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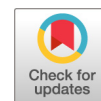
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A Complex Relationship between Immunity and Metabolism in *Drosophila* Diet-Induced Insulin Resistance

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ABSTRACT Both systemic insulin resistance and tissue-specific insulin resistance have been described in *Drosophila* and are accompanied by many indicators of metabolic disease. The downstream mediators of insulin-resistant pathophysiology remain unclear. We analyzed insulin signaling in the fat body studying loss and gain of function. When expression of the sole *Drosophila* insulin receptor (InR) was reduced in larval fat bodies, animals exhibited developmental delay and reduced size in a diet-dependent manner. Fat body InR knockdown also led to reduced survival on high-sugar diets. To look downstream of InR at potential mediators of insulin resistance, transcriptome sequencing (RNA-seq) studies in insulin-resistant fat bodies revealed differential expression of genes, including those involved in innate immunity. Obesity-associated insulin resistance led to increased susceptibility of flies to infection, as in humans. Reduced innate immunity was dependent on fat body InR expression. The peptidoglycan recognition proteins (PGRPs) PGRP-SB2 and PGRP-SC2 were selected for further study based on differential expression studies. Downregulating PGRP-SB2 selectively in the fat body protected animals from the deleterious effects of overnutrition, whereas downregulating PGRP-SC2 produced InR-like phenotypes. These studies extend earlier work linking the immune and insulin signaling pathways and identify new targets of insulin signaling that could serve as potential drug targets to treat type 2 diabetes.

KEYWORDS *Drosophila*, diabetes, innate immunity, insulin receptor, metabolism

Insulin signaling plays an essential role in the control of glucose homeostasis in all animals. After a sugary meal, insulin or insulin-like peptides are secreted into the circulation. These small-peptide hormones bind to (and activate) a highly conserved insulin receptor (InR) in a variety of target tissues. InR activation leads to a signal transduction cascade, including phosphorylation of the kinase Akt and consequent inactivation of the transcription factor FOXO, among other targets. Still, many insulin targets remain unknown. Depending on the target tissue, InR activation can promote glucose uptake, lipogenesis, and glycogen synthesis and can also regulate mitochondrial size and number, cell size, cell division, and differentiation (1–7).

Type 2 diabetes (T2D) is characterized by systemic insulin resistance and exhibits a range of pathophysiology in various target tissues. T2D patients are at increased risk for cardiovascular disease, nonalcoholic fatty liver disease, neuropathy, nephropathy, and retinopathy, although the mechanisms underlying these comorbidities are not fully understood. Another comorbidity in some T2D patients is increased inflammation. Type 2 diabetics exhibit increased infiltration of macrophages into adipose tissue (8, 9) and increased susceptibility to infection. Conversely, patients with some chronic infections

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are more likely to develop insulin resistance (10, 11). To reduce the incidence of these conditions, it is important to understand the genes linking these two pathways.

Drosophila is an efficient and highly sensitive model organism in which to study insulin signaling. Flies express only one InR and one insulin receptor substrate (encoded by *chico*), which are required for both growth and metabolic homeostasis. Insulin-deficient *Drosophila* and insulin-resistant *Drosophila* exhibit reduced size, developmental delay, and hyperglycemia and serve as models of type 1 and type 2 diabetes, respectively (12–14). In *Drosophila*, as in other organisms, a complex relationship between insulin signaling and the immune response exists (15–20). In some settings, increased immunity and increased insulin signaling promote healing: the immune response contributes to the repair of epidermal DNA damage in an InR-dependent manner (21), and epidermal wound healing requires InR (22). In other situations, decreased insulin signaling promotes increased immunity. Chronic FOXO activation (which occurs with decreased insulin signaling) leads to immune activation in the gut (23), and *chico* mutants exhibit increased infection resistance and a reduced bacterial load (24, 25). *Drosophila* FOXO plays a direct role by promoting antimicrobial peptide (AMP) gene expression during starvation, whereas its paralog, Forkhead, directly promotes AMP expression downstream of the target of rapamycin (TOR) (26, 27). Interestingly, overexpressing a constitutively active Toll receptor in the *Drosophila* fat body reduced insulin signaling (17). Therefore, the fly represents a conserved model in which to dissect the interplay between dietary excess, insulin signaling, and the immune response.

Diet-induced insulin resistance can be produced in *Drosophila* by chronic overfeeding using high-calorie diets (28–32). High-sugar (HS)-fed larvae and adult flies develop obesity, dyslipidemia, insulin resistance, decreased fertility, and cardiovascular disease (29–34). Previous studies showed a role for the fat body, a lipid storage tissue akin to mammalian adipose tissue, especially in animals on HS diets. Fat bodies increased lipid storage and developed insulin resistance after HS feeding, compared with control-fed animals. Fat body lipid storage is required for tolerance of HS feeding; genetically lean animals typically exhibit poor survival on HS diets (33, 34). However, roles for the fat body other than lipid storage have been poorly characterized in the context of overnutrition.

In this work, we show a novel role for insulin in controlling the fat body's immune response in the setting of overnutrition. Diet and genotype were manipulated to probe the insulin signaling pathway in the fly. First, a genomic approach identified potential mediators of insulin signaling in the fat body and found a number of differentially expressed (DE) stress, immune, and metabolic genes as well as the transcription factor Seven-up. We show that fat body insulin signaling controls circulating glucose homeostasis, fat storage, and the immune response. In addition, the immune response exerts a surprising influence on diabetes-like phenotypes. Our data support a model in which the fat body serves to balance the competing pathways of inflammation and insulin signaling during overnutrition.

RESULTS

Fat body insulin signaling promotes glucose clearance and lipid homeostasis.

Previously, we and others showed that HS feeding leads to insulin resistance in the *Drosophila* fat body (30–32). Therefore, we took a genetic approach to study the role of fat body insulin signaling during overnutrition by targeting the sole *Drosophila* InR. We used the GAL4/upstream activation sequence (GAL4/UAS) system to elicit transgenic RNA interference (RNAi) in the larval fat body using *r4-GAL4* (38). Fat body *InR* knockdown phenotypes (called *InRⁱ* throughout) were markedly affected by dietary sugar concentrations. A 1 M concentration of sucrose was almost entirely lethal to *r4-GAL4; UAS-InRⁱ* flies (Fig. 1A). Therefore, we focused on 0.7 M sucrose (HS) for our *InRⁱ* overnutrition studies. On control diets (0.15 M sucrose), *r4-GAL4; UAS-InRⁱ* transgenic larvae showed no significant difference in hemolymph glucose concentrations when compared with *w¹¹¹⁸; r4-GAL4* genetically matched controls (Fig. 1B). Upon HS feeding,

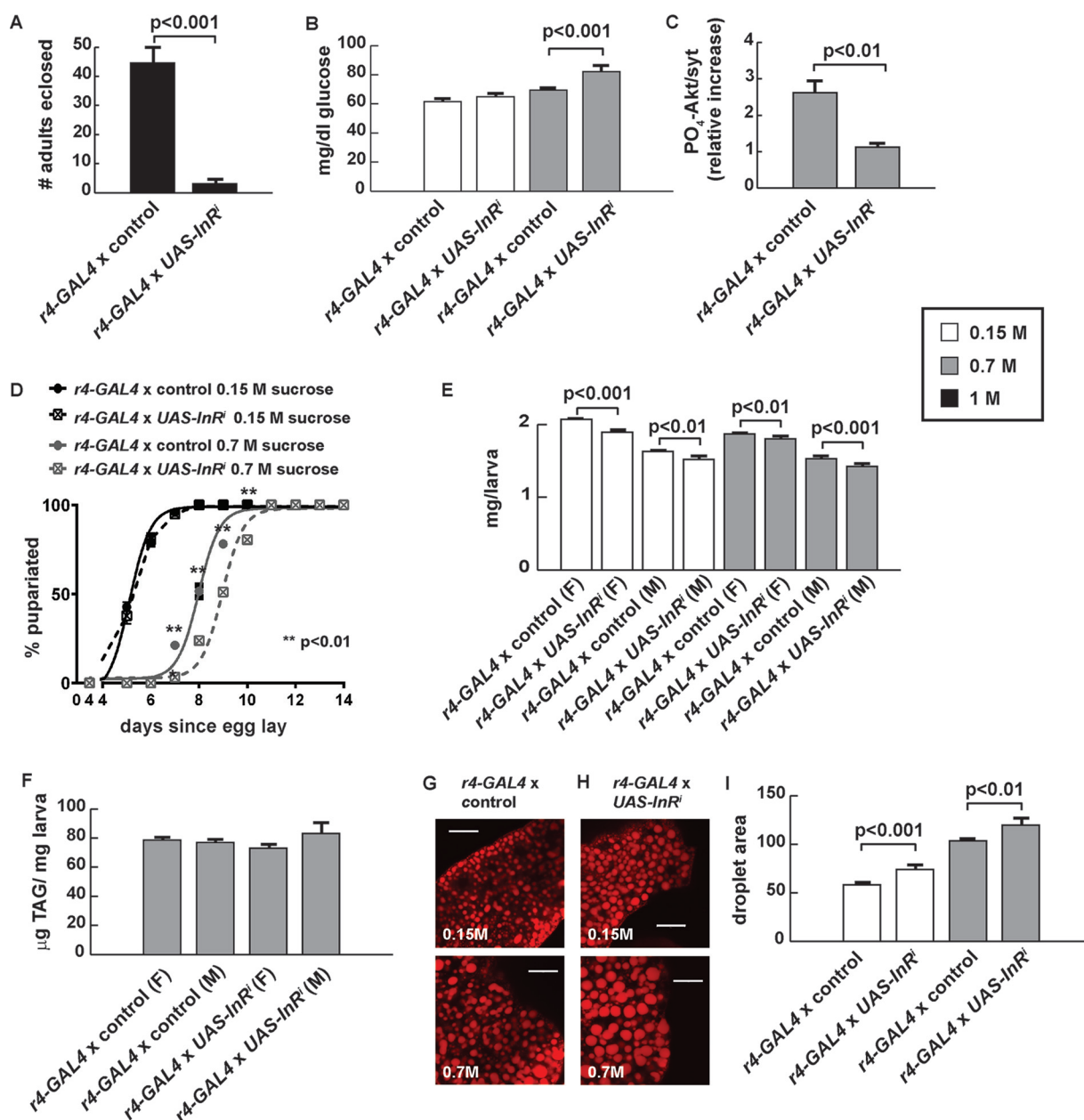
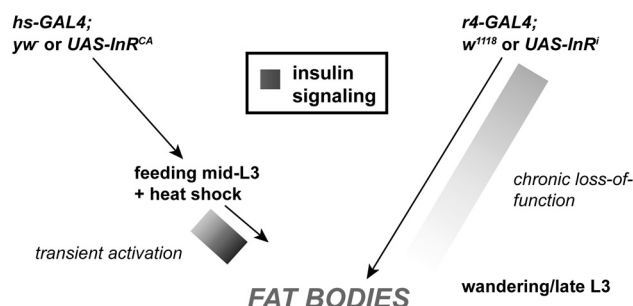


