10-31-2016

Regulation of microvascular flow and metabolism: An overview

Michelle A. Keske

Renee M. Dwyer

Ryan D. Russell
The University of Texas Rio Grande Valley, ryan.russell@utrgv.edu

Sarah J. Blackwood

Aascha A. Brown

See next page for additional authors

Recommended Citation

Keske, M.A., Dwyer, R.M., Russell, R.D., Blackwood, S.J., Brown, A.A., Hu, D., Premilovac, D., Richards, S.M. and Rattigan, S. (2017), Regulation of microvascular flow and metabolism: An overview. Clin Exp Pharmacol Physiol, 44: 143-149. https://doi.org/10.1111/1440-1681.12688

Received Date : 21-Jun-2016

Revised Date : 07-Oct-2016

Accepted Date : 21-Oct-2016

Article type : Symposium Paper - AuPS

Regulation of Microvascular Flow and Metabolism: An Overview

Michelle A. Keske[1], Renee M. Dwyer[2], Ryan D. Russell[1], Sarah J. Blackwood[1], Aascha A. Brown[1], Donghua Hu[1], Dino Premilovac[2], Stephen M. Richards[2], Stephen Rattigan[1]

[1] Menzies Institute for Medical Research, [2]School of Medicine, University of Tasmania, Hobart, Australia.

Clinical and Experimental Pharmacology and Physiology

AuPS Symposia Paper

Word count: 5483

Conflict of interests

There are no conflicts of interest.

Correspondence:

Michelle A. Keske (PhD)

Menzies Institute for Medical Research,

University of Tasmania,
Hobart, Tasmania,
7000, Australia
Ph: (03) 62 26 2669
Fax: (03) 62 26 7704
Email: Michelle.Keske@utas.edu.au

Abstract

Skeletal muscle is an important site for insulin to regulate blood glucose levels. It is estimated that skeletal muscle is responsible for ~80% of insulin-mediated glucose disposal in the post-prandial period. The classical action of insulin to increase muscle glucose uptake involves insulin binding to insulin receptors on myocytes to stimulate glucose transporter 4 (GLUT 4) translocation to the cell surface membrane, enhancing glucose uptake. However, an additional role of insulin that is often under-appreciated is its action to increase muscle perfusion thereby improving insulin and glucose delivery to myocytes. Either of these responses (myocyte and/or vascular) may be impaired in insulin resistance, and both impairments are apparent in type 2 diabetes, resulting in diminished glucose disposal by muscle. The aim of this review is to report on the growing body of literature suggesting that insulin-mediated control of skeletal muscle perfusion is an important regulator of muscle glucose uptake and that impairment of microvascular insulin action has important physiological consequences early in the pathogenesis of insulin resistance. This work was discussed at the 2015 Australian Physiological Society Symposium "Physiological mechanisms controlling microvascular flow and muscle metabolism".

Key words: Insulin action, blood flow, insulin resistance

Introduction

Skeletal muscle makes up a high proportion of body mass (40% of total body weight) due to its important mechanical role in the body. The contractile function of skeletal muscle necessitates increased metabolic activity, the requirements of which are met by alterations in blood flow to facilitate nutrient delivery and waste removal via an intricate vascular network.

Due to its considerable mass, high metabolic rate, and capacity to store glycogen, skeletal muscle is a major site for glucose disposal and thus an important tissue for whole body glucose homeostasis.

Since the early 20th century it has been known that skeletal muscle blood flow increases dramatically during increased metabolic demand. It is becoming clear that increasing total blood flow to muscle does not necessarily improve nutrient exchange by myocytes (1, 2). These consistent findings strongly suggest that total muscle blood flow may not be tightly coupled with metabolism. Rather, the vascular system in muscle has a further level of complexity which involves modulation of blood flow at the microvascular level to control myocyte nutrient exchange. This review highlights the growing body of literature suggesting that insulin-mediated control of the microvasculature in skeletal muscle is an important physiological regulator of muscle glucose uptake, and that loss of microvascular insulin action precedes and potentially drives the development of myocyte insulin resistance.

Insulin and total muscle blood flow

Studies from the 1930s demonstrated that insulin could cause vasodilation and modify blood flow (3). However, these studies were conducted with large doses of insulin that resulted in hypoglycaemia. Thus, whether the vascular activity was due to insulin *per se*, or due to an associated hypoglycaemia-induced epinephrine release was unclear. In the 1990s Baron and colleagues performed studies in humans using the euglycaemic hyperinsulinaemic clamp technique and confirmed that insulin was indeed vasoactive and could dilate the vascular network in skeletal muscle (4, 5). They demonstrated that insulin increases total leg blood flow in a dose-dependent fashion, and that this response was paralleled by increases in muscle glucose uptake. Thus, it was Baron and colleagues who championed the notion that insulin-mediated increases in total muscle blood flow augments the metabolic actions of insulin by enhancing the delivery of glucose and insulin to the myocyte (4, 5). A number of independent investigators have produced similar results showing that insulin stimulates total muscle blood flow in both humans (6-11) and experimental animals (12-14).

