Hypothesis:

‘Vasocrine’ Signalling From Perivascular Fat

- A Mechanism Linking Insulin Resistance and Vascular Disease

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Summary

Adipose tissue expresses cytokines which inhibit insulin signalling pathways in liver and muscle. Obesity also results in impairment of endothelium-dependent vasodilatation to insulin. We propose a vasoregulatory role for local deposits of fat around the origin of arterioles supplying skeletal muscle. Isolated first order arterioles from rat cremaster muscle are under dual regulation by insulin, which activates both endothelin-1 mediated vasoconstriction and nitric oxide mediated vasodilatation. In obese rat arterioles, insulin-stimulated nitric oxide synthesis is impaired, resulting in unopposed vasoconstriction. We propose this to be the consequence of production of the adipocytokine tumour necrosis factor-α from the cuff of fat seen surrounding the origin of the arteriole in obese rats - a depot to which we ascribe a specialist vasoregulatory role. We suggest that this cytokine accesses the nutritive vascular tree to inhibit insulin-mediated capillary recruitment - a mechanism we term ‘vasocrine’ signalling. We also suggest a homology between this vasoactive periarteriolar fat and both periarterial and visceral fat, which may explain relationships between visceral fat, insulin resistance and vascular disease.

(169 words)

Introduction

Obesity is accompanied by resistance to insulin action in skeletal muscle, liver and blood vessels. Insulin resistance in muscle may represent a mechanism to protect muscle from excessive postprandial glucose uptake in conditions of calorie excess. We have suggested that low-grade inflammation, consequent upon the production of proinflammatory cytokines by adipose tissue, may underlie relationships between insulin resistance and vascular disease (1). We now propose that depots of fat around both large and small vessels may be an important source of these cytokines, and that periarteriolar fat plays a physiological role in regulating distribution of blood flow, through outside-to-inside cellular cross talk and through regional vascular signalling - mechanisms we term 'vasocrine.' Moreover, perivascular fat may contribute both to insulin resistance and to macrovascular disease.

Fat and Insulin Resistance - a Circulating Signal?

Obesity decreases insulin action in liver and muscle. It is also associated with impaired endothelium-dependent vasodilatation, in a variety of vascular beds and in response to a variety of vasodilators, including insulin (2). But 'How does the liver/muscle/endothelium know you're fat?' Traditional thinking has been that adipocytes generate insulin resistance by producing non-esterified fatty acids (NEFAs) which inhibit carbohydrate metabolism via substrate competition and impaired insulin signalling (3). The endothelial nitric oxide synthase (eNOS) molecule is inhibited by fatty acids (4), so NEFA may also provide the signal to endothelium. But other potential adipocyte signals are relevant. Mice with a GLUT-4 knockout located specifically to adipose

tissue show insulin resistance in liver and skeletal muscle *in vivo*, but not *in vitro* (5) despite lower circulating NEFA concentrations - implying the existence of other fat-derived insulin resistance signals. Overweight subjects have increased levels of circulating adipocytokines, such as tumour necrosis factor-α (TNF-α) and interleukin-6, which may provide a pathway linking obesity to insulin resistance in liver, muscle and vessels (1).

Fat and Insulin Resistance - a Local Signal?

An alternative explanation has been proposed to explain hepatic and muscle insulin resistance in obesity - that of ectopic fat. Accumulation of hepatocellular fat is associated with hepatic insulin resistance (6). In skeletal muscle, insulin resistance occurs with accumulation of intramyocellular triglyceride (7). These findings raise the possibility that prolonged exposure of liver and muscle to an 'insulin resistant milieu' somehow generates sufficient intrahepatic and intramyocellular fat to produce insulin resistance in these target organs, perhaps through local NEFA generation, or through increased cytokine expression, without any ongoing circulating adipose-derived signal.

We propose that increased adipose mass may also generate cytokine signals to blood vessels through the medium of perivascular fat, also 'ectopic' although within adipocytes. We suggest that the organism's perivascular adipocytes act as an integrated organ responsible for paracrine and 'vasocrine' signalling, which in turn contributes to skeletal muscle insulin resistance.

