

# Whole Egg Consumption Impairs Insulin Sensitivity in a Rat Model of Obesity and Type 2 Diabetes

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## ABSTRACT

**Background:** The literature regarding the relation between egg consumption and type 2 diabetes (T2D) is inconsistent and there is limited evidence pertaining to the impact of egg consumption on measures of insulin sensitivity.

**Objectives:** The aim of this study was to investigate the effect of dietary whole egg on metabolic biomarkers of insulin resistance in T2D rats.

**Methods:** Male Zucker diabetic fatty (ZDF) rats ( $n = 12$ ; 6 wk of age) and age-matched lean controls ( $n = 12$ ) were randomly assigned to be fed a casein- or whole egg-based diet. At week 5 of dietary treatment, an insulin tolerance test (ITT) was performed on all rats and blood glucose was measured by glucometer. After 7 wk of dietary treatment, rats were anesthetized and whole blood was collected via a tail vein bleed. Following sedation, the extensor digitorum longus muscle was removed before and after an intraperitoneal insulin injection, and insulin signaling in skeletal muscle was analyzed by Western blot. Serum glucose and insulin were analyzed by ELISA for calculation of the homeostatic model assessment of insulin resistance (HOMA-IR).

**Results:** Mean ITT blood glucose over the course of 60 min was 32% higher in ZDF rats fed the whole egg-based diet than in ZDF rats fed the casein-based diet. Furthermore, whole egg consumption increased fasting blood glucose by 35% in ZDF rats. Insulin-stimulated phosphorylation of key proteins in the insulin signaling pathway did not differ in skeletal muscle of ZDF rats fed casein- and whole egg-based diets. In lean rats, no differences were observed in insulin tolerance, HOMA-IR and skeletal muscle insulin signaling, regardless of experimental dietary treatment.

**Conclusions:** These data suggest that whole body insulin sensitivity may be impaired by whole egg consumption in T2D rats, although no changes were observed in skeletal muscle insulin signaling that could explain this finding. *Curr Dev Nutr* 2019;3:nzz015.

## Introduction

The increasing prevalence of type 2 diabetes (T2D) is a critical public health issue and insulin resistance is a key contributor to T2D development (1, 2). Insulin resistance is a condition characterized by hyperinsulinemia, hyperglycemia, and impaired glucose and insulin tolerance (3). Diet is an important modifiable risk factor for insulin resistance and the progression of T2D. Therefore, understanding the relation between dietary components, such as whole egg, and insulin resistance is essential for developing future dietary recommendations for the millions of individuals with existing T2D, as well as those who are at high risk for developing T2D.

Insulin mediates its metabolic effects by binding to the insulin receptor, thereby modifying the activity and intracellular location of proteins involved in the insulin signaling pathway. Insulin binding to the insulin receptor triggers autophosphorylation of the insulin receptor (IR)  $\beta$  subunit,



**Keywords:** whole egg, egg consumption, insulin signaling, insulin resistance, diabetes, rat

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Abbreviations used: AS160, Akt substrate 160; EDL, extensor digitorum longus; GLUT4, glucose transporter type 4; HOMA- $\beta$ , homeostatic model assessment of  $\beta$ -cell function; IR, insulin receptor; IRS-1, insulin receptor substrate 1; ITT, insulin tolerance test; p-, phosphorylated; ZDF, Zucker diabetic fatty.

which activates the receptor and initiates a cascade of phosphorylation events (4). Key events in the insulin signaling cascade include the activation of the insulin receptor substrate 1 (IRS-1) via tyrosine phosphorylation; serine/threonine phosphorylation of Akt and its subsequent activation; phosphorylation of Akt substrate 160 (AS160) at serine/threonine residues; and translocation of the glucose transporter type 4 (GLUT4) from intracellular vesicles to the plasma membrane, resulting in increased glucose uptake in skeletal muscle and adipose tissue (5–7). Defects in insulin function through the sequential action of the insulin receptor, IRS-1, Akt, AS160, and GLUT4 have been reported in metabolic disorders associated with insulin resistance, such as obesity and T2D (6, 8). Impaired insulin signaling at any of these key steps reduces the ability of insulin to promote glucose uptake and utilization.

Limited and inconsistent findings have been reported on the relation between egg consumption and T2D. Whereas some studies suggest that egg consumption increases the risk of T2D (9–11), others report a null association or a beneficial impact on T2D risk and outcomes (12–17). A meta-analysis found no association between egg consumption and T2D risk in countries outside of the United States, but found a modest increase in T2D risk that was restricted to US studies, suggesting that these results may be confounded by factors such as dietary behaviors of the US population (18). Results from a recent human study suggest that the apparent association between egg consumption and T2D risk in the US population may be due to an interaction between meat and egg intake, and not egg intake alone (19).