At that time, however, the effect of insulin to stimulate muscle blood flow was viewed as highly controversial. Many studies that reported increases in total blood flow used supra-physiological doses of insulin or required extended exposure times (several hours) before seeing any effect on muscle blood flow (8, 15, 16). These findings raised concerns about the

physiological relevance of insulin-stimulated blood flow, particularly given that insulin levels typically remain elevated for only 90-120 min following a meal in healthy people (17, 18). Yki-Jarvinen and colleagues challenged this theory by demonstrating that insulin-stimulated glucose uptake in skeletal muscle occurred before any augmentation of total muscle blood flow (19-21). These findings raised a number of questions regarding the impact of increased blood flow on insulin-mediated muscle metabolism (19-21). This temporal discordance argument against an important vascular role of insulin is invalid due to the assumption that insulin's vascular action is restricted only to increases in total muscle blood flow. We, together with our collaborators (1, 2), hypothesise that modulation of blood flow within the microvascular network is a more physiologically relevant mode of nutrient and hormone delivery to the myocyte than total limb blood flow.

Insulin and microvascular blood flow

As highlighted above, most studies have focused on insulin's action on total muscle blood flow. However, the effect of insulin to alter the distribution of microvascular blood flow within skeletal muscle may be more physiologically relevant. Total blood flow in skeletal muscle is controlled by constriction/dilation of the 1^{st}-3^{rd} order arterioles. The control of capillary (microvascular) perfusion in skeletal muscle is controlled by the 3^{rd}-5^{th} order arterioles (see Figure 1). At any given point in time, not all capillaries are perfused (22, 23) and this is due vasomotion (24, 25). Vasomotion is a process where blood vessels contract and dilate at regular time intervals to modulate blood flow through different capillary modules to maintain efficient nutrient supply. Therefore, taking a muscle biopsy to count capillaries is not sufficient to understand the extent of microvascular blood flow *in vivo*.

In order to determine the microvascular actions of insulin in skeletal muscle, we developed two techniques for measuring blood flow at this level of the vascular tree *in vivo*. The first technique we developed relies on metabolism of exogenously infused 1-methylxanthine (1-MX) to 1-methylurate (1-MU) by microvascular xanthine oxidase (13, 14, 26-30). 1-MX and 1-MU can be quantified in plasma using high performance liquid chromatography (31). Xanthine oxidase is located primarily on capillary endothelial cells, and not large arteries and veins, or in myocytes (32, 33). The disappearance of 1-MX across the hindleg (A-V difference x femoral artery blood flow) can therefore be used as a biochemical marker for the extent of microvascular blood flow in muscle.

The second technique was an adaptation of an ultrasound imaging technique (contrast-enhanced ultrasound, CEU) to skeletal muscle (14, 34-41). This technique involves infusion of albumin or phospholipid microbubbles (perfluorocarbon gas filled) into the systemic circulation. The microbubbles are a similar in size and rheology to red blood cells, and importantly they remain intravascular (42) making them an excellent perfusion tracer. The microbubbles oscillate in size when exposed to ultrasound, and can be destroyed with high energy pulses of ultrasound. The oscillation and destruction of microbubbles results in the generation of a signal that can be measured as acoustic intensity. Following ultrasonic destruction of microbubbles, the rate of reappearance of microbubbles is a reflection of blood velocity, while the microvascular blood volume can be measured by the plateau of tissue opacification. The contribution of fast filling arteries, arterioles, veins and venules can be background subtracted due to the high flow velocity and thus the residual signal reflects only the microcirculation. Replenishment curves are fitted to $y = A(1 - e^{-\beta t})$, where y is acoustic intensity, t is the time from the destructive ultrasound pulse, A is plateau video intensity (microvascular blood volume), and β is the rate constant, which provides a measure of flow velocity in the microvasculature.

Using both 1-MX and CEU techniques, we were the first to demonstrate that insulin increases microvascular blood flow in skeletal muscle in a similar fashion to muscle contraction (36, 43) (see Figure 1). Importantly, this microvascular action of insulin contributes to ~50% of insulin-stimulated glucose disposal, a finding observed in both humans and experimental animals (13, 14, 35-40). In addition, the microvascular response to insulin can occur independent of its effects on total muscle blood flow, and that even low physiological doses of insulin produced marked increases in microvascular blood flow (14, 30, 36, 39), (44). Interestingly, this insulin-stimulated increase in microvascular blood flow occurs early (by 7 min) in rats and precedes stimulation of the insulin signalling cascade in the myocyte or any significant increase in muscle glucose uptake (39) .