FIG 1 Fat body loss of *InR* exacerbates type 2 diabetes-like phenotypes in overfed *Drosophila*. *r4-GAL4* was used to express double-stranded RNA (dsRNA) encoding a UAS-dependent RNAi transgene, targeting *InR* in the fat body. (A) Wild-type controls survive on 1 M sucrose, whereas very few fat body *InR*ⁱ adults eclose after rearing on this HS diet ($n = 6$ for each genotype). (B) HS (0.7 M) feeding leads to hyperglycemia in fat body *InR* loss-of-function wandering third-instar larvae, compared with controls. No effect on hemolymph glucose concentration is observed when *InR* is reduced in fat bodies of larvae fed the control diet (0.15 M sucrose) ($n \geq 36$ for each genotype and diet combination). (C) Fat body reduction of *InR* expression eliminates insulin-stimulated Akt phosphorylation at serine 505, as expected ($n \geq 9$ for each genotype). (D) HS (0.7 M) feeding leads to developmental delay in fat body *InR* loss-of-function larvae, compared with controls. No developmental delay occurs when *InR*ⁱ larvae are fed the control diet (0.15 M sucrose) ($n \geq 3$ for each genotype and diet combination). (E) *InR* RNAi in fat body reduces whole-animal size in both control and 0.7 M HS-fed wandering third-instar larvae ($n \geq 10$ for each genotype and diet combination). (F) No difference in whole-animal triacylglycerides (TAG) is observed upon fat body *InR* RNAi after rearing on the 0.7 M HS diet ($n \geq 11$ for each genotype). (G to I) Nile Red staining of lipid storage droplets in the larval fat body shows increased droplet size upon fat body *InR* RNAi, compared with controls. Bars, 50 μ m. Thirty-nine different fat body fields were measured for each genotype and diet combination. A two-tailed Student *t* test was used to derive *P* values. Error bars show standard errors of the means (SEM).

however, *r4-GAL4*; UAS-*InR*ⁱ larvae exhibited significant increases in hemolymph glucose compared with *w*¹¹¹⁸; *r4-GAL4* larvae (Fig. 1B). Increased hemolymph glucose or fly “hyperglycemia” correlated with reduced Akt phosphorylation, a downstream marker of *InR* signaling, in insulin-stimulated fat bodies from *r4-GAL4*; UAS-*InR*ⁱ larvae fed HS diets

A. Genetics and sample preparation



B. RNA-seq

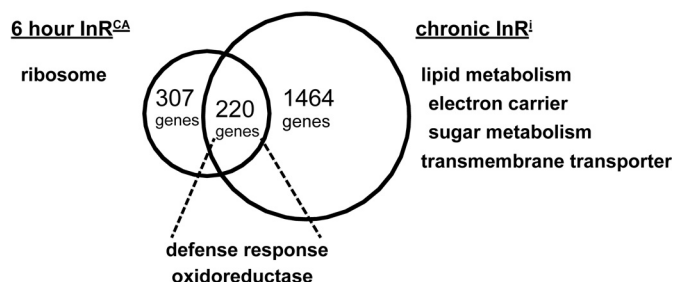


FIG 2 Comparison of *InR* loss- and gain-of function strategies. (A) Fat body loss- and gain-of-function strategies differed in stage and duration. (Left) Constitutively active *InR* was expressed during the mid-third instar via a heat shock promoter and was compared to *InR* in heat-shocked wild-type (*yw*[−]) controls. Samples used for RNA-seq consisted of *yw*[−] or *InR^{CA}* fat bodies isolated from larvae actively feeding on control (5% sugar) diets for 6 h after the heat shock. (Right) *InR* loss-of-function was elicited by dsRNA (*RNAⁱ*) expressed in the fat body throughout larval development on a 0.7 M sucrose diet, compared to fat body driver *r4-GAL4* crossed with *w¹¹¹⁸* controls. Control or chronic *InR^I* fat bodies were isolated from wandering third-instar larvae, when animals could be synchronized due to the developmental delay of *r4-GAL4*; *UAS-InR^I* larvae (*n* = 3 for each genotype). (B) Overlap between the data sets generated by the two strategies, i.e., the common set of *InR* target genes that we considered to be of interest for follow-up studies. DAVID was used to identify potentially interesting genes and pathways in this overlap.

(Fig. 1C). The *r4-GAL4*; *UAS-InR^I* larvae exhibited a significant developmental delay on HS diets (Fig. 1D) as well as a decrease in stage-matched weights on both diets (Fig. 1E), consistent with previously reported phenotypes in insulin-resistant larvae (13, 14, 30, 39). Although no difference in percent body fat was observed (Fig. 1F), fat body lipid droplet size increased when *InR* was reduced (Fig. 1G, H, and I). Thus, decreased insulin signaling in the fat body is sufficient to exacerbate type 2 diabetes-like phenotypes in *Drosophila* challenged with overnutrition.

A genomic approach to identify molecular pathways downstream of *InR*.

Because defects in peripheral tissue insulin signaling during caloric overload are associated with a variety of complications, we set out to discover novel factors or effectors downstream of the insulin receptor. Taking a differential expression approach, we characterized the transcriptomes of control and *UAS-InR^I* fat bodies using transcriptome sequencing (RNA-seq). Conversely, we measured differential expression in control and *UAS-InR^{CA}* fat bodies with transient expression of a constitutively active mutant *InR* (*InR^{A1325D}*) (5) (Fig. 2A). Control and *InR^I*-expressing fat bodies were collected from wandering third-instar larvae after chronic 0.7 M sucrose HS feeding or control feeding, whereas constitutively activated-*InR*-expressing fat bodies were isolated at 6 h after an activating heat shock in *hs-GAL4*; *UAS-InR^{CA}* mid-L3 larvae. By using different paradigms, short term and long term, we predicted that we would identify a core group of insulin-dependent target genes to use as candidates for further testing. Ribo-Zero library prep and Illumina Hi-Seq sequencing followed by genome alignment and EdgeR analysis provided a list of genes exhibiting significant differential expression in each paradigm (Fig. 2B; see also Tables S1 to S3 in the supplemental material). Five hundred

twenty-seven genes were differentially expressed in fat bodies after transient InR activation, with a false-discovery rate of less than 5%. One thousand six hundred eighty-four genes were differentially expressed in InRⁱ fat bodies from larvae fed HS diets, whereas only 176 genes were differentially expressed in InRⁱ fat bodies from larvae on control diets, consistent with the increased severity of phenotypes observed on HS versus control feeding in these larvae. To refine our list of target genes, we considered the overlap between the HS-fed InRⁱ and the InR^{CA} differentially expressed (DE) gene data sets, which consisted of 220 genes (see Table S4 in the supplemental material). Some of these overlapping DE genes encode endoplasmic reticulum (ER) stress response proteins and other proteins with roles in electron transfer as well as in nuclear hormone receptor and adipokinetic hormone (Akh) signaling (Table 1). In particular, *AkhR* expression was increased (1.8-fold) in InRⁱ fat bodies and decreased (−2.3-fold) in InR^{CA} fat bodies. Likewise, expression of the nuclear receptor gene *Seven-up* (*Svp*) was increased (2.4-fold) in InRⁱ fat bodies and decreased (−1.8-fold) in InR^{CA} fat bodies (Table S4). Mechanistic studies have established important roles for both AkhR and Svp in fat body metabolic homeostasis (40), suggesting that our list of targets contains physiologically relevant regulators of insulin signaling in the fat body. Other pathways were highlighted by gene ontology analysis using DAVID (36), including redox biochemistry, metabolism, membrane transport, and the immune response (Fig. 2B). Targets of InR included several metabolic enzymes and translational regulators, similar to what others have shown previously (41–43) (Tables 1 and S4).

