Nitric Oxide and β-Adrenergic Stimulation Are Major Regulators of Preprandial and Postprandial Subcutaneous Adipose Tissue Blood Flow in Humans

Jean-Luc Ardlouze, MD; Barbara A. Fielding, PhD; Jenny M. Currie, BSc; Keith N. Frayn, PhD, ScD; Fredrik Karpe, MD, PhD

Background—Blood flow mediates the metabolic and endocrine roles of adipose tissue. We have previously shown that the postprandial adipose tissue blood flow (ATBF) increase is dependent on insulin sensitivity. However, subcutaneous local insulin delivery had no demonstrable effect on either preprandial or postprandial ATBF. We hypothesized that insulin may act indirectly via sympathetic activation, mainly in the postprandial period, and that nitric oxide may be an overall major regulator of subcutaneous ATBF.

Methods and Results—We investigated the endogenous preprandial and postprandial regulation of ATBF by applying local tissue blockade of β-adrenergic (propranolol), α-adrenergic (phenolamine and yohimbine), and nitric oxide (NOS-1, monomethyl-L-arginine, L-NMMA) regulation of blood flow. Healthy subjects (body mass index, 18 to 31 kg/m²) were challenged with 75 g glucose for endogenous stimulation of ATBF. We used the novel “microinfusion” technique, which allows for simultaneous local delivery of pharmacological agents (or contralateral saline) and measurement of ATBF with the 133Xe washout method. Compared with control, the preprandial ATBF was not affected by propranolol but was increased by 21% (P<0.013) and 15% (P=0.004) with phenolamine and yohimbine, respectively. A decrease of 42% (2.97±0.33 versus 4.75±0.47 mL·min⁻¹·100 g tissue⁻¹, P<0.01) was seen with L-NMMA. The postprandial response was blunted by 58% (0.81±0.42 versus 1.90±0.44 mL·min⁻¹·100 g tissue⁻¹, P<0.004) with propranolol, but neither phenolamine, yohimbine, or L-NMMA altered this response.

Conclusions—Nitric oxide seems to determine the absolute level of ATBF, whereas a major proportion of the postprandial enhancement of ATBF is under β-adrenergic regulation in vivo in humans. (Circulation. 2004;109:47-52.)

Key Words: blood flow ■ receptors, adrenergic, beta ■ nervous system, autonomic ■ nitric oxide synthase ■ insulin

There is a need for tissue-specific regulation of blood flow to meet the very different physiological and metabolic demands of such tissues as liver, skeletal muscle, heart, and adipose tissue. Adipose tissue blood flow (ATBF) seems to exhibit its highest degree of modulation in response to food intake, illustrated by either glucose or mixed-meal ingestion, whereas fat alone does not elicit a blood flow response. The actual stimulus for this nutrient-related increase in ATBF is, however, not fully understood, but the postprandial increase in ATBF coincides with the increase in insulin concentration in plasma and with the suppression of nonesterified fatty acids (NEFAs). Enhancement of ATBF may have importance in metabolic physiology in that the extraction of plasma triglycerides (TGs) increases with increasing blood flow. Furthermore, the possibility exists that increased ATBF facilitates or enables signaling between adipose tissue and other tissues to regulate metabolism.

There is also abundant evidence that β-adrenergic stimulation increases ATBF. This may be induced, for instance, by adrenaline infusion or by local delivery of β-adrenergic stimuli by microdialysis of the tissue using isoprenaline, dobutamine, or isoproterenol. In contrast, experiments using α-adrenergic stimuli such as clonidine and norfenefrine show a predominantly inhibitory effect of α-adrenoceptors on ATBF. We have shown recently that the degree of insulin sensitivity seems to be closely related to ATBF responsiveness, but insulin per se does not seem to stimulate blood flow in adipose tissue as it may do in muscle. We have therefore postulated that the postprandial increase in insulin concentrations may lead to activation of the sympathetic nervous system, with a subsequent enhancement of ATBF.

In concert with the blood flow regulation in many other tissues, nitric oxide (NO) could be a major vasodilator in adipose tissue, or possibly a direct or indirect mediator of insulin vasodilatation. However, NOS-1, monomethyl-L-arginine (L-NMMA), an NO synthase inhibitor, did not seem to alter local ATBF and attenuated the isoproterenol-induced vasodilatation only during adipose tissue microdi-
alysis. These measurements were made by use of the ethanol outflow/inflow ratio, which has an inherently low sensitivity. In fact, very little is known of NO function in adipose tissue in vivo, and to the best of our knowledge, its role in regulating ATBF has never been assessed.

