Update on adipose tissue blood flow regulation

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Submitted 12 July 2011; accepted in final form 3 February 2012

OVER THE LAST DECADES, THE WORLDWIDE PANDEMIC of obesity, metabolic syndrome, and type 2 diabetes mellitus (T2DM) (188) has attracted ever-increasing attention to the role and significance of adipose tissue (AT). Originally regarded as a mere energy store with insulation and protective cushioning functions, AT is now recognized as a bona fide and highly active metabolic and endocrine organ (102). It has also become evident that the white AT is a heterogeneous tissue. From a metabolic point of view, AT can be subdivided into central (abdominal) and peripheral (hips and buttocks) depots (78). Although extra-abdominal fat accumulation does not bring upon higher metabolic and cardiovascular risk, some reports show a protective influence (124). Conversely, a negative metabolic effect has been associated with central fat (102), which in fact comprises two distinct masses (visceral and subcutaneous) with different metabolic and hormonal activities and impacts on metabolic syndrome pathogenesis (95) and blood flow rates (102).

Metabolic processes in fatty tissue require adequate substrate(s) and humoral factor delivery (76). Conversely, adipocytes communicate with other metabolically active tissues through humoral factors (128) as well as through metabolic products (118) serving as energetic substrates. Humoral factors derived from AT arise not only from adipocytes themselves but also from other components of AT such as macrophages and endothelial cells. Moreover, AT is a known conversion site for several hormones or humoral factors, e.g., steroid hormones (156) or components of the renin-angiotensin-aldosterone system (RAAS). All of these functions of AT are tightly linked to intermediary metabolism requires finely tuned perfusion. Because metabolic and vascular processes are so tightly interconnected, any disruption in one will necessarily impact the other. Although altered ATBF is one consequence of expanding fat tissue, it may also aggravate the negative impacts of obesity on the body’s metabolic milieu. This review attempts to summarize the current state of knowledge on adipose tissue vascular bed behavior under physiological conditions and the various factors that contribute to its regulation as well as the possible participation of altered ATBF in the pathophysiology of metabolic syndrome.

obesity; insulin; sympathetic nervous system; nitric oxide; angiotensin II; atrial natriuretic peptide; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide...
In this review, we attempt to illustrate the complexity of ATBF regulation with emphasis on the physiological states. Our review builds upon our joint experiences and summarizes up-to-date information in the field obtained by PubMed searches in the English language bibliography. Although we focused on selected publications from the past 5 years, we also strove not to exclude older publications that are commonly referenced or highly regarded.

**ATBF Under Different Physiological Conditions**

**ATBF variations during fasting and meals.** Importantly, rather than being constant (28, 80), ATBF appears to be acutely regulated, according to nutritional state and AT metabolic activity. As would be expected, ATBF increases during fat storage [triglyceride (TG) clearance into AT in the postprandial period] (149) as well as fat mobilization [release of free fatty acids (FFA) in fasting, the postabsorptive state, or prolonged exercise] (35).

After an overnight fast, abdominal ATBF is typically \( \approx 3-5 \) ml·100 g tissue \(^{-1}\)·min\(^{-1}\), whereas in resting skeletal muscle the value is \( \approx 1.5 \) ml·100 g tissue \(^{-1}\)·min\(^{-1}\) (46). Furthermore, no sex-specific differences have been found in basal ATBF, but ATBF in abdominal region seems to be higher than in femoral adipose tissue (127). Nevertheless, the existence of a rather large intra-individual variation of ATBF has been well recognized (132). ATBF increases steadily during the night, presumably reflecting duration of fasting (79). An extended overnight fast of 14–22 h causes no significant changes in ATBF (104), but when fasting is prolonged to 72 h, ATBF increases \( \approx 1.5\)-fold (136).

In healthy normal-weight subjects, abdominal ATBF increases from two- to threefold (and even \( \approx 4\)-fold) in response to nutritional stimuli. Similar increases also occur in thigh and forearm ATBF in response to feeding (26, 98, 100, 147, 167, 169) and in both sexes (63). The actual onset of physiological postprandial doubling of ATBF occurs some 30–60 min following oral glucose load or ingestion of a mixed meal and lasts 2–2.5 h (27, 35, 100, 101).

Whereas ATBF rises after an oral glucose load or ingestion of a mixed meal, it does not significantly increase following an ingestion of fat alone (53, 167). ATBF after a normal meal peaks earlier than plasma TG levels (167). Similarly, lipoprotein lipase (LPL) action concomitantly with adipose tissue fat deposition occurs later (32, 35). Conversely, ATBF coincides with FFA suppression (42), suggesting that increased postprandial blood flow might serve to deliver a signal, such as insulin, triggering postprandial LPL transcription in preparation for a subsequent peak in plasma TG. Those actions take place to lower FFA delivery to the liver and prevent FFA lowering of endothelial functions (164) by suppression of hormone-sensitive lipase (HSL) activity in the fed state.