As in humans, ER stress may play a role in HS-induced hyperglycemia and insulin resistance. Focusing on the set of 83 genes that were coordinately regulated with increased expression in HS-fed, InRⁱ fat bodies and reduced expression when InR was constitutively active, we noticed two genes encoding proteins with roles in the ER stress response. ER oxidoreductin 1-like (encoded by *CG1333/Ero1L*) helps the ER make disulfide bonds and was increased to 235% in InRⁱ fat bodies and reduced to 46% in InR^{CA} fat bodies (Table 1). P58IPK (encoded by *CG8286*) acts via protein-protein interactions with ER stress kinases and was increased to 198% in InRⁱ fat bodies and reduced to 39% in InR^{CA} fat bodies (Table 1). Because these ER stress response genes were transcriptionally upregulated in previous studies by the ER stressor tunicamycin (44) and ER stress is associated with type 2 diabetes in mammalian adipose tissues (45–47), we set out to define a role for ER stress in HS-induced fly diabetes-like phenotypes. To test a role for ER stress under HS feeding conditions, we used 0.8 M sucrose, which was the maximum that the larvae could tolerate. This 0.8 M HS diet was supplemented with ER stress relief to look for physiological improvements in larvae. The chemical chaperone 4-phenylbutyrate (PBA) was added to fly food at 5 mM, a concentration previously shown to extend life span and ameliorate ER stress in *Drosophila* (48, 49). Larvae were reared to maturity on 0.8 M sucrose HS plus vehicle or PBA food, and we measured insulin-resistant phenotypes. A modest but significant reduction in hemolymph glucose was observed with PBA feeding, consistent with an improvement in insulin signaling (Fig. 3A). In addition, triacylglycerides (TAG) increased modestly in males fed PBA in addition to the 0.8 M HS diet (Fig. 3B). However, no differences in weight were observed between control and PBA-fed HS larvae (Fig. 3C) and we saw no change in insulin signaling at the PO₄-Akt level in isolated fat bodies (Fig. 3D). Next, we used the protein kinase R-like ER kinase (PERK) inhibitor GSK2606414, which has previously been shown to reduce ER stress and to have therapeutic value via dietary supplementation at 10 μ M in flies (50, 51). PERK functions in the ER stress response by phosphorylating the α subunit of eukaryotic initiation factor 2 (eIF2 α) and thereby inhibits translation (52, 53). A modest improvement in hemolymph glucose concentrations was observed in larvae reared on GSK2606414-supplemented food (Fig. 3E). No significant improvements in growth or TAG homeostasis were detected, although a trend toward reduced TAG concentrations was observed (Fig. 3F and G), consistent with a role for ER stress in HS-induced metabolic disease. Finally, we overexpressed an activated form of the transcription factor X-box binding protein 1 (Xbp1s), which protects against ER stress (54, 55), in the fat body. We hypothesized that this might protect tissues from

TABLE 1 Differential expression in fat bodies undergoing short-term InR activation (InR^{CA}) or chronic InR knockdown (InRⁱ)^a

		Fold change	
Gene function and product abbreviation	Gene product full name	lnR ^{CA} /+	lnR ^I /+
ER stress			
P58IPK	P58IPK	−2.56	1.98
Ero1L	Endoplasmic reticulum oxidoreductin 1-like	−2.18	2.35
Hsp70Bb	Heat shock protein 70Bb	5.48	1.91
Immune response, antimicrobial peptides			
Dif	Dorsal-related immunity factor	−1.95	3.45
AttA	Attacin-A	−4.65	5.37
AttB	Attacin-B	−7.19	6.01
IM3	Immune-induced molecule 3	−3.26	377.90
DptB	Diptericin B	−4.97	2.71
CYP450 enzymes			
Cyp28a5	Cyp28a5	−2.56	−2.03
Cyp28d1	Cyp28d1	−2.55	−1.60
Cyp309a1	Cyp309a1	−3.37	−2.79
Cyp4d1	Cytochrome P450-4d1	−2.53	−3.06
Cyp4d14	Cyp4d14	−2.38	1.97
Cyp4g1	Cytochrome P450-4g1	−2.75	2.40
Cyp4p1	Cytochrome P450-4p1	−3.68	1.98
Cyp6a22	Cyp6a22	−3.03	3.08
Cyp6d5	Cyp6d5	−2.02	−2.12
Cyp6t1	Cyp6t1	5.25	−3.21
Cyp9b1	Cytochrome P450-9b1	−3.36	2.68
Cyt-b5-r	Cytochrome b ₅ -related	−2.32	−1.59
Metabolic enzymes, inversely regulated			
IP3K2	Inositol 1,4,5-triphosphate kinase 2	−2.04	1.76
Odc2	Ornithine decarboxylase 2	−2.21	1.73
Pdk	Pyruvate dehydrogenase kinase	−1.91	1.61
Acox57D-p	Acyl coenzyme A oxidase	−2.01	2.00
Ugt58Fa	UDP-glucuronosyltransferase	−2.07	2.31
Pepck	Phosphoenolpyruvate carboxykinase	−2.53	2.45
ATPCL	ATP citrate lyase	1.77	−1.87
Fbp2	Alcohol dehydrogenase	15.53	−1.83
CG3394	Putative fatty acid transporter	2.06	−2.45
CG1969	Glucosamine-phosphate <i>N</i> -acetyltransferase 1	2.69	−1.84
CG30463	Polypeptide <i>N</i> -acetylgalactosaminyltransferase	2.33	−2.22
Metabolic enzymes, coordinately regulated			
Sodh-1	Sorbitol dehydrogenase 1	−2.67	−28.65
Tpi	Triose phosphate isomerase	−2.09	−3.06
Pgm	Phosphogluconate mutase	−2.08	−2.23
Spat	Serine pyruvate aminotransferase	−2.13	−2.36
Got2	Glutamate/oxaloacetate transaminase 2	−2.33	−2.52
Mdh2	Malate dehydrogenase 2	−1.92	−1.85
Mdh1	Malate dehydrogenase 1	−2.11	−1.56
Aldh	Aldehyde dehydrogenase	−2.41	−1.76
AKHR	Adipokinetic hormone receptor	−2.30	1.83
Ribosome			
RpL23	Ribosomal protein L23	−1.84	−1.62
RpL24	Ribosomal protein L24	−2.05	−1.52
RpLP2	Ribosomal protein LP2	−2.10	−2.26
RpL4	Ribosomal protein L4	−2.03	1.65

^aInR target genes in the fat body indicate conserved biology between human and fly insulin resistance, including ER stress, the immune response, redox biochemistry, translation, and key metabolic pathways.

HS-induced metabolic damage, but *r4-GAL4; UAS-Xbp1s* larvae fared poorly. At a concentration of 0.7 M sucrose, we were able to collect significant numbers of these transgenic larvae but observed no improvements (Fig. 3H to J). Thus, while ER stress may play a role in our model of insulin resistance during caloric overload, it does not seem to play a major role in the fat body.

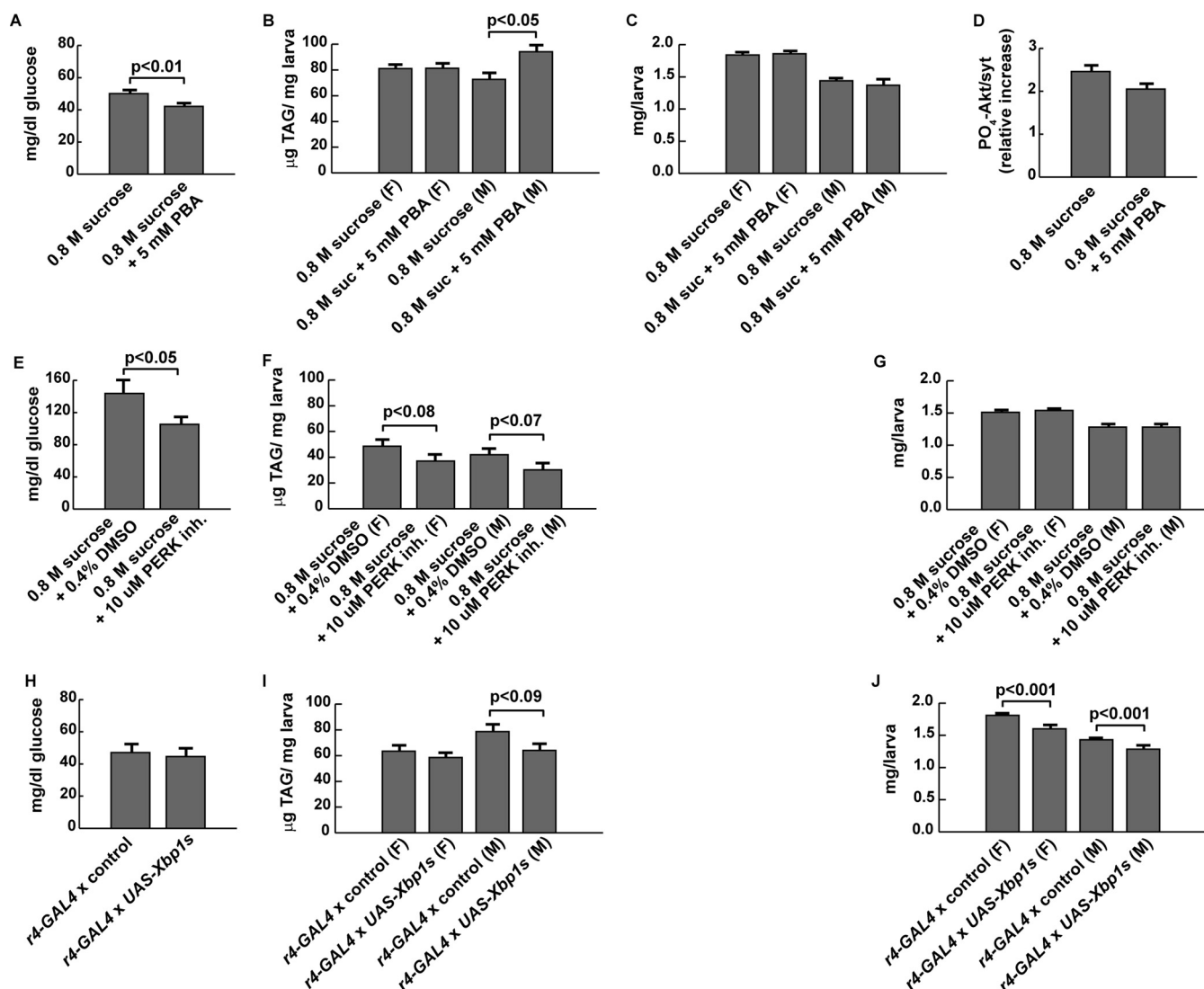


FIG 3 ER stress may play a role in HS-induced hyperglycemia and insulin resistance. Modulation of ER stress during development had mixed effects on wandering third-instar larvae reared on a 0.8 M sucrose HS diet (A to G) or a 0.7 M sucrose HS diet (H to J). (A) Hemolymph glucose concentrations were reduced by feeding HS supplemented with 5 mM 4-phenylbutyrate (PBA) ($n \geq 44$). (B) Percent body fat in female (F) and male (M) larvae reared on both diets ($n \geq 11$). (C) Larval weight at maturity after rearing on HS or PBA-supplemented HS diets ($n \geq 11$). (D) Akt phosphorylation was quantified in the presence of 0.5 μM insulin relative to loading control in fat bodies from larvae reared on HS or PBA-supplemented HS diets ($n = 9$). (E) Hemolymph glucose concentrations in wild-type larvae fed 0.8 M sucrose supplemented with 10 μM PERK inhibitor (inh.) GSK2606414 ($n \geq 11$). (F) PERK inhibitor seems to modestly decrease stored larval TAG ($n \geq 6$). (G) No effect of PERK inhibitor on larval weight was observed ($n \geq 6$). (H) Fat body overexpression of the spliced *Xbp1* cDNA (*Xbp1s*) was unable to reduce hyperglycemia in larvae fed 0.7 M HS diets, compared with driver-crossed controls ($n \geq 24$). (I) Fat body overexpression of *Xbp1s* led to modest effects on TAG accumulation in male larvae ($n \geq 10$). (J) Fat body overexpression of *Xbp1s* reduced larval weight in both sexes ($n \geq 10$). A two-tailed Student *t* test was used to derive *P* values. Error bars show SEM.