It is widely recognized that obesity is a major risk factor for insulin resistance, which precedes the onset of overt diabetes (1–3). We previously reported that a whole egg-based diet attenuates cumulative body weight gain in the Zucker diabetic fatty (ZDF) rat, a well-characterized genetic model of obesity and T2D (20, 21). The observed attenuation in body weight gain was attributed, in part, to an 8% reduction in body fat in ZDF rats consuming a whole egg-based diet (20). Furthermore, we extended this research to a diet-induced model of obesity and demonstrated that whole egg consumption in diet-induced obese rats markedly reduces weight gain compared with that in diet-induced obese rats fed a casein-based diet (CJ Saande, SK Jones, KE Hahn, CH Reed, MJ Rowling, KL Schalinske, unpublished observations, 2017). There is very limited evidence regarding the association between egg consumption and measures of insulin sensitivity (13, 22, 23), and, to our knowledge, the impact of whole egg consumption on insulin signaling has not been examined. Thus, the objective of this study was to investigate whether the previously observed reductions in adiposity in ZDF rats fed a whole egg-based diet are related to improved insulin sensitivity and enhanced insulin signaling.

## Methods

### Rats and diets

All animal studies were approved by the Institutional Animal Care and Use Committee at Iowa State University (IACUC no. 1-18-8674-R; approval date December 1, 2018) and were performed according to the Iowa State University Laboratory Animal Resources Guidelines. Male ZDF (*fa/fa*) rats ( $n = 12$ ) and lean (*fa/+*) control rats ( $n = 12$ ) were purchased at 5 wk of age (Charles River Laboratories). Rats were housed 2/cage with a 12-h light-dark cycle in a temperature-controlled

**TABLE 1** Composition of the casein- and whole egg-based diet fed to lean control and ZDF rats for 7 wk<sup>1</sup>

Ingredient, g/kg	Casein	Whole egg
Casein (vitamin free)	200	0
Dried whole egg	0	413
Corn starch	423	387
Glucose monohydrate	150	150
Mineral mix (AIN 93)	35	35
Vitamin mix (AIN 93)	10	10
Biotin 1%	0	0.4
Corn oil	177	0
Choline bitartrate	2	2
L-Methionine	3	3
Macronutrients, kcal/kg		
Protein	800	800
Lipid	1593	1593
Carbohydrate	2292	2148
Total energy	4685	4541

<sup>1</sup>All ingredients were purchased from Envigo with the exception of dried whole egg (Rose Acre Farms) as well as L-methionine and choline bitartrate (Sigma-Aldrich). ZDF, Zucker diabetic fatty.

<sup>2</sup>The total protein and lipid content provided by 413 g of dried whole egg was 48.4% (200 g) and 42.9% (177 g), respectively.

room. All rats were acclimated to a semipurified diet (AIN-93 G) for 1 wk. Following acclimation, rats were randomly assigned to 1 of 2 experimental diets (Table 1): a casein-based diet ( $n = 12$ ) or a whole egg-based diet ( $n = 12$ ). Both diets provided protein at 20% (wt/wt) and were matched for lipid content (17.7% total lipid) via the addition of corn oil to the casein-based diet to account for the additional lipid contribution of the whole egg. Total protein at 20% (wt/wt) in the whole egg-based diet was achieved by the addition of 413 g/kg dried whole egg, which provided 48.4% (200 g) protein and 42.9% (177 g) lipid. Diets were prepared weekly and rats were given ad libitum access to food and water for a period of 7 wk. Body weight and food intake were recorded 5 d/wk. Prior to killing the animals, food was withheld for 4 h and rats were anesthetized via a single intraperitoneal injection of ketamine:xylazine (90:10 mg/kg body weight). Following sedation, whole blood was collected via a tail vein bleed and blood samples were stored on ice until centrifugation. The extensor digitorum longus (EDL) muscle was removed from one leg prior to an insulin injection to account for basal differences in insulin signaling. All rats were then given an intraperitoneal insulin injection (Sigma; 10 U/kg body weight) and the EDL muscle was removed from the other leg 10 min post-insulin injection to allow sufficient time for insulin signaling to occur (24–27). Immediately following tissue removal, muscle samples were snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for subsequent analysis. The epididymal fat pad was removed and weighed. All 24 rats were killed by exsanguination. Whole blood was centrifuged in separation tubes at  $4,000 \times g$  for 15 min at  $4^{\circ}\text{C}$  and the resultant serum was stored at  $-80^{\circ}\text{C}$ .