The Anatomy and Physiology of Skeletal Muscle Blood Supply

Substantal data from rats (8), and more limited human studies (9,10), have shown insulin to have a marked and rapid effect to increase nutritive blood flow to skeletal muscle and skin, an effect shared, in muscle, with exercise. We propose that this action of insulin helps to coordinate postprandial delivery of substrate and hormone to insulin-sensitive tissues. Insulin's ability to recruit capillaries is likely to depend on its action in dilating precapillary arterioles (11). *In-vitro* studies we have performed in larger (so-called first order) arterioles from rat cremaster muscle, however, demonstrate a dual effect of insulin on arteriolar endothelium. In vessels from healthy rats, insulin has no net effect on vessel diameter, because of a balance between stimulation of two pathways - nitric oxide (NO)-mediated vasodilatation and endothelin-1 (ET-1)-mediated vasoconstriction. Insulin stimulates eNOS activation, the signalling pathway being through insulin receptor substrate-1 (IRS-1), phosphoinositol-3-kinase (PI3-K) and Akt (12,13). However, if this pathway is inhibited, the arteriole constricts, a response mediated, through the extracellular signal-related kinase-1/2 (ERK-1/2) pathway, by endothelin-1 (ET-1) (13). In the cremaster muscle arteriole from a Zucker fatty rat, our findings show that insulin induces vasoconstriction, because of unopposed action of ET-1. These observations imply a dual insulin signalling mechanism - one pathway stimulating nitric oxide (NO) synthesis, the other endothelin-1 release. In obesity, the ET-1 pathway dominates, insulin resistance in these arterioles being limited to the IRS-1/PI3-K/Akt pathway. We propose that this dual mechanism allows for local regulation of insulin action.

The rat cremaster muscle is virtually two-dimensional, allowing observation of structural/functional relationships. The morphology of the muscle differs between lean and obese animals, with the presence of a well-circumscribed depot of fat around the origin of the vessel in the obese Zucker rat (Figure 1). This suggests an elegant regulatory mechanism. We propose that, in situations of chronic calorie excess or inactivity, an organism protects its muscle from substrate over-supply by creating a local fat pad at the vessel origin with specialist vasoregulatory function. Adipocytokines from these pads, such as TNF-α, inhibit the PI3-K signalling pathway of eNOS activation and NO production (14). In vivo, insulin-induced vasodilatation and nutritive capillary recruitment are inhibited by TNF-α (15). These vasoregulatory fat depots, then, have the capacity locally to modulate the systemic vasodilating effects of insulin, instead producing vasoconstriction.

We also suggest a novel mechanism to enhance the signalling function of TNF-α. The effects of insulin to stimulate, and of TNF-α to inhibit, nutritive flow is likely to reside in recruitment or drop-out of muscle capillary networks, consequent upon vasodilatation or constriction in smaller, precapillary arterioles (11). We hypothesise that increased endothelial permeability, consequent upon the cytokine's action, allows its diffusion into the lumen, where high concentrations may exceed the capacity of circulating binding proteins. This might then produce inhibition of insulin-mediated vasodilatation throughout the nutritive vascular tree, down to and including the precapillary arterioles. This could explain the observations reported above, made on arterioles downstream of the fat pad and themselves free of fat (Figure 1). As

these high cytokine concentrations are limited to action on vascular endothelium, and are unlikely to access the systemic circulation, we term this a ‘vasocrine’ signal. Nitric oxide may be another molecule acting in regional vascular beds through similar vasocrine mechanisms (16).

Periarteriolar Fat - Vasoregulatory Function?

We hypothesise, then, that the arterial tree has evolved a sophisticated control mechanism to regulate postprandial substrate partition during conditions of calorie balance and excess. This mechanism depends on the contractile properties of vascular medial smooth muscle cells, which are under dual regulation by insulin. One such mechanism is the synthesis of a vasodilator, NO, the production of which, as elegantly shown by Furchgott (17), requires healthy endothelial cells. We now propose that this mechanism is regionally modulated by adipocytokines secreted from another, adventitial, layer of cells, acting in paracrine and vasocrine fashion to alter insulin-mediated NO/endothelin-1 balance (Figure 2). In lean animals, insulin induces postprandial increases in nutritive flow. However with continued calorie excess, inactivity and obesity, vasoregulatory periarteriolar fat accumulates, resulting in diminished insulin-induced vasodilatation or even vasoconstriction (18), exacerbating muscle insulin resistance and so protecting it from excess substrate delivery.

