Quantity of glucose transporter and appetite-associated factor mRNA in various tissues after insulin injection in chickens selected for low or high body weight

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Zhang W, Sumners LH, Siegel PB, Cline MA, Gilbert ER. Quantity of glucose transporter and appetite-associated factor mRNA in various tissues after insulin injection in chickens selected for low or high body weight. Physiol Genomics 45: 1084–1094, 2013. First published September 17, 2013; doi:10.1152/physiolgenomics.00102.2013.—Chickens from lines selected for low (LWS) or high (HWS) body weight differ by 10-fold in body weight at 56 days old with differences in food intake, glucose regulation, and body composition. To evaluate if there are differences in appetite-regulatory factor and glucose transporter (GLUT) mRNA that are accentuated by hypoglycemia, blood glucose was measured, and hypothalamus, liver, pectoralis major, and abdominal fat collected at 90 days of age from female HWS and LWS chickens, and reciprocal crosses, HL and LH, at 60 min after intraperitoneal injection of insulin. Neuropeptide Y (NPY) and receptor (NPYR) subtypes 1 and 5 mRNA were greater in LWS compared with HWS hypothalamus (P < 0.05), but greater in HWS than LWS in fat (P < 0.05). Expression of NPYR2 was greater in LWS than HWS in pectoralis major (P < 0.05). There was greater expression in HWS than LWS for GLUT1 in hypothalamus and liver (P < 0.05), GLUT2 in fat and liver (P < 0.05), and GLUT9 in liver (P < 0.05). Insulin was associated with reduced blood glucose in all populations (P < 0.05) and reduced mRNA of insulin receptor (IR) and GLUT2 and 3 in liver (P < 0.05). There was heterosis for mRNA, most notably NPYR1 (~78%) and NPYR5 (~81%) in fat and GLUT2 (~70%) in liver. Results suggest that NPY and GLUTs are associated with differences in energy homeostasis in LWS and HWS. Reduced GLUT and IR mRNA after insulin injection suggest a compensatory mechanism to prevent further hypoglycemia.

Chickens selected for low (LWS) or high (HWS) juvenile body weight for more than 55 years now display at selection age (56 days) a 10-fold difference in body weight with correlated responses in food intake regulation, body composition, glucose tolerance, and central insulin sensitivity (17, 18, 33, 41). Some of the LWS chickens are anorexic, and all are lean with very little adipose tissue accumulation by selection age. The HWS chickens are hyperphagic, with selection for high body weight having favored the accumulation of abdominal fat, with a >10-fold difference (as a percentage of body weight) between the lines evident at selection age (6, 18, 26). The lines display differences in food intake and hypothalamic chemistries in response to central administration of food intake-associated neurotransmitters (11–15, 30, 34, 36, 51). Our group demonstrated that HWS chickens exhibit impaired glucose tolerance and hyperglycemia (50), and LWS chicks responded to centrally administered insulin with reduced food intake at a much lower threshold compared with HWS chicks (51). We also showed differential threshold sensitivity in the effects of insulin on blood glucose concentrations. Results from our group also showed that LWS chicks display greater rates of lipolysis and lipogenesis in abdominal fat compared with HWS, with rates of lipolysis exceeding rates of lipogenesis, providing an explanation for why the LWS are extremely lean and accumulate little adipose tissue with age (7). While phenotypic differences between the lines have been documented for >50 generations, the mechanisms underlying differences in appetite, insulin sensitivity, and glucose homeostasis are poorly understood (18, 41).

Birds generally display fasting blood glucose concentrations that are the highest among all vertebrates (37), and chickens are resistant to the hypoglycemic effects of insulin at physiological doses (19). Insulin signaling is functionally conserved in chickens, and insulin immuno-neutralization caused relative hyperglycemia in fed chicks, and while it did not alter activity of early steps in the insulin signaling cascade, phosphorylation of proteins involved in later steps were all decreased after 1 h (20). Other differences in glucose regulation between chickens and mammals include relatively low activity of glucokinase in the liver (39), a key glucose sensor in different tissues, and absence of a glucose transporter 4 (GLUT4) ortholog in the chicken genome (46). In mammals, GLUT4 is the primary insulin-dependent GLUT in skeletal muscle and adipose tissue (3). Relatively low quantities of GLUT isofrom mRNA were detected in chicken skeletal muscle, liver, and adipose tissue (27), and it is unclear if any of those transporters are insulin dependent, although insulin injection was associated with an increase in 2-deoxy-D-[3H]-glucose uptake in skeletal muscle and liver tissues at 10 min after insulin injection in young broiler chicks, demonstrating the presence of insulin-dependent glucose transport in those tissues (56).

