Atropine Improves Insulin Sensitivity in Both Lean and Abdominally Obese Subjects


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Background: Dysregulated autonomic nerve activity may contribute to the development of type 2 diabetes. The aim of this study was to assess the effects of an anticholinergic agent, atropine, and a cholinergic agent, physostigmine, on insulin sensitivity in lean and abdominally obese subjects.

Subjects and Methods: In a single-blinded three-way crossover study, six lean and six abdominally obese nondiabetic subjects [three males and three females in each group; age, 43.8 ± 14.8 vs. 46.8 ± 4.8 yr (mean ± SD); body mass index, 22.6 ± 1.7 vs. 28.8 ± 1.3 kg/m²; and waist circumference, 85 ± 2 vs. 99 ± 6 cm, respectively] were given iv infusions with atropine (15 μg/kg bolus, 4 μg/kg · h infusion), physostigmine (0.12 μg/kg · min) or saline (0.9% NaCl) in a randomized treatment order. Infusions were started 30 min before and continued throughout a 120-min euglycemic (5.6 mM) hyperinsulinemic (40 mU/m² · min) clamp.

Results: Insulin sensitivity (M-value, i.e. glucose infusion rate divided by lean body mass) during the last 60 min of the clamp was higher during infusion with atropine than saline (9.2 ± 1.0 vs. 7.6 ± 1.0 μg/kg lean body mass · min, mean ± SEM; P = 0.015) in all subjects. Physostigmine did not differ significantly from saline (8.2 ± 1.0). M-values were significantly higher in lean vs. obese [atropine, 11.6 ± 1.4 vs. 7.6 ± 1.3; physostigmine, 10.8 ± 1.3 vs. 6.3 ± 1.3; and saline, 9.1 ± 1.4 vs. 6.4 ± 1.3, respectively (all P < 0.05)], but the incremental effect of atropine vs. saline did not differ consistently between groups.

Conclusion: Insulin sensitivity was higher during a short-term atropine infusion compared with saline in both lean and abdominally obese subjects. This insulin-sensitizing effect of cholinergic blockade is unexpected, and the underlying mechanisms should be further investigated. (J Clin Endocrinol Metab 96: E1843–E1847, 2011)
fferent activity on whole-body insulin sensitivity in lean, insulin-sensitive and in abdominally obese, insulin-resis-
tant subjects. The anticholinergic agent atropine and the
cholinergic agent physostigmine were infused and com-
pared with saline in an explorative, single-blinded, place-
bo-controlled, randomized crossover study.

**Subjects and Methods**

**Subjects**

Study participants were recruited through advertisements in
a local newspaper. Fourteen subjects were randomized and in-
cluded into the study, but only 12 subjects completed at least two
of the three experiments (of which one had to be with placebo)
as predefined by the protocol to be included in the data analyses.
One subject was excluded due to bradycardia during the infusion
of physostigmine and one subject due to difficulties in venous
cannulation. Thus, six age-matched nondiabetic lean [body mass
index (BMI) <23 kg/m²] and six abdominally obese (BMI >27
kg/m² and waist circumference >102 cm in men and >88 cm in
women) subjects (six males and six females) without clinically
significant disease and ongoing medication were evaluated. Clin-
ical and biochemical characteristics of study participants at base-
line are shown in Table 1. All the participants gave their informed
consent, and the study was approved by the Ethics Committee of
Gothenburg University and conducted in accordance with the
ethical principles in the Declaration of Helsinki.

**Methods**

In a randomized treatment order with a 2- to 4-wk washout
period between experiments, the subjects were given iv infusions
with atropine (Merck, Whitehouse Station, NJ;15 μg/kg bolus,
4 μg/kg \cdot h infusion) (8), physostigmine (Antilirium; Forest Phar-
maceuticals, St. Louis, MO; 0.12 μg/kg \cdot min) (9) to achieve
maximally effective plasma exposures (8, 9), or placebo [0.9%
saline (NaCl), 10 ml/h]. Intravenous infusions of atropine, phys-
ostigmine, or saline were started 30 min before and then con-
tinued throughout a 120-min euglycemic-hyperinsulinemic
clamp. After initial priming, a constant infusion of short-acting
insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was
administered at 40 mU/m² \cdot min. Blood glucose was determined
at 5-min intervals, and the glucose infusion was adjusted to main-
tain plasma glucose level at 5.6 mmol/liter. Insulin sensitivity was
assessed as glucose uptake (M-value; milligrams per kilogram
lean body mass per minute), i.e. glucose infusion rate (GIR) at
60–120 min, divided by lean body mass (kilograms) to allow for
comparisons despite different degrees of adiposity. During 60–
120 min, a steady state was assumed, but this was not clearly
achieved during atropine infusion. Body composition was de-
termined by bioelectrical impedance (BIA 101-Fitness; RJL Sys-
tems, Detroit, MI).