Functionally, two distinct vascular circuits exist in muscle: a nutritive route supplying nutrients, oxygen and hormones to myocytes, and a non-nutritive one, which may pass through functional shunt vessels in muscle connective

tissue (19). Our hypothesis applies only to nutritive vessels, because insulin-induced vasoconstriction of non-nutritive vessels would increase nutritive flow and thus insulin sensitivity. We propose that nutritive vessels differ functionally from non-nutritive ones in response to insulin (dilatation versus no effect or constriction as the normal response), so that adipocytokines which inhibit insulin-induced vasodilatation will act preferentially on nutritive vessels.

Perivascular fat - What Might It Do?

Perivascular fat is distributed widely throughout the arterial circulation. We speculate that depots around the heart and the major branches of the aorta share homology with the vasoregulatory depots around nutritional arterioles. In conditions of inactivity and calorie excess, these depots enlarge, with potential adverse consequences - including epicardial fat (coronary arteries), perirenal fat (renal arteries) and pelvic fat (ilio-femoral vessels). These depots may have originated during development with a physiological role in regulating blood flow distribution, but may become harmful in excess. Overproduction of adipocytokines and outside-to-inside signalling (20), may lead to inflammation and atherosclerosis, perhaps to coronary calcification and, in renal arteries, to microalbuminuria. Finally, an epidemiological relationship has been noted between epicardial and visceral fat mass (21). Several studies have noted a higher rate of production of adipocytokines from visceral than from subcutaneous fat (22), suggesting a vasoregulatory phenotype analogous to periarteriolar fat. Visceral fat, measured as waist and, perhaps, neck circumference, may, then, simply provide an easily measured index of the body's periarterial and periarteriolar fat.

Testing the Hypothesis

Elegance and symmetry do not, on their own, constitute proof. The rat cremaster muscle provides a valuable model to test the putative vasoregulatory role of periarteriolar fat. The study of vessels from obese rats, with the fat depot in situ or previously removed, and in vessels from lean animals exposed to perfusate from obese vessels, would provide tests of the vasocrine hypothesis. Inhibitors of the major insulin signalling pathways and monoclonal antibodies to cytokines would provide delineation of the signalling molecules and pathways. Additional studies, in other species and vascular beds, might require tissue-specific knock-outs of signalling molecules, and interventions physically to separate blood vessels from perivascular fat. Such interventions would be expected to influence vascular responsiveness, peripheral insulin action, and in the longer term, atherogenesis.

Conclusions

We propose that periarteriolar fat represents a physiological mechanism to regulate distribution of postprandial blood flow within, and perhaps between, organs. This regulation requires a series of locally and regionally acting molecules to modulate the systemic actions of insulin on NO-mediated vasodilatation. However with nutritional excess and indolence, physiology turns to pathology - insulin resistance, microinflammation and macroangiopathy. These proposals remain to be tested.

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Conflict of Interest Statement

JY serves on a Scientific Advisory Board for Glaxo SmithKline.

References

1. Yudkin JS, Stehouwer CDA, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: association with obesity, insulin resistance, and endothelial dysfunction. Arterioscleros Thromb Vasc Biol 1999; 19: 972-978.

2. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. J Clin Invest 1995; 96: 786-792.

3. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. Diabetes Metab Rev 1998; 14: 263-283.

4. Davda RK, Stepniakowski KT, Lu G, Ullian ME and Goodfriend TL. Oleic acid inhibits endothelial nitric oxide synthase by a protein kinase C-independent mechanism. Hypertension 1995; 26: 764-770.

5. Abel ED, Peroni O, Kim JK, Kim YB, Boss O, Hadro E, Minnemann T, Shulman GI, Kahn BB. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature. 2001;409:729-33.

6. Seppala-Lindroos A, Vehkavaara S, Hakkinen A-M, Goto T, Westerbacka J, Sovijarvi A, Halavaara J and Yki-Järvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. J Clin Endo Metab 2002; 87: 3023-3028.

