Inhibition of lipolysis does not affect insulin sensitivity to glucose uptake in the mourning dove☆

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Abstract

Birds have much higher plasma glucose and fatty acid levels compared to mammals. In addition, they are resistant to insulin-induced decreases in blood glucose. Recent studies have demonstrated that decreasing fatty acid utilization alleviates insulin resistance in mammals, thereby decreasing plasma glucose levels. This has yet to be examined in birds. In the present study, the levels of glucose and β-hydroxybutyrate (BOHB), a major ketone body and indicator of fatty acid utilization, were measured after the administration of chicken insulin, acipimox (an anti-lipolytic agent), or insulin and acipimox in mourning doves (Zenaidura macroura). Insulin significantly decreased whole blood glucose levels (19%), but had no effect on BOHB concentrations. In contrast, acipimox decreased blood BOHB levels by 41%, but had no effect on whole blood glucose. In addition to changes in blood composition, levels of glucose uptake by various tissues were measured after the individual and combined administration of insulin and acipimox. Under basal conditions, the uptake of glucose appeared to be greatest in the kidney followed by the brain and skeletal muscle with negligible uptake by heart, liver and adipose tissues. Acipimox significantly decreased glucose uptake by brain (58% in cortex and 55% in cerebellum). No significant effect of acipimox was observed in other tissues. In summary, the acute inhibition of lipolysis had no effect on glucose uptake in the presence or absence of insulin. This suggests that free fatty acids alone may not be contributing to insulin resistance in birds.

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1. Introduction

Carbohydrate stores in avian muscle and liver power take-off and short flights (George and Berger, 1966; Rothe et al., 1987). Beyond that, the energy used by exercising birds is derived from lipid oxidation (Jenni and Jenni-Eiermann, 1998; Jenni-Eiermann et al., 2002; Rothe et al., 1987), a situation akin to human endurance athletics. Therefore, the high ketone levels observed in birds likely arise as byproducts of this metabolism (Jenni-Eiermann et al., 2002; George and Vallyathan, 1964). In fact, migratory shorebirds (i.e. western and semipalmated sandpipers) have large lipid reserves to supply the energy demands of migration (Davis et al., 2005; Driedzic et al., 1993). Moreover, in wind tunnel simulated migratory flight, thrush nightingales were shown to metabolize fat and proteins for energy (Klaassen et al., 2000). For reviews of substrate utilization by migratory birds, see Blem (1980) as well as Jenni and Jenni-Eiermann (1998). Despite this apparent preference for fatty acids as an energy source, birds maintain very high plasma glucose levels compared to mammals of similar body mass and are resistant to the glucose lowering effects of insulin (Chida et al., 2000; Dupont et al., 2004; Hazelwood and Lorenz, 1959; McMurtry et al., 1987; Sitbon et al., 1980; Sweazea and Braun, 2005).

The etiology of insulin resistance in mammals remains to be determined. However, at least two hypotheses have been put forward. One is that it arises from hyperglycemia (for review: Sheetz and King, 2002; Tomás et al., 2002) and a second is that it stems from increases in plasma fatty acids (for review: Boden,
The controversy of whether increased fatty acids or glucose (or both) contribute to the etiology of insulin resistance has yet to be resolved. In isolated rat extensor digitorum longus skeletal muscle preparations, high glucose levels in the pre-incubation medium were shown to decrease basal radiolabeled glucose uptake as well as insulin-stimulated glucose uptake (Kurowski et al., 1999). In contrast, avian skeletal muscles are resistant to insulin-mediated glucose uptake in the presence of physiological levels of glucose (Sweazea and Braun, 2005). Insulin enhances glucose uptake in mammalian tissues by activating a cascade of intracellular signaling events resulting in the translocation of glucose transport proteins from intracellular storage compartments to the plasma membrane (Suzuki and Kono, 1980). In mammals, the decrease in insulin responsiveness in the presence of high plasma glucose stems from blunted activation of intermediates in the insulin-signaling pathway (Kurowski et al., 1999). As birds naturally have high plasma glucose levels, it is possible that insulin resistance in birds is mediated by this mechanism or as a result of the lack of an insulin responsive glucose transporter (GLUT4) (Carver et al., 2001; Duclos et al., 1993; Seki et al., 2003; Sweazea and Braun, 2005, 2006).

