlates in high-fat diet-induced obesity. 2) The effect of different pathological insults associated with obesity on IGF-1R, IR and hybrid expression in human endothelial cells. 3) The effect of IGF-1 and insulin on resistance vessel tone and Akt phosphorylation, and the influence of obesity on these responses.
Methods

Animals & animal procedures. C57BL/6J male mice were purchased from Jackson Laboratories and acclimatised for 7-days before starting experimental procedures. Mice were maintained in a temperature and humidity controlled environment on a 12 h light:dark cycle. Male mice were studied in all experiments which were conducted in accordance with accepted standards of humane animal care under United Kingdom Home Office Project licence No. P144DD0D6.

Diet-induced obesity. Mice were rendered obese by placing them on a 60% high-fat diet (HF; diet D12492, Research Diets Inc. New Brunswick, NJ, USA). Age-matched littermate controls were placed on a 10% low-fat diet (LF; diet D12450B, Research Diets Inc. New Brunswick, NJ, USA). All mice were fed standard chow (Special Diet Services, CRM P(PB), Dietex International) until reaching 6-weeks of age, at which point diets were switched to either HF or LF for 2 weeks, 5-weeks or 16-weeks.

In vivo examination of glucose homeostasis. In vivo metabolic testing was performed as previously described (12, 13); for glucose tolerance tests, mice were fasted for 6 h, followed by intraperitoneal (IP) injection of 2 mg/kg glucose after which blood glucose was determined at 30 minute intervals by tail vein sampling using a portable glucometer (Accu-chek Aviva; Roche Diagnostics, Burgess Hill, U.K.). Blood was sampled at euthanasia from the inferior vena cava. Plasma insulin and IGF-1 were measured using ultrasensitive mouse ELISA kits (CrystalChem, Downers Grove, IL and R&D Systems, Bio-Techne, MN) as previously described (12).

Quantification of Insulin receptors and IGF-1 receptors. Mice were euthanized at 2, 5- or 16-weeks after feeding and aortae harvested and snap frozen. Tissue was processed for analysis by
Western blotting to examine changes in receptor protein expression. Samples were mechanically lysed in cell extraction buffer (Invitrogen, Carlsbad, CA) with inhibitors, using a TissueLyser (QIAGEN, Dusseldorf, Germany). Protein was quantified by the BCA assay (Sigma-Aldrich, St. Louis, MO). Twenty micrograms of protein were resolved on a 4-12% Bis-Tris gel (Bio-Rad, Hertfordshire, UK) and transferred to nitrocellulose membranes. Membranes were probed with antibodies diluted in 5% BSA; 1:1000 insulin receptor-beta (clone 4b8), 1:1000 IGF-1 receptor-beta (clone D23H3) and 1:20000 beta actin (Cell Signaling, Massachusetts, USA), before incubation with appropriate secondary horseradish peroxidase-conjugated antibody (Dako, Glostrup, Denmark). All antibodies are summarised in table 1. Blots were visualised with Immobilon Western Chemiluminescence HRP Substrate (Merck Millipore, Hertfordshire, UK) and imaged with Syngene chemiluminescence imaging system (SynGene, Cambridge, UK) [insert table 1].

**Quantification of Hybrid Receptors.** Hybrid Receptor expression was studied by immunoprecipitation and Western blot analysis. Immunoprecipitation: Total protein was combined with 30 µl protein G agarose beads (Roche Diagnostic, Switzerland), 300 µl buffer (100 mM HEPES, pH 7.8, 100 mM NaCl, 10 mM MgSO4, 0.02% Tween-20) and 1:100 dilution of IGF-1 receptor antibody (D23H3). Ag-Ab immune complexes were allowed to form over 3h at 4°C, after which they were collected using brief centrifugation. Precipitates were washed gently three times in PBS–0.02% Tween-20 before elution with SDS buffer. Western blotting: Samples were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were probed with IR-β antibody; 1:1000 (4b8), followed by appropriate secondary HRP-conjugated antibody to visualise IR/IGF-1R hybrids. Membranes were re-probed with IGF-1R-β antibody to allow hybrid receptors to be reported as relative levels compared with total IGF-1R protein.
**Insulin and IGF-1 receptor gene expression.** Quantitative real-time PCR was used to measure mRNA levels of insulin and IGF-1 receptors. mRNA from aorta was isolated and purified using the RNeasy mini kit (QIAGEN, Dusseldorf, Germany) as per the manufacturers protocol. Reverse transcription was performed using iScript cDNA synthesis kit (Bio-Rad, Hertfordshire, UK). Quantitative PCR was then used to determine IR and IGF-1R mRNA expression using specific TaqMan assays (Invitrogen, IR; Mm01211875_m1, IGF-1R; Mm00802831_m1). Receptor expression was calculated relative to the average of two housekeeping genes; TATA box binding protein (TBP; Mm01277042_m1) and CyclinB (Mm03053893_gH), using the formula $2^{-\Delta\Delta Ct}$.

