Regulation of blood flow in adipose tissue: involvement of the cholinergic system

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The worldwide pandemic of obesity has drawn significant attention to adipose tissue (AT). Originally regarded as a mere energy store, AT is now recognized as a highly active metabolic and endocrine organ whose influx and outflow of nutrients and multiple regulatory factors requires fine tuning.

The regulation of AT blood flow (ATBF) can be divided into two physiological situations (38). Fasting ATBF varies between healthy subjects and assessed the following: 1) the role of Ach in subcutaneous AT during local Ach microinfusion; and 2) the effect of Ach receptor antagonists (atropine and mecamylamine) during local microinfusion; and 3) the responses to cholinergic agents in responder and nonresponder subjects.

Acetylcholine (Ach) has vasodilatory action. We hypothesized that the in situ administration of Ach would increase ATBF dose dependently (3.32 (2.80–5.09), 6.46 (10–3 mol/l, n = 15)) and Ach receptor antagonists (atropine and mecamylamine (nicotinic Ra; 10–5 mol/l; n = 15; 10–4 mol/l, n = 10). Compared with baseline [2.41 (1.36–2.83) ml/100 g · 1·min−1], Ach increased ATBF dose dependently [3.32 (2.80–5.09), 6.46 (4.36–9.51), and 10.31 (7.98–11.52), P < 0.0001], with no difference between R and NR. Compared with control side, atropine (both concentrations) had no effect on fasting ATBF: only atropine 10–4 mol/l decreased post-glucose ATBF [ΔAUC: 1.25 (0.32–2.91) vs. 1.98 (0.64–2.94); P = 0.04]. This effect was further apparent in R. Mecamylamine had no impact on fasting and postglucose ATBF in R and NR subjects. ATBF was manipulated by in situ microinfusion of vasoactive agents (VA) in AT and monitored by the 133Xenon washout technique (both recognized methods) at the VA site and at the control site. We tested incrementally increasing doses of Ach (10–5, 10–4, and 10–3 mol/l; n = 15) and Ach receptor antagonists (R) before and after oral administration of 75-g glucose using atropine (muscarnic Ra; 10–5 mol/l, n = 13; 10–4 mol/l, n = 22) and mecamylamine (nicotinic Ra; 10–5 mol/l, n = 15; 10–4 mol/l, n = 10). Compared with baseline [2.41 (1.36–2.83) ml/100 g · 1·min−1], Ach increased ATBF dose dependently [3.32 (2.80–5.09), 6.46 (4.36–9.51), and 10.31 (7.98–11.52), P < 0.0001], with no difference between R and NR. Compared with control side, atropine (both concentrations) had no effect on fasting ATBF; only atropine 10–4 mol/l decreased post-glucose ATBF (ΔAUC: 1.25 (0.32–2.91) vs. 1.98 (0.64–2.94); P = 0.04). This effect was further apparent in R. Mecamylamine had no impact on fasting and postglucose ATBF in R and NR. Our results suggest that the cholinergic system is implicated in ATBF regulation, although it has no role in the blunting of ATBF response in NR.

adipose tissue blood flow; acetylcholine; muscarinic; nicotinic; atropine

THE WORLDWIDE PANDEMIC OF OBESITY has drawn significant attention to adipose tissue (AT). Originally regarded as a mere energy store, AT is now recognized as a highly active metabolic and endocrine organ whose influx and outflow of nutrients and multiple regulatory factors requires fine tuning.

The regulation of AT blood flow (ATBF) can be divided into two physiological situations (38). Fasting ATBF varies between 2 and 4 ml/100 g · 1·min−1 and is controlled principally by nitric oxide (NO) (4) and, to a lesser extent, by atrial natriuretic peptide (ANP) (19). In contrast, the sympathetic system, which functions through α2-adrenergic receptors (4) and angiotensin II (22), has inhibitory effects on fasting ATBF. Postprandially, ATBF usually increases two- to fourfold as a result of β-adrenergic stimulation (4, 35). However, we estimated that only 50–60% of the postprandial ATBF (ppATBF) increase depends on sympathetic activation (4). Therefore, other regulation factors, such as incretins (glucose-dependent insulinotropic polypeptide) (7) or the parasympathetic/cholinergic system, are believed to be involved in this process.