Given that insulin is released into the circulation in response to a meal, it is plausible that the vascular actions of insulin occur first to facilitate increased delivery of glucose and insulin itself to the myocyte for glucose disposal. Indeed, we have shown that insulin-mediated microvascular responses occur whether insulin is infused intravenously (euglycaemic hyperinsulinaemic clamp) (35, 45) or secreted from the pancreas following the ingestion of a

mixed meal (37). When taken together, these data highlight the sensitive and rapid nature of insulin's microvascular actions in skeletal muscle and emphasise the integral role that the microvasculature has in normal insulin-mediated muscle glucose uptake.

Mechanism of insulin-mediated microvascular recruitment

Local infusion of insulin into the forearm of healthy subjects, at a dose that does not have any significant spill-over to the systemic circulation, can stimulate microvascular blood flow and is paralleled by increased muscle glucose uptake (41). In contrast, central (intracerebroventricular) administration of insulin in healthy rats does not produce any metabolic or haemodymanic effects in skeletal muscle (46). Together, these studies suggest that insulin's effects on microvascular blood flow in muscle are mediated by local, rather than central actions of insulin.

Nitric oxide (NO) has been strongly implicated in insulin-mediated vasodilation (47, 48). Quon and colleagues have elucidated the insulin signalling cascade in cultured vascular endothelial cells which involves the activation of the insulin receptor/IRS-1/PI3K/Akt/eNOS pathway leading to NO production (49-51). The resulting NO then acts to relax adjacent vascular smooth muscle cells thus causing vasodilation. Insulin's ability to stimulate microvascular blood flow in muscle is inhibited by systemic infusion of the nitric oxide synthase inhibitor, Nω-nitro-L-arginine-methyl ester (L-NAME) (39, 48). Importantly, insulin-mediated microvascular recruitment can also be blocked when L-NAME is infused locally, implicating local NOS activation in insulin's vascular actions (52).

Endothelin-1 (ET-1) has also been implicated in the regulation of insulin-mediated microvascular blood flow. In addition to NO, insulin can also stimulate ET-1 release via a MAPK-dependent pathway (53). Systemic infusion of ET-1 into rats can block insulin-mediated microvascular blood flow (54). The vasodilator effects of insulin on large artery dilation are augmented by ET-1 receptor blockade (55, 56). Thus, insulin causes generation of both NO and ET-1, and it is the balance between these two vasoactive agents that contributes to the regulation of microvascular blood flow in skeletal muscle (55, 57).

Epoxyeicosatrienoic acids (EETs) are signalling molecules formed from arachidonic acid. New evidence is emerging to suggest that EETs are involved in insulin-mediated microvascular recruitment in skeletal muscle, particularly when insulin-mediated NO dilation is blunted (58). There is strong evidence that these compounds are one of the endothelial-

derived hyperpolarising factors causing vasodilation of skeletal muscle microvasculature (58). A role for EET production in insulin sensitisation is suggested from a study from Xu and colleagues (59) showing that CYP2J3 gene delivery in vivo increased EET generation, reduced blood pressure, and reversed insulin resistance, albeit by an unknown mechanism.

Impaired microvascular blood flow and insulin resistance

Insulin resistance is defined as a reduced biological response to insulin (60, 61). Given the evidence detailed above regarding insulin's vascular actions, it is logical that the development of insulin resistance also includes a haemodynamic mechanism.

Laakso and colleagues have demonstrated that obese, insulin resistant people display reduced total muscle insulin-mediated vasodilation (4). Others have reported a relationship between insulin resistance and endothelial dysfunction (62-64). While these studies have restricted measurements to large blood vessel responses to insulin, microvascular responses to insulin also appear to be reduced during obesity and insulin resistance. De Jongh and colleagues assessed microvascular function in skin of healthy lean and obese individuals in response to insulin (65). The obese, insulin resistant cohort showed reduced insulin-mediated microvascular blood flow in skin and this correlated with whole body insulin resistance (65).

Vasoconstrictors such as α-methylserotonin (26) and ET-1 (54), inhibit both insulin-stimulated microvascular blood flow and glucose uptake *in vivo***.** The loss of vascular insulin action is also apparent during acute infusion of factors known to be elevated in various insulin resistant states, such as TNFα (29) and elevated free fatty acids (elevated by infusion of Intralipid and heparin) (66). In addition, chronic animal models of insulin resistance, including the high fat-fed (27), Zucker obese (28), and Zucker Diabetic fatty (34) rats display reduced microvascular and metabolic responses to insulin. Activity restricted insulin resistant primates also display impaired muscle microvascular responses to marked insulin secretion elicited by an intravenous glucose tolerance test (67). Insulin infusion in humans (35) or the ingestion of a mixed meal (37) similarly act to increase microvascular blood flow and these microvascular responses are blunted in obese insulin resistant subjects (35, 37).

Together, these studies highlight the important link between microvascular and metabolic actions of insulin in muscle, and implicate the loss of microvascular insulin sensitivity in the development and progression of myocyte insulin resistance.