Type 2 diabetic humans often exhibit increases in fatty acid oxidation resulting in insulin resistance and hyperglycemia due to decreased glucose disposal and increased gluconeogenesis (Fulcher et al., 1992). In fact, elevated plasma fatty acids elicit decreases in basal and insulin-stimulated rates of glucose uptake and phosphorylation as well as inhibition of glycogen synthesis and glycogenolysis (Clerk et al., 2002; Griffin et al., 1999; Thompson et al., 2000). Like elevated glucose levels, fatty acid-induced decreases in insulin-stimulated glucose uptake may be attributed to decreases in activation and expression of insulin signaling pathway intermediates as well as a decrease in glucose transporter activity (Goodyear et al., 1995; Griffin et al., 1999; Rosholt et al., 1994; Zierath et al., 1997).

A recent study using domestic chicks (Gallus gallus) examined the effects of porcine insulin on glucose uptake by various skeletal muscles, cardiac muscle, adipose, liver, kidney, brain, pancreas, and small intestine tissues (Tokushima et al., 2005). However, fatty acid oxidation as a possible inhibitor of insulin-mediated glucose uptake was not examined.

In addition to the above, birds have the highest levels of cytosolic fatty acid binding protein (FABPc) among vertebrates, which may act as a sink to pull fatty acids into cells thereby inducing insulin resistance (Collins and Hargis, 1989; Guglielmo et al., 1998, 2002). The case for fatty acid utilization by birds is strengthened by the fact that they yield more energy per mole than carbohydrates (Abumrad et al., 1999) providing an efficient energy source.

The current study was designed to examine the hypothesis that fatty acids induce insulin resistance in birds, as it is known that birds preferentially utilize free fatty acids to sustain flight (Jenni-Eiermann et al., 2002; George and Vallyathan, 1964). To test this hypothesis, basal and chicken insulin-stimulated glucose uptake by select tissues were measured in the presence and absence of an inhibitor of lipolysis, acipimox (5-methylpyrazine-2-carboxylic acid 4-oxide). Acipimox is an analog of nicotinic acid that inhibits adipose tissue hormone sensitive lipase (Kim et al., 1995; Lee et al., 1996). This inhibition decreases fatty acid oxidation rates, as well as plasma glucose, nonesterified fatty acids, ketone bodies (measured as β-hydroxybutyrate), and triglycerides. In addition, acipimox has been shown to enhance insulin responsiveness to changes in plasma glucose levels in normal rats, obese Zucker diabetic rats, streptozotocin-induced diabetic rats, as well as humans (Blachère et al., 2001; Farrer et al., 1992; Fulcher et al., 1993; Kim et al., 1995; Lee et al., 1996; Lovisolo et al., 1981). To further combat increases in blood glucose, acipimox enhances insulin suppression of hepatic glucose production (gluconeogenesis) along with a compensatory increase in glycogenolysis (Lee et al., 1996).

We suggest that inhibition of lipolysis, using acipimox, will increase the sensitivity of avian tissues to insulin, thereby ameliorating insulin resistance as indicated by increased insulin-induced glucose uptake by peripheral tissues. In addition to the studies of glucose uptake by various avian tissues, plasma ketone bodies (as β-hydroxybutyrate) and glucose were measured to determine the levels of glucose and fatty acid utilization.

2. Materials and methods

2.1. Animals

On the morning of each experiment, adult Mourning doves (Zenaida macroura; 90–110 g body mass (BW)) of both sexes were captured at the University of Arizona Dairy Research Center (Tucson, AZ, USA). Birds were fasted 2–3 h prior to the start of the experiment to allow transport to the lab. Birds were infused with substrates and tissues were extracted as indicated below. All animal protocols were approved by the University Institutional Animal Care and Use Committee.

2.2. Measurement of glucose uptake by tissues

Radiolabeled substrates were purchased from MP Biomedicals (Irvine, CA, USA). The vehicle for the infusates was comprised of 1 mM 2-deoxy-D-glucose (2DG) with the addition of 11.5 μCi/mM [3H]-2DG and 85.2 μCi/mM [U-14C]-mannitol in 0.9% saline. 2-deoxy-D-glucose is used to mimic the uptake of D-glucose as it is recognized by the member transport protein, phosphorylated to 2-deoxy-D-glucose-6-phosphate inside cells, but not metabolized further (Chaisson and Massaro, 1978). Therefore, it is trapped and accumulates inside cells and is an accurate reflection of glucose transport activity (Hansen et al., 1996). Radiolabeled mannitol was present in all infusates as it is not transported into cells and can therefore be used to quantify the extracellular space. Using the specific activity of the isotope, the glucose counts were converted to moles of substrate uptake/mg tissue from which the labeled mannitol counts were subtracted to account for free glucose in the extracellular space. Infusates were administered as a 250 μL bolus via the left brachial vein. The infusate compositions are detailed in the experimental group section and are outlined in Fig. 1. At 10 min post-infusion, a final blood sample was collected and birds were euthanized by carbon dioxide asphyxiation and
tissues were harvested to measure glucose uptake. This time point was chosen as avian insulin has a short half-life (about 5–9 min, McMurtry et al., 1987; Langslow, 1976). In addition, whole blood glucose levels were decreased in Mourning dove at this time point (Table 1).

