Effects of Insulin Exposure Upon the Metabolism of Rat Aortic Media: Influence of Hydrostatic Forces

Loïc Capron, Monique Philippe, Jean-Louis Guilmot, Jean-Noël Fiessinger, and Edward Housset

When added in vitro, insulin exerts little or no effect on the metabolism of arterial media. However, indirect evidence suggests that an in vivo exposure of arterial tissue to insulin stimulates its metabolism. To evaluate the possibility that hemodynamic forces existing only in vivo may account for this difference, we studied the effect of brief (9-minute) exposures to insulin of isolated rat thoracic aorta perfused in situ at low (34 mm Hg = 4.6 kPa) and high (70 mm Hg = 9.4 kPa) static pressures. After perfusion, incorporation of glucose-derived 14C into CO2 and lipids, and production of lactate by incubated aortic tissue, were measured. At low perfusion pressure, insulin did not affect the metabolism of the media but strongly stimulated all aspects of the glucose metabolism of the adventitia. At high pressure, insulin again stimulated the adventitia but now also significantly stimulated (+66%) the synthesis of glucose-derived lipids by the media; production of CO2 and lactate by the media did not increase significantly. These results indicate that insulin directly stimulates at least one pathway of glucose metabolism by the media; and that hemodynamic forces are an important factor in determining the response of aortic media to insulin.

(Arteriosclerosis 1:345-352, September/October 1981)

Smooth muscle cells of the arterial wall participate in the pathogenesis of atherosclerosis. Although diabetes mellitus appears to increase the risk of arterial diseases in man, the actions of insulin on the metabolism of arterial tissue, particularly the media, are still uncertain. No direct metabolic effect of insulin on the media has been firmly established in vitro. However, indirect experimental evidence suggests that a brief in vivo exposure to insulin stimulates glucose metabolism or accumulation of glucose carbon in the media. Longer periods of insulin treatment normalize the depressed metabolic activities of the media found in experimental diabetes. Furthermore, insulin stimulates proliferation and sterol synthesis of cultured arterial smooth muscle cells. None of these observations, however, conclusively demonstrates that smooth muscle cells, when part of arterial tissue, are directly responsive to insulin.

That the presence of a luminal pressure plays a role in causing insulin to act on the cells of the avascular media is an attractive possible explanation for the discrepancy found between in vivo and in vitro exposures to the hormone. To explore this possibility further, we developed a system of in situ perfusion of the isolated rat thoracic aorta that allowed short exposures of the blood vessel to insulin under low and high static pressures. The effects of insulin after its binding to tissues could be evaluated after perfusion by conventional incubation techniques carried out in vitro. The present study shows a stimulation of the glucose metabolism only when the media had been exposed to insulin under a high perfusion pressure, close to the normal mean arterial pressure of the animal.
**Methods**

**Animals**

Male Wistar rats (Iffa Credo, l'Arbresle, France) weighing 150–250 g were used. They were fed regular rat chow containing 59% carbohydrates (rat élevage, Usine d'Alimentation Rationnelle, Villemoison sur Orge, France). Animals fasted for 48 hours before all experiments; this established a baseline metabolic state, because feeding can modify glucose metabolism of the arterial wall. An intraperitoneal injection of thiopental sodium (Nesdonal, Specia, Paris, France) at a dose of 50 mg kg⁻¹ provided deep anesthesia.

**Perfusion**

Our single-pass perfusion system (figure 1) consisted of a perfusion circuit (ABCD), a shunting circuit (FE), a perfusion line (CDEG), and a bypass (DA). The perfusion circuit was composed of: 1) a mixing reservoir (A) where the perfusion medium was stirred and gassed; 2) a peristaltic pump (B) (Cole Parmer, Chicago, Illinois); 3) a perfusion reservoir (C), the height of which (h) could be adjusted to obtain the desired perfusion pressure; 4) the initial part of the perfusion line (CD) connected with the bypass and the distal part of the perfusion line (DEG). The shunting circuit was composed of: 1) a shunting reservoir (F) placed at a fixed height of 120 cm, where the shunting medium was gassed; 2) a shunting line (FE) connected with the distal part (EG) of the perfusion line. The glassware and most of the silicone tubings (Scurasil, Roger Bellon, Neuilly sur Seine, France) of the system were water-jacketed. All parts of the circuit were maintained at a constant temperature by a circulation of heated water (Haake, Karlsruhe, West Germany). The cannulas were shaped from stainless steel tubes (external/internal diameters: 1/0.8 mm for the perfusion cannulas G, and 2/1.6 mm for the pressure cannulas H).