Microvascular-induced muscle insulin resistance

The mechanisms of skeletal muscle insulin resistance are multifactorial, however most investigators have focused on myocyte insulin action. It is important to note that most animal models used to investigate insulin resistance develop both myocyte and microvascular insulin resistance. However, we and others have evidence demonstrating that the microvasculature can become insulin resistant prior to changes in myocyte insulin sensitivity suggesting the vasculature is an early contributor to development of insulin resistance (68-71).

We have recently characterised two dietary animal models that develop microvascular insulin resistance (68, 69). The first of these models is the moderately raised dietary fat model, in which dietary fat in rats is increased from 5% to 9% wt./wt. (68), rather than the more common 5- to 7-fold increase employed in many studies (27, 72, 73). The second model we have characterised is the high salt-fed rat in which dietary NaCl is increased from 0.3% to 8.0% wt./wt (69). After a four-week dietary intervention, both of these animal models develop whole body, skeletal muscle and microvascular insulin resistance *in vivo* when compared to control rats. Insulin-mediated myocyte glucose uptake is normal in both of these animal models when assessed using the constant-flow pump-perfused hindleg technique. In this *ex vivo* preparation the vasculature is fully intact, but does not dilate in response to insulin, enabling insulin and glucose delivery to the myocyte in the absence of insulin-mediated changes in blood flow (68). The reduction in insulin-stimulated muscle glucose uptake *in vivo* therefore is driven by microvascular, rather than myocyte insulin resistance in these two animal models. These data position the loss of normal microvascular function as an early event in the development of muscle insulin resistance.

The endothelial IRS2 knockout mouse displays microvascular insulin resistance and impaired insulin-mediated muscle glucose uptake *in vivo,* and this is associated with reduced activation of eNOS in the vascular endothelium (71). When muscles from these animals are incubated with insulin *in vitro* (where delivery to the myocyte occurs by diffusion and not via the vasculature), myocyte glucose uptake is comparable to control animals. These data implicate reduced eNOS activation by insulin as a contributor to reduced insulin-mediated microvascular recruitment and muscle glucose uptake *in vivo*.

Recent evidence suggests an important role of muscle capillary density in insulin action. Muscle-specific vascular endothelial growth factor (VEGF) knockout animals have reduced

skeletal muscle capillary density and display whole body and muscle insulin resistance *in vivo* (70). However, when muscle samples are isolated and incubated to assess myocyte insulin action, their degree of myocyte insulin sensitivity was similar to control animals. Similarly, humans with reduced skeletal muscle capillary density are also muscle insulin resistant (74). When capillary density in muscle is enhanced with prazosin, muscle glucose uptake improves without any measurable effects on muscle insulin signalling, suggesting enhanced vascular delivery of insulin and glucose to the myocyte (75).

Thus, changes in diet (e.g. elevated dietary fat or sodium), impaired eNOS activation, or even reduced muscle capillary density can impair insulin-mediated microvascular function, even in the presence of normal myocyte insulin sensitivity.

Transport of insulin from the vasculature into the interstitial space

Another potential rate-limiting step for insulin's metabolic actions in skeletal muscle is the movement of insulin from the vasculature to the interstitial space. It has been consistently shown that the concentration of insulin in the interstitial space in skeletal muscle is ~50% lower than the concentration in plasma (76-78). Given that the time-course for insulin-mediated glucose uptake in skeletal muscle is delayed in insulin resistance and type 2 diabetes, there may be a delay in the transit of insulin from the vasculature to the interstitium in these pathologies (79).

In cell culture experiments, insulin transport into the vascular endothelium is insulin receptor mediated (80, 81). In addition, trans-endothelial transport of insulin is dependent on PI3-K, MAPK and cSrc-family tyrosine kinase signalling pathways and activation of eNOS (82). Importantly, inflammatory cytokines such as TNFα and IL-6, which are elevated during states of insulin resistance, impair insulin uptake into the endothelium (83). Thus, these findings suggest that impaired or delayed insulin delivery to the interstitial space in contact with myocytes is a potential mechanism in the development of insulin resistance. However, whether trans-endothelial transport of insulin is a rate limiting step for glucose uptake in insulin resistant muscle still remains to be confirmed *in vivo* (84).

Conclusions

A large body of work over the past two decades has demonstrated that the microvascular effects of insulin are an important component of its capacity to increase glucose disposal in skeletal muscle. Even in the presence of normal myocyte insulin sensitivity, any disruption of

this microvascular action of insulin significantly reduces glucose disposal by skeletal muscle. Thus, microvascular-derived insulin resistance is likely to be one of the first pathogenic changes in the development of insulin resistance, and is therefore an important target for early detection to prevent progression to type 2 diabetes and to inform patient care. An important question that remains is whether correcting this microvascular defect (i.e. restoring insulin-mediated microvascular responses) can effectively treat insulin resistance and type 2 diabetes. This is an area we and others are actively investigating.

Acknowledgements

There are no conflicts of interest. All authors contributed to the writing of this review and approved final version. This work was funded by various grants awarded from the National Health and Medical Research Council of Australia, Australian Research Council, Heart Foundation of Australia, Diabetes Australia, and the National Institutes of Health.