**How ATBF relates to changes in weight and exercise.** Both fasting ATBF and its responsiveness to nutrients are reduced in obese individuals (169). Fasting ATBF negatively correlates with body mass index (BMI), as does postprandial rise in ATBF. Obesity is typically associated with a number of metabolic disorders, including insulin resistance, hypertriglyceridemia, and low HDL cholesterol concentrations. These disorders might be related to alterations in tissue perfusion (168). It has been found that obese insulin-resistant subjects display lower insulin-stimulated glucose uptake and lower blood flow in both visceral and abdominal subcutaneous fat compared with lean subjects (182). To our knowledge, no study has reported on the effects of long-term weight loss and weight maintenance on ATBF. We have found only short-term studies, relative to rapid weight loss, reported in the literature. It has been reported that water content, a potential reflection of nutritive blood flow (112, 142), was increased after a 15.6-kg weight loss over a 9-wk period, suggesting that improved insulin sensitivity may contribute to improve ATBF (110). Recently, rapid weight loss in healthy obese subjects on very low caloric intake diets induced no modification of ATBF when expressed per gram of mass (181).

Physical exercise reduces body weight and increases insulin sensitivity mainly in skeletal muscles (83). Adipose tissue lipolysis and mobilization of fatty acids increase during prolonged exercise of moderate intensity (48). However, the role of fitness or physical activity on ATBF has not been thoroughly assessed, and the results are rather inconsistent. ATBF rises during moderate-intensity exercise (4–6 h) (25, 161). Surprisingly, ATBF did not increase in nontrained young men or in older women (114) who performed two rounds of 60 min on a bicycle (165). Also, whereas no measurable change occurred in subcutaneous ATBF during or after a resistance exercise in young physically active men, skeletal muscle blood flow rose sharply during exercise (135).

The effect of long-term exercise on ATBF is also controversial. One study showed that 16 wk of cycle exercise training did not affect ATBF despite improved maximal oxygen uptake (\( V\text{˙}_\text{O}_2\text{max} \)) in five healthy untrained men (88). Another study compared eight endurance-trained and eight sedentary young men and showed that ATBF in trainees was not significantly higher than in sedentary subjects (160). As well, 11 obese non-diabetic males taking part in a 12-wk aerobic training program improved their \( V\text{˙}_\text{O}_2\text{max} \) but failed to improve ATBF (166). However, long-term exercise is clearly associated with improvement in insulin sensitivity (83, 89). Impaired regulation of ATBF seems to be another facet of the insulin resistance syndrome (100). In this respect it has been shown that, compared with sedentary control subjects, endurance-trained athletes have a greater ATBF in response to infusion of adrenaline (162), the main adrenergic agent contributing to exercise-induced lipolysis (39).

In conclusion, it seems that acute physical activity is not accompanied by an increase of ATBF, possibly as a result of a catecholamine or FFA effect on blood vessels. It was once hypothesized that ATBF may be the rate-limiting step in FFA combustion during exercise caused by the reduction in fatty acid release into the circulation (86). However, more than two decades later it has been shown that, up to very intense exercise, adipose tissue-derived FFAs make up the majority of oxidative fuel used by muscle and that ATBF parallels the process (58). Limitation of adipose tissue to deliver FFAs may reflect some feedback inhibition of lipolysis, perhaps via lactate, or possibly α-adrenergic inhibition of lipolysis and/or blood flow at very high catecholamine concentrations.

It was suggested that the primary determinant of ATBF responsiveness is not obesity per se but the associated insulin resistance in particular within the AT reflected through insulin sensitivity index based on FFA suppression (19). Thus, the inconsistent effect of a long-term exercise and weight loss on
ATBF may reflect their different impact on muscle and fat mass, whole body vs. AT insulin sensitivity, and other factors such as capillary density and/or recruitment, etc.

**ATBF changes in other physiological situations.** Change from the upright to supine position elicits an instantaneous ATBF increment, which is in accord with a decrease in central and local postural sympathetic vasoconstrictor activity (157). The sitting position also decreases ATBF in the subcutaneous adipose tissue adjacent to the anterior tibial muscle (15). Approximately 90 min after the onset of sleep, there is a considerable increment in blood flow that lasts for 100 min (157). It has also been reported that mental stress (56) and filling of the bladder (54) increase ATBF. What these physiological modulations have in common is that they all may be regulated by the sympathetic nervous system (SNS).

### Factors Influencing ATBF

Endothelial and smooth muscle cells express a number of receptors for a wide range of vasoactive compounds, including receptors for insulin with its effect on nitric oxide synthase, endothelin, angiotensin II (ANG II), aldosterone, atrial natriuretic peptide (ANP), and α1-, α2-, and β-adrenergic receptors, to name but a few. The vast majority of these have been tested in relation to ATBF (Table 1 and Figs. 1 and 2). Regulation of ATBF has been studied extensively and reviewed by Frayn et al. (59).