A yin and yang relationship exists between insulin signaling and immunity.

Another pathway highlighted by RNA-seq analyses that has been shown to intersect with insulin signaling in other studies is the immune response (17, 26). Hemolymph from larvae fed control diets melanizes quickly, but HS-fed hemolymph demonstrated a marked reduction in melanization, consistent with a reduced innate immune response (Fig. 4A). Differential expression studies in larval fat body and whole larval and adult *Drosophila* flies also supported a role for the immune response: many immune genes were differentially expressed in the contexts of HS feeding, InR reduction, or InR activation (Table 1; see also Tables S1, S2, S3, S4, S5, and S6 in the supplemental material) (30, 33). Therefore, we asked whether the immune response was affected in HS-fed or *r4-GAL4; UAS-InRⁱ* larvae. We induced septic injury using the Gram-negative

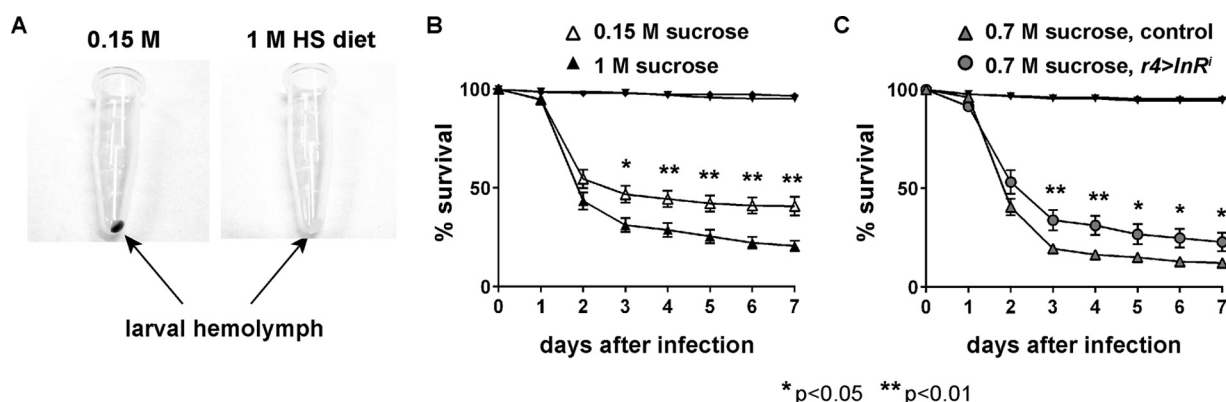


FIG 4 Attenuation of the immune response by HS feeding. (A) Hemolymph isolated from wandering third-instar larvae reared on 0.15 M sucrose melanizes within 5 min at room temperature (left), whereas little color is observed in 5-min-old hemolymph isolated from larvae reared on 1 M sucrose (right). (B) Seven-day survival of adults reared on 0.15 M or 1 M sucrose diets. Three- to 7-day-old wild-type (*w¹¹¹⁸*; *r4-GAL4* transheterozygous) flies were infected with a semilethal dose of *Pseudomonas aeruginosa*, and survival was monitored for 7 days ($n \geq 23$ vials). (C) Seven-day survival of adults reared on a 0.7 M sucrose diet. Three- to 7-day-old control (*w¹¹¹⁸*; *r4-GAL4* transheterozygous) and *InRⁱ* (*r4-GAL4>UAS-InR*) fly survival over 7 days after inoculation with *P. aeruginosa* ($n \geq 19$ vials). Control flies pricked without *P. aeruginosa* are shown at the top of each graph ($n \geq 13$ vials for each). A two-tailed Student *t* test was used to derive *P* values. Error bars show SEM.

bacterium *Pseudomonas aeruginosa* (56) at a concentration at which many but not all animals typically die (see Materials and Methods for details). A significant fraction of larvae died after a clean pricking injury (data not shown) as reported by others (57). Therefore, we continued with adult infection studies. Consistent with a diminished immune response indicated by reduced immune gene expression after HS feeding (Tables S5 and S6), HS-reared adults exhibited increased infection-based mortality after 1 week (79%), compared with control-diet-reared flies (59%) (Fig. 4B), consistent with a reduction in immune function. Clean pricking (without the pathogen) produced a negligible reduction in 7-day adult survival regardless of diet (Fig. 4). Given the finding that loss-of-function in insulin receptor signaling increased the expression of immune response genes (Tables 1, S1, and S2), we hypothesized that the *r4-GAL4; UAS-InRⁱ* loss-of-function flies would be protected from death after infection. Indeed, this was the case, as 23% of *r4-GAL4; UAS-InRⁱ* loss-of-function flies survived acute infection, compared with a 12% survival for control flies fed the same moderately high-sugar diet (0.7 M sucrose) (Fig. 4C). Therefore, reducing *InR* in the fat body can partially rescue the increased infection susceptibility observed during overnutrition.

Peptidoglycan recognition protein RNAi produces insulin-like phenotypes.

Several members of the immune gene family encoding the peptidoglycan recognition proteins (PGRPs) were differentially expressed along with insulin signaling (Table 1). We chose two of these for further study. *PGRP-SB2* is selectively expressed in the larval fat body according to FlyAtlas (58) and is downregulated by HS feeding and by *InR* RNAi in the fat body. We knocked down *PGRP-SB2* and observed a significant improvement in growth and increase in the number of flies surviving 1 M sucrose (Fig. 5A and B). *PGRP-SC2*, in contrast to *PGRP-SB2*, was upregulated by expressing *InR* RNAi in the fat body. Knockdown of *PGRP-SC2* led to the opposite phenotypes of *PGRP-SB2*, displaying sugar-specific lethality and reduced size during caloric overload, compared with controls (Fig. 5C and D). Other HS-associated phenotypes showed less striking influences of PGRP knockdown (see Fig. S1 in the supplemental material). There was not a rescue of the HS-induced developmental delay in *r4-GAL4; UAS-PGRP-SB2ⁱ* larvae (Fig. S1A). Fat body *PGRP-SB2* RNAi did not improve hemolymph glucose homeostasis or triglyceride content (Fig. S1B and C), and *r4-GAL4; UAS-PGRP-SB2ⁱ* fat bodies did not have improved insulin signaling, at least as measured by phospho-Akt level (Fig. S1D). *PGRP-SC2* RNAi did have minor effects on TAG content (Fig. S1E) and infection susceptibility (Fig. S1F). As in fat body *InR* RNAi adult flies, *PGRP-SC2* RNAi improved infection resistance despite reducing growth and survival on HS diets, although the data were significant only at one time point (Fig. S1F). On the other hand, *PGRP-SB2* RNAi adults seemed to exhibit

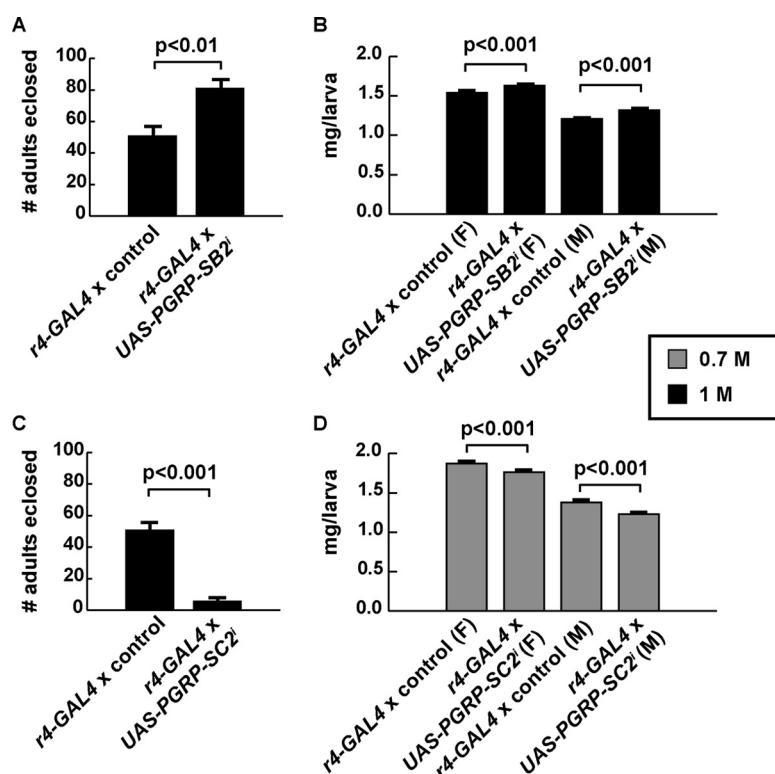


FIG 5 Peptidoglycan recognition proteins (PGRPs) are novel growth regulators downstream of InR. (A) Reducing PGRP-SB2 in the fat body leads to increased survival after rearing on 1 M sucrose HS diets compared with controls (*r4-GAL4* crossed to line number 60100 control genetic background flies) ($n \geq 8$ for each genotype). (B) Reducing PGRP-SB2 also increased larval size at maturity, compared with wild-type controls ($n \geq 15$ for each genotype). (C) PGRP-SC2 RNAi in fat body reduces survival of larvae reared on 1 M sucrose HS diets ($n \geq 5$ for each genotype). Due to the high degree of lethality of 1 M HS diets on these animals, we analyzed them on 0.7 M sucrose HS diets. (D) Larval size is reduced by fat body PGRP-SC2 RNAi ($n \geq 15$ for each genotype). A two-tailed Student *t* test was used to derive *P* values. Error bars show SEM.

a slight impairment in infection resistance, which approached statistical significance only in 0.7 M-reared animals (Fig. S1G and H). These data support a model in which PGRP-SB2 impairs insulin signaling and PGRP-SC2 promotes it.