In addition to its role in glucose regulation, insulin is also an important regulator of appetite. Insulin receptors (IRs) are located on orexigenic neuropeptide Y (NPY)/agouti-related peptide (AGRP) neurons and anorexigenic pro-opiomelanocortin (POMC) neurons in the arcuate nucleus (ARC) of the hypothalamus. The ARC integrates hormonal and nutrient signals from peripheral tissues to regulate food intake and body weight (24), with insulin stimulating and inhibiting NPY/AGRP and POMC neurons, respectively (2). These ARC neurons containing insulin receptors are considered to be the first order in peripheral insulin’s anorexigenic effects. Insulin receptors are detected in the hypothalamus of 5-day-old layer chicks (48), and central injection of insulin was associated with changes in expression of hypothalamic NPY and POMC (49).
Effects on gene expression after central administration of insulin in mammals similarly involve upregulation of POMC mRNA (4) and downregulation of NPY (42).

Our laboratories recently showed that NPY elicits different effects on food intake in LWS and HWS (34). NPY is one of the most potent orexigenic (food-intake stimulating) factors identified to date in birds and mammals (55). Recent studies in rodents show that NPY also plays a role in adipogenesis, lipogenesis, and brown fat activation (9, 28, 40, 58). Thus, NPY may play a role in food intake regulation and body composition in chickens genetically selected for low and high body weight and information on expression of NPY and its receptor subtypes in different tissues may provide clues about functions of the NPY system in avian species.

Identifying the underlying mechanisms of differences in food intake, glucose regulation, and body composition between LWS and HWS chickens may provide insight on their role in appetite and metabolic disorders across species. Hence, the objective of the present study was to determine the effect of exogenous insulin on mRNA abundance of canonical mediators of appetite and GLUTs in the hypothalamus, abdominal fat, liver, and skeletal muscle of chickens from lines LWS and HWS and their reciprocal crosses.

**MATERIALS AND METHODS**

**Animals**

All animal protocols were approved by the Institutional Animal Care and Use Committee at Virginia Tech. The chickens used in this experiment were progeny of S$_{90}$ generation matings within parental lines selected for high (HWS) or low (LWS) body weight at 56 days of age and reciprocal crosses between them. For the reciprocal line crosses, sire parent line is denoted first and the dam parental line second (HWS male $\times$ LWS female = HL, LWS male $\times$ HWS female = LH). Progeny were produced in a single hatch from breeders between 53 and 54 wk of age.

Chicks were reared in starter and developer battery pens with free access to feed and water under a continuous photoperiod and thermo-neutral conditions. The antibiotic-free mash diets fed were those used for investigation of heterosis for those traits. For genes (POMC, AGRP, IAPP, TH, and PC2) that were evaluated only in the hypothalamus, the statistical model included the main effects of treatment (insulin vs. vehicle), and the interaction between them. The statistical model included the main effects of genetic population (HWS, LWS, HL, and LH), treatment (insulin vs. vehicle), and the interaction between them.

**Model for blood glucose concentrations:** $y = \mu + \alpha + \beta + (\alpha\beta) + \xi_{|k}$, where $\mu$ is grand mean response, $\alpha$ is effect of treatment (insulin vs. vehicle), $\beta$ is effect of population (HWS, LWS, HL, and LH), $(\alpha\beta)$ is interaction effect between treatment and population, and $\xi_{|k}$ is random errors—normal ($0, \sigma$).

**Data Analyses**

Chicken was defined as the experimental unit. Blood glucose data were analyzed by ANOVA using the Glimmix procedure of SAS 9.3 (SAS Institute, Cary, NC). The statistical model included the main effects of genetic population (HWS, LWS, HL, and LH), treatment (insulin vs. vehicle), and the interaction between them. The statistical model included the main effects of treatment (insulin vs. vehicle), and the interaction between them. For expression of all other genes, which were measured in all tissues, the model included the main effects of insulin treatment, line and tissue, and the interactions between them. Tissue was included in the model as a repeated measure, with Type=cs and dfm=bw selected based on variance and correlation among different tissues. Post hoc pairwise comparisons were carried out with Tukey’s test. All data are presented as least-squares means ± SE. Differences were determined by 10.220.32.246 on June 22, 2017 http://physiolgenomics.physiology.org/ Downloaded from
considered significant at $P < 0.05$. The statistical models are as follows. 

**Model for hypothalamic gene expression.** $y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$, where $\mu$ is grand mean response, $\alpha_i$ is effect of treatment, $\beta_j$ is effect of line, $(\alpha\beta)_{ij}$ is interaction effect between treatment and line, and $\epsilon_{ijk}$ is interaction effect between tissue and treatment, $(\alpha\beta)_{ij}$ is interaction effect between treatment and population, and $(\alpha\beta)_{ij}$ is interaction effect between tissue and population, $\epsilon_{ijk}$ is interaction effect between tissue and treatment, population, and $(\alpha\beta)_{ij}$ is random errors—normal $(0, \delta\epsilon)$. 

**Heterosis**

For genes that showed significant differences in expression between HWS and LWS lines in various tissues, expression was measured in reciprocal crosses and heterosis was calculated from mRNA abundance data as follows: \% heterosis $= [(\text{cross-line average} - \text{pure-line average})/\text{pure-line average}] \times 100$. 