**Insulin.** ATBF seems to exhibit its highest degree of modulation in response to food intake, which is illustrated by either glucose (26) or mixed-meal (35) ingestion. Postprandial rise in ATBF coincides with a plasma insulin increase and suppression of FFA. Animal studies suggested insulin as a factor controlling vascular tone, especially through nitric oxide (NO) and endothelin-1 (ET-1) production in endothelial cells (180). Impact of insulin on the vascular response and energy metabolism are tightly coregulated, and it has been shown that stimulation of endothelial NO synthase (eNOS) shares the same postreceptor pathway with glucose uptake. Very high concentrations of insulin seem to induce a NO-mediated vasodilatation in skeletal muscle (163) through an insulin-mediated increase of eNOS expression (108). Elevated insulin levels in insulin-resistant subjects, as well as acute endogenous and exogenous insulin elevations in normal and insulin-resistant subjects, have been shown to be related to an elevated level of circulating ET-1, thus reinforcing the link between insulin and ET-1 production and release (122). In contrast to skeletal muscle perfusion (17), studies failed to prove a direct insulin effect on postprandial ATBF (98). NO has been shown to have no role in postprandial ATBF regulation, corroborating this finding (11). It may be that postprandial ATBF is merely concomitant with insulin surge, with the impact of insulin being mediated by an as-yet-unknown mechanism. One possible and indirect effect of insulin on ATBF could be suppression of FFA production. FFAs have been shown to impair endothelial function and vasodilatory response in skeletal muscle as well as insulin resistance with elevated levels of FFAs. This could be one of the way through which insulin and hyperinsulinemia influence ATBF (64, 164). Another indirect mechanism is looked for between insulin and the SNS. It is well known that food intake (137) or imposed hyperinsulinemia (3, 184) induces sympathetic activation. One sign of this is increased plasma concentration of noradrenaline, which is likely to spill over from muscle and AT (137). Contrary to the idea of increased sympathetic activation induced by hyperinsulinemia, a correlation between increased plasma noradrenaline and increased ATBF, but not increased plasma insulin, has been shown (98). Nevertheless, the authors of these studies speculate on the nonlinear relationship between hyperinsulinemia (3, 21) and the degree of sympathetic activation as well as a possible sharp downregulation of adipose tissue adrenergic receptors pursuant to prolonged activation of the SNS by hyperinsulinemia.

In summary, although insulin per se does not seem to directly affect ATBF, its physiological and pathophysiological effects are mediated by other mechanisms such as sympathetic activation or endothelial dysfunction.

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ATBF, adipose tissue blood flow; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; ANP, atrial natriuretic peptide; eNOS, endothelial nitric oxide synthase; ET-1, endothelin-1; GIP, glucose-dependent insulinoitropic polypeptide; GLP-1, glucagon like peptide-1; IR, insulin resistance; NO, nitric oxide; PSNS, parasympathetic nervous system; SNS, sympathetic nervous system. ↑ Stimulation; ↓ inhibition.
SNS. Despite some apparently contradictory reports mentioned above, the SNS is regarded as the major factor controlling postprandial ATBF. Indeed, evidence abounds that \(\beta\)-adrenergic stimulation increases ATBF. The increase is inducible, for instance, by adrenaline infusion (148) or local delivery of \(\beta\)-adrenergic stimuli by microdialysis using dobutamine (16) or isoproterenol (127). Adrenaline and noradrenaline levels increase in response to oral glucose ingestion and may be linked to increased ATBF stimulated by glucose (26). Moreover, sympathetic receptor blockers have been shown to reduce the postprandial response by 58% (11). A study of paraplegic individuals showed that, during an exercise, ATBF rises according to circulating catecholamines rather than sympathetic nerve activity (165).

Several mechanisms have been proposed to explain postprandial SNS activation. Splanchnic bed glucoreceptors have been suggested as one possible trigger because ATBF response is greater when glucose is administered orally vs. intravenously during an euglycemic hyperinsulinemic clamp (98). Conversely, hyperglycemia per se does not directly affect ATBF. Direct hypothalamic stimulation by insulin has also been suggested as another possible trigger of SNS activation. Finally, splanchnic vascular bed distension may also contribute, at least partially. Indeed, intestinal blood flow increases in response to oral but not intravenous glucose. It is interesting to note that SNS activation also occurs following fat ingestion (68), but as discussed earlier, a pure fat meal does not induce a rise in ATBF.

Basal, i.e., preprandial ATBF, is under the influence of the SNS as well. Experiments using \(\alpha\)-adrenergic stimuli, such as clonidine (66) and norfenefrine (57), confirmed the predominantly inhibitory effect of \(\alpha\)-adrenoceptors on ATBF. Comparison of various agonists and antagonists showed that \(\alpha_2\)-adrenoceptor activation leads to more pronounced vasoconstriction, with little importance of \(\alpha_1\)-receptors in AT microcirculation (11). In contrast, \(\beta\)-adrenergic stimulation had no effect on preprandial ATBF (11).

The impact of the SNS on AT vasculature is tightly coregulated with its metabolic role. \(\alpha_2\)-Adrenergic stimulation leads to vasoconstriction as well as lipolysis suppression (66); conversely, \(\beta_2\)-adrenergic stimulation promotes vasodilatation together with lipolysis. Sympathetic coordination of lipolysis with ATBF is disrupted by the antilipolytic effect of insulin, but this occurs only in the early postprandial period (66).