Identification of novel potential InR-dependent promoters. To search for genomic elements that confer insulin sensitivity, we characterized potential regulatory mechanisms for two gene lists: (i) genes decreased in InRⁱ fat bodies and increased in InR^{CA} fat bodies (45 genes) and (ii) genes decreased in InR^{CA} fat bodies and increased in InRⁱ fat bodies (83 genes). Known TF binding sites, as well as *de novo* 5- to 12-base sequences, were identified in promoters (−700 to +100 with respect to the transcriptional start site [TSS]) of the genes in each of the two lists. Several such sequence elements were identified as enriched in these data and may serve as sensors of insulin signaling (Fig. 6).

DISCUSSION

In this work, we link two established functions of the fat body, insulin signaling and the immune response, and show that they vary reciprocally to one another at the transcriptional level. Increasing insulin signaling led to reduced immune gene expression, and decreasing insulin signaling led to increased immune gene expression and increased resistance to infection. Surprisingly, we found that HS diet-induced obesity and growth defects could be regulated by fat body misexpression of immune response genes. This cell-autonomous molecular relationship complements previous studies in both flies and mammals (16, 19, 20, 59) and supports a counterregulatory model in which insulin signaling and the immune response negatively regulate each other to maintain energy balance.

Motif	p value	% of targets	Best match
GATGCCAGCTAA	1 e-12	12.99	POLO10
ATAAGCAACAT	1 e-10	11.69	Forkhead
CAACACTCTT	1 e-10	18.18	Unknown
GCTGATAAGA	1 e-10	29.87	Unknown
CCGCTGGGCAGC	1 e-10	10.39	POLO08
CATCCAGAC	1 e-9	19.51	Paired
ATCTCAAAAT	1 e-9	14.63	Caudal

FIG 6 Promoter elements common to InR target genes. The top 5 predicted sites were found within a predicted promoter region (−700 to +100 with respect to the TSS) in genes with increased expression in InRⁱ fat bodies and decreased expression in InR^{CA} fat bodies. The bottom two consensus sequences were common to the promoter regions of genes with decreased expression in InRⁱ fat bodies and increased expression in InR^{CA} fat bodies. Only the first site (GATGCCAGCTAA) was considered a strong and significant match; the other six motifs are weakly enriched.

Using differential expression to search for potential downstream regulators of insulin signaling in the fat body, we focused on selected gene ontology categories based on the literature. A number of known FOXO targets were identified, as expected (60–62). Ribosomal subunits were also overrepresented, consistent with previous studies showing a direct relationship between insulin signaling and ribosome biogenesis (41, 63). This DE gene list was therefore likely to include additional, novel targets of physiological importance. Most (58%) of the overlapping DE genes were inversely regulated between constitutively active and reduced InR function. Many, however, were regulated the same direction in both paradigms, which was unexpected. The former are likely to be more-direct targets and/or sensors of insulin pathway activity. The latter group of genes are likely to have more-complicated relationships with the insulin signaling pathway, such as stage- or diet-specific roles. Changes in gene expression might represent downstream effects of insulin signaling or counterregulatory attempts to fix cellular growth or metabolic defects during insulin resistance. One counterregulatory attempt might be the observed increase in fat body *PGRP-SC2* expression in InRⁱ fat bodies, which might be expected to improve downstream aspects of insulin signaling. Another potential compensatory mechanism for insulin resistance in InRⁱ fat bodies is the gene *ilp6*, which encodes a growth promoter during metamorphosis (64) and increases in expression in InRⁱ fat bodies on both control and HS diets (351% and 385%, respectively; for both comparisons, $P < 0.001$). The promoter elements identified using bioinformatics analysis may serve as key markers for and/or mediators of insulin signaling. Future studies will explore these possibilities.

Many fat body DE genes represent conserved pathways that are of clinical importance in the pathophysiology of type 2 diabetes. Both HS and InR knockdown in the fat body increased the expression of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting step of gluconeogenesis (Table 1) (33). The insulin-resistant fat body may therefore act somewhat like the insulin-resistant liver, which exhibits an increase in hepatic gluconeogenesis in type 2 diabetes in some rodent and human studies (65, 66). InRⁱ also led to increased pyruvate dehydrogenase kinase (PDK) and decreased ATP citrate lyase expression, both of which could inhibit glycolytic flux. Reducing glycolysis contributes to hyperglycemia and insulin resistance in some genetic forms of type 2 diabetes (67, 68) and is the basis of the competitive fatty acid versus glucose utilization model known as the Randle cycle (69, 70). In addition, we observed significant changes in the expression of cytochrome P450 enzymes and well as regulators of fatty acid and polyamine metabolism, protein glycosylation, and the immune response. We set out to understand which of these InR DE genes represented physiological changes in insulin-resistant flies.

First, we looked at ER stress, also known as the unfolded protein response. During

ER stress, the accumulation of aberrant proteins leads to compensatory increases in degradation or refolding chaperones. ER stress is relieved by clearing the ER lumen of misfolded proteins and by reducing translation (71). ER stress increases in obese, high-calorie-fed, T2D patients, and reducing ER stress can improve T2D in rodents (45, 47, 72). We saw a transcriptional upregulation of genes that promote the ER stress response along with a downregulation of ribosomal genes in insulin-resistant fat bodies. Surprisingly, there was only a minor role for ER stress in fat body insulin signaling, if any. One possibility is that the upregulation of ER stress response genes can offset an early increase in ER stress, such that healthy levels of stress can be maintained.

Both differential expression and septic injury studies pointed to an important and conserved relationship between insulin resistance and the immune response. Type 2 diabetic patients exhibit increased susceptibility to infection, and some infections increase insulin resistance (11, 73–75). Likewise, in the *Drosophila* HS diet-induced T2D model, systemic insulin resistance and obesity caused by HS feeding also corresponded with reduced survival after septic injury. This parallel between flies and humans is consistent with a recently established model in which diet-induced obesity paradoxically increases inflammation but reduces immune function (8, 9, 18). The increase in immune gene expression in *InR*ⁱ fat bodies corresponded to improved resistance to infection in these animals, which is perhaps a surprising result, considering the increased susceptibility to infection in HS-reared flies. Reducing *InR* in this context seems to negate the detrimental effects of an HS diet, such that genetically engineered insulin resistance acted protectively, boosting the immune response. Our results are consistent with a previously reported role for the transcription factor FOXO, a negative regulator of insulin signaling, which can directly induce immune gene expression in flies and mammalian cells (26, 76). Interestingly, previous studies in *Drosophila* have shown that infection or activation of the tumor necrosis factor (TNF) receptor or Toll pathway can inhibit insulin secretion and signaling (16, 17, 77), whereas eliminating macrophages improves insulin sensitivity (78). Our results support a negative-feedback model in which insulin signaling inhibits innate immunity and immunity inhibits insulin signaling, a relationship that is strained on an HS diet. This mechanism would help to maintain metabolic homeostasis when nutrients are limited via reallocation of fat body resources to fighting infection when necessary instead of to promotion of growth or energy storage. Such a model has been proposed (18, 79).

A wide variety of antimicrobial peptides and peptidoglycan recognition proteins (PGRPs) are secreted by the fat body during the *Drosophila* immune response, which typically recognizes the presence of infection via the Toll or IMD pathways, converging on the transcription factor NF- κ B to promote pathogen recognition and elimination, as in humans (80, 81). Taking a candidate gene approach, we focused on two genes in the PGRP family, *PGRP-SB2* and *PGRP-SC2*. *PGRP-SB2* expression was increased in *InR*^{CA} fat bodies, whereas *PGRP-SC2* mRNA was increased in *InR*ⁱ fat bodies, suggesting opposing functions for these two proteins. Reducing *PGRP-SB2* in the fat body led to increased growth and survival when challenged with HS feeding, suggesting that it exerts a negative effect on insulin signaling. In contrast, fat body RNAⁱ targeting *PGRP-SC2* gave phenotypes similar to those induced by *InR* RNAⁱ, including increased lethality and reduced size on HS diets, suggesting that *PGRP-SC2* is protective against HS-induced metabolic toxicity. An increase in infection resistance, albeit at a single time point (Fig. S2F), is consistent with previous reports that *PGRP-SC2* negatively regulates IMD/Relish-dependent immune gene expression (23, 82). Interestingly, other studies have found no role for *PGRP-SB* or *PGRP-SC* in susceptibility to infection despite their effects on immune gene expression (23, 83, 84). Although the mechanisms underlying the different phenotypes in these *PGRP-SB2*ⁱ and *PGRP-SC2*ⁱ flies are still poorly understood, they are likely to fit into the narrative established by previous investigators showing a complex relationship between immunity and insulin signaling. Future studies will explore the roles of these and other *InR* target genes and putative *InR* target promoters in the insulin response.

These studies represent a simple paradigm in which to identify insulin targets in

different cells, organs, and tissues that exhibit insulin-dependent phenotypes, such as the heart, eye, brain, gut, gonad, or muscle. Because the complications of type 2 diabetes affect many organs and systems, probing transcriptional targets of InR in these and other tissues may reveal additional, tissue-specific genes and mechanisms that contribute to insulin resistance.