### Insulin tolerance tests

Insulin tolerance tests (ITTs) were performed at week 5 of experimental dietary treatment. Rats were fasted for a period of 4 h prior to insulin tolerance testing and given an intraperitoneal insulin injection (0.5 U/kg body weight). Blood samples were collected from the tail vein immediately prior to the insulin challenge, as well as 15, 30, 45, and

**TABLE 2** Body and adipose tissue weights and total food intake of lean and ZDF rats fed a casein- or whole egg-based diet for 7 wk<sup>1</sup>

	Lean		ZDF		P		
	Casein	Whole egg	Casein	Whole egg	Genotype	Diet	Genotype x diet
Initial body weight, <sup>2</sup> g	157 ± 5 <sup>a</sup>	155 ± 6 <sup>a</sup>	191 ± 6 <sup>b</sup>	191 ± 4 <sup>b</sup>	<0.001	0.877	0.824
Final body weight, <sup>2</sup> g	329 ± 7 <sup>a</sup>	334 ± 5 <sup>a</sup>	378 ± 4 <sup>b</sup>	371 ± 8 <sup>b</sup>	<0.001	0.897	0.368
Epididymal fat pad weight, <sup>2</sup> g/100 g body weight	0.47 ± 0.04 <sup>a</sup>	0.50 ± 0.08 <sup>a</sup>	0.86 ± 0.08 <sup>b</sup>	0.83 ± 0.03 <sup>b</sup>	<0.001	0.992	0.642
Total food intake, <sup>3</sup> g	990 ± 28 <sup>a</sup>	930 ± 29 <sup>a</sup>	1843 ± 135 <sup>b</sup>	1732 ± 163 <sup>b</sup>	<0.001	0.449	0.818
Total energy intake, <sup>3</sup> kcal	4639 ± 130 <sup>a</sup>	4224 ± 132 <sup>a</sup>	8636 ± 632 <sup>b</sup>	7865 ± 740 <sup>b</sup>	<0.001	0.265	0.729

<sup>1</sup>ZDF, Zucker diabetic fatty.<sup>2</sup>Data are means ± SEMs; n = 6. Data within the same row without a common letter differ (*P* < 0.05).<sup>3</sup>Data are means ± SEMs; n = 3. Total food intake per cage (2 rats/cage). Data within the same row without a common letter differ (*P* < 0.05).

60 min thereafter. Blood sampling was performed by making a nick with a sterilized razor blade toward the end of the tail and blood glucose was measured with the use of a glucometer (Bayer Healthcare). When blood glucose was above the detection limit (600 mg/dL), the maximum value of 600 mg/dL was used.

### Serum glucose and serum insulin

Serum collected on the final day of the study was used for analysis of fasting glucose, fasting insulin, and calculation of HOMA-IR. Serum glucose was measured with the use of a commercially available colorimetric kit (Wako Diagnostics). Analysis of serum insulin was measured by a commercially available immunoassay kit for the detection of insulin in rat sera (EMD Millipore).

### Western blot analysis

EDL muscles were homogenized in 800 μL of lysis buffer [Tris-hydrochloric acid (pH 7.8, 50 mM), EDTA (1 mM), EGTA (1 mM), glycerol (10%, wt/vol), Triton-X 100 (1%, wt/vol), dithiothreitol (1 mM)] containing phosphatase (Sigma) and protease (Thermo Scientific) inhibitors. Samples were then centrifuged at 4000 × *g* for 15 min at 4°C and the supernatant was collected. Protein concentrations were determined via a bicinchoninic acid assay (Pierce) according to the manufacturer's instructions. A total of 20 μg protein was loaded and run on a 4–15% gradient sodium dodecyl sulfate polyacrylamide gel (Bio-Rad). Following separation, proteins were transferred onto a polyvinylidene difluoride membrane (EMD Millipore) and blocked at room temperature for 1 h in Tris-buffered saline with 0.05% Tween and 5% nonfat dry milk. Membranes were incubated in phosphorylated (p)-IGF1 Receptor β<sup>Tyr1135/1136</sup>/Insulin Receptor β<sup>Tyr1150/1151</sup>, p-Akt<sup>Ser473</sup>, Akt, and p-AS160<sup>Thr642</sup> antibodies (Cell Signaling) at 1:1000 overnight at 4°C. Following incubation with primary antibody, membranes were washed and incubated with an antirabbit secondary antibody (Cell Signaling) at 1:5000 for 1 h at room temperature. Membranes were incubated in enhanced chemiluminescent substrate (SuperSignal West Pico PLUS Sensitivity Substrate or SuperSignal West Femto Maximum Sensitivity Substrate; Thermo Scientific) for 5 min prior to imaging with the ChemiDoc XRS detection imaging system (Bio-Rad). Densitometry was determined with the use of Image Lab software (BioRad) and raw data was normalized to total protein.

### Statistical analysis

All data were evaluated for statistically significant differences (*P* < 0.05) with the use of SPSS Statistics Software version 23 (IBM). Body and epididymal fat pad weights, food intake, and serum parameters were analyzed with the use of a 2-factor ANOVA (diet × genotype). An analysis of main effects was performed when the interaction between diet and genotype was not statistically significant. Insulin tolerance test data was analyzed by a 3-factor, repeated-measures ANOVA (time × diet × genotype) and statistically significant 2-factor interactions were followed by an analysis of simple main effects. Western blot data was analyzed with the use of a 3-factor mixed ANOVA to determine the effects of insulin, diet, and genotype on insulin signaling. All pairwise comparisons were performed with the use of Fisher's least significant difference post-hoc test.