We hypothesized that the regulation of human ATBF is largely a result of sympathetic activation and NO modulation. Therefore, α- and β-adrenoceptor antagonists and L-NMMA were used to evaluate the endogenous ATBF regulation before and after oral glucose intake. Experiments were performed by use of a novel microinfusion technique, which quantitatively assesses the local effect of vasoactive compounds on ATBF.

Methods

Subjects
Seventeen healthy volunteers (10 female) participated in the experiments. Median age was 26 years (range, 19 to 53 years), and mean body mass index (BMI) was 21 kg/m² (range 18 to 31). The Oxfordshire Clinical Research Ethics Committee approved the studies, which conformed to the Declaration of Helsinki, and all subjects gave informed consent.

Study Design
Subjects were asked to refrain from strenuous exercise or alcohol intake 24 hours before the experiment and, after an overnight fast, were studied at rest. On arrival at the Clinical Research Unit, after 15 minutes of rest, blood pressure was measured in triplicate. Arterialized venous (heated-hand) blood samples were taken during the experiment. After 2 baseline samples had been taken (at -20 minutes and 0 minutes), samples were taken at 30-minute intervals and immediately placed on ice. The plasma was separated at +4°C and frozen within 15 minutes. Pulse rates and blood pressures were recorded before each blood sample. At 60 minutes, 75 g of glucose in water was ingested to stimulate endogenous ATBF.

Microinfusion Protocol
The microinfusion technique is based on 133Xe washout and allows for simultaneous monitoring and manipulation of local ATBF. An experimental outline is shown in Figure 1. Right and left sides were studied simultaneously on each subject to allow direct comparison of the effects of the vasoactive compound on one side with a contralateral control saline side. To achieve this, small catheters, 6 mm long, with an internal diameter of 0.38 mm and outer diameter of 1.5 mm and a dead-space volume of 60 μL (Quick-set infusion set, MiniMed, Applied Medical Technology Ltd) were inserted 8 to 10 cm on either side of the midline into the abdominal subcutaneous tissue, and a saline infusion was started at 2 μL/min. After 20 minutes had been allowed for the tissue to recover, 133Xe was injected through the port in the hub of the catheter, and a γ-counter probe (Oakfield Instruments) was placed over the infusion device. 133Xe (0.5 to 1.0 MBq) was injected at each site. Next, the catheter was perfused for 1 minute at 60 μL/min with saline to wash the dead space and for a further 40 to 60 minutes at 2 μL/min to allow for equilibration. At time zero, at 1 of the 2 sites, right or left, chosen at random, the saline was switched, by disconnection at the hub of the infusion set, to the vasoactive compound while the infusion rate was maintained at 2 μL/min. The blood flow recording over the next 60 minutes assessed the effect of treatment per se while the saline infusion was continued on the contralateral site. At time +60 minutes, the subject was given 75 g oral glucose in 200 mL of lemon-flavored water, and infusions were continued for a further 120 minutes.

In analysis of the difference between the right and left sides under control conditions, the ATBF coefficient of variation was 17.3%.

Pharmacological Agents
The effect of propranolol, a nonspecific β-adrenoceptor antagonist, was investigated in 13 people. Solutions (10⁻⁵ mol/L, ie, 0.034 μg/μL) of propranolol (Inderal, 1 mg/mL, Zeneca) were prepared by dilution in saline just before the experiment. The total amount of propranolol administered during the 3-hour microinfusion period was 12.2 μg, a dose corresponding to 1 ten-thousandth of the conventional daily treatment of angina pectoris.

Dose-response (10⁻³, 10⁻⁴, 10⁻⁵ mol/L) experiments (n=5) were also performed with propranolol. For that purpose, 3 of the 4 sites were randomly infused with 1 of the 3 concentrations; the fourth was a saline control site.

The effects of phentolamine (10⁻³ mol/L, n=10), a nonspecific α-antagonist (Rogitine, 10 mg/mL, Alliance) and yohimbine (10⁻³ mol/L, n=6), a specific α₁-blocker (Sigma), were tested.

The L-arginine analogue L-NMMA (10⁻³ mol/L, Sigma) was prepared by sterile filtration-dilution and tested in 6 subjects.