Although sympathetic stimulation increases vascular permeability within AT (145), this does not occur in other tissues such as the skin or skeletal muscle. The unique ability of AT to adjust its vascular permeability has been confirmed recently (158). Increase in permeability is inhibited by \(\alpha\)-receptor-blocking agents (158), and it has been suggested that \(\alpha\)-receptors, when stimulated, cause contraction of smooth muscle cells and dilation of pores between endothelial cells. Even during sympathetically induced vasoconstriction, the product of permeability and capillary surface area for solutes rises (61). This may have important implications on the metabolic pro-
cesses that occur in AT during states of high levels of catecholamines (e.g., during exercise).

Therefore, it is clear that sympathetic control of AT is crucial to the regulation of tissue mass and function. The α₂-adrenoceptor is the main vasoconstrictor in the preprandial period, whereas the β-adrenoceptor is a strong regulator postprandially. The SNS is responsible for fine-tuning ATBF and metabolism so that adequate energetic fuel is available whenever necessary and so that energy is stored postprandially. Its physiological importance is further underlined by the finding that autonomic dysfunction affects basal and postprandial ATBF (64).

Parasympathetic nervous system. There is an apparent contradiction between neuroanatomic and functional studies concerning the parasympathetic innervation in white AT. In 2002, neuroanatomic results by Kreier et al. (107) suggested a parasympathetic nervous system in the white AT of rats. These were largely questioned (18) since results from other studies failed to provide corroboration (14, 69). On the other hand, functional studies showed that significant changes in AT lipolysis occurred after the administration of parasympathetic stimulating or blocking agents (7). Indeed, it was found that nicotinic receptor stimulation increases lipolysis in humans, whereas muscarinic receptor stimulation decreases lipolysis (6). Interestingly, the study by Kreier et al. (107) showed a drop in insulin-mediated glucose and FFA extraction as well as a rise in HSL activity after vagal denervation of fat pads.

No effect of either nicotine or carbachol (a muscarinic receptor agonist) on ATBF has been found when administered locally or systemically (7) using the ethanol escape technique under microdialysis condition. Although this method has been described as rather insensitive for ATBF measurement (99), the finding that systemic nicotine administration stimulates β-adrenergic receptors (7) may at least in part account for discrepancies between anatomic and physiological studies. Several reports of cross-talk point to alternative pathways possibly involved in the parasympathetic influences on AT, such as acetylcholine (ACh) in combination with NO and/or vasoactive intestinal peptide (VIP) or NO with VIP but without ACh (117, 134, 176).

Taken together, results from functional studies suggest dual autonomic control of white AT with anabolic parasympathetic and catabolic sympathetic influences. Nevertheless, the principle of such dual regulation of peripheral tissues is not universal (blood vessels, sweat glands), and neuroanatomic reports have not yet confirmed parasympathetic nervous system in AT so far.

NO. The formation of NO is known to occur in human adipocytes and preadipocytes (8, 143) and to be released in white AT by preadipocytes, adipocytes, vascular endothelial cells, and vascular smooth muscle cells (VSMC) (68, 119, 143). The enzymes responsible are endothelial NO synthase in vascular endothelial cells and adipocytes as well as the inducible NO synthase in all cell types when appropriately stimulated. There is constitutive low-level expression of eNOS in white AT. In vitro, NO formation in AT is upregulated by lipopolysaccharides, cytokines such as tumor necrosis factor-α (TNFα) and interferon-γ, noradrenaline, insulin, and ANG II (82). NO activates soluble guanylate cyclase, resulting in 3’,5’-cyclic monophosphate (cGMP) formation (68) and the subsequent cGMP-dependent protein kinase promotion, leading to the biological effects of NO (91). mRNA expression of several subunits of the soluble guanylate cyclase and both isoforms of the cGMP-dependant protein kinase was reported in isolated human adipocytes and preadipocytes (51), and NO has been shown to modulate lipid metabolism of adipocytes (138).

In addition to its effects on adipocytes, NO is a strong vasodilator in VSMC (119) and takes part in signaling processes that lead to angiogenesis in vascular endothelial cells (138). In accord with blood flow regulation in many other tissues, NO could be a major vasodilator in AT and possibly a direct or indirect mediator of insulin vasodilation (163). After NO-mediated vasodilatation in muscles was described during a euglycemic hyperinsulinemic clamp (163), it was shown in vitro that eNOS is regulated at the level of expression and activity by insulin (108). However, it has been pointed out that the vasodilatory effect of insulin occurs only at pharmacological insulin doses (97). Moreover, N⁴-monomethyl-L-arginine, a NO synthase inhibitor, does not seem to alter local ATBF (8) and attenuates the isoproterenol-induced vasodilation (97) only during AT microdialysis, suggesting that NO is not a mediator of ATBF. Although these measurements were made using the ethanol outflow/inflow ratio, which has an inherently low sensitivity (99), using the ³¹³Xenon washout method it was later shown that N⁴-monomethyl-L-arginine has a vasoconstricting effect in the preprandial period (11). These results are in agreement with the well-established vasodilatory effect of NO in other tissues. Although postprandial ATBF has been shown to be independent of NO, NO activity nevertheless determines the level at which this vasodilatory response takes place (11). Studies on the regulation of ATBF at the transcriptional level suggest that NO production is involved in the response of ATBF to nutrient intake. Expression of eNOS mRNA strongly and positively associates with postprandial but not with fasting ATBF (139). The strong association found between postprandial ATBF and eNOS mRNA content was not affected by body weight, suggesting a genetic influence on ATBF independent of obesity (139). In that study, the expression of eNOS mRNA also decreased in the overweight/obese group, suggesting an “environmental” influence on ATBF. However, these findings contrast with those of previous studies where increased eNOS gene and protein expression was shown in obese subjects (47, 50). It is also noteworthy that thiazolidinediones and other insulin-sensitizing agents enhance resting ATBF (171) and flow-mediated vasodilatation in people with insulin resistance (29, 186), whereas rosiglitazone does not appear to improve flow-mediated vasodilatation in coronary artery of patients without diabetes (154). This may suggest a common mechanism for increasing NO bioavailability in response to insulin sensitization.