**Blood chemistry**

Plasma glucose during clamps was determined by the HemoCue
glucose system (HemoCue AB, Ängelholm, Sweden) and plasma
free fatty acids with an enzymatic colorimetric method (Wako
Chemicals GmbH, Neuss, Germany). Serum insu-
lin, C-peptide, and other blood chemistry were analyzed at the
Department of Clinical Chemistry, Sahlgrenska University Hos-
pital, Gothenburg.

**Statistical methods**

Baseline and biochemical characteristics were analyzed by
descriptive statistics and presented as mean ± SD or SEM as indi-
cated. M-values were analyzed by a mixed-model ANOVA with
treatment, treatment sequence, and study period as fixed factors
and subject nested within treatment sequence as a random factor.
The level of significance was 0.05. Inference was made on the
estimated treatment contrast of atropine vs. saline (placebo).
Exploratory simple linear regressions analyses of placebo-ad-
justed M-values of atropine vs. baseline characteristic variables
were performed. Twelve evaluated subjects give at least 80%
power assuming a treatment difference of 20% and a SD of 2
mg/kg lean body mass \cdot min for the primary comparison of in-
sulin sensitivity (M-value) during atropine vs. saline (placebo)
infusion. SAS version 9.1.3 (SAS Institute Inc. Cary, NC) was
used for all statistical analyses.

**Results**

In all subjects, insulin sensitivity assessed as M-value
(GIR) at 60–120 min was significantly higher during in-
fusion with atropine than saline (P = 0.015). Physostig-
mime did not differ significantly from saline (P = 0.36), as
shown in Table 2 and Fig. 1. The difference in M-value
(GIR) between atropine vs. saline was present already af-
fter 40 min of the clamp, i.e. 70 min after start of atropine
infusion, as shown in Fig. 1. M-values were overall higher in
lean vs. obese subjects and higher during infusion with
atropine than saline in both subgroups (lean and obese) as

![graphic]

**TABLE 1. Baseline characteristics of study participants (n = 12) during clamp (60–120 min)**

<table>
<thead>
<tr>
<th></th>
<th>Lean subjects (n = 6)</th>
<th>Obese subjects (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>43.8 ± 14.8</td>
<td>46.8 ± 4.8</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6 ± 1.7</td>
<td>28.8 ± 1.3b</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>85 ± 2</td>
<td>99 ± 6b</td>
</tr>
<tr>
<td>OGGT plasma glucose (mmol/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>5.1 ± 0.4</td>
<td>5.1 ± 0.5</td>
</tr>
<tr>
<td>120 min</td>
<td>5.3 ± 1.1</td>
<td>6.7 ± 1.4</td>
</tr>
<tr>
<td>OGGT serum insulin (mU/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 minute</td>
<td>4.0 ± 1.0</td>
<td>7.5 ± 2.7a</td>
</tr>
<tr>
<td>120 minute</td>
<td>34 ± 24</td>
<td>77 ± 50</td>
</tr>
<tr>
<td>Total cholesterol (mmol/liter)</td>
<td></td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/liter)</td>
<td></td>
<td>2.8 ± 1.5</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/liter)</td>
<td></td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>Triglycerides (mmol/liter)</td>
<td>1.0 ± 0.4</td>
<td>1.7 ± 0.8</td>
</tr>
</tbody>
</table>

Data are mean ± sd, fasting serum samples. HDL, High-density
lipoprotein; LDL, low-density lipoprotein; OGGT, oral glucose tolerance
test (75 g glucose).

a Differences between groups, lean vs. obese subjects: * P < 0.05;
b P < 0.001.
shown in Table 2. The incremental effect of atropine vs. saline did not differ significantly between subgroups (not shown). The (placebo-adjusted) incremental effect of atropine on glucose uptake was near-significantly and inversely associated with age (r = −0.53; P = 0.08) but not with any of the other reported baseline characteristics, e.g., BMI, waist circumference, and lipids.