Tissues that are known to take up glucose (in mammals) were chosen for these studies: skeletal muscle (extensor digitorum communis, pectoralis major, quadriceps, latissimus dorsi), heart, liver, kidney, visceral fat, cerebellum, and cortex. Tissues were extracted at 10 min post-infusion, snap-frozen in liquid nitrogen, weighed (to 0.1 mg), and digested at 60 °C in 500 μL of 0.5 N NaOH in liquid scintillation vials placed in a water bath for two hours. To account for background emission from NaOH, a 500 μL aliquot of 0.5 N NaOH was counted. Scintillation cocktail (EcoLite®) was added to each vial and the samples were dark-adapted for three hours prior to counting to prevent photo luminescence.

2.3. Experimental groups

2.3.1. Group 1

Controls (n=5): Glucose uptake was measured 10 min after the infusion of the vehicle only (1 mM 2-deoxy-D-glucose, 11.5 μCi/mM [3H]-2-deoxy-D-glucose, 85.2 μCi/mM [U-14C]-mannitol in 0.9% saline) (Fig. 1).

2.3.2. Group 2

The effects of chicken insulin (Litron Laboratories, Rochester, NY, USA; Purity >95%): Glucose uptake was measured 10 min after the infusion of the vehicle with the addition of NaOH, a 500 μL aliquot of 0.5 N NaOH was counted. Scintillation cocktail (EcoLite®) was added to each vial and the samples were dark-adapted for three hours prior to counting to prevent photo luminescence.

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whole blood glucose (mM/L)</th>
<th>Control (pretreatment)</th>
<th>Experimental % of Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acipimox (300 mg/kg</td>
<td>19.9±2.2</td>
<td>20.6±1.4</td>
<td>103%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>80 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (80 μg/kg</td>
<td>17.6±1.3</td>
<td>14.3±0.9</td>
<td>81%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>10 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acipimox + insulin</td>
<td>20.0±0.9</td>
<td>16.7±0.9</td>
<td>84%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>(10 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data expressed as mean±SEM of 5 animals and were analyzed using nonparametric repeated measures ANOVA. Each animal served as its own control (within subjects statistical design). Refer to Fig. 1 for an outline of the experimental procedures. Data for 2-min blood samples are not shown as they did not differ significantly from 10 min (p>0.05).

2.3.3. Group 3

The acute effects of acipimox (n=5): Animals were administered an oral dose of 300 mg/kg BW acipimox (5-methylpyrazine-2-carboxylic acid 4-oxide; BioMol International, LP (Plymouth Meeting, PA, USA)) 80 min prior to infusion. At time zero, animals were infused with the vehicle only and after 10 min, tissues were extracted and glucose uptake was measured. This drug was used to determine whether inhibition of lipolysis would increase basal and/or insulin-stimulated glucose uptake. In the present experiments, the effectiveness of acipimox was assessed by collecting blood samples and measuring β-hydroxybutyrate (BOHB) as an indication of ketone body levels (Table 2).

Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whole blood BOHB (mM/L)</th>
<th>Control (pretreatment)</th>
<th>Experimental % of Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acipimox (300 mg/kg 80 min)</td>
<td>1.12±0.10</td>
<td>0.46±0.06</td>
<td>41%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Insulin (80 μg/kg 10 min)</td>
<td>0.66±0.16</td>
<td>0.71±0.18</td>
<td>108%</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Acipimox + insulin (10 min)</td>
<td>0.56±0.07</td>
<td>0.51±0.08</td>
<td>91%</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Data for ketones (as β-hydroxybutyrate (BOHB)) expressed as mean±SEM of 5 animals. Each animal served as its own control. Refer to Fig. 1 for an outline of the experimental procedures. Paired Student’s t-Test was used as only two time points were compared for each bird.