References

1. Barrett EJ, Rattigan S. Muscle perfusion: its measurement and role in metabolic regulation. *Diabetes* 2012; **61**:2661-8.

2. Vincent MA, Clerk LH, Rattigan S, Clark MG, Barrett EJ. Active role for the vasculature in the delivery of insulin to skeletal muscle. *Clin Exp Pharmacol Physiol* 2005; **32**:302-7.

3. Abramson DI, Schkloven N, Margolis MN, Mirsky IA. Influence of massive doses of insulin on peripheral blood flow in man. *Am. J. Physiol.* 1939; **128**:124-32.

4. Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. *J. Clin. Invest.* 1990; **85**:1844-52.

5. Laakso M, Edelman SV, Olefsky JM, Brechtel G, Wallace P, Baron AD. Kinetics of in vivo muscle insulin-mediated glucose uptake in human obesity. *Diabetes* 1990; **39**:965-74.

6. Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J. Clin. Invest.* 1991; **87**:2246-52.

7. Dela F, Larssen JJ, Mikines KJ, Galbo H. Normal effect of insulin to stimulate leg blood flow in NIDDM. *Diabetes* 1995; **44**:221-6.

8. Raitakari M, Nuutila P, Ruotsalainen U, et al. Evidence for dissociation of insulin stimulation of blood flow and glucose uptake in human skeletal muscle - Studies using [15 O]H 2 O, [18 F]fluoro-2-deoxy-D-glucose, and positron emission tomography. *Diabetes* 1996; **45**:1471-7.

9. Tack CJ, Lutterman JA, Vervoort G, Thien T, Smits P. Activation of the sodium-potassium pump contributes to insulin-induced vasodilation in humans. *Hypertension* 1996; **28**:426-32.

10. Utriainen T, Malmstrom R, Makimattila S, Yki-Jarvinen H. Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. *Diabetologia* 1995; **38**:555-64.

11. Vollenweider P, Tappy L, Randin D, et al. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J. Clin. Invest.* 1993; **92**:147-54.

12. Renaudin C, Michoud E, Rapin JR, Lagarde M, Wiernsperger N. Hyperglycaemia modifies the reaction of microvessels to insulin in rat skeletal muscle. *Diabetologia* 1998; **41**:26-33.

13. Rattigan S, Clark MG, Barrett EJ. Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 1997; **46**:1381-8.

14. Vincent MA, Dawson D, Clark AD, et al. Skeletal muscle microvascular recruitment by physiological hyperinsulinemia precedes increases in total blood flow. *Diabetes* 2002; **51**:42-8.

15. Baron AD, Brechtel-Hook G, Johnson A, Hardin D. Skeletal muscle blood flow. A possible link between insulin resistance and blood pressure. *Hypertension* 1993; **21**:129-35.

16. Bonadonna RC, Saccomani MP, Del Prato S, Bonora E, Defronzo RA, Cobelli C. Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. *Circulation* 1998; **98**:234-41.

17. Fugmann A, Sarabi M, Karlstrom B, Berne C, Lithell H, Lind L. Blood flow is an important determinant of forearm glucose uptake following a mixed meal. *Acta Diabetol.* 2003; **40**:113-7.

18. Vincent MA, Clerk LH, Lindner JR, et al. Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am. J. Physiol. Endocrinol. Metab.* 2006; **290**:E1191-E7.

19. Nuutila P, Raitakari M, Laine H, et al. Role of blood flow in regulating insulin-stimulated glucose uptake in humans. Studies using bradykinin, [15O]water, and [18F]fluoro-deoxy- glucose and positron emission tomography. *J. Clin. Invest.* 1996; **97**:1741-7.

20. Vicini P, Bonadonna RC, Utriainen T, et al. Estimation of blood flow heterogeneity distribution in human skeletal muscle from positron emission tomography data. *Ann. Biomed. Eng.* 1997; **25**:906-10.

21. Utriainen T, Nuutila P, Takala T, et al. Intact insulin stimulation of skeletal muscle blood flow, its heterogeneity and redistribution, but not of glucose uptake in non-insulin-dependent diabetes mellitus. *J. Clin. Invest.* 1997; **100**:777-85.

22. Sweeney TE, Sarelius IH. Arteriolar control of capillary cell flow in striated muscle. *Circulation Research* 1989; **64**:112-20.

23. Honig CR, Odoroff CL, Frierson JL. Active and passive capillary control in red muscle at rest and in exercise. *American Journal of Physiology* 1982; **243**:H196-H206.

24. Intaglietta M. Vasomotor activity, time-dependent fluid exchange and tissue pressure. *Microvasc Res* 1981; **21**:153-64.

25. Intaglietta M, Gross JF. Vasomotion, tissue fluid flow and the formation of lymph. *International Journal of Microcirculation: Clinical and Experimental* 1982; **1**:55-65.