In 2002, neuroanatomical studies suggested parasympathetic innervation of the retroperitoneal and subcutaneous white AT (WAT) in rats (29). Other studies failed to reproduce these results. However, studies have shown that the administration of parasympathetic stimulating or blocking agents significantly altered AT lipolysis (2, 3). These contradictions between negative neuroanatomical and positive functional studies could be reconciled by the existence of a ubiquitous nonneuronal cholinergic system (nNCS), in other words, autocrine or paracrine communication between cells based on acetylcholine (Ach) signaling outside the nervous system (for review, see Ref. 43).

In contrast to the metabolic action of cholinergic agents described above and to the well-known vasodilatory effect of Ach (23), ATBF has been found to be unchanged after the systemic (3) or local (2) administration of nicotine (nicotinic receptor agonist), carbachol (muscarnic receptor agonist), and their antagonists using the ethanol escape technique under microdialysis conditions (2, 3). This method has since been reported to be rather insensitive for ATBF measurements (25). Thus, using an accurate method (24, 37), we decided to reexamine the role of the cholinergic system in ATBF regulation. We hypothesized that the in situ administration of Ach into AT has a vasodilatory effect.

We also thought that these conflicting results could be related to the ppATBF responder/nonresponder phenomenon, which we reported previously in healthy subjects (6). In responders, ppATBF increases by >50% from the baseline fasting blood flow, a physiological feature; however, in nonresponders, the ATBF response is blunted and ≤50% (6). The nonresponder status is close to the conditions of so-called normal-weight metabolically obese subjects. In our report, the prevalence of nonresponders was 32%. Therefore, we also hypothesized that nonresponders could have biased previous studies on the cholinergic regulation of ATBF.

To test our hypotheses, we performed experiments in healthy subjects and assessed the following: 1) the role of Ach in subcutaneous AT during local Ach microinfusion; 2) the effect of Ach receptor antagonists (atropine and mecamylamine) during local microinfusion; and 3) the responses to cholinergic agents in responder and nonresponder subjects.

Sotorník R, Baillargeon JP, Gagnon-Auger M, Ménard J, Brassard P, Ardilouze JL. Regulation of blood flow in adipose tissue: involvement of the cholinergic system. Am J Physiol Endocrinol Metab 309: E55–E62, 2015. First published May 12, 2015; doi:10.1152/ajpendo.00016.2015.—Acetylcholine (Ach) has vasodilatory action. However, data are conflicting about the role of Ach in regulating blood flow in subcutaneous adipose tissue (ATBF). This may be related to inaccurate ATBF recording or to the responder/nonresponder (R/NR) phenomenon. We showed previously that healthy individuals are R (ATBF increases postprandially by >50% of baseline BF) or NR (ATBF increases ≤50% postprandially). Our objective was to assess the role of the cholinergic system on ATBF in R and NR subjects. ATBF was manipulated by in situ microinfusion of vasoactive agents (VA) in AT and monitored by the 133Xenon washout technique (both recognized methods) at the VA site and at the control site. We tested incrementally increasing doses of Ach (10–5, 10–4, and 10–3 mol/l; n = 15) and Ach receptor antagonists (Ra) before and after oral administration of 75-g glucose using atropine (muscarnic Ra; 10–5 mol/l, n = 13; 10–4 mol/l, n = 22) and mecamylamine (nicotinic Ra; 10–5 mol/l, n = 15; 10–4 mol/l, n = 10). Compared with baseline [2.41 (1.36–2.83) ml/100 g · 1·min−1], Ach increased ATBF dose dependently [3.32 (2.80–5.09), 6.46 (4.36–9.51), and 10.31 (7.98–11.52), P < 0.0001], with no difference between R and NR. Compared with control side, atropine (both concentrations) had no effect on fasting ATBF; only atropine 10–4 mol/l decreased post-glucose ATBF (ΔAUC: 1.25 (0.32–2.91) vs. 1.98 (0.64–2.94); P = 0.04). This effect was further apparent in R. Mecamylamine had no impact on fasting and postglucose ATBF in R and NR. Our results suggest that the cholinergic system is implicated in ATBF regulation, although it has no role in the blunting of ATBF response in NR.