2.3.4. Group 4

The effects of insulin after acute acipimox treatment (n=5): Animals were administered an oral dose of acipimox 80 min

Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Average (nM/mg tissue±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensor digitorum communis</td>
<td>−0.102±0.03a</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>0.024±0.04</td>
</tr>
<tr>
<td>Pectoralis</td>
<td>0.292±0.12</td>
</tr>
<tr>
<td>Latissimus dorsi</td>
<td>0.362±0.13</td>
</tr>
<tr>
<td>Heart</td>
<td>−0.520±0.23a</td>
</tr>
<tr>
<td>Liver</td>
<td>−1.30±0.11b</td>
</tr>
<tr>
<td>Kidney</td>
<td>15.4±3.2</td>
</tr>
<tr>
<td>Cortex</td>
<td>2.35±0.37b</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>2.51±0.23b</td>
</tr>
<tr>
<td>Adipose</td>
<td>−0.367±0.09a</td>
</tr>
</tbody>
</table>

Data analyzed using nonparametric ANOVA.

a Differs significantly from kidney (p≤0.05).
b Differs significantly from liver (p≤0.05). All other comparisons were not statistically significant. As radiolabeled mannitol was used to account for the extracellular space, negative values reflect a greater amount of glucose presence in the extracellular space than in the cells and data are presented as the amount of radiolabeled glucose in the cells.
prior to infusion with the vehicle containing insulin (80 μg/kg BW). Ten min later, tissues were extracted and glucose uptake was measured to determine whether acipimox enhances insulin sensitivity to glucose uptake in birds.

2.4. Determination of plasma glucose and ketone bodies (as β-hydroxybutyrate (BOHB))

Blood was sampled from the brachial vein of unanesthetized birds as sodium pentobarbital anesthesia was found to greatly reduce blood glucose levels. Similar data have been reported in studies of domestic chickens (Edens and Siegel, 1975). Blood samples were collected into heparinized capillary tubes and kept on ice for analysis. A within-subjects statistical design was used as samples were taken from the same birds over time (groups 2–4), allowing each bird to serve as its control. Blood was not sampled for Group 1 as no manipulations were performed on this group. For the other groups, blood samples were taken at the beginning of each experiment (−80 min for acipimox studies and 0 min for insulin only) and again at 2 and 10 min post-treatment. A five-minute collection was not performed as preliminary studies on unanesthetized birds showed no difference from the 10-min collections. Glucose levels were measured at all time points whereas ketone bodies (as BOHB) were measured at the beginning and end of each experimental protocol to verify the
effectiveness of acipimox. β-hydroxybutyrate is a major ketone body that was measured in the present study as it is also the predominant metabolic product in ketoacidosis (Trachtenbarg, 2005) and has been shown to be significantly reduced in response to acipimox treatment suggesting an inhibition of lipolysis (Farrer et al., 1992).

Samples were analyzed using PTS PANELS glucose and ketone test strips (specific for BOHB) and read with a CardioChek P•A meter (Polymer Technology Systems, Indianapolis, IN, USA). In studies by Guerci et al. (2003) the results obtained using this method strongly correlated with those of spectrophotometric methods.

2.5. Determination of plasma insulin

Plasma insulin was determined on a separate group (untreated) of Mourning dove as previously described (McMurtry et al., 1983). All samples were analyzed in a single assay to eliminate interassay variation. An intra-assay coefficient of variation for the same samples was calculated to be 2.4%. The effectiveness of chicken insulin at significantly lowering whole blood glucose levels in dove suggests that avian insulin is well-conserved.

2.6. Data analysis

Blood glucose data were analyzed using nonparametric repeated measures ANOVA as samples were taken at different time points from the same birds, therefore each bird served as its control (a within-subjects statistical design). When significance was indicated, a Friedman post hoc analysis was used. Blood ketone levels (as BOHB) were assessed using paired Student’s t-Test as only two time points were compared, per bird, for each protocol. Differences in glucose uptake by control tissues as well as uptake by tissues in the various protocols were analyzed using one way ANOVA and, when significant, Fisher LSD Method post hoc analysis was used. Data are expressed as mean±SEM. Significance was determined at p ≤ 0.05.

3. Results

Tables 1 and 2 give the glucose and BOHB levels, respectively, for the experimental protocols. The control levels of insulin in dove were 1.03±0.1 ng/mL. Chicken insulin infusion decreased plasma glucose levels by 19%, but had no effect on plasma BOHB (Tables 1 and 2). Acipimox was effective at decreasing plasma BOHB, as expected, but had no effect on plasma glucose levels in the presence of insulin suggesting it...
may inhibit insulin-induced glucose disposal (Tables 1 and 2). When administered alone, acipimox had no effect on blood glucose levels (Table 1).