Biochemical Measurements
Plasma glucose was measured the same day in samples stored at +4°C by an enzymatic method. The remaining plasma was stored at -20°C for measurement of NEFA (WAKO NEFA C kit, Alpha Laboratories) and TG (Randox Laboratory) concentrations by enzymatic methods. Plasma insulin was measured by a double-antibody radioimmunoassay (Pharmacia and Upjohn).

Figure 1. Time line diagram of experiment. Microinfusion catheters were inserted into abdominal subcutaneous tissue, and a saline infusion was started. After 20 minutes had been allowed for tissue to recover, 133Xe was injected through catheter. Next, catheter was perfused for a further 40 to 60 minutes to allow for equilibration. At time zero, at 1 of 2 sites, right or left, chosen at random, saline was switched to pharmacological agent. At time +60 minutes, subject was given a 75-g oral glucose drink while infusions continued for a further 120 minutes. Accordingly, preprandial effect of pharmacological agent was recorded between 0 and 60 minutes, and effect on postprandial ATBF was recorded from 60 to 180 minutes. Thick arrows represent blood sample and blood pressure time points.
Calculations and Statistics
Statistical analyses were performed with SPSS for Windows Release 11.5. Analytical data are expressed as mean±SEM or median and interquartile range. Differences between groups were assessed by the Mann-Whitney U test. Xenon counts were recorded continuously, and blood flow was calculated as the mean of consecutive 10-minute time periods, as described previously. At the saline sites, the stability of ATBF during the baseline and the preglucose period (calculated as the mean of all time points from +5 to +55 minutes) was tested with a paired t test. Responses to pharmacological agents were evaluated within individuals by comparison with the saline control site. The evaluation of the effect of the treatment per se was made by averaging the 3 consecutive time points (+35, +45, and +55 minutes) just before glucose intake after subtraction of the baseline (−15 and −5 minutes). The peak ATBF value was calculated as the mean of the 3 contiguous points (including the maximum) that gave the highest mean value within each subject. The “response to the meal” was analyzed by calculating the ATBF area under the curve (AUC) by the trapezoidal rule or the incremental area under the curve (iAUC). The baseline for calculating the iAUC was taken as the ATBF measurement at the time of ingestion of glucose (+65 minutes). Both AUCs were time-averaged, ie, the AUC and the iAUC were divided by the time under which they were calculated (divided by 110 minutes). Statistical comparisons of drug effects within individuals were made using paired t tests or the Wilcoxon signed rank test when appropriate. The overall effects of treatments compared with control were also analyzed by repeated-measures ANOVA to identify time effects, drugs effects, and time and drug interactions.

Insulin sensitivity was calculated by the homeostasis model assessment (HOMA). 21

Results
Systemic Responses
Fasting plasma glucose (5.02±0.07 mmol/L), insulin (4.9±0.3 mU/L), TGs (0.83±0.08 mmol/L), and NEFAs (551±39 μmol/L) were stable during the baseline period and up to 60 minutes. After ingestion of glucose, plasma concentrations of glucose and insulin increased, whereas NEFAs decreased. Concentrations and responses corresponded to those expected from healthy subjects and did not differ during different experiments (Figure 2, A through C). Mean blood pressure was unchanged during the course of the study (data not shown).

ATBF Regulation
At the saline sites, ATBF was stable during the baseline and preglucose periods (4.09±0.27 versus 4.02±0.19 mL·min⁻¹·100 g tissue⁻¹, P=0.65). There were no differences in baseline ATBF between saline and treatment side (also saline during the baseline period) (Table). In response to glucose, the mean blood flow increase on the control side was 75% (4.1±0.3 versus 7.2±0.6 mL·min⁻¹·100 g tissue⁻¹, P<0.001) (Figure 2D).

During the preprandial period, microinfusion of propranolol did not affect ATBF (Figure 3A). In contrast, phentolamine induced a 21% increase in ATBF toward the end of the preprandial period (P=0.013) (Figure 3B). A corresponding effect (+15%) was observed with yohimbine (P=0.004) (Figure 3C). Local administration of L-NMMA induced a rapid and marked decrease of ATBF by 42% (P=0.01) (Figure 4).