**Insulin and IGF-1 stimulated Akt phosphorylation in vivo.** Mice were injected subcutaneously with either vehicle, native human insulin (Novo Nordisk, Malov, Denmark) or recombinant human IGF-1 (Ipsen, Slough, UK). Dosage was calculated based on the average weight of all lean mice (assuming blood volume does not significantly alter in obese mice). Plasma levels of human IGF-1 and human insulin in the mice were measured using ELISAs (insulin; Novo Nordisk, Malov, Denmark. IGF-1; Immunodiagnostic Systems, Tyne & Wear, UK) as described previously (14), in order to confirm equivalent dosing levels between HF and LF mice. After 15 min stimulation, mice were euthanized and the aorta rapidly harvested and snap-frozen. Twenty micrograms of protein were processed for Western blotting. Nitrocellulose membranes were probed with antibodies diluted in 5% BSA; 1:1000 Akt, 1:2000 phosphorylated Akt (Ser473) and 1:20000 beta actin (Cell Signaling, Massachusetts, USA).

**In vitro assessment of receptor and hybrid expression in human umbilical vein endothelial cells.** Cryopreserved human umbilical vein endothelial cells (HUVECs) were purchased from Promocell (Stourbridge, UK) and maintained in culture in endothelial cell growth medium at 37°C in a humidified atmosphere with 5% CO₂. At ~70% confluency, cells were treated with the following; 100
nM human recombinant insulin (Sigma-Aldrich, Dorset, UK), 100 nM human recombinant IGF-1 (GroPep, Adelaide, Australia), 10 ng/ml TNF-\(\alpha\) (PeproTech, London, UK) 1 \(\mu\)M angiotensin II (Sigma-Aldrich, Dorset, UK), 50 \(\mu\)M hydrogen peroxide (\(\text{H}_2\text{O}_2\); Sigma-Aldrich, Dorset, UK)), 25 mM glucose +/- 10nM insulin (Sigma-Aldrich, Dorset, UK) for 24 h in low serum (0.5%) medium. Whole cell lysates were prepared in cell extraction buffer and samples processed for Western blot analysis of insulin receptor, IGF-1 receptor and hybrid receptors.

**Resistance vessel vasomotor function in response to insulin and IGF-1**

Two millimetre segments of 1st order mesenteric arteries were harvested from LT HF and LF mice, and mounted in a wire myograph (Danish Myo Technology A/S, Aarhus, Denmark) containing physiological buffer (mM): KCl 7.4; NaCl 118; NaHCO\(_3\) 15; KH\(_2\)PO\(_4\) 1.2; MgSO\(_4\) 1.2; glucose 11; CaCl\(_2\) 2.5; EDTA 0.023 at 37\(^\circ\)C, 5\%CO\(_2\) and 95\% O\(_2\). Vessels were equilibrated at a resting lumen diameter of 0.9 x L\(_{100}\) (L\(_{100}\) represents vessel diameter under passive transmural pressure of 100 mmHg) in buffer for 30 min. Three potassium-induced constrictions were performed using high potassium buffer and vessels constricting less than 1 mN were excluded from the study. Vessels were pre-constricted with phenylephrine, at a dose yielding approximately 40% constriction obtained with high potassium buffer, and left to stabilise for 10 min. Relaxation to cumulative addition of either insulin (0.001 nM / 1 pM to 1 \(\mu\)M) or IGF-1 (0.001nM to 10nM) was assessed in pre-constricted vessels. A time-matched control recording was also performed following the same protocol, without the addition of insulin or IGF-1. The contractile force of a vessel segment was recorded using PowerLab 4/25-LabChart7 acquisition system (ADInstruments, Oxford, UK).

