THE EFFECT OF INSULIN ON NONESTERIFIED FATTY ACID
RELEASE FROM THE HUMAN LEG*

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In recent years much interest has been focused
on the plasma level of nonesterified fatty acid
(NEFA), or unesterified fatty acid (UFA), and
on its role as an energy substrate, particularly for
muscle and liver.

Both insulin and glucose produce a rapid fall
in serum NEFA level (1, 2), which is related to
a decreased output of NEFA from the adipose cell
(3, 4). The concept has arisen that in the fasting
state NEFA is continuously mobilized from fat
stores, and when glucose becomes available, this
mobilization ceases and the blood level falls (5).

Several years ago Bell and Burns (6) showed
that the intra-arterial injection of small doses of
insulin into the leg of a human subject caused a
prompt widening of the arteriovenous (A-V) dif-
ference for glucose across the injected limb as
compared to the opposite noninjected limb. This
apparent fixation of the insulin in the injected
limb and the resulting differential metabolic effect
seemed to offer an opportunity to study further the
effect of insulin on NEFA handling by the periph-
eral tissues.

METHODS

Studies have been carried out on six normal fasting
human subjects. Indwelling Cournand needles were
placed in one femoral artery and in both femoral veins
under procaine anesthesia. Simultaneous control samples
were drawn from each of these three sites, followed by
a small dose of glucagon-free insulin (one-half to one
unit) injected intra-arterially. Thus insulin was injected
directly into one limb, reaching the other limb after at
least one circuit and in smaller concentration.

Care was taken to insure simultaneous withdrawal of

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all samples. Immediately after withdrawal, 5 ml. of
blood was transferred from each syringe into fluoride
tubes for glucose determination and the remainder was
transported under refrigeration to the laboratory for
centrifugation and determination of NEFA level ac-
cording to the method of Dole (1). The analysis was
done as rapidly as possible after withdrawal. In addition
to analyses for sugar and NEFA, serum cholesterol
was determined by the method of Abell, Levy, Brodie
and Kendall (7) and triglycerides were determined by the
method of Van Handel and Zilversmit (8). Blood
sugar was done by the method of Somogyi (9, 10).

The insulin used for the study was hyperglycemic fac-
tor-free crystalline zinc insulin freshly prepared in saline
for each experiment. The time required for injection
was less than 10 seconds.

Six subjects were studied, observations being made on
six injected limbs and six control limbs. All studies
were carried out on lower extremities except for one
control limb (Subject P). In this instance the arm was
used instead of the leg because of difficulty in obtain-
ing blood from the femoral vein.

RESULTS

The results are listed in Tables I through V. The
changes to be emphasized are as follows.

1. Arterial NEFA level

The arterial level of NEFA at the control pe-
riod ranged from 339 to 915 μMoles per L., all
within the accepted normal range. The mean ar-
terial level of NEFA was significantly decreased
after insulin injection (Table I). Although the
relaxation of the subjects after the placement of
the needles cannot be ruled out as a cause of the
drop in this study (11, 12), such a drop in ar-
terial NEFA has not been seen in other subjects
receiving arterial injections of materials not af-
flecting NEFA release.

2. A-V NEFA difference

Table II shows, for each subject, the A-V
NEFA differences, both before and after insulin,
The tremendous responsiveness of the peripheral tissues of the leg to small doses of insulin is well shown in these studies. They confirm the reports of Bell and Burns (6) that insulin injected in small quantity into the artery supplying a single limb has a major effect on glucose uptake in this limb as compared to the remainder of the body; they show in addition that these effects extend to the NEFA as well as to glucose. It is felt that these studies show a primary effect of insulin on peripheral tissues, presumably adipose tissues, in regulating the level of NEFA as well as in regulation of blood glucose. The exit of NEFA from adipose tissues of the injected limb is reduced in contrast to the exit of NEFA from the uninjected limb.

It should be noted that at 20 minutes after injection, the change in A-V difference from the control condition was also significantly different.

3. Glucose

The A-V glucose difference changes were as described by Bell and Burns (6): a widening in the injected limb, a narrowing in the uninjected limb (Tables IV and V).

4. Triglyceride and cholesterol

There was no significant alteration in triglyceride or cholesterol levels during the course of the study.

DISCUSSION

for both the injected and noninjected limbs. In the injected leg, the initially negative A-V NEFA difference was narrowed at 10 minutes and, in four cases, reversed at 20 minutes. Changes in the A-V differences from the control period were large and uniform enough to be statistically significant (p being less than 0.05 at 10 minutes and less than 0.01 at 20 minutes after insulin injection). The noninjected limb showed no significant change in A-V differences.

Table III provides two further evidences that, regarding NEFA, insulin acted chiefly in the injected limb. Twenty minutes after insulin, the A-V NEFA change was significantly different in the two limbs. Further, during the first 10 minutes, the change in A-V difference from the control condition was also significantly different.