The values for glucose uptake by all control tissues are shown in Table 3 and Fig. 2. Extensor digitorum communis, heart, liver, and adipose tissues display significantly less glucose uptake than the kidney \((p<0.05)\). Brain tissues display significantly higher basal glucose uptake than liver tissues \((p<0.05)\). It appears that the greatest uptake of glucose occurs in kidney and brain tissues. In addition, insulin had no effect on glucose uptake by any tissue examined even in the presence of acipimox, an inhibitor of fatty acid lipolysis (Figs. 2–5). As acipimox was effective at decreasing plasma BOHB, it is believed that lipolysis was effectively decreased in birds using this compound. Unlike mammals, however, decreased fatty acid lipolysis had no effect on insulin responsiveness in birds as measured by sensitivity to insulin-mediated glucose uptake. In fact, no significant differences in glucose uptake were observed by tissues after any treatment, other than brain (Figs. 3–5). Of note, acipimox alone, and co-administered with insulin, significantly decreased glucose uptake by brain tissues (cerebellum and cortex; Fig. 5).

4. Discussion

Birds are hyperglycemic by mammalian standards (Ummin-ger, 1975), an observation supported by the findings of high plasma glucose levels in Mourning doves in the present study. The plasma glucose concentrations of the Mourning dove are comparable to glucose levels in goldfinches (18.3 mM/L; Marsh and Dawson, 1982), Vesper sparrows (20.3 mM/L; Swain, 1987), pigeons (15.3 mM/L; Gayathri et al., 2004) and chickens (13.8–15.7 mM/L; Langslow et al., 1970; Riesenfeld et al., 1982). The levels of BOHB (Table 2) were also found to be much greater than in humans 35.5 (fed)–90.2 (fasting (μM/L)); Diem, 1970).

There is a striking difference in the basal levels of glucose uptake by the various avian tissues examined in this study (Table 3, Fig. 2). Extensor digitorum communis, heart, liver, and adipose tissues display significantly less glucose uptake than the kidney \((p<0.05)\). Significantly higher basal glucose uptake occurs in brain tissues compared to liver \((p<0.05)\). Overall, the uptake of glucose was greatest in the kidney followed by the brain. There were no significant differences in glucose uptake among the skeletal muscles (Figs. 2 and 3). These contrasts in levels of tissue glucose uptake may reflect the varying reliance on glucose as a metabolic substrate by the tissues examined.

In mammals, insulin is responsible for lowering blood glucose when levels are increased, such as after a meal. It acts by inducing the translocation of glucose transport proteins from intracellular compartments to the plasma membrane (Suzuki and Kono, 1980). Defects in this pathway lead to insulin resistance. The levels of insulin in Mourning dove (1.03 ±0.1 ng/mL) were similar to what has been previously reported in chickens (1.09–1.98 ng/mL, Colca and Hazelwood, 1976; Simon, 1980). Birds are also hyperglycemic, as they have approximately two-to-three fold higher plasma glucose levels compared to mammals. Despite these high plasma glucose concentrations, basal plasma insulin levels in birds are comparable to those observed in mammals (Simon, 1989).

Birds have a higher tolerance for insulin than mammals as estimated by the dose required to produce convulsions across various avian and mammalian species. For example, a dose of 29.4 mg/kg mammalian insulin was required to produce convulsions in pigeons, whereas only 970 μg/kg produced the same effect in dogs (Chen et al., 1945). There are reports of the transient glucose lowering effects of insulin in birds as well as resistance to insulin-mediated glucose uptake by avian tissues (Chida et al., 2000; Dupont et al., 2004; Hazelwood and Lorenz, 1959; McMurtry et al., 1987; Sweazea and Braun, 2005).

Using methodology similar to that employed in the present study, porcine insulin administered at a dose of 40 μg/kg BW increased glucose uptake by tissues in developing domestic chicks (Tokushima et al., 2005). However, in adult Mourning dove, a similar dose of chicken insulin produced no effect on plasma glucose levels. Rather, a dose of 80 μg/kg BW chicken insulin was required to induce a glucose-lowering response in Mourning dove (19%; Table 1). This decrease in plasma glucose without a corresponding increase in tissue glucose uptake (Figs 2–5) may be a result of disposal by the kidney. However, urine samples were not measured for glucose content in the present study. Insulin had no effect on plasma BOHB levels (Table 2).