Significance of heterosis was evaluated with nonorthogonal contrasts between the F1 and the average of the parental lines. Comparison of expression between the populations was as follows. 

**Model for gene expression across all populations.** $y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$, where $\mu$ is grand mean response, $\alpha_i$ is effect of treatment, $\beta_j$ is effect of population (HWS, HL, LH, and LWS), $(\alpha\beta)_{ij}$ is random interaction effect between treatment and population, and $\epsilon_{ijk}$ is random errors—normal $(0, \delta\epsilon)$. 

**RESULTS**

**Body Weights and Blood Glucose Concentrations**

The mean body weights of HWS, HL, LH, and LWS were 2,000, 1,200, 1,190, and 200 g, respectively. There was an interaction of population and insulin treatment on blood glucose concentration ($P = 0.01$). In vehicle-treated chickens, there were no significant differences in blood glucose among the populations, whereas concentrations were significantly lower in all insulin-injected than their vehicle controls, with LWS insulin-injected chickens having the lowest blood glucose of all groups (Table 2).

**Appetite-Associated Factor and GLUT mRNA Abundance in the LWS and HWS Lines**

Abundance of mRNA was measured first in different tissues of HWS and LWS, the rationale being that for genes that were different between the lines, heterosis would later be explored using the samples obtained from the reciprocal crosses. Hence, data were first summarized for effects in the parental lines, with main effects and $P$ values for the two-way interactions. Three-way interactions were not significant for any gene and hence removed from the model. Significant two-way interactions are displayed graphically. Thereafter, comparisons among LWS and HWS and their reciprocal crosses are discussed. Among the genes evaluated in only the hypothalamus, there was no effect of insulin treatment or line on gene expression (Table 3).

**Abundance of NPY and NPVR Subtype mRNA in all Tissues**

There were no effects of insulin injection on mRNA abundance of NPY or its receptor subtypes (Table 4). Expression of NPY was lower in all insulin-injected than their vehicle controls, with LWS insulin-injected chickens having the lowest blood glucose of all groups (Table 2).
mRNA was not detected in the pectoralis major. There was also a tissue-specific expression compared with fat and liver, respectively. Neuropeptide P (NPY) expression in other tissues was similar between the lines. (Tukey’s pairwise comparisons). ND, mRNA not detected. Interactions refers to 2-way interactions on mRNA abundance. T, treatment (insulin or vehicle).

Table 3. Expression of appetite-associated factor mRNA in the hypothalamus at 90 days in parental line LWS and HWS chickens

<table>
<thead>
<tr>
<th>Effect</th>
<th>Gene</th>
<th>POMC</th>
<th>AGRP</th>
<th>IAPP</th>
<th>TH</th>
<th>PC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Insulin</td>
<td>2.26</td>
<td>1.83</td>
<td>1.39</td>
<td>1.24</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>1.15</td>
<td>1.50</td>
<td>1.06</td>
<td>0.83</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.63</td>
<td>0.85</td>
<td>0.18</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.24</td>
<td>0.79</td>
<td>0.21</td>
<td>0.20</td>
<td>0.37</td>
</tr>
<tr>
<td>Line</td>
<td>HWS</td>
<td>1.73</td>
<td>0.50</td>
<td>1.19</td>
<td>0.86</td>
<td>1.27</td>
</tr>
<tr>
<td></td>
<td>LWS</td>
<td>1.68</td>
<td>2.82</td>
<td>1.25</td>
<td>1.21</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.63</td>
<td>0.84</td>
<td>0.18</td>
<td>0.22</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.96</td>
<td>0.07</td>
<td>0.81</td>
<td>0.28</td>
<td>0.20</td>
</tr>
</tbody>
</table>

P values

T X line | 0.42 | 0.86 | 0.57 | 0.24 | 0.71 |

Values represent least-squares means ± SE (n = 5) and associated P values for main effects, and P values for the 2-way interaction. T X line, 2-way interaction of treatment by genetic line. High-weight and low-weight chickens are denoted as HWS and LWS, respectively.

greater (P < 0.0001) in the hypothalamus compared with the other tissues (50-fold greater than in fat or muscle, 500-fold greater than in liver), and abundance of mRNA was greater (P < 0.05) in abdominal fat and muscle compared with liver. There was an interaction of tissue and line on NPY mRNA (P < 0.0001) where in the hypothalamus, expression was greater in LWS than in HWS (P < 0.05), while in abdominal fat abundance was greater in HWS than in LWS chickens (P < 0.05, Fig. 1A). Expression in other tissues was similar between the lines.

Similarly, NPYR1 mRNA was greater (P < 0.0001) in the hypothalamus than in the other tissues (Table 4), with ~45-fold greater (P < 0.0001) and 450-fold greater (P < 0.0001) expression compared with fat and liver, respectively. Neuropeptide YR1 mRNA was not detected in the pectoralis major. There was also an interaction of tissue and line on NPYR1 expression, identical to that observed for NPY, where in hypothalamus, expression was greater (P < 0.01) in LWS than in HWS, whereas in abdominal fat, abundance was greater (P < 0.01) in HWS than in LWS (Fig. 1B).