**Media**

The shunting medium was Krebs Henseleit bicarbonate buffer with glucose 5 mmoles liter⁻¹ (KHBG). The perfusion medium was KHBG con-

---

**Figure 1.** Perfusion system of rat thoracic aorta. Perfusion circuit (ABCD): mixing reservoir (A), peristaltic pump (B), perfusion reservoir (C), the height of which (h) can be adjusted to obtain the desired perfusion pressure; shunting circuit (FE): shunting reservoir (F), shunting line (FE); bypass (DA); perfusion line (CDEG). Thoracic aorta perfused between perfusion (G) and pressure (H) cannulas; pressure transducer (PT); innominate (IA), left common carotid (LCCA), left subclavian (LSCA), intercostal (ICA), and subcostal (SCA) arteries; diaphragm (Dia). Single-headed arrows indicate the direction of flow. Media were gassed with 95% O₂–5% CO₂ (O₂–CO₂). Water-jacketing of the three reservoirs and of most tubings (AB, BC, CD, DA, FE) is not represented.
taining either highly purified regular bovine insulin (Endopancrine Monopic, Organon, St. Denis, France) 100 mU · ml⁻¹ for insulin exposure experiments, or only an equivalent amount of the solving buffer of this commercial insulin preparation (glycerol 0.14 mmole · liter⁻¹, phenol 0.05 mmole · liter⁻¹, and glyccolull 0.50 mmole · liter⁻¹, final concentrations in KHBG) for control experiments. Media were equilibrated at pH 7.4 by gassing with a mixture of O₂ and CO₂ (95/5, v/v), and heated in the circuit for 30 to 60 minutes before starting perfusion. To avoid contamination of control medium with insulin, different sets of glassware, tubings, and cannulas were used for control and insulin experiments.

In the steady state of the system — during equilibration of the media — the perfusion circuit was bypassed and the shunting circuit closed by clamping DE and EG. The left common carotid artery was exposed. Its distal portion was ligated, and its proximal part was clamped. The perfusion cannula was inserted between ligature and clamp. The abdominal cavity was opened and the aorta ligated just above the renal arteries. Clamps of the shunting circuit and carotid artery were removed, and the thorax was opened. The pressure in the aorta fell due to hemorrhage until it reached the pressure of the shunting circuit (approximately 80 mm Hg = 10.7 kPa); from then on, the aorta was perfused by the shunting medium. The aortic arch between the innominate and left common carotid arteries, and the left subclavian artery were ligated. The pressure cannula was inserted into the distal end of the thoracic aorta just below the subcostal arteries, and connected with an electronic pressure transducer (Hewlett Packard quartz transducer 1290 A). The bypass and shunting line were clamped, and the perfusion line was simultaneously opened by removing the clamp in DE. The abdominal and thoracic organs were removed. The perfusion medium leaked through the unligated intercostal and subcostal arteries. The perfusion lasted for 9 minutes. Pressure was monitored continuously.

The shunting circuit had three purposes: 1) it permitted all blood contained in the aorta to be washed out completely before starting experimental perfusion; 2) it avoided a total collapse of the aorta, which might have damaged the endothelium and modified its permeability; and 3) it allowed perfusion with the experimental medium to begin only after all vascular connections of the aorta with organs such as the liver, brain, and pancreas had been interrupted.

In preliminary experiments, the actual temperature of the perfusion of the aorta was measured. Instead of the pressure cannula, a thermocouple probe connected with an electronic thermometer (Bailey Instruments, Saddie Brook, New Jersey) was placed into the distal end of the thoracic aorta. Temperature was constantly measured during a trial perfusion period.

**Incubation**

The incubation medium for all experiments was KHBG containing D-glucose [¹⁴C(U)] (New England Nuclear, Boston, Massachusetts) with a specific activity of 0.1 mCi · mmole⁻¹. Before incubation the medium was equilibrated at 37°C and pH 7.4 by gassing with 95% O₂-5% CO₂ for 30 minutes.