MATERIALS AND METHODS

Fly lines. Controls and RNAi lines were obtained from the Vienna Drosophila Resource Center (VDRC). VDRC's *w¹¹¹⁸* line (stock line number 60000) and RNAi-TK landing site control (60100) were used as genetic background controls for RNAi transgenes targeting InR (number 992), PGRP-SB2 (number 106538), and PGRP-SC2 (number 104578). *hs-GAL4* (number 2077) and *UAS-InR.A1325D* (number 8263) lines were from the Bloomington Drosophila stock center. The *r4-GAL4* line was from reference 38, and the *UAS-Dcr2* line was used in all crosses to amplify RNAi (85). Offspring of the control cross between line number 60100 and the *UAS-Dcr2*; *r4-GAL4* line were used to test the ER stress drugs (Fig. 3). *UAS-Xbp1s* flies were a gift from Diego Rincon-Limas (54).

Drosophila husbandry. The lab maintenance diet was a standard corn meal-dextrose-agar diet. Experimental diets consisted of Bloomington's Semidefined food with sucrose used for all sugar at concentrations of 0.15 M (5%), 0.7 M (24%), 0.8 M (27%), and 1 M (34% sucrose), as used in previous studies (30, 33); 5 mM 4-phenylbutyrate (PBA; Sigma number P21005) and 10 μ M PERK inhibitor (GSK2606414) were added to the diet as indicated, and these diets were compared to diets using an equivalent amount of the appropriate solvent or diluent (water or dimethyl sulfoxide [DMSO]). Heat shock treatments were done for 30 min in empty fly vials using a 37°C water bath. Six hours of recovery after heat shock gave robust phosphorylation of Akt, and this duration was therefore used for the RNA-seq experimental samples.

Gene expression. RNA-seq experiments were done as described previously (33) using three biological replicates for each genotype and/or diet. Briefly, fat bodies were separated from the rest of the animal after bisecting, inverting, and placing the insect into phosphate-buffered saline (PBS). Fat bodies were dislodged by pipetting and separated by centrifugation; the purity of fat body tissue was checked on a slide before freezing in TRIzol. RNA was extracted and DNase and RiboZero treated, and then libraries were prepared and Illumina Hi-seq sequenced and data were processed by the Washington University Genome Technology Access Center. The Tuxedo Suite (86) and EdgeR (35) were used to detect differentially expressed transcripts with a *P* value of <0.01 and a false-discovery rate of <0.05. DAVID was used to characterize the gene ontology of differentially expressed gene lists with a *P* value cutoff of <0.05 (36). Microarray studies on adult Canton-S flies were done as previously described (30).

Hemolymph glucose assays. Hemolymph glucose assays were done as described previously (33). Briefly, larvae were rinsed in a sieve to remove any food from the cuticle surface and then dried. Jeweler's fine forceps were used to injure the animal, and hemolymph was collected and then added to frozen Infinity glucose reagent (Thermo number TR15421) to suppress melanization and trehalase activity. The optical density at 340 nm (OD_{340}) was read after 10 min at 37°C using a microplate spectrophotometer and corrected to a blank well, and then the concentration of glucose was determined using a standard curve.

Triglyceride assays. Triglyceride assays were done as described previously (33). Briefly, wandering third-instar larvae were rinsed and dried and then weighed and frozen at -80°C until the assay. PBS–0.1% Tween was used to homogenize larvae. Tubes were incubated at 65°C for 5 min and then cooled to room temperature. An aliquot was added to Thermo Infinity triglyceride reagent (number TR22421), and the mixture was incubated at 37°C for 5 to 10 min and then quantified using a microplate spectrophotometer at OD_{540} and a standard curve and blank well.

Insulin stimulation and Western blotting. Insulin stimulation experiments were done as previously described (33). Briefly, larvae were rinsed, bisected, inverted, and placed in Schneider's medium. Insulin (1 μ M; Sigma I2643) or dilution buffer (10 mM HEPES) was added, and larvae were cultured for 15 min. Fat bodies were harvested and frozen in sample buffer until Western blotting was performed. The PO_4 -Akt band intensity was normalized to syntaxin as a loading control. Primary antibodies used were from Cell Signaling (anti- PO_4 -Akt at Ser505, number 4054) and the Developmental Studies Hybridoma Bank (antisyntaxin, number 8C3).

Nile Red staining and lipid droplet quantification. Larvae were inverted to expose fat bodies, fixed for 30 min in 4% paraformaldehyde in PBS, and then washed 3 times, 10 min each time, in PBS and 0.1% Triton X (PBS-TX) and stained in PBS-TX + 0.001% Nile Red (Acros number 200007-168) for 1 to 1.5 h. Larvae were washed again in PBS-TX and mounted for confocal microscopy using a laser excitation of 543 nm. ImageJ software was used to quantify droplet sizes.

Infection assays. *Pseudomonas aeruginosa* strain PA14 was grown overnight and diluted to an A_{600} of 0.005, which corresponded to approximately 3.0×10^7 CFU/ml. Adults were anesthetized in CO_2 and injured using a 0.001-mm-tip tungsten needle (Fine Science Tools number 10130-05) dipped in culture and then allowed to wake, and viability was monitored for 7 days. Larval infection susceptibility was measured by septic injury during the wandering third instar, and then viability was monitored for 10 days, spanning metamorphosis and eclosion. Animals stabbed without inoculum were used as a control and to determine the extent of death not associated with the pathogen.

Bioinformatics motif discovery. We used the MatInspector program (37, 87) in Genomatix Genome Analyzer (GGA) v3.2, to identify known transcription factor binding sites common to at least 85% of the sequences in each gene list. We searched the putative promoter sequences (lengths optimized by GGA)

against Genomatix's Matrix Family Library version 9.1, including all vertebrate matrices, insect matrices, and Core Promoter Elements (where a match required 0.75 similarity to the core, most conserved positions of the binding site, as well as similarity above a matrix-specific cutoff). We used the HOMER (Hypergeometric Optimization of Motif EnRichment) suite of tools (88) to perform *de novo* motif discovery, i.e., identify novel binding sites within the putative promoter sequences of genes in each list. We defined the promoter sequences as bases -700 to $+100$ of the TSS and looked for motifs of length 5 through 12 that are enriched relative to the rest of the genome.

Accession number(s). Complete RNA-seq and microarray data are accessible at GEO (<https://www.ncbi.nlm.nih.gov/geo/>) via accession numbers [GSE97447](#) (InR⁺ data), [GSE96763](#) (InR^{CA} data), and [GSE105448](#) (adult *Canton-S* data).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/MCB.00259-17>.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.

SUPPLEMENTAL FILE 3, XLSX file, 0.2 MB.

SUPPLEMENTAL FILE 4, XLSX file, 0.1 MB.

SUPPLEMENTAL FILE 5, XLSX file, 0.1 MB.

SUPPLEMENTAL FILE 6, XLSX file, 0.1 MB.

SUPPLEMENTAL FILE 7, XLSX file, 0.1 MB.

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REFERENCES

- Liu H-Y, Yehuda-Shnaidman E, Hong T, Han J, Pi J, Liu Z, Cao W. 2009. Prolonged exposure to insulin suppresses mitochondrial production in primary hepatocytes. *J Biol Chem* 284:14087–14095. <https://doi.org/10.1074/jbc.M807992200>.
- Parra V, Verdejo HE, Iglewski M, Del Campo A, Troncoso R, Jones D, Zhu Y, Kuzmich J, Pennanen C, Lopez-Crisosto C, Jaña F, Ferreira J, Noguera E, Chiong M, Bernlohr DA, Klip A, Hill JA, Rothermel BA, Abel ED, Zorzano A, Lavandro S. 2014. Insulin stimulates mitochondrial fusion and function in cardiomyocytes via the Akt-mTOR-NFκB-Opa-1 signaling pathway. *Diabetes* 63:75–88. <https://doi.org/10.2337/db13-0340>.
- Das R, Sebo Z, Pence L, Dobens LL. 2014. *Drosophila* tribbles antagonizes insulin signaling-mediated growth and metabolism via interactions with Akt kinase. *PLoS One* 9:e109530. <https://doi.org/10.1371/journal.pone.0109530>.
- Luo J, Liu Y, Nässel DR. 2013. Insulin/IGF-regulated size scaling of neuroendocrine cells expressing the bHLH transcription factor Dimmed in *Drosophila*. *PLoS Genet* 9:e1004052. <https://doi.org/10.1371/journal.pgen.1004052>.
- DiAngelo JR, Birnbaum MJ. 2009. Regulation of fat cell mass by insulin in *Drosophila melanogaster*. *Mol Cell Biol* 29:6341–6352. <https://doi.org/10.1128/MCB.00675-09>.
- Saucedo LJ, Gao X, Chiarelli DA, Li L, Pan D, Edgar BA. 2003. Rheb promotes cell growth as a component of the insulin/TOR signalling network. *Nat Cell Biol* 5:566–571. <https://doi.org/10.1038/ncb996>.
- Bouskila M, Hirshman MF, Jensen J, Goodyear LJ, Sakamoto K. 2008. Insulin promotes glycogen synthesis in the absence of GSK3 phosphorylation in skeletal muscle. *Am J Physiol Endocrinol Metab* 294:E28–E35. <https://doi.org/10.1152/ajpendo.00481.2007>.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808. <https://doi.org/10.1172/JCI200319246>.
- Lumeng CN, Bodzin JL, Saltiel AR. 2007. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 117:175–184. <https://doi.org/10.1172/JCI29881>.
- Hsieh M-C, Wang SSW, Hsieh Y-T, Kuo F-C, Soon M-S, Wu D-C. 2013. *Helicobacter pylori* infection associated with high HbA1c and type 2 diabetes. *Eur J Clin Invest* 43:949–956. <https://doi.org/10.1111/eci.12124>.
- Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J. 2003. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. *Gastroenterology* 125:1695–1704. <https://doi.org/10.1053/j.gastro.2003.08.032>.
- Rulifson EJ, Kim SK, Nusse R. 2002. Ablation of insulin-producing neurons in flies: growth and diabetic phenotypes. *Science* 296:1118–1120. <https://doi.org/10.1126/science.1070058>.
- Bohni R, Riesgo-Escovar J, Oldham S, Brogiolo W, Stocker H, Andrus B, Beckingham K, Hafen E. 1999. Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1–4. *Cell* 97:865–875. [https://doi.org/10.1016/S0092-8674\(00\)80799-0](https://doi.org/10.1016/S0092-8674(00)80799-0).
- Shingleton AW, Das J, Vinicius L, Stern DL. 2005. The temporal requirements for insulin signaling during development in *Drosophila*. *PLoS Biol* 3:e289. <https://doi.org/10.1371/journal.pbio.0030289>.
- Dionne M. 2014. Immune-metabolic interaction in *Drosophila*. *Fly (Austin)* 8:75–79. <https://doi.org/10.4161/fly.28113>.
- Dionne MS, Pham LN, Shirasu-Hiza M, Schneider DS. 2006. Akt and FOXO dysregulation contribute to infection-induced wasting in *Drosophila*. *Curr Biol* 16:1977–1985. <https://doi.org/10.1016/j.cub.2006.08.052>.
- DiAngelo JR, Bland ML, Bambina S, Cherry S, Birnbaum MJ. 2009. The immune response attenuates growth and nutrient storage in *Drosophila*.