In other studies, much higher doses of insulin were required to produce a glucose lowering effect which brings into question the physiological relevance of such high doses. For example, 40–170 μg/kg human insulin was necessary to decrease blood glucose in molting starlings (Remage-Healey and Romero, 2002) whereas 150–330 μg/kg BW of bovine insulin was required in chickens (Chida et al., 2000; Vives et al., 1981). These studies, in conjunction with the present study, using a lower insulin dose, highlight the apparent insulin resistance of birds as well as a possible reduced affinity of avian insulin receptors for mammalian insulin in adult birds.

The lack of insulin effectiveness at increasing tissue uptake of glucose in our studies, in contrast to those of others (Tokushima et al., 2005), may also stem from the use of an adult bird of flight as opposed to domestic chicks, which would necessarily have different energy requirements. It is important to note that our data are reported as nanomoles per mg tissue, and therefore are not directly comparable to the data from Tokushima et al. (2005) as they report uptake in dpm/mg tissue.

It is known that birds preferentially utilize free fatty acids for energy to sustain flight (Jenni and Jenni-Eiermann, 1998; Jenni-Eiermann et al., 2002), which may induce insulin resistance via inhibition of various components of the insulin signaling pathway as well as down-regulation of the insulin receptor. As BOHB levels (by-products of fatty acid oxidation) were also notably higher in Mourning dove in comparison to mammals, this led us to examine the hypothesis that fatty acids are possible inhibitors of insulin-mediated glucose uptake in birds, which may act to sustain the high glucose levels. If free fatty acids induce insulin resistance in birds then the inhibition of lipolysis using acipimox, an inhibitor of adipose tissue hormone sensitive lipase, should result in increased sensitivity to insulin-mediated glucose uptake by tissues.
The effectiveness of acipimox at inhibiting lipolysis was demonstrated by a decrease in blood BOHB levels (Table 3). In the presence of insulin, this effect of acipimox was abolished suggesting a counter-regulatory nature of the drug in birds. Acipimox had no effect on blood glucose levels in the absence of insulin, a finding that has been demonstrated in studies on mammals (Lovisolo et al., 1981). It reversed the effects of insulin on plasma glucose suggesting that it may inhibit insulin-mediated glucose lowering in birds (Table 1) as opposed to mammals where it enhances insulin-mediated glucose disposal. Responsiveness to acipimox was observed in tissues that demonstrated high levels of basal glucose uptake. Specifically, acipimox significantly decreased glucose uptake by brain tissues (Figs. 2 and 5). At first glance, this decrease in glucose uptake by brain tissues in the presence of acipimox-induced decreases in blood glucose and BOHB seems paradoxical as these substrates are the main source of energy for the brain. However, birds are the only known vertebrate to have a glycogen body (corpus gelatinosum) made up of ~75% glycogen and located in the lumbosacral region of the spinal cord. The glycogen body has been suggested to play a role in glucose homeostasis by the central nervous system as glycogen has been detected in the cerebrospinal fluid as well as in the blood vessels stemming from this glycogen-containing body (Möller and Kummer, 2003). Therefore, it is possible that with the decrease of whole blood BOHB, as well as glucose levels, stored glycogen may be released from the glycogen body negating the necessity to take up new glucose.

The sensitivity of mammalian skeletal muscle to insulin is dependent on the abundance of insulin responsive glucose transport proteins, GLUT4 (Kern et al., 1990), which are also expressed in cardiac muscle and adipose tissues. The reported lack of GLUT4 expression in avian muscles (cardiac and skeletal) and adipose tissues may be a contributing factor to insulin resistance in these tissues (Carver et al., 2001; Duclos et al., 1993; Seki et al., 2003; Sweazea and Braun, 2005, 2006). In support of this, glucose handling in birds is comparable to that of GLUT4-null mice which have decreased whole body insulin sensitivity. Unlike birds, however, these mice are growth-retarded and exhibit cardiac hypertrophy as well reduced adipose tissue deposits which is associated with decreased longevity (Katz et al., 1995).

The results of the present studies suggest that despite the preference for free fatty acids as an energy source for prolonged flight, the inhibition of lipolysis using acipimox had no effect on plasma glucose levels or basal- and insulin-stimulated glucose uptake by avian tissues. This suggests that fatty acids may not be maintaining insulin resistance in birds, a finding that is in contrast to what occurs in mammals administered acipimox. Therefore, the high plasma glucose concentrations in avian plasma, which may be attributed to the absence of an insulin-responsive glucose transporter, may in fact be a cause of the observed insulin resistance in birds.

Acknowledgments

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