At the end of perfusion the segment of aorta located between the left subclavian and the subcostal arteries was removed, opened longitudinally, and dissected into intima-media and adventitia layers as described by Wolinsky and Daly at 37°C. No effort was made to remove the peri-adventitious fat. All aortas were dissected by the same person, and the dissection time gave a rough estimate of the uniformity of dissection. The strips of media and the adventitia were quickly blotted on filter paper and transferred separately into 10 ml Erlenmeyer flasks containing 1 ml of incubation medium. Flasks were stoppered by serum caps fitted with plastic wells (Kontes, Vineland, New Jersey), placed into a shaking metabolic incubator (Heto, Birkerød, Denmark), set at 37°C and 70 linear oscillations per minute, and flushed for 2 minutes with 95% O₂-5% CO₂. For all experiments, the incubation time was 1 hour; at the end, 0.2 ml of (p-disobutyl — cresoxyethoxyethyl) dimethylbenzylammonium hydroxide) 1 mole · liter⁻¹ in methanol (Hydroxide of hyamine 10-X, Packard, Downers Grove, Illinois) was injected into the center well to trap CO₂, and 0.1 ml of 4N H₂SO₄ into the main compartment to inhibit further tissue metabolism, and shaking was continued for 30 minutes more.

**Analytical Procedures**

**¹⁴CO₂**

Each well containing hydroxide of hyamine was placed in a plastic counting vial containing 10 ml of scintillation solution (Econfluor, New England Nuclear) and the radioactivity was counted.

**¹³C Lipids**

Tissues were blotted and washed for 2 to 5 minutes in 2 ml of NaCl 154 mmoles · liter⁻¹. Tissues were then blotted again, weighed (wet weight), and lipids were extracted overnight with 5 ml of 2/1 (v/v) chloroform-methanol. The lipid extract was then washed three times by the procedure of Folch et al., and evaporated to dryness under a stream of nitrogen in a glass.
counting vial. Ten ml of Econofluor was added and the radioactivity measured. Preliminary experiments had shown that the extraction of lipids was essentially complete under these conditions.

Lactate
Total lactate in the medium was determined according to the method of Noll\textsuperscript{17} using the reagents of the "Test Combination Lactate Fully Enzymatic" (Boehringer, Mannheim, West Germany).

Deoxyribonucleic Acid (DNA)
The delipidated tissues were homogenized in 2 ml of 0.5 N HClO\textsubscript{4}, and DNA contents were measured according to Burton\textsuperscript{18} using calf thymus DNA (Type I, Sigma, St. Louis, Missouri) as standard.

For each experimental session, 0.1 ml of the incubation medium was dissolved in 10 ml of Pico-Fluor 15 (Packard) to measure the actual specific activity. Radioactivity was counted in a liquid scintillation spectrometer (Tri-Carb, Model 3380, Packard).

Experimental Groups
In the main experiment, four groups of 12 animals were studied. The first two groups were perfused at low pressure (LP), the perfusion reservoir being placed at a height (h) of 65 cm. One group was perfused with control medium (CLP), the other with insulin containing medium (ILP). The last two groups were perfused at high pressure (HP, perfusion reservoir placed at \( h = 135 \) cm) with either control or insulin containing medium. Smaller groups of animals were used in preliminary and additional experiments (temperature studies), as indicated in the results.

Units and Statistics
Results are expressed as pmole of glucose-equivalent produced (\(^{14}\text{CO}_2\)) or accumulated (\(^{14}\text{C} \text{lipids}\)), or as pmole produced (lactate) per \( \mu \text{g DNA} \) per minute of incubation: (pmole/\( \mu \text{g DNA} \cdot \text{min}^{-1}\)). The amount of glucose converted to CO\(_2\) and lipids was calculated by dividing the disintegrations in the product per minute by the specific activity of the glucose in the medium.

The stimulation of Group X compared with Group Y is expressed by its ratio calculated as: stimulation ratio (\%) = \( 100 \times (X - Y)/Y \), where X and Y are the mean values of Groups X and Y.

Results are given as mean ± 2 standard errors of the mean (SEM). We used the analysis of variance for the overall comparisons of the four groups in the main experiments. When \( F \) was significant (\( F > 2.82, p < 0.05 \)) we did three pre-planned pairwise comparisons (CLP vs CHP, ILP vs CLP, and IHP vs CHP) with the method of Bonferroni,\textsuperscript{19} which in this case required a \( t \) value of 2.51 to achieve significance (\( p < 0.05 \)). Unpaired Student's \( t \) test was used for comparisons between two means in preliminary and additional experiments.

Results
Animal Characteristics

Table 1 gives the characteristics of animal groups in the main experiment. No significant differences among the four groups (\( F < 1.89 \)) were found for body weight, time of dissection, wet weight of media and adventitia, and DNA content of media. For the DNA content of adventitia, an unexpected, slight but significant difference (\( F = 4.36 \)) existed between the CLP and I\(_{LP}\) groups (123 ± 18 vs 88 ± 14 \( \mu \text{g} \), \( t = 3.24 \)).