## Results

### Body and relative adipose tissue weights

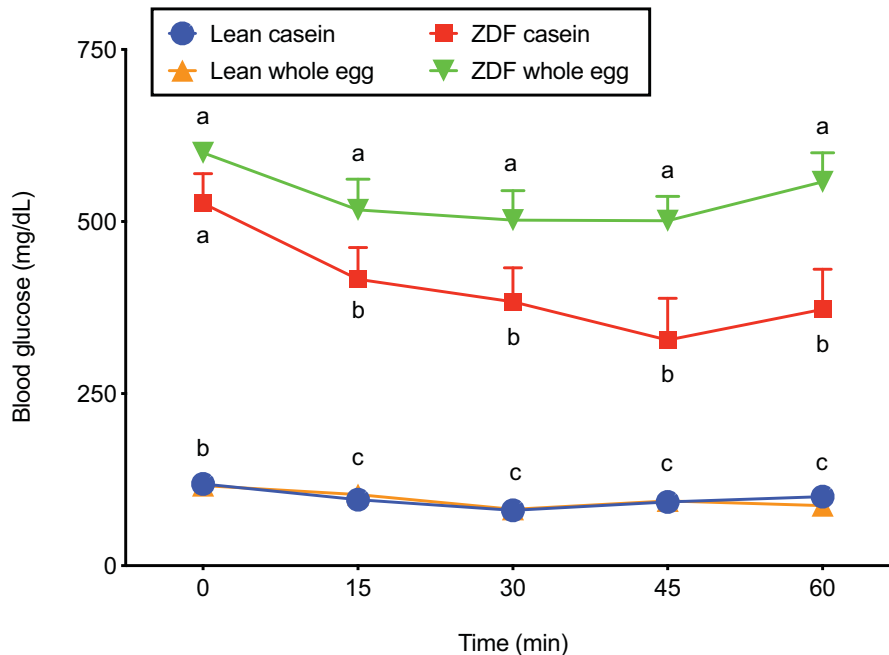
As expected, there was a significant main effect of genotype on initial and final body weight. ZDF rats had a higher mean initial body weight than their lean counterparts, and body weight was 13% higher in ZDF rats than in lean rats on the final day of the study. Diet was without effect on final body weight in both lean and ZDF rats (Table 2). Likewise, there was a significant main effect of genotype on relative adipose tissue weight [epididymal fat pad weight (g/100 g body weight)]. The ZDF genotype was associated with a 74% higher mean relative adipose tissue weight than the lean genotype. No significant differences in relative adipose tissue weight were observed across diets within lean or ZDF rats (Table 2).

### Food intake

Main effects analysis indicated a significant effect of genotype on food intake and total energy intake. ZDF rats exhibited an 86% higher mean total food intake than lean rats (Table 2). Likewise, total energy intake was 86% higher in ZDF rats than in lean rats (Table 2). There was no effect of diet on total food intake or total energy intake.

### ITT

Analysis of ITT blood glucose concentrations revealed a significant effect of time on circulating glucose concentrations, demonstrating that insulin effectively lowered blood glucose. There was also a significant



**FIGURE 1** ITT blood glucose in lean and ZDF rats fed a casein- or whole egg-based diet for 5 wk. Data are means  $\pm$  SEMs;  $n = 3-6$ . Data within the same time point without a common letter differ ( $P < 0.05$ ). Three-factor repeated-measures ANOVA: time,  $P < 0.001$ ; diet,  $P = 0.027$ ; genotype,  $P < 0.001$ ; time  $\times$  diet,  $P = 0.662$ ; time  $\times$  genotype  $P = 0.031$ ; diet  $\times$  genotype  $P = 0.025$ ; Time  $\times$  diet  $\times$  genotype,  $P = 0.572$ . ITT, insulin tolerance test; ZDF, Zucker diabetic fatty.

effect of genotype and diet, as well as significant diet  $\times$  genotype and time  $\times$  genotype 2-factor interactions. As expected, there was a simple main effect of genotype ( $P < 0.001$ ) on blood glucose, indicating markedly higher blood glucose in ZDF rats than in lean rats at each time point (Figure 1). A simple main effect of time was also observed in the ZDF genotype ( $P = 0.001$ ), but not in the lean genotype ( $P = 0.836$ ). Lastly, a simple main effect of diet was observed in the ZDF genotype ( $P < 0.001$ ), but not in the lean genotype ( $P = 0.987$ ). With the exception of baseline blood glucose, ZDF rats fed the whole egg-based diet exhibited  $\sim 38\%$  higher blood glucose concentrations from the 15–60 min time points than ZDF rats fed the casein-based diet. In contrast, blood glucose did not differ between dietary treatment groups in lean rats at any of the time points (Figure 1).