### TABLE I

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control period</th>
<th>10 min. after insulin</th>
<th>Change during 10 min.</th>
<th>20 min. after insulin</th>
<th>Change during entire 20 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>703</td>
<td>676</td>
<td>-27</td>
<td>525</td>
<td>-178</td>
</tr>
<tr>
<td>P</td>
<td>876</td>
<td>769</td>
<td>-107</td>
<td>504</td>
<td>-372</td>
</tr>
<tr>
<td>E</td>
<td>339</td>
<td>275</td>
<td>-64</td>
<td>206</td>
<td>-133</td>
</tr>
<tr>
<td>L</td>
<td>354</td>
<td>306</td>
<td>-48</td>
<td>306</td>
<td>-48</td>
</tr>
<tr>
<td>McI</td>
<td>485</td>
<td>446</td>
<td>-39</td>
<td>451</td>
<td>-92</td>
</tr>
<tr>
<td>McD</td>
<td>915</td>
<td>775</td>
<td>-140</td>
<td>648</td>
<td>-267</td>
</tr>
<tr>
<td>Mean</td>
<td>612</td>
<td>541</td>
<td>-71</td>
<td>440</td>
<td>-172</td>
</tr>
</tbody>
</table>

| “t” value | 3.96 | 3.23 |
| p         | <0.02 | <0.05 |

### TABLE II

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control period</th>
<th>10 min. after insulin</th>
<th>20 min. after insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injected limb</td>
<td>Noninjected limb</td>
<td>Injected limb</td>
</tr>
<tr>
<td>N</td>
<td>-163</td>
<td>-190</td>
<td>-69</td>
</tr>
<tr>
<td>P</td>
<td>-71</td>
<td>+48*</td>
<td>+15</td>
</tr>
<tr>
<td>E</td>
<td>-73</td>
<td>-73</td>
<td>+25</td>
</tr>
<tr>
<td>L</td>
<td>-64</td>
<td>-84</td>
<td>-53</td>
</tr>
<tr>
<td>McI</td>
<td>-40</td>
<td>-21</td>
<td>-46</td>
</tr>
<tr>
<td>McD</td>
<td>-231</td>
<td>+3</td>
<td>-65</td>
</tr>
<tr>
<td>Mean</td>
<td>-107.00</td>
<td>-52.83</td>
<td>-32.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>10 min. after insulin</th>
<th>20 min. after insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected limb</td>
<td>Noninjected limb</td>
</tr>
<tr>
<td>N</td>
<td>-69</td>
</tr>
<tr>
<td>P</td>
<td>+15</td>
</tr>
<tr>
<td>E</td>
<td>+25</td>
</tr>
<tr>
<td>L</td>
<td>-53</td>
</tr>
<tr>
<td>McI</td>
<td>-46</td>
</tr>
<tr>
<td>McD</td>
<td>-65</td>
</tr>
<tr>
<td>Mean</td>
<td>-32.17</td>
</tr>
</tbody>
</table>

* Brachial vein.
Injection of insulin, the mean arterial level of NEFA usually exceeded the mean venous level of NEFA in the injected limb. This is not felt to represent an increased uptake of NEFA but a suppressed release of it which allowed the utilization of NEFA to exert a predominant effect on the venous level.

### SUMMARY

1. The tissues of the leg exhibited extreme responsiveness to injection of small doses of insulin.
2. Arterial nonesterified fatty acid (NEFA) level dropped significantly within 20 minutes after intra-arterial injection of small doses of insulin.
3. The arteriovenous (A-V) NEFA difference in the injected limb changed significantly after insulin injection, from a negative A-V difference to a frequently positive A-V difference.
4. The A-V NEFA difference in the control limb did not change after insulin injection.

### TABLE III

*Arteriovenous (A-V) nonesterified fatty acid difference (micromoles per liter): Comparison of change in injected and noninjected limbs*

<table>
<thead>
<tr>
<th></th>
<th>A-V difference</th>
<th>Change from control level</th>
<th>A-V difference</th>
<th>Change from control level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected limb</td>
<td>-107.0</td>
<td>+74.83</td>
<td>+30.67</td>
<td>+137.67</td>
</tr>
<tr>
<td>Noninjected limb</td>
<td>-52.82</td>
<td>-26.01</td>
<td>-50.67</td>
<td>+2.15</td>
</tr>
<tr>
<td>Difference</td>
<td>-54.18</td>
<td>+100.84</td>
<td>+81.33</td>
<td>+135.52</td>
</tr>
<tr>
<td>&quot;t&quot; value</td>
<td>1.75</td>
<td>3.02</td>
<td>2.62</td>
<td>2.22</td>
</tr>
</tbody>
</table>

(Not significant) (Not significant) (p<0.05) (Not significant)

5. At 20 minutes after insulin injection the change in A-V NEFA difference in the injected limb was significantly different from that of the control limb and demonstrates a distinct action in the injected limb apart from the rest of the body.

### TABLE IV

*Arterial glucose (milligrams per 100 milliliters): Change after insulin*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Control period</th>
<th>10 min. after insulin</th>
<th>20 min. after insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>84.2</td>
<td>78.0</td>
<td>74.0</td>
</tr>
<tr>
<td>P</td>
<td>77.8</td>
<td>74.0</td>
<td>72.2</td>
</tr>
<tr>
<td>E</td>
<td>73.7</td>
<td>59.8</td>
<td>49.1</td>
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<tr>
<td>L</td>
<td>82.2</td>
<td>82.8</td>
<td>76.8</td>
</tr>
<tr>
<td>McI</td>
<td>96.0</td>
<td>83.9</td>
<td>75.1</td>
</tr>
<tr>
<td>McD</td>
<td>96.2</td>
<td>86.8</td>
<td>75.4</td>
</tr>
<tr>
<td>Mean</td>
<td>85.0</td>
<td>77.7</td>
<td>70.4</td>
</tr>
</tbody>
</table>

Mean change from control to 10 min. after insulin = (-7.30 ("t" = 3.213, p < 0.05).

Mean change from 10 to 20 min. after insulin = (-7.28 ("t" = 5.009, p < 0.01).

### REFERENCES