**Ex vivo analysis of insulin and IGF-1 induced Akt phosphorylation in resistance arteries**

First order mesenteric artery segments of 5mm length were placed into Krebs Ringer solution and stimulated with insulin or IGF-1 at different concentrations (0.001nM to 1\(\mu\)M) for 15min at 37\(^\circ\)C.
Stimulated vessels were snap-frozen, then lysed and sonicated. Samples were analysed by SDS-PAGE and Western blotting.

**Statistical Methods**

Data were analysed using GraphPad Prism software (version 7). For animal studies, one-way ANOVA was used to compare the mean value across groups, followed by Tukey's multiple comparisons test. Where differences between two groups were analysed, an unpaired t-test was used with Welch’s correction. To study differences in vitro, a paired two-way t-test was utilised. The results are given as mean ± standard error of the mean (SEM). In this study, differences with a P value of <0.05 were considered statistically significant. Multivariate and Univariate analysis Uni- and multi-variate linear regression analysis was performed using SPSS version 21 (IBM Corporation, Armonk, NY) to determine the association between receptor abundance and selected covariates. Standardised regression (beta) coefficients are presented, with * denoting statistical significance at P<0.05.
Results

**Progressive decline in insulin and IGF-1 sensitivity in obesity.** We fed mice a HF, obesogenic diet for 2, 5 or 16 weeks; this led to progressive metabolic impairment in comparison to LF fed controls (summarised in table 2) [insert table 2].

**IGF-1R, IR and IGF-1R/IR hybrid receptor expression in aorta during obesity.** We studied changes in IR, IGF-1R and hybrid receptor expression in aortic lysates from mice after 2, 5 and 16-weeks of HF and LF. In tissue samples, IR was observed as a double band migrating at 80-100kD. We observed IR as a single or double band in tissue but not cell lysates. We suggest this is due to the varying degrees of IR glycosylation in different cell types and tissues, resulting in two migrating populations of IR. We did not observe any discernible difference in the two populations of IR when comparing HF and LF. The level of hybrid receptors was studied by immunoprecipitating IGF-1R and detecting IR in the hybrid receptor by Western blot. The relative level of IR compared to total IGF-1R was determined. The effect of an obesogenic diet was studied over time. After 2 weeks feeding, IR, IGF-1R and hybrid receptor protein expression was unchanged (figure 1). After 5 weeks HF, IR expression in aorta was unchanged (figure 1A), whereas IGF-1R expression had declined by 30% (figure 1B) and hybrid receptor expression increased by 38% (figure 1C). After 16 weeks of HF IR expression had declined by 24% (figure 1A), IGF-1R expression had declined further by 34% (figure 1B) and hybrid receptor expression increased by 62% (figure 1C).

To determine whether the reduction in receptor expression was due to transcriptional changes, real-time PCR was performed on RNA isolated from aorta of mice after 16-weeks of feeding. No changes were observed in IR (figure 1D) or IGF-1R (figure 1E) relative mRNA expression between HF and LF fed mice [insert figure 1].

Univariate correlates of hybrid receptor expression were plasma insulin, plasma IGF-1, fasting glucose, body weight, dietary fat content and duration of diet, all P<0.05. In multivariate analysis,
independent predictors of hybrid expression were dietary fat content, duration of diet ingestion and plasma IGF-1, all P<0.05 (table 3) [insert table 3].

IGF-1R, IR and IGF-1R/IR hybrid expression in vitro in response to different components of the obesity phenotype. We cultured human umbilical vein endothelial cells in conditions aiming to recapitulate different components of the obesity phenotype including elevated: insulin, IGF-1, glucose with and without insulin, angiotensin II, hydrogen peroxide and TNF-alpha for 24 h. Only insulin and IGF-1 reduced expression of their respective receptors; despite this hybrid receptor expression remained unchanged (figure 2) [insert figure 2].