Still, the lack of postprandial ATBF responsiveness in insulin resistance remains unexplained. One possible explanation is the β-adrenergic receptor downregulation during chronic sympathetic stimulation in chronic hyperinsulinemia. SNS overactivity has also been shown to promote oxidative stress (81). High oxygen free radical production consumes NO, thus blunting normal insulin-mediated vasodilatation (150). Even individuals without diabetes but with impaired fasting glucose demonstrate significantly lowered endothelium-dependent vasodilatation compared with control subjects (178). Central
nervous (hypothalamic) insulin resistance is suggested as another possible mechanism of impaired postprandial ATBF.

In skeletal muscle, it has been shown that glucose uptake requires not only vasodilatation but also capillary recruitment (17). Thus dysfunction of capillary recruitment may contribute, at least in part, to insulin resistance in muscle together with insufficient vasodilatation or lower capillary density (92). The process is NO dependent and influenced by insulin (33). In addition, recent reports suggest that capillary recruitment may take place in physiological AT perfusion (174) and that this process is impaired in T2DM (175). It is possible that similar mechanisms controlling vasodilatation in AT also contribute to capillary recruitment, but further studies are needed to fully address this presumption as well as the role of NO and insulin.

RAAS. AT possesses all components of the ANG II synthetic pathway, the expression of which has also been confirmed during adipocyte differentiation. Conversely, AT is fully equipped to bind and respond to ANG II. The link between AT and systemic renin-angiotensin system (RAS) may dwell in secretion of angiotensinogen (AGT), ANG II, and other products of RAS into circulation and conversion of plasmatic precursor of ANG II, thus contributing to overall RAS activity, or in receiving signals from systemic RAS.

Angiotensinogen is expressed abundantly in AT. In experimental models, it has been shown that glucocorticosteroids, androgens, FFAs (146), TNFα (13), and overfeeding (60) all separately lead to higher AGT mRNA expression. The influence of insulin is controversial, and β-adrenergic stimulation probably exerts an indirect effect through FFA increase during lipolysis. In animal models, it has been shown that higher ANG II levels negatively influence liver AGT at the expression level. Nevertheless, in AT, a positive feedback loop has been found. As mentioned above, AT produces ANG II. Adipocyte-derived ANG II may enter the circulation and exert its systemic effect, and conversely, systemic ANG II may affect AT growth, metabolism, and perfusion. Goossens et al. (74) showed that basal plasmatic levels of AGT and ANG II were similar in both lean and obese subjects. During β-adrenergic stimulation, plasmatic levels of ANG II increased significantly in obese compared with lean subjects. No release of ANG II has been shown from abdominal AT to the circulation either under basal conditions or after stimulation, showing that locally produced ANG II does not exert endocrine effects. (74).

Conversely, systemic ANG II may enter AT and influence its metabolism and blood flow. Local ANG II administration by microdialysis decreases basal ATBF and lipolysis. Postprandial ATBF is not influenced by ANG II (72). The inhibitory effect of ANG II on ATBF was prevented by an ANG II type I receptor blocker but not by an angiotensin-converting enzyme inhibitor. It has been suggested that plasmatic as opposed to locally produced ANG II reduces ATBF, assuming that ANG II acts in an autocrine or tightly paracrine manner with no effect on surrounding blood vessels (75). Nevertheless, in humans, an alternative angiotensin-converting enzyme-independent pathway of ANG II production has been found; thus the possibility that ANG II may regulate vascular tone cannot be entirely discarded (87). The inhibitory effect of ANG II on ATBF may, to a certain extent, be linked to its stimulatory effect on the NADPH oxidase enzymatic complex. This highly regulated membrane-bound enzyme complex catalyzes the production of reactive oxygen species (ROS), resulting in lower NO availability with subsequent endothelial dysfunction, loss of vasodilator capacity, increased monocyte/macrophage infiltration, and increased procoagulant activity. Nevertheless, a study by Goossens et al. (75) showed that the effect of ANG II on ATBF was NO independent.

Metabolic and growth effects of ANG II have been tested as well. ANG II influences adipocyte secretion of several products, such as prostacyclin, leptin, plasminogen activator inhibitor-1, and NO (2). ANG II not only binds to receptors on adipocyte plasma membranes but also to presynaptic nerve endings and blood vessels (52). Thus, it may influence AT metabolism either directly or via regulation of tissue perfusion and local SNS activity (36).