Abundance of NPYR2 mRNA was also greater (P < 0.0001) in hypothalamus than in the other tissues (Table 4), with fivefold greater expression compared with fat (P < 0.01) and skeletal muscle (P < 0.01) and 50-fold greater expression than in liver (P < 0.0001). There was also a line by tissue interaction (P = 0.003), where in pectoralis major, mRNA abundance was greater in LWS than in HWS (P < 0.05, Fig. 1C).

Quantities of NPYR5 mRNA were greater in LWS than in HWS chickens (P = 0.009, Table 4). Expression was greater in hypothalamus than in other tissues (P < 0.0001), being ~30- and 150-fold greater than in fat (P < 0.001) and liver (P < 0.001), respectively, with no detectable expression in pectoralis major. There was also a line by tissue interaction (P = 0.001), similar to NPY and NPYR1, where expression in hypothalamus was greater in LWS compared with HWS (P < 0.01) and expression in fat was greater in HWS than in LWS (P < 0.01, Fig. 1D). Similar to NPY and the other receptor subtypes, quantities of NPYR6 mRNA were greater in hypothalamus than in all other tissues (P < 0.0001), with expression more than fivefold greater in fat (P < 0.01), 15-fold greater than in liver (P < 0.01), and more than twofold greater abundance compared with pectoralis major (P < 0.05).

mRNA Abundance of FOXO1, GLUTs, and IR

Expression of FOXO1 (Table 4), GLUTs 1, 2, 3, 8, and 9, and IR (Table 5) were summarized for different tissues of vehicle- and insulin-injected HWS and LWS chickens. Abundance of FOXO1 mRNA was greater (P = 0.02) in HWS compared with LWS chickens. There was also a tissue-specific distribution (P < 0.0001), where expression was greater (at

Table 4. Expression of NPY, NPY receptor subtype, and FOXO1 mRNA in different tissues of HWS and LWS chickens

<table>
<thead>
<tr>
<th>Effect</th>
<th>Gene</th>
<th>NPY</th>
<th>NPYR1</th>
<th>NPYR2</th>
<th>NPYR5</th>
<th>NPYR6</th>
<th>FOXO1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Insulin</td>
<td>0.27</td>
<td>0.28</td>
<td>0.33</td>
<td>0.29</td>
<td>0.49</td>
<td>5.68</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>0.24</td>
<td>0.32</td>
<td>0.33</td>
<td>0.28</td>
<td>0.43</td>
<td>4.43</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.40</td>
<td>0.25</td>
<td>1.00</td>
<td>0.78</td>
<td>0.35</td>
<td>0.25</td>
</tr>
<tr>
<td>Line</td>
<td>HWS</td>
<td>0.22</td>
<td>0.27</td>
<td>0.35</td>
<td>0.22</td>
<td>0.47</td>
<td>6.39</td>
</tr>
<tr>
<td></td>
<td>LWS</td>
<td>0.29</td>
<td>0.34</td>
<td>0.32</td>
<td>0.35</td>
<td>0.45</td>
<td>3.72</td>
</tr>
<tr>
<td></td>
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<td>0.02</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.06</td>
<td>0.05</td>
<td>0.21</td>
<td>0.009</td>
<td>0.81</td>
<td>0.02</td>
</tr>
<tr>
<td>Tissue</td>
<td>Hypothalamus</td>
<td>0.98 ± 0.06</td>
<td>0.89 ± 0.05</td>
<td>0.9 ± 0.04</td>
<td>0.82 ± 0.06</td>
<td>1.09 ± 0.09</td>
<td>1.0 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>0.02 ± 0.001</td>
<td>0.02 ± 0.002</td>
<td>0.2 ± 0.04</td>
<td>0.03 ± 0.003</td>
<td>0.2 ± 0.05</td>
<td>1.19 ± 0.07</td>
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<td>Liver</td>
<td>0.002 ± 0.001</td>
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<td>0.02 ± 0.01</td>
<td>0.005 ± 0.003</td>
<td>0.07 ± 0.01</td>
<td>4.16 ± 0.2</td>
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<tr>
<td></td>
<td>Muscle</td>
<td>0.02 ± 0.001</td>
<td>ND</td>
<td>0.15 ± 0.01</td>
<td>ND</td>
<td>0.47 ± 0.07</td>
<td>13.9 ± 2.1</td>
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<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td></td>
</tr>
</tbody>
</table>

P values

T X tissue | 0.82 | 0.52 | 0.97 | 0.91 | 0.25 | 0.69 |
| Line X tissue | <0.0001 | 0.01 | 0.003 | 0.001 | 0.17 | 0.002 |
| T X line | 0.48 | 0.33 | 0.38 | 0.89 | 0.85 | 0.64 |

Data are expressed as least-squares means ± SE (n = 5). Different letters within a column for the main effect of tissue indicate a difference at P < 0.05 (Tukey’s pairwise comparisons). ND, mRNA not detected. Interactions refers to 2-way interactions on mRNA abundance. T, treatment (insulin or vehicle).
least 3-fold) in skeletal muscle compared with the other tissues ($P < 0.0001$), and greater in liver than in fat ($P < 0.05$) or hypothalamus ($P < 0.05$). There was an interaction of line and tissue on mRNA abundance ($P = 0.002$), where in pectoralis major and liver, expression was greater in HWS compared with LWS ($P < 0.05$, Fig. 2A).