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METHODS

Study population. Experiments were performed at the Clinical Research Centre of the Centre Hospitalier Universitaire de Sherbrooke (CHUS). We recruited healthy Caucasian subjects. The subjects were asked to refrain from strenuous exercise and from alcohol and caffeine intake for 48 h before the experiment, and after an overnight fast, they were studied during bed rest. On arrival, the blood pressure (BP; 5 series Upper Arm Blood Pressure Monitor; Omron) and pulse rate were measured three times at 3-min interval (and averaged) while the subjects were sitting, and these values were then recorded throughout the study while the subjects were in a recumbent position. Anthropometric characteristics and personal and family medical histories were recorded. Weight was measured barefoot in light clothing on a body composition analyzer (Tanita, TBF-300A; Tanita, Arlington Heights, IL) to the nearest 0.1 kg and height with a stadiometer to the nearest 0.01 m. Body mass index (BMI) was calculated as weight (kg) divided by square of height (m²). Waist was measured at a level midway between the lowest rib and the iliac crest. Blood samples were collected, and a microinfusion setup was installed.

Fasting glucose and triacylglycerols were measured by the enzymatic method (Cobus Analyzer, Roche Diagnostics) and glycated hemoglobin (A1C) by HPLC in the biochemistry laboratory of the CHUS.

The studies were approved by the Université de Sherbrooke Research Ethics Committee and were in compliance with the Declaration of Helsinki; all subjects gave informed consent to participate.

Microinfusion technique. We recently described in detail the microinfusion technique (37). The method was designed for the local delivery of potentially vasoactive agents (VA) without systemic effects and with a simultaneous measurement of ATBF. A combination of techniques is used and based on 133Xenon (133Xe) washout (34). In principle, VA is directly infused into the exact location into which 133Xe has been deposited, allowing the recording of 133Xe disappearance in relation to changes in the local tissue environment. To achieve this condition, we used small, 6-mm-long cannulas that were designed for the continuous delivery of insulin in diabetic subjects (Inset II Infusion Set; Animas, LifeScan). The cannulas were inserted into the abdominal subcutaneous tissue on the right and left sides 6–8 cm from the umbilicus when needed), and the insertion needles were removed. The tissue was allowed to recover for 20 min while saline was infused at 2 μl/min; then, 133Xe (Lanteus Medical Imaging) diluted in sterile saline was injected directly through the hub of the devices (1–2 MBq/site). The cannulas were then perfused for 60 min at 2 μl/min to allow for equilibration. At −60 min, the baseline ATBF (b-ATBF) was recorded for 1 h using γ-counter probes placed over the infusion devices and connected to the Mediscint System (Oakfield Instruments, Eynsham, UK). At time 0, 2 μl/min VA infusions were started at one site while saline (control side, same infusion rate) was continuously infused at the contralateral side; each subject was its own control. We demonstrated that there is no difference in ATBF, fasting or postprandially, between the right and left abdominal sides (5); thus, our design enables direct comparison of the VA and saline control sides at the same abdominal level.

Pharmacological agents. Stock solutions of acetylcholine (endogenous neurotransmitter; an ester of acetic acid and choline) (10⁻¹ mol/l) and mecamylamine (10⁻² mol/l) were prepared by sterile dilution and filtration of drug powder (Sigma-Aldrich) and stored at −20°C. The solution used in tests was prepared by sterile dilution in saline of the stock solutions just before the experiments. Solutions of atropine (0.4 mg/ml atropine; Sandoz) were prepared by sterile dilution in saline just before the experiment. The total amount of atropine administered during the 3 h of the microinfusion period did not exceed 27.5 μg, a dose corresponding to one thirty-six of the recommended daily dose in clinical practice.