#### Serum glucose, serum insulin, HOMA-IR, and homeostatic model assessment of $\beta$ -cell function

There was a significant main effect of genotype on serum glucose, serum insulin, and the HOMA-IR. As expected, mean serum glucose, serum insulin, and HOMA-IR values were 244%, 629%, and 234% higher, respectively, in the ZDF genotype than in the lean genotype (Table 3). Diet was without effect on serum glucose concentrations within the lean genotype; however, serum glucose concentrations were increased by 35% in ZDF rats fed the whole egg-based diet than in ZDF rats fed the casein-based diet (Table 3). No differences in serum insulin concentrations were observed across dietary groups within the lean genotype, whereas serum insulin was 68% higher in ZDF rats fed the casein-based diet than in ZDF rats fed the whole egg-based diet. There was no effect of diet on the HOMA-IR within the lean or ZDF genotype (Table 3). Lastly, there was a significant main effect of diet on

the homeostatic model assessment of  $\beta$ -cell function (HOMA- $\beta$ ). The whole egg-based diet was associated with a mean decrease of 44% in HOMA- $\beta$  compared with the casein-based diet (Table 3).

#### Insulin signaling pathway

Insulin increased phosphorylation of the IR  $\beta^{\text{Tyr}1150/1151}$  by 291% in lean rats fed the whole egg-based diet compared with IR  $\beta^{\text{Tyr}1150/1151}$  phosphorylation prior to insulin (Figure 2); however, post-insulin IR  $\beta^{\text{Tyr}1150/1151}$  phosphorylation did not reach statistical significance ( $P = 0.215$ ) in lean casein-fed rats compared with pre-insulin phosphorylated insulin receptor (p-IR)  $\beta^{\text{Tyr}1150/1151}$ . No differences in p-IR  $\beta^{\text{Tyr}1150/1151}$  were observed pre- or post-insulin in ZDF rats, regardless of dietary treatment (Figure 2). In lean rats fed the casein- and whole egg-based diets, the post-insulin ratio of p-Akt<sup>Ser473</sup>:total Akt was increased 17-fold and 18-fold, respectively, compared with the pre-insulin ratio (Figure 3). Pre- and post-insulin p-Akt<sup>Ser473</sup>:total Akt did not differ in ZDF rats, regardless of dietary treatment. However, in ZDF rats fed the whole egg-based diet, the post-insulin p-Akt<sup>Ser473</sup>:total Akt ratio did not statistically differ from the lean genotype (Figure 3). No differences in post-insulin p-AS160<sup>Thr642</sup> were observed, regardless of diet or genotype (data not shown).

#### Discussion

The relation between egg consumption and T2D remains contradictory and evidence is limited regarding potential mechanisms that may explain the reported associations between dietary egg intake, glycemic control, and incident diabetes. The present study aimed to examine the

**TABLE 3** Fasting serum glucose, fasting serum insulin, HOMA-IR and HOMA- $\beta$  of lean and ZDF rats fed a casein- or whole egg-based diet for 7 wk<sup>1</sup>

	Lean		ZDF		P		
	Casein	Whole egg	Casein	Whole egg	Genotype	Diet	Genotype x diet
Serum glucose, mg/dL	124 $\pm$ 13 <sup>c</sup>	189 $\pm$ 19 <sup>c</sup>	457 $\pm$ 31 <sup>b</sup>	618 $\pm$ 86 <sup>a</sup>	<0.001	0.026	0.317
Serum Insulin, ng/mL	0.3 $\pm$ 0.1 <sup>c</sup>	0.4 $\pm$ 0.1 <sup>c</sup>	3.2 $\pm$ 0.4 <sup>a</sup>	1.9 $\pm$ 0.6 <sup>b</sup>	<0.001	0.116	0.078
HOMA-IR	2.1 $\pm$ 0.46 <sup>b</sup>	4.0 $\pm$ 1.2 <sup>b</sup>	82 $\pm$ 9.3 <sup>a</sup>	59 $\pm$ 20 <sup>a</sup>	<0.001	0.344	0.267
HOMA- $\beta$ , %	51 $\pm$ 13 <sup>a,b</sup>	32 $\pm$ 13 <sup>b</sup>	72 $\pm$ 13 <sup>a</sup>	37 $\pm$ 12 <sup>a,b</sup>	0.331	0.046	0.554

<sup>1</sup>Data are means  $\pm$  SEMs; *n* = 6. Data within the same row without a common letter differ (*P* < 0.05). HOMA- $\beta$ , HOMA- $\beta$ , homeostatic model assessment of  $\beta$ -cell function; ZDF, Zucker diabetic fatty.

effects of egg consumption on insulin tolerance and insulin signaling in vivo through the use of a rat model of obesity and T2D. Although egg consumption impaired glycemic control in ZDF rats during an ITT, no differences were observed in skeletal muscle insulin signaling between ZDF rats fed casein- and whole egg-based diets. Although skeletal muscle is the primary site of insulin-stimulated glucose disposal, glucose metabolism by the liver and adipose tissue also contributes to whole body glucose homeostasis (28–30). The relative contribution of these tissues to systemic glucose metabolism, as well as differences in timing between insulin tolerance testing and skeletal muscle collection for insulin signaling analysis, may explain the differential results observed between whole body insulin tolerance and skeletal muscle insulin signaling.