- by reducing insulin signaling. *Proc Natl Acad Sci U S A* 106:20853–20858. <https://doi.org/10.1073/pnas.0906749106>.
18. Hotamisligil GS. 2017. Inflammation, metaflammation and immunometabolic disorders. *Nature* 542:177–185. <https://doi.org/10.1038/nature21363>.
 19. Rynes J, Donohoe CD, Frommolt P, Brodessa S, Jindra M, Uhlirva M. 2012. Activating transcription factor 3 regulates immune and metabolic homeostasis. *Mol Cell Biol* 32:3949–3962. <https://doi.org/10.1128/MCB.00429-12>.
 20. Clark RI, Tan SWS, Péan CB, Roostalu U, Vivancos V, Bronda K, Pilátová M, Fu J, Walker DW, Berdeau R, Geissmann F, Dionne MS. 2013. MEF2 is an in vivo immune-metabolic switch. *Cell* 155:435–447. <https://doi.org/10.1016/j.cell.2013.09.007>.
 21. Karpac J, Younger A, Jasper H. 2011. Dynamic coordination of innate immune signaling and insulin signaling regulates systemic responses to localized DNA damage. *Dev Cell* 20:841–854. <https://doi.org/10.1016/j.devcel.2011.05.011>.
 22. Kakanj P, Moussian B, Grönke S, Bustos V, Eming SA, Partridge L, Leptin M. 2016. Insulin and TOR signal in parallel through FOXO and S6K to promote epithelial wound healing. *Nat Commun* 7:12972. <https://doi.org/10.1038/ncomms12972>.
 23. Guo L, Karpac J, Tran SL, Jasper H. 2014. PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell* 156:109–122. <https://doi.org/10.1016/j.cell.2013.12.018>.
 24. Libert S, Chao Y, Zwiener J, Pletcher SD. 2008. Realized immune response is enhanced in long-lived puc and chico mutants but is unaffected by dietary restriction. *Mol Immunol* 45:810–817. <https://doi.org/10.1016/j.molimm.2007.06.353>.
 25. McCormack S, Yadav S, Shokal U, Kenney E, Cooper D, Eleftherianos I. 2016. The insulin receptor substrate Chico regulates antibacterial immune function in *Drosophila*. *Immun Ageing A* 13:15. <https://doi.org/10.1186/s12979-016-0072-1>.
 26. Becker T, Loch G, Beyer M, Zinke I, Aschenbrenner AC, Carrera P, Inhester T, Schultze JL, Hoch M. 2010. FOXO-dependent regulation of innate immune homeostasis. *Nature* 463:369–373. <https://doi.org/10.1038/nature08698>.
 27. Varma D, Bülow MH, Pesch Y-Y, Loch G, Hoch M. 2014. Forkhead, a new cross regulator of metabolism and innate immunity downstream of TOR in *Drosophila*. *J Insect Physiol* 69:80–88. <https://doi.org/10.1016/j.jinsphys.2014.04.006>.
 28. Birse RT, Choi J, Reardon K, Rodriguez J, Graham S, Diop S, Ocorr K, Bodmer R, Oldham S. 2010. High-fat-diet-induced obesity and heart dysfunction are regulated by the TOR pathway in *Drosophila*. *Cell Metab* 12:533–544. <https://doi.org/10.1016/j.cmet.2010.09.014>.
 29. Na J, Musselman LP, Pendse J, Baranski TJ, Bodmer R, Ocorr K, Cagan R. 2013. A *Drosophila* model of high sugar diet-induced cardiomyopathy. *PLoS Genet* 9:e1003175. <https://doi.org/10.1371/journal.pgen.1003175>.
 30. Musselman LP, Fink JL, Narzinski K, Ramachandran PV, Hathiramani SS, Cagan RL, Baranski TJ. 2011. A high-sugar diet produces obesity and insulin resistance in wild-type *Drosophila*. *Dis Model Mech* 4:842–849. <https://doi.org/10.1242/dmm.007948>.
 31. Pasco MY, Leopold P. 2012. High sugar-induced insulin resistance in *Drosophila* relies on the lipocalin Neural Lazarillo. *PLoS One* 7:e36583. <https://doi.org/10.1371/journal.pone.0036583>.
 32. Morris SNS, Coogan C, Chamseddin K, Fernandez-Kim SO, Kolli S, Keller JN, Bauer JH. 2012. Development of diet-induced insulin resistance in adult *Drosophila melanogaster*. *Biochim Biophys Acta* 1822:1230–1237. <https://doi.org/10.1016/j.bbadis.2012.04.012>.
 33. Musselman LP, Fink JL, Ramachandran PV, Patterson BW, Okunade AL, Maier E, Brent MR, Turk J, Baranski TJ. 2013. Role of fat body lipogenesis in protection against the effects of caloric overload in *Drosophila*. *J Biol Chem* 288:8028–8042. <https://doi.org/10.1074/jbc.M112.371047>.
 34. Musselman LP, Fink JL, Baranski TJ. 2016. CoA protects against the deleterious effects of caloric overload in *Drosophila*. *J Lipid Res* 57:380–387. <https://doi.org/10.1194/jlr.M062976>.
 35. Robinson MD, McCarthy DJ, Smyth GK. 2010. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26:139–140. <https://doi.org/10.1093/bioinformatics/btp616>.
 36. Huang DW, Sherman BT, Lempicki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4:44–57. <https://doi.org/10.1038/nprot.2008.211>.
 37. Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T. 2005. MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics* 21:2933–2942. <https://doi.org/10.1093/bioinformatics/bti473>.
 38. Lee G, Park JH. 2004. Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone-encoding gene in *Drosophila melanogaster*. *Genetics* 167:311–323. <https://doi.org/10.1534/genetics.167.1.311>.
 39. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. 2001. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110. <https://doi.org/10.1126/science.1057987>.
 40. Gronke S, Muller G, Hirsch J, Fellert S, Andreou A, Haase T, Jackle H, Kuhnlein RP. 2007. Dual lipolytic control of body fat storage and mobilization in *Drosophila*. *PLoS Biol* 5:e137. <https://doi.org/10.1371/journal.pbio.0050137>.
 41. Essers P, Tain LS, Nespital T, Goncalves J, Froehlich J, Partridge L. 2016. Reduced insulin/insulin-like growth factor signaling decreases translation in *Drosophila* and mice. *Sci Rep* 6:30290. <https://doi.org/10.1038/srep30290>.
 42. Mounier C, Posner BI. 2006. Transcriptional regulation by insulin: from the receptor to the gene. *Can J Physiol Pharmacol* 84:713–724. <https://doi.org/10.1139/y05-152>.
 43. O'Brien RM, Streeter RS, Ayala JE, Stadelmaier BT, Hornbuckle LA. 2001. Insulin-regulated gene expression. *Biochem Soc Trans* 29:552–558. <https://doi.org/10.1042/bst0290552>.
 44. Girardot F, Monnier V, Tricoire H. 2004. Genome wide analysis of common and specific stress responses in adult *Drosophila melanogaster*. *BMC Genomics* 5:74. <https://doi.org/10.1186/1471-2164-5-74>.
 45. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, Gorgun CZ, Hotamisligil GS. 2006. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science* 313:1137–1140. <https://doi.org/10.1126/science.1128294>.
 46. Gregor MF, Yang L, Fabbrini E, Mohammed BS, Eagon JC, Hotamisligil GS, Klein S. 2009. Endoplasmic reticulum stress is reduced in tissues of obese subjects after weight loss. *Diabetes* 58:693–700. <https://doi.org/10.2337/db08-1220>.
 47. Boden G, Duan X, Homko C, Molina EJ, Song W, Perez O, Cheung P, Merali S. 2008. Increase in endoplasmic reticulum stress-related proteins and genes in adipose tissue of obese, insulin-resistant individuals. *Diabetes* 57:2438–2444. <https://doi.org/10.2337/db08-0604>.
 48. Kang HL, Benzer S, Min KT. 2002. Life extension in *Drosophila* by feeding a drug. *Proc Natl Acad Sci U S A* 99:838–843. <https://doi.org/10.1073/pnas.022631999>.
 49. Brown MK, Chan MT, Zimmerman JE, Pack AI, Jackson NE, Naidoo N. 2014. Aging induced endoplasmic reticulum stress alters sleep and sleep homeostasis. *Neurobiol Aging* 35:1431–1441. <https://doi.org/10.1016/j.neurobiolaging.2013.12.005>.
 50. Kim H-J, Raphael AR, LaDow ES, McGurk L, Weber RA, Trojanowski JQ, Lee VM-Y, Finkbeiner S, Gitler AD, Bonini NM. 2014. Therapeutic modulation of eIF2 α phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nat Genet* 46:152–160. <https://doi.org/10.1038/ng.2853>.
 51. Guthrie LN, Abiraman K, Plyler ES, Sprengle NT, Gibson SA, McFarland BC, Rajbhandari R, Rowse AL, Benveniste EN, Meares GP. 2016. Attenuation of PKR-like ER kinase (PERK) signaling selectively controls endoplasmic reticulum stress-induced inflammation without compromising immunological responses. *J Biol Chem* 291:15830–15840. <https://doi.org/10.1074/jbc.M116.738021>.
 52. Harding HP, Zhang Y, Ron D. 1999. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 397:271–274. <https://doi.org/10.1038/16729>.
 53. Shi Y, Vattam KM, Sood R, An J, Liang J, Stramm L, Wek RC. 1998. Identification and characterization of pancreatic eukaryotic initiation factor 2 α -subunit kinase, PEK, involved in translational control. *Mol Cell Biol* 18:7499–7509. <https://doi.org/10.1128/MCB.18.12.7499>.
 54. Casas-Tinto S, Zhang Y, Sanchez-Garcia J, Gomez-Velazquez M, Rincon-Limas DE, Fernandez-Funez P. 2011. The ER stress factor XBP1s prevents amyloid-beta neurotoxicity. *Hum Mol Genet* 20:2144–2160. <https://doi.org/10.1093/hmg/ddr100>.
 55. Sone M, Zeng X, Lares J, Ryoo HD. 2012. A modified UPR stress sensing system reveals a novel tissue distribution of IRE1/XBP1 activity during normal *Drosophila* development. *Cell Stress Chaperones* 18:307–319. <https://doi.org/10.1007/s12192-012-0383-x>.
 56. Lau GW, Goumnerov BC, Walendziewicz CL, Hewitson J, Xiao W, Mahajan-Miklos S, Tompkins RG, Perkins LA, Rahme LG. 2003. The *Drosophila melanogaster* toll pathway participates in resistance to infection by the gram-negative human pathogen *Pseudomonas aeruginosa*. *In-*