Ach experiments. For Ach experiments, two catheters were applied. At time 0, at one randomly chosen site the infusion was switched from
saline to incrementally increasing doses of Ach (10⁻⁵, 10⁻³, and 10⁻¹ mol/l) in fasting subjects. ATBF was monitored for 40 min following the switch to each consecutive concentration (Ach). The saline infusion was concomitantly continued on the other side to monitor potential systemic effects of the Ach microinfusion. The participants fasted until the end of the study and also participated in atropine or mecamylamine experiments, thus allowing us to separate responders from nonresponders. An experimental outline is shown in Fig. 1A.

Atripe and mecamylamine experiments. For atropine (naturally occurring alkaloid; competitive antagonist of muscarinic receptors) and mecamylamine (an aliphatic amine; nonspecific antagonist of nicotinic receptors) experiments, four catheters were placed: two above and two below the umbilicus. At time 0, at both levels, saline was switched to atropine (10⁻⁶ or 10⁻⁵ mol/l) or mecamylamine (10⁻³ or 10⁻⁴ mol/l) at one of the two sides of the abdomen, which was chosen at random, and the effects of VA on the fasting ATBF (IVA-ATBF) were recorded for the next hour. At time +60 min, subjects were given 75 g of glucose orally, the best ATBF-stimulating factor (38), and the post-glucose ATBF (pG-ATBF) was monitored for the next 120 min while all microinfusions (VA and saline) were maintained at 2 µl/min. Thus, the test in one subject usually provided independent results for two VA. The ATBF response to glucose was monitored on the control (saline) side, allowing us to separate responders from nonresponders. An experimental outline is shown in Fig. 1B.

The choice of VA concentrations was guided by previous studies by our research group (4, 6) and others (3) and by six pilot experiments.

Calculations. The Mediscint System provides counts per 20 s. ATBF is calculated every 10 min as the product of 1) the exponential rate constant/λ (k, in counts/s, calculated from the semilog plot of disappearance of counts vs. time) and 2) the tissue-blood coefficient of partition (λ, in ml/g) of the isotope: ATBF = k × λ × 100 (g) × 60 (s). The results are expressed in ml/100 g tissue-1 min⁻¹.

No ATBF difference between men and women was reported in the literature (30). To confirm this, we evaluated baseline and pG-ATBF according to sex difference. We did not find any significant difference between men and women either in the whole cohort or in responders (data not shown). Accordingly, we performed the subsequent analyses without considering sex difference.

For all tests, b-ATBF was calculated as the mean of the last two values of the baseline period (i.e., the last 20 min).

For Ach experiments, the ATBF during infusions of increasing concentrations of Ach was calculated as the mean of the last two values of the respective period. The effect of Ach for each concentration was compared with b-ATBF.

For atropine and mecamylamine experiments, IVA-ATBF (or saline on the control side) was calculated as the mean of the last two values of the period. The effect of the Ach antagonist on the fasting ATBF was calculated as IVA-ATBF minus b-ATBF, and this difference was compared with the value for the corresponding period on the contralateral saline side. pG-ATBF was evaluated using the following: 1) the difference between the peak ATBF value (pk-ATBF; the mean of 3 points: the maximum value and the 2 adjacent points) and b-ATBF, a difference that was expressed in percentage as well and used to define responders (ATBF increase >50% of baseline ATBF) and nonresponders (ATBF increase ≤50% postprandially); and 2) the time-averaged (divided by time after glucose ingestion, i.e., 120 min) incremental ATBF area under the curve (ATBF-iAUC) with the ATBF reading at +65 min as the baseline value. These calculation methods were used in our previous publications (4, 6).

Statistical analyses. These analyses were performed with GraphPad Prism 6.04 (GraphPad Software). Analytical data are expressed as the mean ± SD or median and interquartile range, as appropriate.

The effect of Ach (and that of saline on the control side) was evaluated within the same infusion site by Friedman test with Dunn’s multiple comparison post hoc test. Responses to Ach blockers were compared with the saline control site within the same individual and were tested by a paired t-test or Wilcoxon matched-pairs signed rank test if the data were not normally distributed. Differences between responder and nonresponder groups were assessed by an unpaired t-test or Mann-Whitney U-test if the data were not normally distributed. Differences among groups, defined according to VA and concentration, were tested by one-way ANOVA or median and interquartile range (IQR) and evaluated with Kruskal-Wallis test if not normally distributed. Ach, acetylcholine; DBP, diastolic blood pressure; SBP, systolic blood pressure; Mecam, mecamylamine; TAG, triacylglycerols. Groups are defined according to vasoactive agent and concentration tested. P values are not significant.