Very few studies have investigated the effect of egg consumption on direct measures of insulin sensitivity (22). In the present study, we report higher blood glucose during an ITT in ZDF rats consuming a whole egg-based diet than in ZDF rats fed a casein-based diet. In support of this finding, egg consumption was inversely associated with insulin sensitivity and the metabolic clearance rate of insulin in a cross-sectional analysis of a nondiabetic population, although these associations became insignificant after adjustment for BMI and dietary cholesterol (22). Likewise, Djousse et al. (13) reported an increase in fasting blood glucose and insulin resistance, as measured by HOMA-IR, across varying amounts of egg consumption in a prospective cohort of older adults. However, the authors noted that the magnitude of difference, although statistically significant, was not likely to be of clinical significance (13). Here, we report higher fasting blood glucose in ZDF rats after 7 wk of dietary treatment with the whole egg-based diet, but no differences in HOMA-IR, a model used to quantify insulin resistance, between ZDF rats fed casein- and whole egg-based diets.

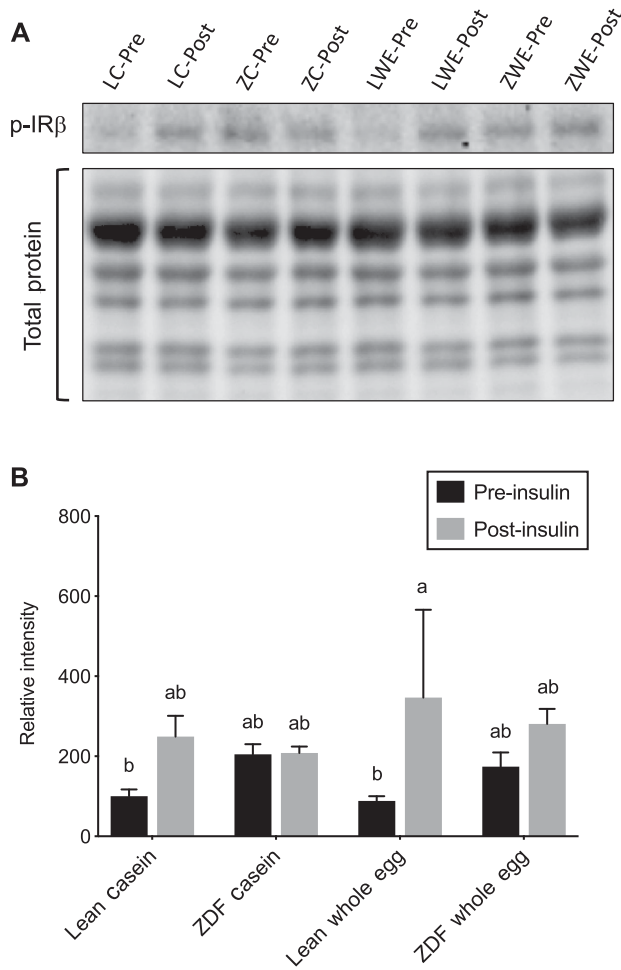
In the early stages of insulin resistance, enhanced pancreatic insulin secretion attempts to compensate for reduced responsiveness to insulin in peripheral tissues as a means to maintain normal glucose tolerance. A physiologic approach to accomplish this goal is by enhanced  $\beta$ -cell mass and activity (31, 32). As insulin resistance progresses, compensatory hyperinsulinemia is unable to maintain normal blood glucose concentrations. Insulin secretion is continuously stimulated by hyperglycemia, and  $\beta$ -cell structure and function becomes compromised, ultimately leading to apoptosis (32). In ZDF rats,  $\beta$ -cell mass decreases between 6 and 12 wk of age, and is significantly reduced at 12 wk (33–35). The observed loss of  $\beta$ -cell mass has been attributed an increase in cell death (33, 34).  $\beta$ -cell dysfunction in ZDF rats is accompanied by a progressive decline in circulating insulin concentrations, beginning at 7 wk of age (33, 35). We report significantly lower serum insulin, concomitant with higher serum glucose, in ZDF rats fed the whole

egg-based diet compared with ZDF rats fed the casein-based diet after 7 wk of dietary treatment (13 wk of age). Additionally, consumption of a whole egg-based diet was associated with decreased HOMA- $\beta$ , an index of  $\beta$ -cell function, suggesting impaired insulin production and secretion in rats fed the whole egg-based diet. It is possible that ZDF rats fed the whole egg-based diet exhibit a higher rate of decline in  $\beta$ -cell function, potentially explaining these differences. In cultured  $\beta$ -cells, cholesterol accumulation results in apoptosis and impaired glucose-stimulated insulin secretion (36–39). The cholesterol content of whole egg may play a role in the observed reduction in serum insulin; however, whether whole egg consumption affects  $\beta$ -cell function in ZDF rats remains to be determined.