During the postprandial period, the ATBF response to the meal was blunted by 58% (iAUC 0.81±0.42 versus 1.90±0.44 mL·min⁻¹·100 g tissue⁻¹, P=0.004) by propranolol (Figure 3A). We have previously shown that the ATBF response to glucose ingestion is dependent on insulin
sensitivity. The 13 subjects were divided into low- and high-insulin-sensitivity groups (n=7 and n=6, respectively) according to the HOMA median and by high and low BMI. As expected, the absolute ATBF response (iAUC) was greater in the group with higher insulin sensitivity (2.67±0.57 versus 1.27±0.45 mL·min⁻¹·100 g tissue⁻¹, P=0.046). However, there was no difference in the relative reduction of ATBF by propranolol depending on insulin sensitivity (median [interquartile range], –52% [38–80] versus –45% [36–80]). Essentially identical results were obtained when the group was divided according to BMI (data not shown).

There were no statistically significant differences of AUC or iAUC with either phentolamine or yohimbine. However, comparing the relative increases in ATBF between yohimbine and phentolamine, the increase was slightly and statistically significantly higher (P=0.028) with yohimbine (Figure 3, B and C). With L-NMMA, the ATBF response was almost parallel but substantially downshifted compared with the control side. The postprandial enhancement of ATBF was essentially preserved, but the level from which the response occurred was lower (Figure 4). This is illustrated by a difference in ATBF AUC (3.67±0.47 versus 6.12±0.79 mL·min⁻¹·100 g tissue⁻¹, P=0.004) and iAUC (0.98±0.57 versus 1.62±0.57 mL·min⁻¹·100 g tissue⁻¹, P=0.11) for L-NMMA and control side, respectively.

**Propranolol Dose-Response Experiments**

The 3 propranolol concentrations used (10⁻³, 10⁻⁴, and 10⁻⁵ mol/L) showed no difference in blunting of the ATBF enhancement brought about by the glucose ingestion (Figure 5). This indicates that the lowest propranolol concentration (10⁻³ mol/L) achieved a near-maximal effect.

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**Baseline Preprandial and Postprandial ATBF in Response to Blockade of β-Adrenergic (Propranolol) and α-Adrenergic (Phentolamine and Yohimbine) Receptors and to NO Synthase (L-NMMA) by Microinfusion**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Preprandial</th>
<th>Postprandial AUC</th>
<th>Postprandial I/AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol (n=13)</td>
<td>3.31±0.34</td>
<td>3.03±0.31</td>
<td>3.92±0.34*</td>
<td>0.89±0.41†</td>
</tr>
<tr>
<td>Control</td>
<td>3.65±0.35</td>
<td>3.31±0.18</td>
<td>4.85±0.57</td>
<td>1.90±0.44</td>
</tr>
<tr>
<td>Phentolamine (n=10)</td>
<td>3.98±0.50</td>
<td>4.83±0.61*</td>
<td>5.62±0.83</td>
<td>1.13±0.53</td>
</tr>
<tr>
<td>Control</td>
<td>4.23±0.49</td>
<td>4.21±0.35</td>
<td>5.36±0.62</td>
<td>1.49±0.4</td>
</tr>
<tr>
<td>Yohimbine (n=6)</td>
<td>4.01±0.17</td>
<td>4.60±0.17†</td>
<td>6.04±0.62</td>
<td>1.63±0.41</td>
</tr>
<tr>
<td>Control</td>
<td>3.74±0.32</td>
<td>3.73±0.34</td>
<td>5.34±0.63</td>
<td>1.25±0.44</td>
</tr>
<tr>
<td>L-NMMA (n=6)</td>
<td>4.47±0.87</td>
<td>2.97±0.33†</td>
<td>3.67±0.47†</td>
<td>0.98±0.57</td>
</tr>
<tr>
<td>Control</td>
<td>4.37±0.67</td>
<td>4.75±0.47</td>
<td>6.12±0.79</td>
<td>1.62±0.57</td>
</tr>
</tbody>
</table>

Control indicates infusion of saline on the contralateral side. Oral glucose (75 g) was given at time +60 minutes (preprandial period) to stimulate the endogenous postprandial enhancement of ATBF. The assessment of the effect of the treatment per se was made during the preprandial period. The effect on ATBF in the postprandial period was assessed by calculation of AUC and iAUC. Data are given as mean±SEM, in mL/min per 100 g tissue. AUC and iAUC are time-averaged, ie, the AUC and the iAUC were divided by the time under which they were calculated.

*P<0.05 vs control; †P<0.01 vs control. The differences between saline and pharmacological agents were also tested by repeated-measures ANOVA. Without exception, these results confirmed the statistical differences found by use of the AUC comparisons.

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**Discussion**

We describe the adrenergic regulation of preprandial and postprandial ATBF together with regulation by NO. It seems that fasting ATBF is primarily under NO tone and to some extent under α-adrenergic control. However, the postprandial enhancement of ATBF is controlled principally by the β-adrenergic system, but the level from which this enhancement takes place is strongly influenced by the NO tone.