26. Rattigan S, Clark MG, Barrett EJ. Acute vasoconstriction-induced insulin resistance in rat muscle in vivo. *Diabetes* 1999; **48**:564-9.

27. St-Pierre P, Genders AJ, Keske MA, Richards SM, Rattigan S. Loss of insulin-mediated microvascular perfusion in skeletal muscle is associated with the development of insulin resistance. *Diabetes Obes Metab* 2010; **12**:798-805.

28. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark AD, Clark MG. Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. *Diabetes* 2002; **51**:3492-8.

29. Youd JM, Rattigan S, Clark MG. Acute impairment of insulin-mediated capillary recruitment and glucose uptake in rat skeletal muscle in vivo by TNF-alpha. *Diabetes* 2000; **49**:1904-9.

30. Zhang L, Vincent MA, Richards SM, et al. Insulin sensitivity of muscle capillary recruitment in vivo. *Diabetes* 2004; **53**:447-53.

31. Rattigan S, Appleby GJ, Miller KA, et al. Serotonin inhibition of 1-methylxanthine metabolism parallels its vasoconstrictor activity and inhibition of oxygen uptake in perfused rat hindlimb. *Acta Physiologica Scandinavica* 1997; **161**:161-9.

32. Hellsten-Westing Y. Immunohistochemical localization of xanthine oxidase in human cardiac and skeletal muscle. *Histochemistry* 1993; **100**:215-22.

33. Jarasch ED, Bruder G, Heid HW. Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiologica Scandinavica Supplementum* 1986; **548**:39-46.

34. Clerk LH, Vincent MA, Barrett E, Lankford MF, Lindner JR. Skeletal muscle capillary responses to insulin are abnormal in late-stage diabetes and are restored by angiotensin converting enzyme inhibition. *Am.J.Physiol.Endocrinol.Metab.* 2007; **293**:E1804-E9.

35. Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes* 2006; **55**:1436-42.

36. Dawson D, Vincent MA, Barrett EJ, et al. Vascular recruitment in skeletal muscle during exercise and hyperinsulinemia assessed by contrast ultrasound. *Am J Physiol Endocrinol Metab* 2002; **282**:E714-20.

37. Keske MA, Clerk LH, Price WJ, Jahn LA, Barrett EJ. Obesity blunts microvascular recruitment in human forearm muscle after a mixed meal. *Diabetes Care* 2009; **32**:1672-7.

38. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab* 2003; **285**:E123-9.

39. Vincent MA, Clerk LH, Lindner JR, et al. Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. *Diabetes* 2004; **53**:1418-23.

40. Vincent MA, Clerk LH, Lindner JR, et al. Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am J Physiol Endocrinol Metab* 2006; **290**:E1191-7.

41. Coggins M, Lindner J, Rattigan S, et al. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 2001; **50**:2682-90.

42. Jayaweera AR, Edwards N, Glasheen WP, Villanueva FS, Abbott RD, Kaul S. In vivo myocardial kinetics of air-filled albumin microbubbles during myocardial contrast echocardiography. Comparison with radiolabeled red blood cells. *Circulation Research* 1994; **74**:1157-65.

43. St-Pierre P, Keith LJ, Richards SM, Rattigan S, Keske MA. Microvascular blood flow responses to muscle contraction are not altered by high-fat feeding in rats. *Diabetes Obes Metab* 2012; **14**:753-61.

44. Eggleston EM, Jahn LA, Barrett EJ. Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake. *Diabetes* 2007; **56**:2958-63.

45. Sjoberg KA, Holst JJ, Rattigan S, Richter EA, Kiens B. GLP-1 increases microvascular recruitment but not glucose uptake in human and rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 2014; **306**:E355-62.

46. Bradley EA, Willson KJ, Choi-Lundberg D, Clark MG, Rattigan S. Effects of central administration of insulin or l-NMMA on rat skeletal muscle microvascular perfusion. *Diabetes Obes Metab* 2010; **12**:900-8.

47. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* 1994; **94**:1172-9.

48. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S. Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am. J. Physiol. Endocrinol. Metab.* 2003; **285**:E123-E9.

49. Montagnani M, Ravichandran LV, Chen H, Esposito DL, Quon MJ. Insulin receptor substrate-1 and phosphoinositide-dependent kinase-1 are required for insulin-stimulated production of nitric oxide in endothelial cells. *Mol Endocrinol* 2002; **16**:1931-42.

50. Zeng GY, Quon MJ. Insulin-stimulated production of nitric oxide is inhibited by wortmannin - Direct measurement in vascular endothelial cells. *J. Clin. Invest.* 1996; **98**:894-8.

51. Zeng G, Nystrom FH, Ravichandran LV, et al. Roles for insulin receptor, PI3-kinase, and Akt in insulin-signaling pathways related to production of nitric oxide in human vascular endothelial cells. *Circulation* 2000; **101**:1539-45.