Aberrant insulin signaling in skeletal muscle and adipose tissue impairs insulin-mediated translocation of GLUT4 and subsequent glucose uptake. To our knowledge, there are no previous studies examining the effect of egg consumption on insulin signaling. In the present study, phosphorylation of IR  $\beta^{\text{Tyr1150/1151}}$  was not significantly increased in ZDF rats following an insulin injection, regardless of experimental dietary treatment. This result is consistent with findings from numerous human studies, which show reduced tyrosine phosphorylation of the insulin receptor and its subsequent kinase activity in states of insulin resistance (40–45). The serine/threonine kinase Akt is activated by insulin-stimulated phosphorylation at both Thr308 and Ser473 and plays a key role in the regulation of glucose uptake into insulin-responsive tissues (46). As expected, we report a marked increase in the ratio of p-Akt<sup>Ser473</sup>:total Akt in lean rats in response to insulin. Conversely, the p-Akt<sup>Ser473</sup>:total Akt ratio was not significantly increased by insulin in ZDF rats fed both casein- and whole egg-based diets. In agreement with this finding, several studies report defective Akt phosphorylation and kinase activity in insulin-resistant subjects compared with lean controls (47–51). Phosphorylation of AS160, a downstream substrate of Akt, links insulin signaling to GLUT4 translocation and impaired insulin-stimulated AS160 phosphorylation has been reported in the skeletal muscle of diabetic human subjects (51, 52). In contrast to these findings, we did not observe differences in post-insulin p-AS160<sup>Thr642</sup> between lean and ZDF rats, regardless of dietary treatment group.

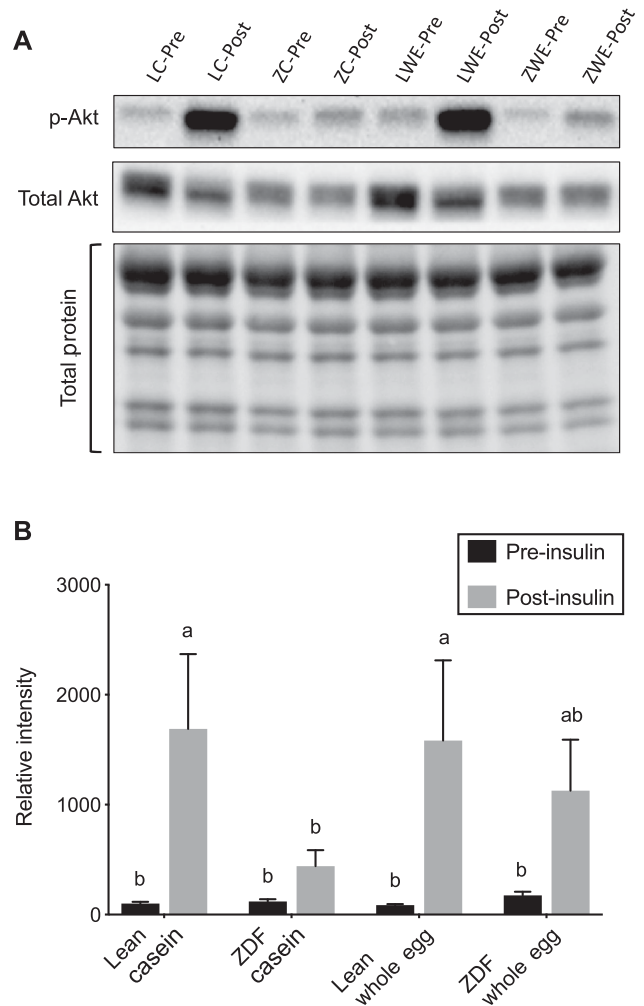
Eggs are a source of high-quality protein, and several human studies report an association between egg consumption, increased satiety, and reduced caloric intake (53–56). Egg consumption has also been shown to promote weight loss in a limited number of human studies (57, 58). In contrast to our previous findings (20, 21), we did not observe a reduction in body weight gain in ZDF rats fed a whole egg-based diet. Moreover, relative adipose tissue weight not differ between ZDF rats, regardless of dietary treatment. It is well documented that weight loss is





**FIGURE 2** Skeletal muscle p-IR  $\beta^{\text{Tyr}1150/1151}$  (A) and representative Western blot images of skeletal muscle p-IR  $\beta^{\text{Tyr}1150/1151}$  and total protein (B) pre- and post-insulin injection in lean and ZDF rats fed a casein- or whole egg-based diet for 7 wk. Data are expressed relative to pre-insulin p-IR  $\beta^{\text{Tyr}1150/1151}$  in lean rats fed the casein-based diet. Data are means  $\pm$  SEMs;  $n = 5-6$ . Bars without a common letter differ ( $P < 0.05$ ). Three-factor mixed ANOVA: insulin,  $P = 0.029$ ; diet,  $P = 0.492$ ; genotype,  $P = 0.874$ ; insulin  $\times$  diet,  $P = 0.297$ ; insulin  $\times$  genotype  $P = 0.169$ ; diet  $\times$  genotype  $P = 0.723$ ; insulin  $\times$  diet  $\times$  genotype,  $P = 0.837$ . LC-Pre, lean casein pre-insulin; LC-Post, lean casein post-insulin; ZC-Pre, ZDF casein pre-insulin; ZC-Post, ZDF casein post-insulin; LWE-Pre, lean whole egg pre-insulin; LWE-Post, lean whole egg post-insulin; ZWE-Pre, ZDF whole egg pre-insulin; ZWE-Post, ZDF whole egg post-insulin. p-IR, phosphorylated insulin receptor; ZDF, Zucker diabetic fatty.