There were interactions of line and tissue on mRNA abundance of GLUT1 (Fig. 2B), 2 (Fig. 2C), and 9 (Fig. 2D), and interactions of treatment and tissue on gene expression of GLUT2 (Fig. 3A) and 3 (Fig. 3B, Table 5). Expression of GLUT 1, 2, and 9 was greater ($P < 0.05$) in HWS compared with LWS chickens. Abundance of GLUT1 mRNA was greater in hypothalamus than in the other tissues ($P < 0.001$), and abundance was greater in skeletal muscle than in fat and liver ($P < 0.01$, Table 5). Expression of GLUT1 was greater in HWS than LWS in hypothalamus and liver ($P < 0.05$) and was similar for both lines in other tissues (Fig. 2B). For GLUT2, expression was greater (>200-fold) in liver than in the other tissues ($P < 0.0001$), and expression was greater in muscle and fat than in the hypothalamus ($P < 0.05$). In the liver and fat,

Table 5. Abundance of GLUT and IR mRNA in different tissues of HWS and LWS chickens

<table>
<thead>
<tr>
<th>Gene</th>
<th>GLUT1</th>
<th>GLUT2</th>
<th>GLUT3</th>
<th>GLUT8</th>
<th>GLUT9</th>
<th>IR</th>
</tr>
</thead>
<tbody>
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<td>Treatment</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>0.40</td>
<td>221.33</td>
<td>0.42</td>
<td>1.00</td>
<td>3.46</td>
<td>0.75</td>
</tr>
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<td>Vehicle</td>
<td>0.40</td>
<td>344.40</td>
<td>0.41</td>
<td>1.09</td>
<td>3.40</td>
<td>0.89</td>
</tr>
<tr>
<td>SE</td>
<td>0.02</td>
<td>25.24</td>
<td>0.02</td>
<td>0.04</td>
<td>0.33</td>
<td>0.07</td>
</tr>
<tr>
<td>$P$ value</td>
<td>0.98</td>
<td>0.003</td>
<td>0.69</td>
<td>0.14</td>
<td>0.91</td>
<td>0.20</td>
</tr>
<tr>
<td>Line</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HWS</td>
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<td>0.42</td>
<td>1.09</td>
<td>4.41</td>
<td>0.91</td>
</tr>
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<td>LWS</td>
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<td>235.63</td>
<td>0.40</td>
<td>1.00</td>
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<td>0.73</td>
</tr>
<tr>
<td>SE</td>
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<td>25.24</td>
<td>0.02</td>
<td>0.04</td>
<td>0.33</td>
<td>0.07</td>
</tr>
<tr>
<td>$P$ value</td>
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<td>0.02</td>
<td>0.59</td>
<td>0.18</td>
<td>0.0007</td>
<td>0.10</td>
</tr>
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<td>Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>0.96 ± 0.04a</td>
<td>1.20 ± 0.17b</td>
<td>1.06 ± 0.05a</td>
<td>1.0 ± 0.02b</td>
<td>0.9 ± 0.09b</td>
<td>0.2 ± 0.03c</td>
</tr>
<tr>
<td>Fat</td>
<td>0.13 ± 0.008c</td>
<td>4.46 ± 1.60bc</td>
<td>0.1 ± 0.005b</td>
<td>0.33 ± 0.02c</td>
<td>0.44 ± 0.22c</td>
<td>0.54 ± 0.03c</td>
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<tr>
<td>Liver</td>
<td>0.11 ± 0.014c</td>
<td>1.12 ± 0.71a</td>
<td>0.1 ± 0.005b</td>
<td>1.3 ± 0.08a</td>
<td>12.2 ± 0.97b</td>
<td>0.93 ± 0.03a</td>
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<tr>
<td>Muscle</td>
<td>0.39 ± 0.05b</td>
<td>4.3 ± 0.8b</td>
<td>ND</td>
<td>1.6 ± 0.1a</td>
<td>0.2 ± 0.03a</td>
<td>1.60 ± 0.20a</td>
</tr>
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<td>$P$ value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Interactions</td>
<td></td>
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<tr>
<td>T × tissue</td>
<td>0.96</td>
<td>0.01</td>
<td>0.005</td>
<td>0.15</td>
<td>0.64</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Line × tissue</td>
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<td>0.02</td>
<td>0.71</td>
<td>0.32</td>
<td>0.0008</td>
<td>0.31</td>
</tr>
<tr>
<td>T × line</td>
<td>0.38</td>
<td>0.40</td>
<td>0.26</td>
<td>0.84</td>
<td>0.07</td>
<td>0.54</td>
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</tbody>
</table>