Aldosterone is an integral part of the RAAS and has been shown to be an important pathophysiological factor in metabolic syndrome pathogenesis (115). The relationship between aldosterone and ATBF has not yet been examined, but it is tempting to suppose an additional inhibitory effect. Mineralocorticoid receptors have been found on both endothelial and smooth muscle cells (115). Through a combination of genomic and nongenomic effects, aldosterone worsens the availability of NO and thus endothelium-derived vasodilatation (115). This effect is mediated by activation of NADPH oxidase, similarly to ANG II. Vascular damage leads to smooth muscle proliferation, fibrous protein deposition, and remodeling of vascular walls. Aldosterone also exerts unfavorable metabolic effects such as an increase in insulin resistance (159).

Altogether, AT expresses all of the components of the RAS synthetic pathway and probably participates in global RAS activation, especially through AGT production. AT-derived ANG II does not seem to participate substantially on systemic RAS activation. On the other hand, AT is able to respond to ANG II. Locally produced ANG II takes part in AT metabolic and growth processes. Nevertheless, ATBF seems to be affected predominantly by systemic ANG II. In addition to NO, ANG II is involved in the regulation of fasting ATBF, suggesting that the balance between ANG II-suppressive and NO-stimulatory effects may be an important determinant of vascular tone and thus ATBF under fasting conditions (75).

Other Possible Regulators of ATBF

ET-1. ET-1 is produced in vascular endothelial cells through cleavage of proendothelin to big endothelin and subsequently by endothelin-converting enzyme (103). ET-1, a peptide of 21 amino acid residues, is the most potent vasoconstrictor substance known (31, 123). ET-1 release is stimulated by a number of factors (e.g., ANG II, thrombin, various cytokines, ROS, and shearing forces) and inhibited by NO, prostacyclin, and ANP. Control of vascular tone depends on the balance between both vasodilating and vasoconstricting factors, namely NO and ET-1 (177). However, the true impact of ET-1 on ATBF and its relationship with NO and other vasoactive factors within AT remains to be determined.

Gastrointestinal hormones. The ATBF response is markedly higher after a 75-g oral glucose load than after intravenous glucose and insulin infusions. This highlights the potential relationship between the gastrointestinal tract and ATBF. Obvious mediator candidates are incretin hormones, glucagon-like-peptide-1 (GLP-1), and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 is the most important incretin in...
Humans (1, 125). Its receptors have been detected in AT on human adipocytes (126, 179). In addition, GLP-1 has a vasodilatory effect on hepatic and pulmonary arteries and mediates a concentration-dependent vasorelaxation of rat aortas (76). The vasodilative effect is mediated either indirectly through activation of GLP-1 receptors, leading to NO production in endothelial cells, or directly through GLP-1 receptors present on VSMC (144). One study performed in humans to test whether GLP-1 acted on ATBF was inconclusive, which was probably due to the fact that the ethanol escape technique under microdialysis condition was used (22, 111). Aside from its physiological glucose-dependent insulinoergic effects, GIP also acts in combination with hyperinsulinemia and hyperglycemia to increase blood flow, glucose uptake, and FFA reesterification, resulting in increased TG deposition in abdominal AT. Nevertheless, there is no demonstrated effect of GIP per se on net lipid metabolism in the splanchnic area either during fasting conditions or in combination with hyperinsulinemia and hyperglycemia (12). Thus, the role of GLP-1 and GIP on ATBF regulation remains to be established.

Natriuretic peptides. Natriuretic peptides (NP) are another important group of factors influencing and possibly coordinat- ing lipolysis and ATBF. Their vascular effect is mediated mainly through the type A receptor (NPRA) (67) present on smooth muscle cells (109). ANP comes from the circulation and is also formed locally by endothelial cells (106). Local administration of ANP leads to increased ATBF and lipolysis to an extent similar to catecholamines and independently of them. ANP seems to be an important factor activating lipolysis and FFA release from AT during exercise (129). Paradoxically, this effect is amplified when exercise is performed under β-blockade treatment because of an enhanced cardiac release of ANP (129). The precise role of NP in basal and meal-induced ATBF has not been reported so far to our knowledge.

Leptin. Leptin as an AT hormone (189) participates in energy balance regulation (84), influences immune function (55, 120), and also exerts direct autocrine/paracrine effects (63). Leptin receptors are found in the vasculature, and long-term infusion of leptin increases arterial blood pressure (153) and leads to endothelial dysfunction (105) as well as angiogenesis (155). Leptin is also proposed to be involved in the control of vascular tone and blood pressure by simultaneously producing a neurogenic pressor effect and an opposing NO-mediated depressor effect (62). Studies addressing direct leptin effect on AT perfusion are lacking. Although some investigators found an inverse correlation between leptin levels and abdominal ATBF (181), this association lost significance after controlling for BMI (4).

C-peptide. C-peptide, a hormone derived from the catabolism of proinsulin, displays some vasodilatory properties in cutaneous tissue by stimulating eNOS activity (185). Its effect on postprandial ATBF is unlikely since C-peptide plasma concentration decreased during intravenous euglycemic insulin-glucose regimen (98).