### Table 1. Characteristics of participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (males/females)</th>
<th>Ach Atropine, 10⁻⁴ mol/l</th>
<th>Ach Atropine, 10⁻³ mol/l</th>
<th>Mecam, 10⁻³ mol/l</th>
<th>Mecam, 10⁻¹ mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (males/females)</td>
<td>15 (0/15)</td>
<td>13 (1/12)</td>
<td>22 (2/20)</td>
<td>15 (3/12)</td>
<td>10 (2/8)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>30.9 ± 5.7</td>
<td>28.6 ± 5.4</td>
<td>28.9 ± 5.6</td>
<td>32.7 ± 4.9</td>
<td>30.4 ± 4.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.5 ± 2.8</td>
<td>22.0 ± 2.5</td>
<td>22.7 ± 2.3</td>
<td>23.2 ± 2.6</td>
<td>22.7 ± 2.0</td>
</tr>
<tr>
<td>Body fat, kg</td>
<td>18.5 ± 6.1</td>
<td>13.4 ± 2.8</td>
<td>16.0 ± 4.8</td>
<td>17.6 ± 4.8</td>
<td>16.2 ± 7.2</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>43.6 (40.8–45.2)</td>
<td>43.2 (41.3–52.8)</td>
<td>44.2 (41.6–47.6)</td>
<td>44.1 (42.1–46.5)</td>
<td>45.0 (42.2–48.2)</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD and evaluated with 1-way ANOVA or median and interquartile range (IQR) and evaluated with Kruskal-Wallis test if not normally distributed. Ach, acetylcholine; DBP, diastolic blood pressure; SBP, systolic blood pressure; Mecam, mecamylamine; TAG, triacylglycerols. Groups are defined according to vasoactive agent and concentration tested. P values are not significant.

### Table 2. Effect of Ach on ATBF

<table>
<thead>
<tr>
<th>Site</th>
<th>Baseline</th>
<th>10⁻⁴ Ach</th>
<th>10⁻³ Ach</th>
<th>10⁻¹ Ach</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ach</td>
<td>2.41 (1.36–2.83)</td>
<td>3.32 (2.80–5.09)</td>
<td>6.46 (4.36–9.51)*</td>
<td>10.31 (7.98–11.52)††</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Saline</td>
<td>2.62 (1.62–3.64)</td>
<td>2.19 (1.68–3.80)</td>
<td>2.43 (1.75–3.63)</td>
<td>2.52 (1.79–4.53)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Data are presented as median and IQR; n = 15. ATBF, adipose tissue blood flow in ml/100 g-1·min⁻¹. Differences between baseline ATBF and ATBF measured under each concentration of Ach (active site) or saline (control site) were evaluated using Dunn’s multiple-comparison post hoc test. *ATBF changes were evaluated using Friedman test. †P = 0.0001; ††P < 0.0001 vs. baseline ATBF; ‡P = 0.0001 vs. 10⁻⁵ Ach. 10⁻⁵, 10⁻³, and 10⁻¹ Ach refer to 10⁻⁵, 10⁻³, and 10⁻¹ mol/l Ach concentrations, respectively.

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concentration, were tested by one-way ANOVA or Kruskal-Wallis test if not normally distributed. \( P \) values < 0.05 were considered statistically significant for all analyses.