Data are expressed as least-squares means ± SE ($n = 5$). Different letters within a column for the main effect of tissue indicate a difference at $P < 0.05$ (Tukey’s pairwise comparisons).
expression of GLUT2 was greater in HWS than in LWS ($P < 0.05$), while expression was similar for both lines in the other two tissues (Fig. 2C). Insulin treatment was associated with reduced expression of GLUT2 in the liver (Fig. 3A). GLUT3 mRNA was not detected in pectoralis major. Expression was 10-fold greater in the hypothalamus than in abdominal fat and liver ($P < 0.0001$). Similar to GLUT2, insulin injection was associated with decreased expression of GLUT3 in the liver compared with vehicle-injected birds ($P < 0.05$, Fig. 3B). GLUT8 mRNA was greatest in the liver and skeletal muscle, intermediate in hypothalamus, and lowest in abdominal fat ($P < 0.05$). Expression of GLUT9 was $>10$-fold greater in liver compared with other tissues ($P < 0.0001$) and was greater in hypothalamus than in fat or muscle ($P < 0.05$). In the liver, GLUT9 mRNA was twofold greater in HWS compared with LWS ($P < 0.0001$), while expression was similar in both lines in other tissues (Fig. 2D).

IR expression was greatest in pectoralis major compared with the other tissues ($P < 0.001$, pectoralis major $> $ liver $> $ abdominal fat $> $ hypothalamus). There was an interaction of insulin and tissue on mRNA quantities ($P < 0.0001$) where in the liver, insulin injection was associated with decreased abundance compared with the vehicle-injected counterparts ($P < 0.0001$, Fig. 3C).

Heterosis for mRNA Abundance

To investigate heterosis for genes that showed significant differences in mRNA abundance between the HWS and LWS parental lines, we evaluated expression of those genes in HL and LH reciprocal crosses and calculated heterosis (Table 6). There were no differences in response to insulin treatment on gene expression in the reciprocal crosses. In the abdominal fat, heterosis was negative for all GLUTs ($P = 0.02$). In the liver, heterosis was significant for all GLUTs and was positive for GLUT1 (34%) and negative for GLUT2 (−70%) and GLUT9 (−20%). In the hypothalamus, heterosis

Fig. 3. Quantities of glucose transporter 2 (GLUT2) (A), GLUT3 (B), and insulin receptor (IR) (C) mRNA in the hypothalamus, abdominal fat, pectoralis major, and liver of vehicle- or insulin-injected 90-day-old chickens selected for high and low body weight. Two-way interactions of tissue and genetic line on mRNA abundance ($P < 0.05$). Values represent least-squares means ± SE ($n = 10$). Differing letters above bars indicate significant difference at $P < 0.05$ (Tukey’s pairwise comparisons).
was negative for NPYR1 (P = 0.001). Most of the genes showed less expression in the reciprocal crosses than in parental lines except GLUT1 in the liver and GLUT1 and NPY in the hypothalamus.

**DISCUSSION**

**Abundance of Appetite-Associated Factor mRNA in the Hypothalamus**

Expression of POMC and several other hypothalamic genes was similar between LWS and HWS, results consistent with those reported at 4 days posthatch (25). There was greater expression of two major orexigenic factors, NPY and AGRP, in the hypothalamus of the relatively hypophagic LWS chickens. As AGRP has been described as a lipogenic factor in chickens (57), and NPY has a role in adipose tissue metabolism in mammals (40), their expression and function could be related. Abundance of appetite-associated factors after insulin injection. 

In the present study, there were no changes in mRNA abundance of appetite-associated factors after insulin injection. Blood glucose concentrations were reduced, and there were differences in blood glucose between LWS and HWS chickens; however, there was not an association between reductions in blood glucose at 1 h and mRNA abundance. In the hypothalamus and brain stem of 3- to 4-day-old chicks, there was increased POMC and reduced NPY at 15 and 30 min, respectively, postintracerebroventricular (icv) injection of insulin (49). It is possible that because effects of insulin on food intake are most prominent within the first 30 min (51), the transcriptional effects on appetite-associated factors occurred and disappeared within the first hour after injection. One hour was selected in order to capture changes associated with different blood glucose concentrations. Other studies showing effects of insulin on gene expression in the hypothalamus were conducted in icv-injected animals (4, 42, 49), thus it is possible that both route of injection and duration of study influence effects on gene expression of appetite-associated factors in the hypothalamus.

**NPY and Receptor Subtype mRNA Abundance**

Of the genes evaluated in the hypothalamus, NPY, NPYR1, and NPYR5 mRNA were greater in LWS than in HWS chickens. These results are in contrast with those reported by Ka et al. (25), where NPY mRNA abundance was lower in 4-day-old LWS females than in HWS. Because we measured expression at 90 days, it is possible that accumulation of adipose tissue is associated with changes in the regulation of appetite and energy metabolism. Abundance of NPY mRNA in the hypothalamus is enhanced in response to food deprivation and in genetic and diet-induced rodent models of obesity (53), thus in the present study the effect of genetic line could be influenced by adiposity as well as the 16 h fast that preceded insulin injection.