Mean heart rate was higher during the clamp (60–120 min) for atropine compared with physostigmine and saline infusions (74 ± 11 vs. 63 ± 10 and 60 ± 9 beats/min, P < 0.001 for both). The incremental increase in heart rate during atropine did not correlate to the increase in M-value (not shown). No significant differences in mean systolic or diastolic blood pressure during the clamp (60–120 min) were found between the different treatments (data not shown). No differences in plasma levels of glucose, free fatty acids, C-peptide, and insulin during clamps (60–120 min) were seen between treatments and subgroups as shown in Table 2.

### Discussion

In this study we show that whole-body insulin sensitivity was higher during a short-term atropine compared with saline/placebo or physostigmine infusion in lean insulin-sensitive and in abdominally obese insulin-resistant subjects. This insulin-sensitizing effect of cholinergic blockade was unexpected.

In contrast to our findings, a previous in vitro study has demonstrated that activation of the M₃ muscarinic acetylcholine receptor in L6 skeletal muscle cells with acetylcholine stimulates glucose uptake via a CaMKK (calmodulin-dependent protein kinase kinase)-AMPK (AMP-activated protein kinase)-dependent mechanism, of the insulin-stimulated pathway (10). However, administration of atropine in an in vivo study induced a significant decrease in plasma insulin without change in plasma glucose in obese ob/ob mice (11). In the same study, the muscarinic agonist bethanicol, mimicking cholinergic activity, aggravated whole-body insulin resistance and increased gluconeogenesis (11). Overall, those latter findings are in agreement with our present results, although we did not specifically address the effect on hepatic glucose output.

The effect of insulin on whole-body glucose disposal is lower in the fasted compared with the fed state as has been shown in animal studies. This is consistent with the HIRS hypothesis implicating a postprandial cholinergic mechanism enhancing insulin action (12). In accordance, a dose-dependent decrease in glucose disposal rate during atropine administration has been shown in a cat study (6). In humans, a submaximal bolus dose of atropine (0.5 mg) postprandially also reduced insulin sensitivity (13), and in rats postprandial insulin sensitivity and decrease in glucose dis-

### TABLE 2. Metabolic measurements during clamp (60–120 min)

<table>
<thead>
<tr>
<th>Metric</th>
<th>Lean subjects (n = 6)</th>
<th>Obese subjects (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atropine</td>
<td>Physostigmine</td>
</tr>
<tr>
<td>Plasma glucose (mmol/liter)</td>
<td>5.4 ± 0.18</td>
<td>5.5 ± 0.15</td>
</tr>
<tr>
<td>Serum C-peptide (nmol/liter)</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Plasma FFA (mmol/liter)</td>
<td>0.05 ± 0.03</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Serum insulin (mU/liter)</td>
<td>82 ± 3.1</td>
<td>92 ± 5.4</td>
</tr>
<tr>
<td>M-value (mg/kg lbm · min)</td>
<td>11.6 ± 1.4a</td>
<td>10.8 ± 1.3</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. FFA, Free fatty acids; lbm, lean body mass.

- Differences between treatments, atropine vs. physostigmine vs. saline within groups, lean vs. obese: P < 0.05.
- Differences between groups, lean vs. obese subjects: P < 0.05.
Mediated by muscarinic M1 or M3 receptors that are abundant in the brainstem, amygdala, hippocampal formation, and cerebral cortex (21). Effects of vagal afferents from the gastrointestinal tract to the brain may be yet another possible explanation for the insulin-sensitizing effect of atropine. This could also involve an interaction with systemic or local effects of gut peptides (22).

Of note, there can be major differences between acute pharmacological effects as shown in this study compared with chronic, long-term effects of parasympathetic efferent activity. Thus, in previous cross-sectional studies, reduced parasympathetic reactivity has been associated with insulin resistance and visceral adiposity (1). Because those studies are cross-sectional, a casual relationship cannot be established.

A limitation of this study is the lack of a clear mechanism explaining the insulin-sensitizing mechanism of atropine. Additional studies are warranted to assess effects on both systemic and tissue blood flow. Also, the time course and duration of the effect of atropine needs further exploration. If possible, long-term interventions modulating parasympathetic activity and effects would be of interest to identify novel therapeutic approaches in insulin-resistant conditions.

In conclusion, we found that insulin sensitivity assessed by euglycemic-hyperinsulinemic clamp was higher during a short-term atropine infusion compared with infusion with saline/placebo. Findings were similar in both lean and abdominally obese subjects. This insulin-sensitizing effect of acute cholinergic blockade was unexpected, and the possible underlying mechanisms, e.g., related to systemic and local tissue blood flow, as well as the possible clinical implications should be further investigated.

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