a highly effective strategy to improve insulin sensitivity and glycemia, both in the prevention and treatment of T2D (59, 60). Furthermore, numerous human studies report improved glycemic control in type 2 diabetics following adherence to low-carbohydrate, low-glycemic index, and high-protein diets (61, 62). Indeed, beneficial impacts of egg consumption on blood glucose control have been shown in human subjects when combined with energy or carbohydrate restriction (12, 23, 63, 64). For example, Pearce et al. (12) reported improvements in glycemic



**FIGURE 3** The ratio of skeletal muscle p-Akt $^{\text{Ser}473}$ :total Akt (A) and representative Western blot images of skeletal muscle p-Akt $^{\text{Ser}473}$ , total Akt, and total protein (B) pre- and post-insulin injection in lean and ZDF rats fed a casein- or whole egg-based diet for 7 wk. Data are expressed relative to the pre-insulin p-Akt $^{\text{Ser}473}$ :total Akt ratio in lean rats fed the casein-based diet. Data are means  $\pm$  SEMs;  $n = 5-6$ . Bars without a common letter differ ( $P < 0.05$ ). Three-factor mixed ANOVA: insulin,  $P < 0.001$ ; diet,  $P = 0.53$ ; genotype,  $P = 0.157$ ; insulin  $\times$  diet,  $P = 0.571$ ; insulin  $\times$  genotype  $P = 0.11$ ; diet  $\times$  genotype  $P = 0.535$ ; insulin  $\times$  diet  $\times$  genotype,  $P = 0.609$ . LC-Pre, lean casein pre-insulin; LC-Post, lean casein post-insulin; ZC-Pre, ZDF casein pre-insulin; ZC-Post, ZDF casein post-insulin; LWE-Pre, lean whole egg pre-insulin; LWE-Post, lean whole egg post-insulin; ZWE-Pre, ZDF whole egg pre-insulin; ZWE-Post, ZDF whole egg post-insulin. p-Akt, phosphorylated Akt; ZDF, Zucker diabetic fatty.

and lipid profiles in type 2 diabetics following consumption of a hypoenergetic, high-protein diet containing 2 eggs/d. In individuals with metabolic syndrome, Blesso et al. (23) found a reduction in HOMA-IR following consumption of a carbohydrate-restricted diet including 3 eggs/d. In the current study, rodent diets were matched for macronutrient content and there were no differences in final body weight between ZDF rats fed casein- and whole egg-based diets. Taken together,

these findings suggest that reported improvements in glycemic control associated with egg consumption may be related to changes in dietary macronutrient content or improved body weight management, and not a direct effect of egg consumption on skeletal muscle insulin signaling.

A limitation of this study is the quantity of dried whole egg used in the whole egg-based diet, which exceeds the amount of whole egg that would typically be consumed in a human diet. The quantity of dried whole egg was determined such that the casein- and whole egg-based diets were matched for protein content. Additionally, analysis of  $\beta$ -cell mass and glucose-stimulated insulin secretion would provide insight into whether  $\beta$ -cell function declines more rapidly in ZDF rats fed the whole egg-based diet. Lastly, insulin signaling was only analyzed in the EDL muscle. The EDL is frequently used in analysis of skeletal muscle insulin signaling (7, 65–68). However, it is possible that sensitivity for phosphoregulation by insulin may differ in other muscle groups. Future studies will include analysis of skeletal muscle groups composed of different fiber types, as well as additional tissues, to provide a more comprehensive examination of insulin signaling.

In summary, these data suggest that whole egg consumption may impair insulin sensitivity in T2D rats. Although consumption of a whole egg-based diet adversely affected whole body insulin sensitivity in ZDF rats, we were unable to identify changes in skeletal muscle insulin signaling that could explain this finding. Future studies investigating the impact of whole egg consumption on  $\beta$ -cell function may offer a potential explanation for the reduction in fasting serum insulin in ZDF rats fed a whole egg-based diet. Furthermore, dose-response studies are warranted to determine whether the observed impairment in insulin sensitivity is maintained at a lower dose of whole egg.

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The authors' responsibilities were as follows: CJS: designed the study and performed all aspects of animal maintenance, preparation of experimental diets, insulin tolerance testing and laboratory experiments, and drafted the original version of this manuscript; MAS: assisted in animal maintenance and preparation of experimental diets; JLW assisted with insulin tolerance testing; RJV, MJR, and KLS: assisted with the study design; and all authors read and approved the final manuscript.

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