The LWS are hypophagic with anorexics in the population and data indicate that NPY, which encodes one of the more potent orexigenic factors identified to date in mammals, is more highly expressed in these chickens. Although 5-day-old LWS chicks did not respond to exogenous NPY with increased food intake, the HWS chicks responded at the lowest dose tested, and hypothalamic nucleus activation between lines was similar (34). Those findings, coupled with our observation that expression of NPY and two of its receptor subtypes is greater in the hypothalamus of LWS chickens, suggests that the lack of response to NPY is due to an effect downstream of NPY bind-
ing to its receptors rather than a deficiency or dysfunction in NPY or receptors per se. Preliminary bioinformatics analyses suggest that there are no polymorphisms or deletions that would alter the amino acid sequence and hence functionality of NPY or its receptors.

The role of chicken NPY receptor subtypes in food intake and other biological functions is unclear, thus in the present study mRNA was measured for multiple subtypes, including the poorly characterized YR6. In mammals, subtypes YR1, 2, and 5 are involved in food intake regulation (8). Differential expression of NPY and its receptors between LWS and HWS in abdominal fat may indicate a role for NPY in fat deposition. Treatment of 3T3-L1 cells (murine preadipocytes) with NPY induced PPAR-γ expression, differentiation into adipocytes, and lipid accumulation (40) and inhibited α-MSH-induced lipolysis (5). The NPY YR2 and YR5 antagonists inhibited the stimulatory effect of NPY on adipocyte differentiation, whereas treatment with receptor agonists enhanced differentiation and lipogenesis, demonstrating that effects of NPY on adipocyte differentiation were mediated through YR2 and YR5. The YR1 is also present in human adipocytes and mediated the antilipolytic effect of NPY on human adipocytes (47). On the basis of these reports suggesting a role for NPY in regulating energy storage via white adipose tissue, greater expression of NPY and receptor subtypes 1, 2, and 5 in HWS abdominal fat could be involved in their enhanced rate of abdominal fat mass deposition. That heterosis was highly negative for both YR1 and YR5 mRNA in the adolescent fat suggests that the encoded receptors play a role in fat deposition in LWS and HWS.

**FOXO1 Expression in Different Tissues**

Expression of FOXO1, which encodes a major transcriptional regulator that is highly expressed in insulin-sensitive tissues, was also evaluated (29). Expression of FOXO1 was measured because it was hypothesized that differential blood glucose concentrations in LWS and HWS may be associated with differences in expression of genes associated with insulin resistance and glucose intolerance in humans. The FOXO1 regulates expression of genes associated with gluconeogenesis, energy metabolism, and oxidative stress (22). In the present study, FOXO1 mRNA was greater in the pectoralis major and liver of HWS than of LWS. In insulin-resistant individuals, FOXO1 overexpression in skeletal muscle is associated with hyperglycemia and glucose intolerance (23), thus expression of FOXO1 could be related to differences in energy metabolism between LWS and HWS. Greater rates of adipose tissue lipolysis in LWS (7) could be related to differences in oxidative activity, although these data have not been reported for LWS and HWS in adipose tissue and skeletal muscle.

**Tissue Distribution of GLUTs**

Phylogenetic analysis of human and chicken GLUT amino acid sequences revealed that chicken GLUT genes align with their respectively numbered gene in humans, and there does not appear to be a chicken GLUT gene that is similar to human GLUT4 (Fig. 4). Because mechanisms controlling glucose uptake in insulin-sensitive tissues of chickens are unclear and there are likely differences in nutrient uptake and utilization between HWS and LWS, we evaluated expression of GLUT genes in different tissues.

The GLUTs showed distinct tissue specificities, with GLUT1 mRNA most abundant in the hypothalamus, GLUT2 and 9 in the liver, GLUT3 absent from the skeletal muscle, and GLUT8 similarly expressed across all tissues. GLUT9 was reported to mediate the uptake of uric acid and glucose in mammals, with greatest expression in expression in liver (16); however, its substrate specificity in chickens is unclear. Expression of GLUT2 was >200-fold greater in the liver than in other tissues examined. In mammals, GLUT2 is reported to be a low-affinity, high-capacity transporter that mediates uptake of glucose, galactose, and fructose across a wide range of physiological concentrations, playing an important role in maintaining glucose flux in liver cells (31). Both GLUT1 and GLUT3 are described as being high-affinity, low-capacity transporters that are responsible for basal glucose uptake in the central nervous system of mammals (43), and GLUT8 is described as an intracellular GLUT that is ubiquitously expressed across most tissues (44), thus tissue distribution patterns of the GLUTs in chickens are similar to those reported in mammalian species. The differences between HWS and LWS were also tissue specific, with GLUT1 greater in HWS than in LWS in the hypothalamus, and GLUT2 and 9 greater in HWS in the liver. These results suggest that HWS have a greater capacity for glucose uptake in those tissues, consistent with a greater metabolic demand and glucose load in the relatively hyperphagic and obese HWS chickens. These data may appear counterintuitive, as previous research showed that HWS were relatively hyperglycemic and glucose intolerant. It remains to be determined the relative contribution of different GLUTs to overall glucose uptake in peripheral tissues of chickens and the cellular mechanisms underlying hyperglycemia and glucose regulation in HWS.
Effect of Insulin on mRNA Abundance in the Liver