Kinin. Kinins are derived from kininogen by the action of kallikrein (152). It is still unresolved whether kininogen and kallikrein are acquired from the circulation if they are locally produced. The circulating kallikrein kinin system can be activated on vascular endothelial cells, but studies with rat adipocytes also indicate a local production (151). Kinins elicit NO and prostacyclin secretion from vascular endothelial cells, and therefore, they are potent vasodilators (119). They also increase the permeability of the endothelium and lead to a marked enhancement of adipocytes’ insulin sensitivity (30). The presence of circulating RAS components makes kinins a potential vasoactive regulator in ATBF.

Others. A positive correlation has been found between ATBF in humans and the levels of thyroid hormones (187). In hypothyroidism, the absent postprandial ATBF rise has been found, and it seems to be a major factor of insufficient insulin-stimulated glucose uptake and TG clearance (44). Conversely, hyperthyroidism is accompanied with markedly increased basal ATBF, which does not rise further after meal and which paradoxically masks metabolic defects in glucose uptake and suppression of lipolysis caused by insulin resistance in AT (43). Fatty acid-binding protein 4 regulates proliferation of endothelial cells, and its circulating levels are positively associated with endothelial dysfunction (9), but no study exists regarding its association with ATBF. Relation to ATBF was excluded for several other cytokines: adiponectin (42), interleukin-4, -6, and -10, TNFα, and interferon-γ (181).

Genetic Background of ATBF

It was shown that ATBF can be determined by genetic factors as well. Peroxisome proliferator-activated receptor-γ gene polymorphism influences not only metabolic functions, including the extent of postprandial FFA suppression and insulin sensitivity, etc., but also postprandial ATBF (172). It was also confirmed that the extent of expression of vasodilating factor eNOS and NPRA is genetically determined and significantly correlates with postprandial ATBF independently of BMI (139). As mentioned above, ANP plays and important role in lipolysis and ATBF regulation, and thus changes in NPRA expression may affect its influence, independently of obesity, and participate in the development of T2DM as a polygenetic disorder.

ATBF in Obesity and Insulin Resistance States

It is well established that, in obese individuals, fasting ATBF is lower than in healthy normal-weight subjects, and the rise in postprandial ATBF is impaired, i.e., peaks, under 50% above baseline blood flow (169). A significant relationship between fasting and postprandial ATBF exists (169), and both processes negatively correlate with BMI (169) or subcutaneous and visceral fat mass (4). Altered fasting and postprandial ATBF were found in all stages of impaired glucose tolerance, from impaired fasting glucose to overt T2DM, even in nonobese first-degree relatives of subjects with T2DM (169). This study showed significant association of postprandial ATBF with insulin sensitivity. Insulin resistance is a confirmed determinant of lower vascular responsiveness superior to obesity (19), but some studies have failed to show a significant relationship between ATBF and insulin resistance (169). In young, healthy, normal-weight individuals with normal glucose tolerance, we demonstrated that postprandial ATBF did not increase in 32% of subjects; i.e., their ATBF behaved the same as in obese people (10). According to our pilot results, low responsiveness may be a function of a higher ratio of fat mass despite normal BMI and indices of insulin sensitivity (169). One explanation for the discrepancy in relation of ATBF to obesity vs. insulin resistance may stem from two causes. J) Insulin itself does not
exert a direct vasodilatory effect in AT and probably works only as a mediator, and 2) other insulin-independent mechanisms, within or outside of AT, contribute to disturbed AT perfusion.

**Disturbed postprandial ATBF.** As mentioned above, β-adrenergic action has been assigned a primary role in the regulation of postprandial ATBF. Obese, insulin-resistant subjects display increased muscle sympathetic activity in the fasting state and a lower SNS response to elevation of insulin (183). Accordingly, physiological regulation of postmeal ATBF may be disturbed at several levels depending upon the degree of insulin resistance (Fig. 3A). First, reception of the insulin signal in the hypothalamus could be desensitized. This is supported by the impaired muscle sympathetic nerve activity observed in response to hyperinsulinemia in obese people. Second, long-standing hyperinsulinemia may give rise to desensitization with respect to increased sympathetic output in the target tissue, i.e., downregulation of β-adrenoceptors in the vascular bed of AT. Our finding of significantly lower responses to β-adrenergic stimulation in healthy low responders compared with responders supports this hypothesis (169). Third, rather than increasing total blood flow, insulin may regulate AT distribution pattern and capillary recruitment (169), effects that are not captured by classical techniques of ATBF measurements.

In T2DM, incretin insufficiency contributes to the manifestation of disease and may be involved in insufficient postprandial ATBF. Later, autonomic dysfunction may hamper communication between the gastrointestinal tract and the central nervous system, and taking into account the possible role of gastrointestinal tract glucoreceptors, it may further compromise the appropriate increase in postprandial ATBF (Fig. 3A).