Table 1. Characteristics of Animal Groups in Main Experiment

<table>
<thead>
<tr>
<th>Animal Group*</th>
<th>Body weight (g)</th>
<th>Dissection time (sec)</th>
<th>Media</th>
<th>Total DNA (( \mu \text{g} ))</th>
<th>Adventitia</th>
<th>Total DNA (( \mu \text{g} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>202 ± 20\textsuperscript{†}</td>
<td>123 ± 20</td>
<td>14.8 ± 1.0</td>
<td>32.9 ± 1.5</td>
<td>70.7 ± 15.4</td>
<td>123 ± 18</td>
</tr>
<tr>
<td>Insulin</td>
<td>199 ± 20</td>
<td>112 ± 16</td>
<td>14.1 ± 1.0</td>
<td>31.0 ± 3.0</td>
<td>58.1 ± 12.4</td>
<td>88 ± 14\textsuperscript{‡}</td>
</tr>
<tr>
<td>High Pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>204 ± 13</td>
<td>133 ± 24</td>
<td>14.4 ± 0.8</td>
<td>31.3 ± 2.2</td>
<td>66.3 ± 9.8</td>
<td>114 ± 16</td>
</tr>
<tr>
<td>Insulin</td>
<td>210 ± 16</td>
<td>129 ± 18</td>
<td>14.3 ± 0.8</td>
<td>28.6 ± 3.3</td>
<td>73.0 ± 7.0</td>
<td>120 ± 13</td>
</tr>
</tbody>
</table>

*Perfusion with medium containing either no insulin (control) or insulin at a concentration of 100 mU-ml\(^{-1}\) for 9 minutes at low or high pressure.

\textsuperscript{†}Each value is the mean ± 2 SEM of 12 animals.

\textsuperscript{‡}Insulin is significantly different from control (\( p < 0.05 \), analysis of variance and Bonferroni method for multiple simultaneous comparisons).

DNA = deoxyribonucleic acid.
**Physical Results**

Figure 2 displays the pressure recordings in the four experimental groups. Pressure always declined somewhat during the 9 minutes of perfusion. The drop was of about 10 mm Hg (1.3 kPa) at low pressure and about 25 mm Hg (3.3 kPa) at high pressure and did not differ for the two experimental solutions at each pressure level. The mean perfusion pressures calculated by averaging the mean values of each time point were 35 mm Hg (4.7 kPa) for CLP, 33 mm Hg (4.4 kPa) for ILP, and 70 mm Hg (9.4 kPa) for both CHP and IHP. Note that the high pressure was close to the physiological pressure and that the low pressure was markedly below normal pressure.

The difference between these measured pressures and the heights of the perfusion reservoir in each situation must be due to resistance losses in the system.

Preliminary experiments had shown that, as expected, the flow of medium through the aorta depended on the pressure, being much higher at high (about 7 ml·min⁻¹) than at low (about 2 ml·min⁻¹) pressure. As a consequence, the temperature at the distal end of the aorta remained somewhat higher at high than at low pressure. For the main experiment, the thermostatic circuit was set at 42°C, at which setting the temperatures inside the aorta during the 9-minute perfusion at low and high pressures were respectively 29.5 ± 1.4 and 32.2 ± 1.0°C (mean of six experiments). This 2.7°C difference was significant (p < 0.02). The temperature in the aorta tended to decrease slightly (variation of less than 2°C) during the 9-minute perfusion.

**Metabolic Results**

**Adventitia**

Intentionally, most of the fat was retained in the samples of adventitia used for incubation. It served as a highly insulin-sensitive tissue in assessing whether our experimental protocol provided an efficient exposure of aorta to insulin. Perfusion with an insulin-containing medium significantly stimulated all aspects of the glucose metabolism of the adventitia whether the aorta was perfused at low or at high pressure (table 2). The greatest percentage changes occurred for ¹⁴C lipids (+1249%; t = 4.06 at LP, and +1376%; t = 5.71 at HP); ¹⁴CO₂ showed less change (+691%, t = 8.54 at LP, and +413%, t = 7.22 at HP); and lactate showed the least (+220%, t = 8.67 at LP, and +192%, t = 6.19 at HP). The pressure of perfusion did not significantly influence the metabolism of control adventitias (CHP vs CLP; t < 0.80) and did not consistently modify the stimulating effect of the insulin exposure.