The liver was the only tissue where there was an effect of insulin treatment on mRNA, where GLUT2, GLUT3, and IR expression all decreased after insulin injection. These results implicate GLUT2 and GLUT3 as potential insulin-dependent GLUTs in chickens. Insulin treatment was associated with an increase in 2-deoxy-glucose uptake and abundance of GLUT1 mRNA and protein in chicken embryonic myoblasts (59), providing evidence for the presence of insulin-stimulated GLUTs.

The majority of GLUT2 protein is localized to the plasma membrane in the basal (noninsulin stimulated) state (10), and perfusion of rat liver with insulin was associated with decreased plasma membrane-bound GLUT2 (1). As suggested by the results in the present study, transcriptional downregulation of GLUT2 may serve a similar function in reducing glucose flux across the hepatocyte plasma membrane during the hypoglycemic response.

Gene expression was measured at 1 h postinjection, with blood glucose concentrations reduced in all insulin-injected chickens compared with the vehicle-injected controls. As GLUT2 mediates bidirectional transport in hepatocytes, downregulation of GLUT2 and GLUT3 may serve as mechanisms to reduce glucose uptake from the blood, thus preventing further hypoglycemia, while downregulation of the IR may also serve as a compensatory mechanism to prevent further utilization of glucose during hypoglycemia. As mentioned earlier, the lack of effects on gene expression of GLUTs in other tissues may indicate relatively less sensitivity in those tissues or may represent a time-dependent effect. It is possible that the major transcriptional events in skeletal muscle and adipose tissue in response to the insulin occurred before 1 h, during the initial decline in blood glucose concentrations, and following glucose clearance, the response shifted to the liver to prevent further decreases in blood glucose. It is also possible that transcriptional changes occurred after 1 h in response to the onset of hypoglycemia.

Heterosis for NPY, NPYRs, and GLUTs in Different Tissues

Transcriptional diversity at specific sets of genes influences heterosis for different traits (54). According to the heterosis analysis, the expression of most of the genes was biased toward the LWS line. For NPY mRNA in the abdominal fat and hypothalamus, the average of the parental lines was similar to the reciprocal crosses, while all of the NPYRs were different, with NPYR1 and NPYR5 mRNA exhibiting much greater heterosis than NPYR2. Given the role of NPY and its receptors in the hypothalamus and abdominal fat in promoting food intake and fat deposition, the reciprocal crosses may have an advantage in dealing with excess energy by having reduced expression of NPYR1 and NPYR5. In the hypothalamus, NPYR1 but not NPY was significantly reduced in the reciprocal lines compared with the parental lines, indicating that the receptor may play a more important role in regulation of food intake. All GLUTs had negative heterosis except GLUT1 in the liver, suggesting that the HWS line is more efficient in utilizing glucose than all other lines, consistent with their superior feed efficiency (18).

Appetite factors were shown to have high heritability in humans (21, 32); however, there is no report on the heritability of appetite regulation in these lines. Moreover, methods for estimating heritability may include nonadditive and/or additive genetic variation. As single genes can each have a heterozygous effect, there can be an average across them, thus demonstrating why multiple genes effects can be masked at the phenotypic level. The relevance of heterosis for NPY, NPY receptor, and GLUT mRNA may have important implications for appetite and metabolic disorders.

Conclusions and Implications

In conclusion, results from this study indicate that there are differences in expression of NPY and its receptor subtypes in the hypothalamus and white adipose tissue of chickens selected for high or low body weight. We also observed differences in GLUT expression between the lines in hypothalamus, abdominal fat, and liver, suggestive of more efficient glucose assimilation in the high-weight chickens. Insulin injection was associated with a more pronounced effect on blood glucose in LWS chickens after 1 h, although effects on gene expression were not different between the lines. The liver was the only organ affected by insulin injection, with a downregulation of GLUTs and IR, suggestive of a compensatory mechanism to prevent further utilization of glucose in the liver during insulin-induced hypoglycemia.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: W.Z. performed experiments; W.Z. analyzed data; W.Z., P.B.S., M.A.C., and E.R.G. interpreted results of experiments; W.Z. prepared figures; W.Z. drafted manuscript; W.Z., L.H.S., P.B.S., M.A.C., and E.R.G. approved final version of manuscript; L.H.S., P.B.S., M.A.C., and E.R.G. edited and revised manuscript; W.Z., L.H.S., P.B.S., M.A.C., and E.R.G. conception and design of research.

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