**RESULTS**

**Characteristics of subjects.** The anthropometric and biochemical characteristics of participants are shown in Table 1. We recruited Caucasian subjects, who were young (30 ± 5 yr) and lean (22.9 ± 2.6 kg/m\(^2\)), although seven were slightly

![Graph A](image1)

**A:** ATBF response to local adipose tissue stimulation with 3 incrementally increasing concentrations of Ach. Each concentration was infused for 40 min. On the control side, saline was infused. **B:** ATBF is expressed in corresponding box plots. The effect of each concentration is calculated as the mean of the last 2 values for each 40-min period. ATBF changes were evaluated using Friedman test \((P < 0.0001)\). Differences between b-ATBF and ATBF following each concentration of Ach were evaluated using Dunn’s multiple-comparison post hoc test. **C:** ATBF response to Ach in 7 responders (R) and 8 nonresponders (NR). Data are expressed as median (interquartile range).

<table>
<thead>
<tr>
<th>Table 3. Effect of ( 10^{-4} ) (n = 13) and ( 10^{-3} ) mol/l (n = 15) atropine and ( 10^{-4} ) and ( 10^{-3} ) mol/l (n = 10) Mecam on glucose-stimulated ATBF.</th>
<th>P Values</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ph-b</strong></td>
<td>Saline</td>
<td>3.14 ± 2.47</td>
</tr>
<tr>
<td><strong>%ATBF</strong></td>
<td>Saline</td>
<td>49.5 (12.6–161.0)</td>
</tr>
<tr>
<td><strong>ATBF-( i )-AUC</strong></td>
<td>Saline</td>
<td>0.78 (0.14–2.70)</td>
</tr>
</tbody>
</table>

Normally distributed data are presented as mean ± SD and evaluated with paired \( t \)-test. Data not normally distributed are presented as median and IQR and evaluated with Wilcoxon matched-pairs signed-rank test. Ph-b, difference between peak and baseline ATBF value; %ATBF, percent ATBF variation between baseline and peak ATBF; \( i \)-AUC, incremental area under the curve.
overweight (26.8 ± 1.2 kg/m²). No subjects were diabetic according to the fasting glycemia and A₁c values. Baseline cardiovascular parameters (BP, heart rate) were within the normal range and did not change significantly (data not shown) during the administration of each VA.

**Ach experiments.** Ach was tested in 15 subjects (Table 2 and Fig. 2). Ach increased ATBF dose dependently. ATBF rose fourfold (+328%) with a statistically significant change from the baseline starting at the 10⁻³ mol/l concentration. At the saline site, ATBF did not change in the fasting subjects, thus excluding both a systemic effect of the Ach microinfusion and a time effect.

As expected (6), nonresponders (n = 8) had a nonsignificantly lower b-ATBF [2.00 (1.15–2.53) vs. 2.55 (2.41–4.12) ml·100 g⁻¹·min⁻¹, P = 0.12] than responders (n = 7). There was a trend toward a lower ATBF response to Ach in nonresponders [6.31 (5.52–7.87) vs. 7.80 (7.33–11.30) ml·100 g⁻¹·min⁻¹; P = 0.07] only for the highest Ach concentration. The relative increase from baseline was comparable in both groups (348 ± 163 vs. 378 ± 182%, P = 0.74; Fig. 2C).

**Atropine experiments.** Atropine was tested at the 10⁻⁴ and 10⁻⁵ mol/l concentrations in 13 and 22 subjects, respectively (Tables 3 and 4). The effect of each concentration was evaluated in responders (10⁻⁴ mol/l atropine: n = 10, 10⁻⁵ mol/l atropine: n = 14) and nonresponders.

**ATBF at the control site.** There was no difference in b-ATBF between sites [2.16 (1.90–3.11) vs. 2.15 (1.65–3.16) ml·100 g⁻¹·min⁻¹, P = 0.18]. ATBF was also stable during the b-ATBF and fVA-ATBF periods [b-ATBF vs. fVA-ATBF: 2.15 (1.65–3.16) vs. 2.09 (1.73–3.06) ml·100 g⁻¹·min⁻¹, P = 0.74].

In response to glucose, ATBF increased by 92% [b-ATBF vs. fVA-ATBF: 2.18 (1.85–2.91) vs. 3.62 (2.71–4.23) ml·100 g⁻¹·min⁻¹, P < 0.0001]. This increase was pronounced (+89%) in responders [2.18 (1.56–2.79) vs. 4.12 (3.36–5.72) ml·100 g⁻¹·min⁻¹; P < 0.0001] and nonsignificant (+23%) in nonresponders [2.20 (1.91–3.02) vs. 2.71 (2.21–3.77) ml·100 g⁻¹·min⁻¹; P = 0.16].