**Media**

The metabolic results for media are displayed numerically in table 2 and graphically in figure 3. At a low perfusion pressure, insulin exposure did not significantly modify any of the three aspects of glucose metabolism of the media (ILP vs CLP: f = 0.03 for ¹⁴CO₂, t = 1.47 for ¹⁴C lipids, and t = −0.08 for lactate). At high perfusion pressure, insulin exposure did significantly stimulate (+66%, t = 5.10) the synthesis of glucose-derived lipids. The stimulation of glucose-derived CO₂ production was substantial (+29%) but it just fell short of being significant (t = 2.33). The stimulation of lactate production was negligible (+14%) and not significant (t = 1.02). Although the glucose metabolism of the control medias was always slightly more active after perfusion at high than at low pressure, none of the differences was significant (all CHP vs CLP: t < 1.51).

**Effect of Perfusion Temperature**

The 2.7°C difference in final perfusate temperature might account for the observed differ-
Table 2. Glucose Metabolism of Rat Thoracic Aorta

<table>
<thead>
<tr>
<th>Group</th>
<th>(^{14}\text{CO}_2^*)</th>
<th>(^{14}\text{C} \text{Lipids}^†)</th>
<th>Lactate$\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.4 ± 3.0</td>
<td>1.85 ± 0.53</td>
<td>59 ± 13</td>
</tr>
<tr>
<td>Insulin</td>
<td>82.3 ± 17.3§</td>
<td>24.96 ± 10.17§</td>
<td>189 ± 39§</td>
</tr>
<tr>
<td>High pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>14.7 ± 2.9</td>
<td>2.36 ± 0.55</td>
<td>48 ± 7</td>
</tr>
<tr>
<td>Insulin</td>
<td>75.4 ± 15.8§</td>
<td>34.84 ± 12.45§</td>
<td>140 ± 9§</td>
</tr>
<tr>
<td>Media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.47 ± 0.38</td>
<td>0.232 ± 0.026</td>
<td>86 ± 20</td>
</tr>
<tr>
<td>Insulin</td>
<td>2.48 ± 0.42</td>
<td>0.282 ± 0.014</td>
<td>74 ± 20</td>
</tr>
<tr>
<td>High pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.04 ± 0.40</td>
<td>0.267 ± 0.028</td>
<td>104 ± 24</td>
</tr>
<tr>
<td>Insulin</td>
<td>3.32 ± 0.82</td>
<td>0.443 ± 0.088§</td>
<td>119 ± 20</td>
</tr>
</tbody>
</table>

$^{14}\text{CO}_2$ units: (pmole equivalent glucose produced) · (μg DNA)$^{-1}$ · (min.incubation)$^{-1}$.

$^{14}\text{C}$ lipids units: (pmole equivalent glucose accumulated) · (μg DNA)$^{-1}$ · (min.incubation)$^{-1}$.

$^†$Lactate units: (pmole produced) · (μg DNA)$^{-1}$ · (min.incubation)$^{-1}$.

$§$Insulin is significantly different from control ($p < 0.05$, analysis of variance and Bonferroni method for multiple simultaneous comparisons).

See table 1 for explanation of groups. Each value is the mean ± 2 SEM of 12 animals.

Under these conditions, insulin exposure at low pressure (32 mm Hg = 4.3 kPa for the six rats perfused with control medium C; 35 mm Hg = 4.6 kPa for the six rats perfused with insulin-containing medium I) still did not significantly stimulate the synthesis of glucose-derived lipids by the media (0.296 ± 0.090 for C vs 0.240 ± 0.065

![Figure 3. Glucose metabolism of the aortic media after 9 minutes of perfusion with either control (C, open circles) or insulin containing (I, solid circles) medium at either low (LP) or high (HP) pressure. Bars indicate the mean of 12 experiments ± 2 SEM. Incubation time = 1 hour. Units: pmole of glucose equivalent produced (\(^{14}\text{CO}_2^\)) or accumulated (\(^{14}\text{C} \text{lipids}\)), or pmole produced (lactate) per μg DNA per minute of incubation: pmole-μg DNA$^{-1}$-min$^{-1}$.](http://atvb.ahajournals.org/)
pmole $\cdot \mu$g DNA$^{-1} \cdot$min$^{-1}$ for I, $p > 0.30$), although it again provoked an important increase of lipid accumulation in the adventitia ($2.29 \pm 0.65$ for C vs $18.78 \pm 5.41$ pmole $\cdot \mu$g DNA$^{-1} \cdot$min$^{-1}$ for I, $p < 0.001$). Clearly, the difference in perfusion temperature in the main experiment was not responsible for the lack of response of the media to an insulin exposure at low pressure. Rather, the pressure increase must be the major, if not exclusive, basis for the insulin effects on the media seen in the $I_{hp}$ group.