52. Bradley EA, Richards SM, Keske MA, Rattigan S. Local NOS inhibition impairs vascular and metabolic actions of insulin in rat hindleg muscle in vivo. *Am J Physiol Endocrinol Metab* 2013; **305**:E745-50.

53. Potenza MA, Marasciulo FL, Chieppa DM, et al. Insulin resistance in spontaneously hypertensive rats is associated with endothelial dysfunction characterized by imbalance between NO and ET-1 production. *Am. J. Physiol. Heart Circ. Physiol.* 2005; **289**:H813-H22.

54. Ross RM, Kolka CM, Rattigan S, Clark MG. Acute blockade by endothelin-1 of haemodynamic insulin action in rats. *Diabetologia* 2007; **50**:443-51.

55. Cardillo C, Nambi SS, Kilcoyne CM, et al. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation* 1999; **100**:820-5.

56. Cardillo C, Campia U, Bryant MB, Panza JA. Increased activity of endogenous endothelin in patients with Type II diabetes mellitus. *Circulation* 2002; **106**:1783-7.

57. Eringa EC, Stehouwer CD, Walburg K, et al. Physiological concentrations of insulin induce endothelin-dependent vasoconstriction of skeletal muscle resistance arteries in the presence of tumor necrosis factor-{alpha} dependence on c-Jun N-terminal kinase. *Arteriosclerosis, Thrombosis, and Vascular Biology* 2005; **26**:274-80.

58. Shim CY, Kim S, Chadderdon S, et al. Epoxyeicosatrienoic acids mediate insulin-mediated augmentation in skeletal muscle perfusion and blood volume. *Am. J. Physiol. Endocrinol. Metab.* 2014; **307**:E1097-104.

59. Xu X, Zhao CX, Wang L, et al. Increased CYP2J3 expression reduces insulin resistance in fructose-treated rats and db/db mice. *Diabetes* 2010; **59**:997-1005.

60. Defronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* 1991; **14**:173-94.

61. Reaven GM. Insulin resistance and compensatory hyperinsulinemia: role in hypertension, dyslipidemia, and coronary heart disease. *Am. Heart J.* 1991; **121**:1283-8.

62. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *Journal of Clinical Investigation* 1996; **97**:2601-10.

63. Hashimoto M, Akishita M, Eto M, et al. The impairment of flow-mediated vasodilatation in obese men with visceral fat accumulation. *International Journal of Obesity and Related Metabolic Disorders* 1998; **22**:477-84.

64. Westerbacka J, Vehkavaara S, Bergholm R, Wilkinson I, Cockcroft J, Yki-Jarvinen H. Marked resistance of the ability of insulin to decrease arterial stiffness characterizes human obesity. *Diabetes* 1999; **48**:821-7.

65. de Jongh RT, Serne EH, Ijzerman RG, De Vries G, Stehouwer CD. Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation* 2004; **109**:2529-35.

66. Clerk LH, Rattigan S, Clark MG. Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes* 2002; **51**:1138-45.

67. Chadderdon SM, Belcik JT, Smith E, et al. Activity restriction, impaired capillary function, and the development of insulin resistance in lean primates. *Am J Physiol Endocrinol Metab* 2012; **303**:E607-13.

68. Premilovac D, Bradley EA, Ng HL, Richards SM, Rattigan S, Keske MA. Muscle insulin resistance resulting from impaired microvascular insulin sensitivity in Sprague Dawley rats. *Cardiovasc Res* 2013; **98**:28-36.

69. Premilovac D, Richards SM, Rattigan S, Keske MA. A vascular mechanism for high-sodium-induced insulin resistance in rats. *Diabetologia* 2014; **57**:2586-95.

70. Bonner JS, Lantier L, Hasenour CM, James FD, Bracy DP, Wasserman DH. Muscle-specific vascular endothelial growth factor deletion induces muscle capillary rarefaction creating muscle insulin resistance. *Diabetes* 2013; **62**:572-80.

71. Kubota T, Kubota N, Kumagai H, et al. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 2011; **13**:294-307.

72. Turner N, Kowalski GM, Leslie SJ, et al. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* 2013; **56**:1638-48.

73. Kraegen EW, James DE, Storlien LH, Burleigh KM, Chisholm DJ. In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. *Diabetologia* 1986; **29**:192-8.

74. Gavin TP, Stallings HW, 3rd, Zwetsloot KA, et al. Lower capillary density but no difference in VEGF expression in obese vs. lean young skeletal muscle in humans. *J Appl Physiol (1985)* 2005; **98**:315-21.

75. Akerstrom T, Laub L, Vedel K, et al. Increased skeletal muscle capillarization enhances insulin sensitivity. *Am J Physiol Endocrinol Metab* 2014; **307**:E1105-16.

76. Yang YJ, Hope I, Ader M, Poulin RA, Bergman RN. Dose-response relationship between lymph insulin and glucose uptake reveals enhanced insulin sensitivity of peripheral tissues. *Diabetes* 1992; **41**:241-53.