7. Kelley DE and Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 2000; 49: 677-683.

8. Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG and Barrett EJ. Insulin sensitivity of muscle capillary recruitment in vivo. Diabetes 2004; 53: 447-453.

9. Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S and Barrett E.
Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. Diabetes 2001 ; 50 : 2682-2690.
.

10. Serné EH, IJzerman RG, Gans RO, Nijveldt R, De Vries G, Evertz R, Donker AJ, Stehouwer CD. Direct evidence for insulin-induced capillary recruitment in skin of healthy subjects during physiological hyperinsulinemia. Diabetes 2002 ; 51 : 1515-1522..

11. Greene AS, Tonellato PJ, Lui J, Lombard JH and Cowley AW. Microvascular rarefaction and tissue vascular resistance in

hypertension. Am J Physiol (Heart Circ Physiol) 1989; 256: H126-H131.

12. Rattigan S, Clark MG and Barrett EJ. Hemodynamic actions of insulin in rat skeletal muscle. Evidence for capillary recruitment. Diabetes 1997; 46: 1381-1388.

13. Eringa EC, Sthouwer CDA, van Nieuw Amerongen GP, Ouwehand L, Westerhof N and Sipkema P. Vasoconstrictor effects of insulin in skeletal muscle arterioles are mediated by ERK1/2 activation in endothelium. Am J Physiol Heart Circ Physiol (Aprl 1, 2004). 10.1152/ajpheart.00067.2004.

14. Mohamed F, Monge JC, Gordon A, Cernacek P, Blais D and Stewart DJ. Lack of role for nitric oxide (NO) in the selective destabilisation of endothelial NO synthase mRNA by tumor necrosis factor-alpha. Arterioscler Thromb Vasc Biol 1995; 15**:** 52-57.

15. Zhang L, Wheatley CM, Richards SM, Barrett EJ, Clark MG, Rattigan S. TNF-alpha acutely inhibits vascular effects of physiological but not high insulin or contraction. Am J Physiol Endocrinol Metab. 2003; 285: E654-60.

16. Schechter AN and Gladwin MT. Hemoglobin and the paracrine and endocrine functions of nitric oxide. N Engl J Med. 2003; 348:1483-5.

17. Furchgott RF and Zawadaki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373-376.

18. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark AD and Clark MG. Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. Diabetes 2002; 51: 3492-3498.

19. Newman JMB, Steen JT and Clark MG. Vessels supplying septa and tendons as functional shunts in perfused rat hindlimb. Microvasc Res 1997; 54: 49-57.

20. Mazurek T, Zhang LF, Zalewski A, Mannion JD, Diehl JT, Arafat H, Sarov-Blat L, O'Brien S, Keiper EA, Johnson AG, Martin J, Goldstein BJ and Shi Y. Human epicardial adipose tissue is a source of inflammatory mediators. Circulation 2003; 108: 2460-2466.

21. Iacobellis G, Ribaudo MC, Assael F, Vecci E, Tiberti C, Zappaterreno A, Di Mario U and Leonetti F. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. J Clin Endo Metab 2003; 88: 5163-5168.

22. Arner P. Regional differences in protein production by human adipose tissue. Biochem Soc Trans 2001; 29 (Pt 2): 72-75.

Figure 1a: Isolated cremaster muscle from Zucker fatty rat

1b: Enlargement of origin of arteriole

a

b

<u>Figure 2</u>: Vasocrine signalling from perivascular fat

Adipocytokines secreted from perivascular adipocytes inhibit the PI3-kinase pathway of insulin signalling, leaving unopposed vasoconstrictor effects of endothelin-1. High concentrations of tumour necrosis factor-α access the vascular lumen, resulting in inhibition of endothelial PI3-kinase pathway insulin signalling in downstream vessels. Reduced insulin-mediated enhancement of muscle nutritive blood flow will contribute to insulin resistance. EC - endothelial cell; VSMC - vascular smooth muscle cell; eNOS - endothelial nitric oxide synthase; NO - nitric oxide; PI3-K - phosphoinositol-3-kinase; TNF-α - tumour necrosis factor-α; IL-6 - interleukin-6; NEFA - non-esterified fatty acids; ERK - extracellular signal-related kinase; ET-1 -

endothelin-1