**Discussion**

Our aim in these studies was to answer two questions: Does insulin **directly** stimulate glucose metabolism of the aortic media? Do hydrostatic forces acting upon the arterial wall influence the efficiency of an insulin exposure? Our results suggest that the answer to both questions is yes. However, such conclusions should be accepted with caution. Our perfusion system is indeed still quite far from reproducing the physical conditions under which an artery normally functions. Among the most obvious discrepancies are: 1) the existence of a static instead of an oscillatory pressure, which may greatly affect the penetration of large molecules into the arterial wall; 2) utilization of an artificial perfusion medium with viscosity, oncotic pressure, oxygen carrying and delivering capacities, and temperature different from those of blood; 3) elimination of normal mediastinal pressure variations induced by respiration; and 4) the unphysiologically high concentration of insulin in the perfusion medium (100 mU $\cdot$ml$^{-1}$). The threshold level for insulin action can and should now be tested in this system. It must be stressed, however, that even the large amounts of insulin used here have previously failed to alter the metabolism of the arterial media in vitro$^{2,3}$ and failed to do so even in our system when studied at low perfusion pressure.

Under these experimental conditions, a short (9 minute) exposure to insulin caused a marked stimulation of the adventitia's metabolism at both perfusion pressures studied. This pattern of stimulation was grossly similar to that observed with pure adipose tissue$^1$ and may be due to the high fat content of our adventitious preparation. Our experimental protocol clearly provided an efficient exposure of adventitia to insulin that was not influenced by pressure. This would appear to rule out any contribution by the perfusion pressure to the access of insulin to the adventitia through the vasa vasorum. It is also quite possible that leakage of perfusion fluid into the thorax might account for the stimulation of adventitia by insulin.

Glucose metabolism of the media was stimulated to a much lesser extent and only after insulin exposure at high pressure (i.e., a pressure close to the physiological arterial pressure of the rat). The synthesis of glucose-derived lipids was significantly stimulated, whereas the synthesis of CO$_2$ and lactate showed only an insignificant increase. These observations suggest that insulin can directly stimulate the glucose metabolism of the media because the exposure to the hormone was performed in an isolated system, with all possible contributions of organs such as liver, brain, and pancreas eliminated. Such a conclusion had previously been supported only inferentially by in vivo studies$^{4-6}$ or studies on cultured smooth muscle cells.$^{12}$ Interestingly, these studies showed stimulation ratios for lipid synthesis from glucose,$^4$ accumulation of glucose carbon,$^6$ or sterol synthesis from acetate$^{4,5,12}$ varying between 50% and 120%, a range that includes the 66% ratio found here.

No modification of the metabolism of the media was observed after an insulin exposure at low pressure, which establishes the complete separation of the media from the adventitia by the dissection procedure of Wolinsky and Daly.$^{15}$ More important is the inference that hemodynamic forces can play a key role in the penetration of insulin into the media in vivo. With our protocol, however, it is not yet possible to dissociate the effect of luminal pressure from that of luminal flow. The media of rat thoracic aorta is totally avascular, and its nutrition depends upon the filtration of plasma components through the inner layers of arterial wall.$^{22}$ The role of hemodynamic forces in this filtration process$^{20,21,24}$ lends further support to this interpretation of our findings.

Our observation that the effects of at least one peptide hormone, insulin, on the glucose metabolism of aortic media depend on hydrostatic conditions opens up many intriguing questions about interactions between humoral and hemodynamic factors. Most obvious from our studies is the question of insulin's role in the lipid accumulation found in atherosclerotic lesions.$^{25}$ However, much remains to be learned about normal arterial physiology before complex problems of pathological processes can be addressed. Our experimental system could provide a means for systematic exploration of these important issues.

**Acknowledgment**

We are grateful to Harvey Wolinsky for his criticism of the manuscript.
References

5. Stout RW. Insulin stimulation of cholesterol synthesis by arterial tissue. Lancet 1968;2:467-468

Index Terms: glucose metabolism • insulin • aorta • hemodynamics • diabetes mellitus
Effects of insulin exposure upon the metabolism of rat aortic media: influence of hydrostatic forces.
L Capron, M Philippe, J L Guilmot, J N Flessinger and E Housset

doi: 10.1161/01.ATV.1.5.345

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/1/5/345

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/