77. Yang YJ, Hope ID, Ader M, Bergman RN. Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest* 1989; **84**:1620-8.

78. Sjostrand M, Holmang A, Lonnroth P. Measurement of interstitial insulin in human muscle. *Am J Physiol* 1999; **276**:E151-4.

79. Nolan JJ, Ludvik B, Baloga J, Reichart D, Olefsky JM. Mechanisms of the kinetic defect in insulin action in obesity and NIDDM. *Diabetes* 1997; **46**:994-1000.

80. Barrett EJ, Eggleston EM, Inyard AC, et al. The vascular actions of insulin control its delivery to muscle and regulate the rate-limiting step in skeletal muscle insulin action. *Diabetologia* 2009; **52**:752-64.

81. Barrett EJ, Liu Z. The endothelial cell: an "early responder" in the development of insulin resistance. *Rev Endocr Metab Disord* 2013; **14**:21-7.

82. Wang H, Wang AX, Liu Z, Barrett EJ. Insulin signaling stimulates insulin transport by bovine aortic endothelial cells. *Diabetes* 2008; **57**:540-7.

83. Wang H, Wang AX, Barrett EJ. Caveolin-1 is required for vascular endothelial insulin uptake. *Am J Physiol Endocrinol Metab* 2011; **300**:E134-44.

84. Lee WL, Klip A. Endothelial Transcytosis of Insulin: Does It Contribute to Insulin Resistance? *Physiology (Bethesda)* 2016; **31**:336-45.

FIGURE LEGEND

Figure 1: Under basal conditions ~ 1/3rd of capillaries are perfused in skeletal muscle. During contraction arterioles (1^{o} through to 5^{o}) dilate and capillaries fully recruit in an intensity-dependent fashion. In the presence of insulin, approximately 2/3rd of capillaries are perfused, changes in bulk flow may also occur during hyperinsulinemia. During states of insulin resistance, insulin-mediated capillary perfusion of skeletal muscle is markedly impaired.

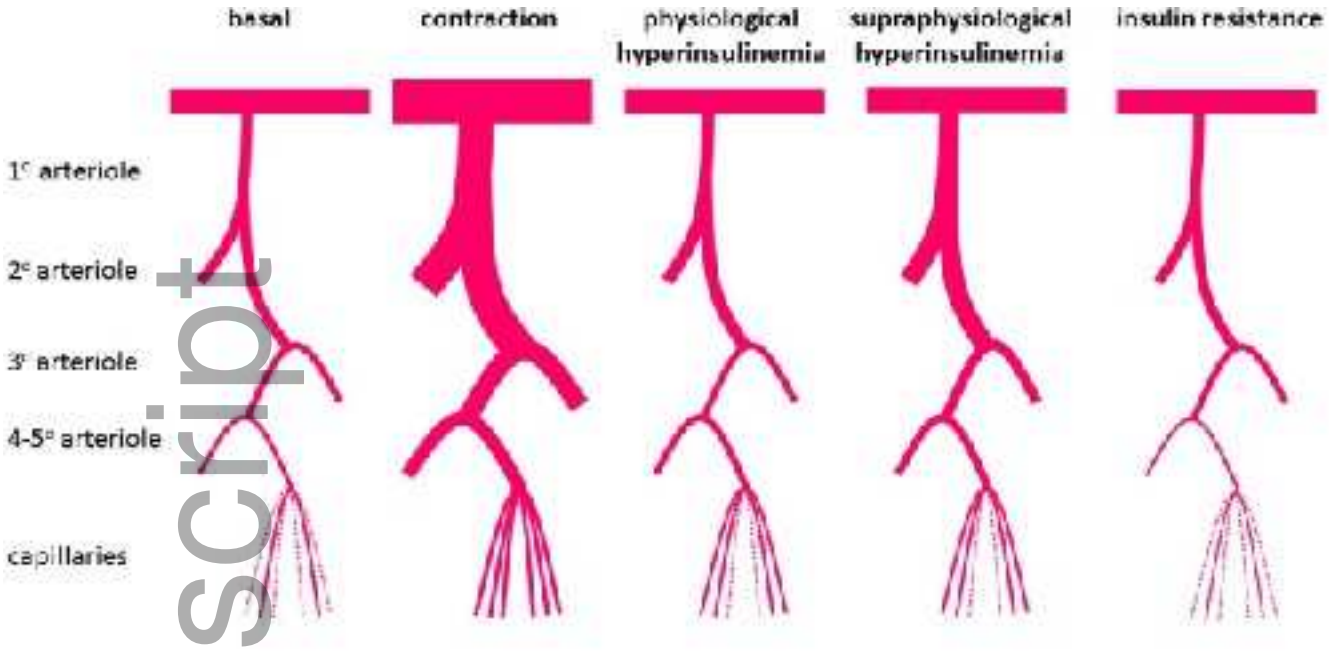

Figure 1:

cep_12688_f1.jpg