**ATBF at the mecamylamine site.** Mecamylamine at both concentrations had no significant effect on fVA-ATBF [10⁻³ mol/l mecamylamine: 0.04 (−0.19 to 0.29) vs. −0.03 (−0.24 to 0.04) ml·100 g⁻¹·min⁻¹, P = 0.60; 10⁻⁴ mol/l mecamylamine: −0.08 ± 0.31 vs. 0.14 ± 0.21 ml·100 g⁻¹·min⁻¹, P = 0.09]. There was also no effect on pG-ATBF in any of the subjects (Table 3) or separately in responders or nonresponders (data not shown).

**DISCUSSION**

Our in vivo study demonstrated the functional presence of nNCS in the AT microvasculature of healthy humans. The maximal stimulatory effect of the pharmacological dose of Ach on ATBF was comparable with that of isoproterenol, a strong agonist of β-adrenergic receptors (6, 24). pG-ATBF decreased in response to atropine at the highest concentration, whereas no effect was induced by mecamylamine. The study also denies the role of this nNCS in the blunting of the ATBF response in nonresponders.

The cholinergic influence on AT has been suggested by metabolic studies in both animals (44) and humans (2, 3) and was supported by the finding of cholinergic receptors in human AT (44) and mature adipocytes (12). Nevertheless, it seems that WAT does not receive parasympathetic innervation (8, 9, 21). Conversely, a growing body of evidence has proposed that nNCS is an important auto- and paracrine regulatory factor in multiple tissues (43), e.g., airways (31), immune cells (27), or the islets of Langerhans (33). Our ATBF data show that AT is another tissue that is affected by nNCS.

<table>
<thead>
<tr>
<th>Table 4. Effect of 10⁻⁴ (n = 10) and 10⁻⁵ mol/l atropine (n = 14) on glucose-stimulated ATBF in responders only</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atropine, 10⁻⁴ mol/l</strong></td>
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<tr>
<td>---</td>
</tr>
<tr>
<td>Pk-b</td>
</tr>
<tr>
<td>%ATBF</td>
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<tr>
<td>ATBF-iAUC</td>
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</table>

*Normally distributed data are presented as means ± SD and evaluated with paired t-test. aData not normally distributed are presented as median and IQR and evaluated with Wilcoxon matched-pairs signed-rank test.*
To our knowledge, the role played by the cholinergic system in the regulation of ATBF has been poorly explored in humans. However, Ach is a powerful vasodilator factor (23, 36). In clinical research, Ach is frequently employed in tests assessing endothelium-dependent vasodilation (16). Moreover, components of the Ach synthesis pathway and both nicotinic (42) and muscarinic (39) cholinergic receptors have been found in endothelial cells (28). The Ach vasodilator effect has been demonstrated in animal fat pads (17) and in human AT arterioles that were perfused ex vivo with increasing Ach concentrations (23). These studies strongly suggest that Ach affects ATBF, but this effect had not been demonstrated; only one in vivo human study using the microdialysis technique failed to show any vasodilatory effect of Ach (2). This disappointing finding may be related to the methods used in the study. ATBF was assessed by the ethanol escape technique, which has been reported to be rather insensitive for ATBF measurements (25).

In our work, we employed the xenon-washout method, a robust and accurate method for ATBF measurement (25, 37). Using Ach concentrations comparable with those in the cited microdialysis study (2), we were able to show that direct Ach microinfusion clearly and dose-dependently increased ATBF. The 10⁻³ mol/l concentration increased ATBF significantly. The 10⁻⁵ mol/l concentration increased ATBF by only 38%, a nonsignificant difference most likely due to lower statistical power. We also demonstrated that blockade of muscarinic receptors with 10⁻⁴ mol/l atropine reduced pG-ATBF, whereas the 10⁻³ mol/l concentration had no effect. Likewise, we found no effect of either concentration of atropine on fasting ATBF.