- fect Immun 71:4059–4066. <https://doi.org/10.1128/IAI.71.7.4059-4066.2003>.
57. Kenmoku H, Hori A, Kuraishi T, Kurata S. 2017. A novel mode of induction of the humoral innate immune response in *Drosophila* larvae. *Dis Model Mech* 10:271–281. <https://doi.org/10.1242/dmm.027102>.
 58. Robinson SW, Herzyk P, Dow JAT, Leader DP. 2013. FlyAtlas: database of gene expression in the tissues of *Drosophila melanogaster*. *Nucleic Acids Res* 41:D744–D750. <https://doi.org/10.1093/nar/gks1141>.
 59. Ganeshan K, Chawla A. 2014. Metabolic regulation of immune responses. *Annu Rev Immunol* 32:609–634. <https://doi.org/10.1146/annurev-immunol-032713-120236>.
 60. Alic N, Andrews TD, Giannakou ME, Papatheodorou I, Slack C, Hoddinott MP, Cochemé HM, Schuster EF, Thornton JM, Partridge L. 2011. Genome-wide dFOXO targets and topology of the transcriptomic response to stress and insulin signalling. *Mol Syst Biol* 7:502. <https://doi.org/10.1038/msb.2011.36>.
 61. Puig O, Marr MT, Ruhf ML, Tjian R. 2003. Control of cell number by *Drosophila* FOXO: downstream and feedback regulation of the insulin receptor pathway. *Genes Dev* 17:2006–2020. <https://doi.org/10.1101/gad.1098703>.
 62. Bai H, Kang P, Hernandez AM, Tatar M. 2013. Activin signaling targeted by insulin/dFOXO regulates aging and muscle proteostasis in *Drosophila*. *PLoS Genet* 9:e1003941. <https://doi.org/10.1371/journal.pgen.1003941>.
 63. Teleman AA, Hietakangas V, Sayadian AC, Cohen SM. 2008. Nutritional control of protein biosynthetic capacity by insulin via Myc in *Drosophila*. *Cell Metab* 7:21–32. <https://doi.org/10.1016/j.cmet.2007.11.010>.
 64. Slaidina M, Delanoue R, Gronke S, Partridge L, Léopold P. 2009. A *Drosophila* insulin-like peptide promotes growth during nonfeeding states. *Dev Cell* 17:874–884. <https://doi.org/10.1016/j.devcel.2009.10.009>.
 65. Pagliassotti MJ, Prach PA. 1997. Increased net hepatic glucose output from gluconeogenic precursors after high-sucrose diet feeding in male rats. *Am J Physiol* 272:R526–R531.
 66. Kreutner W, Springer SC, Sherwood JE. 1975. Resistance of gluconeogenic and glycogenic pathways in obese-hyperglycemic mice. *Am J Physiol* 228:663–671.
 67. Stoffel M, Duncan SA. 1997. The maturity-onset diabetes of the young (MODY1) transcription factor HNF4alpha regulates expression of genes required for glucose transport and metabolism. *Proc Natl Acad Sci U S A* 94:13209–13214. <https://doi.org/10.1073/pnas.94.24.13209>.
 68. Froguel P, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clément K, Fougère F. 1992. Close linkage of glucokinase locus on chromosome 7p to early-onset non-insulin-dependent diabetes mellitus. *Nature* 356:162–164. <https://doi.org/10.1038/356162a0>.
 69. Hue L, Taegtmeyer H. 2009. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab* 297:E578–E591. <https://doi.org/10.1152/ajpendo.00093.2009>.
 70. DeFronzo RA. 2004. Pathogenesis of type 2 diabetes mellitus. *Med Clin North Am* 88:787–835, ix. <https://doi.org/10.1016/j.mcna.2004.04.013>.
 71. Xu C, Bailly-Maitre B, Reed JC. 2005. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 115:2656–2664. <https://doi.org/10.1172/JCI26373>.
 72. Zhang W, Hietakangas V, Wee S, Lim SC, Gunaratne J, Cohen SM. 2013. ER stress potentiates insulin resistance through PERK-mediated FOXO phosphorylation. *Genes Dev* 27:441–449. <https://doi.org/10.1101/gad.201731.112>.
 73. Yki-Järvinen H, Sammalkorpi K, Koivisto VA, Nikkilä EA. 1989. Severity, duration, and mechanisms of insulin resistance during acute infections. *J Clin Endocrinol Metab* 69:317–323. <https://doi.org/10.1210/jcem-69-2-317>.
 74. Shah BR, Hux JE. 2003. Quantifying the risk of infectious diseases for people with diabetes. *Diabetes Care* 26:510–513. <https://doi.org/10.2337/diacare.26.2.510>.
 75. Magliano DJ, Harding JL, Cohen K, Huxley RR, Davis WA, Shaw JE. 2015. Excess risk of dying from infectious causes in those with type 1 and type 2 diabetes. *Diabetes Care* 38:1274–1280. <https://doi.org/10.2337/dc14-2820>.
 76. Seiler F, Hellberg J, Lepper PM, Kamyschnikow A, Herr C, Bischoff M, Langer F, Schäfers H-J, Lammert F, Menger MD, Bals R, Beisswenger C. 2013. FOXO transcription factors regulate innate immune mechanisms in respiratory epithelial cells. *J Immunol* 190:1603–1613. <https://doi.org/10.4049/jimmunol.1200596>.
 77. Agrawal N, Delanoue R, Mauri A, Basco D, Pasco M, Thorens B, Léopold P. 2016. The *Drosophila* TNF Eiger is an adipokine that acts on insulin-producing cells to mediate nutrient response. *Cell Metab* 23:675–684. <https://doi.org/10.1016/j.cmet.2016.03.003>.
 78. Woodcock KJ, Kierdorf K, Pouchelon CA, Vivancos V, Dionne MS, Geissmann F. 2015. Macrophage-derived upd3 cytokine causes impaired glucose homeostasis and reduced lifespan in *Drosophila* fed a lipid-rich diet. *Immunity* 42:133–144. <https://doi.org/10.1016/j.immuni.2014.12.023>.
 79. Kotas ME, Medzhitov R. 2015. Homeostasis, inflammation, and disease susceptibility. *Cell* 160:816–827. <https://doi.org/10.1016/j.cell.2015.02.010>.
 80. Kurata S. 2014. Peptidoglycan recognition proteins in *Drosophila* immunity. *Dev Comp Immunol* 42:36–41. <https://doi.org/10.1016/j.dci.2013.06.006>.
 81. Dziarski R, Gupta D. 2010. Review: mammalian peptidoglycan recognition proteins (PGRPs) in innate immunity. *Innate Immun* 16:168–174. <https://doi.org/10.1177/1753425910366059>.
 82. Bischoff V, Vignal C, Duvic B, Boneca IG, Hoffmann JA, Royet J. 2006. Downregulation of the *Drosophila* immune response by peptidoglycan-recognition proteins SC1 and SC2. *PLoS Pathog* 2:e14. <https://doi.org/10.1371/journal.ppat.0020014>.
 83. Paredes JC, Welchman DP, Poidevin M, Lemaitre B. 2011. Negative regulation by amidase PGRPs shapes the *Drosophila* antibacterial response and protects the fly from innocuous infection. *Immunity* 35:770–779. <https://doi.org/10.1016/j.immuni.2011.09.018>.
 84. Zaidman-Rémy A, Poidevin M, Hervé M, Welchman DP, Paredes JC, Fahlander C, Steiner H, Mengin-Lecreux D, Lemaitre B. 2011. *Drosophila* immunity: analysis of PGRP-SB1 expression, enzymatic activity and function. *PLoS One* 6:e17231. <https://doi.org/10.1371/journal.pone.0017231>.
 85. Dietzl G, Chen D, Schnorrer F, Su KC, Barinova Y, Fellner M, Gasser B, Kinsey K, Oppel S, Scheiblaue S, Couto A, Marra V, Keleman K, Dickson BJ. 2007. A genome-wide transgenic RNAi library for conditional gene inactivation in *Drosophila*. *Nature* 448:151–156. <https://doi.org/10.1038/nature05954>.
 86. Ghosh S, Chan C-KK. 2016. Analysis of RNA-Seq data using TopHat and Cufflinks. *Methods Mol Biol* 1374:339–361. https://doi.org/10.1007/978-1-4939-3167-5_18.
 87. Quandt K, Frech K, Karas H, Wingender E, Werner T. 1995. MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res* 23:4878–4884. <https://doi.org/10.1093/nar/23.23.4878>.
 88. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, Cheng JX, Murre C, Singh H, Glass CK. 2010. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38:576–589. <https://doi.org/10.1016/j.molcel.2010.05.004>.