During the preprandial period, we demonstrated that in subcutaneous adipose tissue, exposure of the extracellular space to propranolol had no effect on ATBF, whereas phentolamine promoted an increase of the fasting blood flow. These findings are in agreement with studies using microdialysis in which clonidine (an α₂-adrenoceptor agonist) induced vasoconstriction and inhibited lipolysis. Close examination of the difference between the nonspecific α-blockade (by phentolamine) and the α₂-specific blockade (by yohimbine) revealed some interesting differences. The vasodilatory effect of yohimbine tended to be sustained compared with that of phentolamine. The response curve was almost parallel and supershifted above the saline side, and compared with phentolamine, the magnitude of the effect was in favor of yohimbine. This suggests that α₂-adrenoceptor activation leads to vasoconstriction and that α₁-receptors have little effect in the adipose tissue microcirculation.

The postprandial enhancement of ATBF was severely blunted by local administration of propranolol. This is in accordance with previous investigations using intravenous administration of propranolol that showed a much reduced ATBF response to a meal. Because the effect on postprandial enhancement of ATBF by propranolol is similar with intravenous administration and local delivery (this study), we conclude that the β-adrenergic stimulation of postprandial ATBF is not of humoral origin. The dose-response study of...
propranolol showed that the $10^{-5}$ mol/L concentration achieved a near-maximal blockade of the $\beta$-adrenergic–stimulated postprandial enhancement of ATBF. A 100-fold higher concentration ($10^{-3}$ mol/L) did not achieve greater blockade, indicating that factors other than $\beta$-adrenergic stimulation may also be involved in the postprandial enhancement of ATBF.

In accord with the well-established vasodilatory effect of NO in other tissues, the preprandial vasoconstriction induced by L-NMMA was not unexpected. The role of NO in the regulation of ATBF seems to be very distinct. Abrogation of NO function by local administration of L-NMMA leads to rapid and substantial vasoconstriction. Interestingly, when ATBF was stimulated by oral glucose intake, ie, through a $\beta$-adrenergic stimulation, the ATBF response was almost completely preserved. This shows that the postprandial enhancement in ATBF is independent of NO, but the NO activity determines the level from which this response takes place.

The present findings highlight the integrated response of adipose tissue. Although we did not quantify lipolysis from adipose tissue, it is well established that $\alpha_2$-adrenergic stimulation increases lipolysis, whereas $\beta_2$-adrenergic stimulation has an antilipolytic effect. Accordingly, it seems that ATBF regulation and lipolysis are coregulated, ie, antilipolysis by $\alpha_2$-adrenergic receptor stimulation goes with vasoconstriction in adipose tissue, whereas the lipolytic response of $\beta_2$-adrenergic receptor stimulation goes with vasodilatation.
of physiological situations in which coregulation has been demonstrated are induced mental stress and prolonged physical exercise. Obviously, other regulatory systems control adipose tissue lipolysis, and in the early postprandial period, the antilipolytic action of insulin overrides the lipolytic stimulus of the sympathetic activation. Accordingly, shortly after meal intake, but probably only in that period, lipolysis and blood flow regulation are not coregulated. Of note, we have previously shown that insulin as such plays no local role in the regulation of ATBF. It could be speculated that increased blood flow is needed in connection with a lipolytic stimulus to protect the tissue’s vascular endothelium or interstitial environment against harmful concentrations of NEFAs.

The pathophysiological consequences of a blunted meal-induced ATBF response and the potential implications of a dynamic ATBF may be of importance. First, the insulin-induced ATBF response and the potential implications of a NEFAs or interstitial environment against harmful concentrations of lipolytic stimulus to protect the tissue increased blood flow is needed in connection with a lipolytic stimulus of the sympathetic activation. Accordingly, shortly after meal intake, but probably only in that period, lipolysis and blood flow regulation are not coregulated. Of note, we have previously shown that insulin as such plays no local role in the regulation of ATBF. It could be speculated that increased blood flow is needed in connection with a lipolytic stimulus to protect the tissue’s vascular endothelium or interstitial environment against harmful concentrations of NEFAs.

In summary, we have described the major factors regulating human ATBF in that NO seems to determine the absolute level of ATBF, whereas a major proportion of the postprandial enhancement of ATBF is under β-adrenergic regulation.

Acknowledgment
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References
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