Temporal effects of obesity on IGF-1 and insulin stimulated Akt phosphorylation. To examine the temporal effect of obesity on insulin and IGF-1 mediated phosphorylation of the key signalling kinase Akt, we performed in vivo administration of either insulin or IGF-1 to HF and LF fed mice after 2, 5 and 16-weeks. To determine the optimum dose of IGF-1, we first performed a study in lean mice to examine the effect of equimolar and equipotent (as determined by blood glucose lowering ability) doses of IGF-1 and insulin on aortic Akt phosphorylation. When equimolar concentrations of insulin (4.5 nmol/kg) or IGF-1 (4.5 nmol/kg) were administered, insulin led to a greater decrement in blood glucose and greater increment in phosphorylation of Akt in aorta than IGF-1 (figure 3A and 3B). An IGF-1 dose of 90 nmol/kg stimulated similar blood glucose lowering and Akt phosphorylation as 4.5 nmol/kg insulin. Therefore, in subsequent studies, we used equipotent doses; insulin at 4.5 nmol/kg and IGF-1 at 90 nmol/kg. To ensure that plasma exposure levels would be comparable between LF and HF mice, doses for all mice were calculated based on the average bodyweight of the LF mice. Plasma exposure levels of human insulin and IGF-1 was assessed with specific ELISAs for insulin and IGF-1 and we found comparable levels between the LF and HF groups (figure 3C and 3D).
After 2 weeks HF, despite no change in receptor expression, both insulin and IGF-1 (figure 3E) mediated Akt phosphorylation were blunted. After 5 weeks HF both insulin and IGF-1 (figure 3F) mediated Akt phosphorylation were blunted. By 16 weeks, however, while insulin mediated Akt phosphorylation remained blunted, IGF-1 mediated Akt phosphorylation was similar in LF and HF fed mice (figure 3G), possibly reflecting an increase in hybrid receptor expression [insert figure 3].

**Resistance vessel relaxation and Akt phosphorylation in response to insulin and IGF-1.** We previously demonstrated that 8 weeks HF led to blunting of both insulin and IGF-1 mediated vasorelaxation of the aorta (2), however, this study did not examine the effect of obesity on resistance vessel function. Here we show that both insulin and IGF-1 led to vasorelaxation of 1st order mesenteric arteries (figure 4A, 4B and 4C). IGF-1, however, was more potent than insulin (figure 4D and 4E). HF resulted in blunted insulin-mediated vasorelaxation (figure 4F) but IGF-1 mediated responses were unaffected (figure 4G). A dose-dependent increase in phosphorylation of Akt was observed with increasing concentrations of insulin and IGF-1 (figure 4H & 4I), however, IGF-1 treatment led to a greater maximal response (figure 4J and 4K). HF blunted insulin mediated Akt phosphorylation in 1st order mesenteric arteries (figure 4H), but IGF-1-mediated Akt phosphorylation was unaffected by HF (figure 4I) [insert figure 4].
Discussion

This report describes a number of novel findings of relevance to our understanding of obesity, metabolic disease and the insulin/IGF-1 system, including: 1) IR/IGF-1R hybrid protein level in aorta does not appear to be a primary function of mRNA IR and IGF-1R levels. 2) Independent in vivo correlates of hybrid expression are plasma IGF-1, HF and duration of HF feeding period. 3) IGF-1 is a more potent activator of Akt and vasorelaxation in resistance vessels than insulin; whereas we have shown previously that the opposite is true in larger conduit vessels. 4) After 16 weeks HF diet, IGF-1 mediated responses in resistance vessels and conduit vessels are preserved whereas insulin induced responses are blunted.

Hybrid receptor expression does not appear to be simply dependent on the molar fraction of each receptor

Energy and nutrient homeostasis in mammals requires tight regulation and integration of multiple systems, which during periods of cellular and whole organism stress, couple nutrient delivery to energy storage, cell growth and tissue repair (15). Integral to nutrient homeostasis is the insulin/IGF-1 system, the development and evolution of which occurred before the relatively unusual environmental circumstances of caloric excess experienced by 21st century humans (16). As a result the insulin/IGF-1 system is unable to effectively adapt to the challenge posed by chronic calorie excess and gradually deteriorates giving rise to insulin resistant type 2 diabetes mellitus and its lethal complications, many of which involve the cardiovascular system (17).