Concurrently, we have shown a lack of an effect of a nicotinic blocker, mecamylamine, on ATBF. Our data suggest that the Ach vasodilatory action in AT is not mediated by nicotinic receptors. These results are consistent with previous in vitro animal studies demonstrating that muscarinic receptors are primarily responsible for vasodilatation (11, 20).

Overall, it seems that the cholinergic receptors can be stimulated directly in vivo by high concentrations of Ach or agonists (our results) or ex vivo in experimental conditions (23) and that they elicit changes in AT perfusion. However, in physiological conditions, the cholinergic effect depends also on local Ach production and seems less prominent than that of classical vasodilators. This finding is in line with the general concept of the regulation of tissue microperfusion; i.e., the role of classical vasodilation factors such as NO or prostaglandins declines with decreasing vascular diameter (13). In microvessels (arteriolar diameters <100 μm), the involvement of distinct and more important factors, which are collectively named the endothelium-derived hyperpolarizing factor, is presumed (for review, see Ref 16). Moreover, endothelial-endothelial and endothelial-smooth muscle gap junctions operate as a very efficient functional unit that synchronizes vasodilatation within the microvascular tree (13). Because Ach is released in response to shear stress (32), endothelial nNCS probably potentiates the effect of other systemic and local factors by the mechanisms of auto- and paracrine activation of endothelial cells and makes a part of complex regulation of AT perfusion.

Reduced fasting and postprandial ATBF is a hallmark of obesity (1) and type 2 diabetes (14). This reduction is related to insulin resistance (26, 41), β-adrenergic resistance (10), or endothelial dysfunction (18). Surprisingly, these ATBF features are present in 32% of healthy, nonobese subjects, who are called nonresponders (6). Nonresponders are characterized by ATBF resistance to local β-adrenergic stimulation, suggesting a defect in β-adrenergic receptors. Our present study showed no difference in Ach-stimulated ATBF between responders and nonresponders. These findings suggest that nNCS has no role in the blunting of pG-ATBF in nonresponders. The lack of an effect of Ach on ATBF in a previous negative study using microdialysis (2) was not caused by the nonresponder phenomenon but was most probably related to the recording method.

Our study has many strengths. This study was performed in vivo in humans. We were able to recruit a large number of healthy subjects, allowing a comparison between responders and nonresponders. Moreover, we used the microinfusion technique, which is considered the gold standard for ATBF manipulation, and we used concentrations of VA that were previously shown to induce metabolic effects in AT. Nevertheless, our study also has limitations. First, the study was limited to abdominal subcutaneous AT. As a consequence, the results cannot be generalized to other AT depots (femoral, visceral) due to well-known differences in metabolic activity, innervation, and vascularization. To assess these depots, another technique such as positron emission tomography should be used (40, 41). Another limitation is related to the mecamylamine group. Compared with the atropine group, the proportion of nonresponders was higher in the mecamylamine group (41 vs. 35%), and even in responders the pG-ATBF response was lower (p-b-ATBF difference: 154 vs. 89%). This finding might have reduced the possibility of showing a subtle role of nicotinic receptors in vasodilation, which was suggested by some in vivo animal studies (15, 45). Finally, our data were obtained in Caucasian subjects and might not be applicable to other populations.

CONCLUSION

Our study reveals the functional involvement of the cholinergic system in ATBF regulation. The Ach vasodilatory action is mediated by muscarinic receptors. However, the observed ATBF changes were obtained using high doses of stimulating agents. Thus, in physiological conditions, nNCS likely plays a complementary role. The blunted postprandial ATBF surge in healthy, nonobese, nonresponder subjects does not seem to be related to nNCS.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

R.S., P.B., and J.-L.A. conception and design of research; R.S., M.G.-A., and J.M. performed experiments; R.S., J.-P.B., and J.-L.A. analyzed data; R.S., J.-P.B., and J.-L.A. interpreted results of experiments; R.S. prepared figures; R.S. drafted manuscript; R.S., J.-P.B., and J.-L.A. edited and revised manuscript; J.-L.A. approved final version of manuscript.

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