**Disturbed basal ATBF and physical activity stimulation.** Even during the postabsorptive state, inadequate perfusion may bring about a poorer exchange of signal and nutritional substances with AT. Moreover, inadequate perfusion implies a disturbed basal level of postprandial ATBF (169). A number of disturbances may disrupt appropriate tone and reactivity of the AT vascular bed (Fig. 3B).

**Expansion of AT**

In obesity, fatty tissue mass expands without a concomitant increase in capillary density, thus leading to a longer diffusion distance (71) and lower blood flow per cell (41, 94) and per amount of AT because of increased adipocyte cell size (41). Therefore, expansion of AT and enlargement of adipose cells may result in a state of relative hypoperfusion with insufficient basal ATBF.

**Endothelial Dysfunction and Insulin Resistance**

Endothelial dysfunction in obesity may hamper vasomotoric function of AT microvessels. We have shown that NO is an important factor responsible for fasting ATBF level (169), and others have demonstrated the relationship between fasting ATBF and markers of endothelial dysfunction (5, 169).

Obesity-associated insulin resistance in the endothelium contributes to endothelial dysfunction and is a suggested precursor of T2DM (169, 173). Resistance to insulin leads to disequilibrium between vasodilator and vasoconstrictor factors, namely between ET-1 and NO action, which is a hallmark of endothelial dysfunction (170). Moreover, insulin exerts a priming effect for other signaling molecules such as eNOS (116), and its defective action disturbs their activity. Thus, although insulin is not a verified vasodilator in AT, insulin resistance may have a great impact on ATBF.

Increased oxidative stress has been demonstrated in expanded AT (65) and may interconnect several manifestations of obesity, such as AT inflammation, insulin resistance (90), T2DM, endothelial dysfunction, and cardiovascular disease (20). High-oxygen free radical production consumes NO, thus interfering with normal insulin-mediated vasodilation (150). Even individuals without diabetes but with impaired fasting glucose demonstrate significantly lower endothelium-dependent vasodilation compared with control subjects (178). SNS overactivity has also been shown to promote oxidative stress (81).

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**Fig. 3.** Possible mechanisms of impacts of obesity on postprandial (A) and basal ATBF (B). β-IR, β-IR receptor; FFA, free fatty acids; IR, insulin resistance; RAAS, renin-angiotensin-aldosterone system; SNS, sympathetic nervous system; T2DM, type 2 diabetes mellitus.
Activated RAAS

Activated RAAS in obesity is also suggested to play an important role in the development of endothelial dysfunction, hypertension, and insulin resistance (37, 73). Fatty tissue contributes to activation of RAAS by several mechanisms, including higher levels of AGT (49), presumed AT-derived mineralocorticoid-releasing factor (113), and epoxy-keto derivative of linoleic acid, reflecting higher levels of FFA and oxidative stress (70) stimulating aldosterone production. Conversely, higher levels of ANG II and aldosterone promote inflammatory adipokine expression (77), endothelial dysfunction, and oxidative stress in endothelial cells (34) and suppress insulin metabolic signaling and glucose uptake even in vascular smooth muscle cells (85). Apart from its effect on endothelial dysfunction, ANG II directly lowers fasting ATBF in humans in a dose-dependent manner (75).

Other Possible Causes of Perturbed ATBF

Postprandial, insulin-mediated suppression of FFAs is not an accepted mechanism of postprandial vasodilation in AT (75). On the other hand, higher levels of FFA do alter ATBF since they promote endothelial dysfunction in obesity (164).

Leptin might be another obesity-associated factor contributing to endothelial dysfunction (105, 164), to reduced ATBF, and, as a consequence of a concomitant increase in blood pressure, to increased cardiovascular morbidity and mortality in obesity (181).

ANP is one of the two most important agents regulating lipolysis and ATBF during exercise (131, 164). A negative correlation between ANP levels and BMI was found in obese people. Moreover, although obesity negatively impacts ANP’s effect in AT, this can be restored by endurance training, providing yet another possible link between expanded fat and altered ATBF (130, 164).

Consequences of Altered ATBF

Skeletal muscle is the major site for insulin-stimulated glucose uptake in vivo (40, 133, 164). Besides myocytes, adipocytes are also highly insulin-responsive cells. In terms of glucose disposal, glucose uptake and blood flow in AT exhibit insulin resistance in obesity, but because of the enlarged fatty mass, AT does not seem to contribute substantially to reduced insulin-stimulated whole body glucose uptake in obesity (181).

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This work was supported by the Canadian Institutes of Health Research (CIHR), Fonds de la Recherche en Santé du Québec (FRSQ), and Diabète Québec. J.-L. Ardlouze is a CIHR scholar (2006 CIHR New Investigator Award) and is member of the FRSQ-funded Centre de Recherche Clinique Étienne-Le Bel.

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

AUTHOR CONTRIBUTIONS
R.S. and P.B. prepared the figures; R.S., P.B., E.M., P.Y., A.C.C., and J.-L.A. drafted the manuscript; R.S., P.B., E.M., P.Y., A.C.C., and J.-L.A. edited and revised the manuscript; R.S., P.B., A.C.C., and J.-L.A. approved the final version of the manuscript; J.-L.A. did the conception and design of the research; J.-L.A. analyzed the data; J.-L.A. interpreted the results of the experiments.

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