A hallmark of type 2 diabetes is the increased expression of IR/IGF-1R hybrids which are thought to restrict insulin signalling in favour of IGF-1 (5), a scenario we (18, 19), and others, have demonstrated may be present in the endothelium (20) and vasculature (21). Understanding how hybrid receptors are regulated and activated in the vasculature is hence of importance to our understanding of obesity related perturbation of insulin signalling and vascular dysfunction. In the
present study, and consistent with cross-sectional studies in humans (10), increased hybrid receptor expression was preceded by insulin (and IGF-1) resistance. We also show that expression of hybrid receptors is closely linked to the duration of high fat diet ingestion and plasma IGF-1 level. In contrast to elegant studies from Federici et. al., we did not demonstrate independent correlations between hybrid receptor expression and blood glucose (22) or insulin concentration (23), rather IGF-1 concentration more closely correlated with hybrid expression. It is possible that this is due to the presence of obesity rather than the primary hyperinsulinaemia described by Federici et. al. (23). An additional explanation could be the use of different tissues – muscle samples as studied by Federici et al may show more sensitivity to hybrid formation following perturbations in insulin and glucose, whereas vascular tissue as in the present study may be more sensitive to changes in IGF-1 levels. After 16 weeks of HF, we observed no change in receptor mRNA levels yet found that IR and IGF-1R protein decreased in obese mice. Despite this reduction in total receptor level, the relative expression of hybrid receptors increased. This suggests that regulation of IR and IGF-1R occurs at the translational level or it could be speculated that the internalization/degradation pathways of the receptors are distinct from hybrid receptors and they are more readily influenced by hormone exposure levels.

**Insulin and IGF-1 in resistance vessel function.** We previously showed that obesity leads to resistance to both IGF-1 and insulin mediated activation of eNOS and relaxation of the aorta (2). Studies in humans have shown that IGF-1 increases forearm blood flow consistent with an effect on resistance vessels (24). McCallum et. al. showed that IGF-1 mediated vasodilatation of aorta is blunted in hypertensive rats (25) and Hasdai et. al. showed that arteriolar vasorelaxation to IGF-1 is attenuated in experimental hypercholesterolaemia (26). The effect of obesity on insulin and IGF-1 mediated responses in resistance vessels has been unclear. Here we show that IGF-1 relaxes resistance vessels and is more potent than insulin. We also show the intriguing finding that obesity
blunts insulin mediated resistance vessel relaxation and Akt phosphorylation while IGF-1 mediated vasorelaxation and Akt phosphorylation remained intact. These findings reveal a potentially important divergence between insulin and IGF-1 responses in resistance vessels with preservation of IGF-1 responses, when we previously showed that obesity leads to IGF-1 resistance in aorta (2).

**Study limitations.** A number of limitations should be discussed: we used the semi-quantitative approach of expression levels of receptors to estimate receptor numbers so we cannot comment on the exact numerical relationship between IR and IGF-1R in relation to hybrid receptor formation. In resistance vessels we were unable to quantify receptor expression due to limited amounts of protein available; it would be of interest in the future to examine receptor expression in resistance vessels as obesity progresses.

**Conclusion.** We have provided a number of insights into changes in the IR/IGF-1R/hybrid receptor system as obesity progresses, showing that after longer term obesity, IGF-1 mediated Akt phosphorylation is preserved in aorta and resistance vessels. Moreover, we show that IGF-1 is a more potent vasodilator of resistance vessels than insulin, and after 16 weeks of high fat diet, while insulin mediated resistance vessel function is blunted, IGF-1 responses are maintained. These data raise the intriguing possibility that using IGF-1 or manipulating hybrid expression may be an approach to treat obesity related vascular dysfunction, a possibility that warrants future work.

**Declaration of Conflicting Interests.** The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References


7. **Slaaby R, Schaffer L, Lautrup-Larsen I, et al.** Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. J. Biol. Chem. 2006; **281**:25869-74.


9. **Slaaby R.** Specific insulin/IGF1 hybrid receptor activation assay reveals IGF1 as a more potent ligand than insulin. Sci. Rep. 2015; **5**:7911.


