A *Caenorhabditis elegans* nutrient response system partially dependent on nuclear receptor NHR-49

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Appropriate response to nutritional stress is critical for animal survival and metabolic health. To better understand regulatory networks that sense and respond to nutritional availability, we developed a quantitative RT-PCR strategy to monitor changes in metabolic gene expression resulting from short-term food deprivation (fasting) in *Caenorhabditis elegans*. Examining 97 fat and glucose metabolism genes in fed and fasted animals, we identified 18 genes significantly influenced by food withdrawal in all developmental stages. Fasting response genes fell into multiple kinetic classes, with some genes showing significant activation or repression just 1 h after food was removed. As expected, fasting stimulated the expression of genes involved in mobilizing fats for energy production, including mitochondrial β-oxidation genes. Surprisingly, however, we found that other mitochondrial β-oxidation genes were repressed by food deprivation. Fasting also affected genes involved in mono- and polyunsaturated fatty acid synthesis: four desaturases were induced, and one stearoyl-CoA desaturase (SCD) was strongly repressed. Accordingly, fasted animals displayed considerable changes in fatty acid composition. Finally, nuclear receptor *nhr-49* played a key role in nutritional response, enabling induction of β-oxidation genes upon food deprivation and facilitating activation of SCD in fed animals. Our characterization of a fasting response system and our finding that *nhr-49* regulates a sector within this system provide insight into the mechanisms by which animals respond to nutritional signals.

fasting | fat metabolism | HNF4 | stearoyl-CoA desaturase

Precise control of energy storage and consumption is essential for surviving periods of food deprivation. Regulatory networks that govern fat and glucose metabolism are optimized to expend carbohydrates and accumulate fat when food intake is abundant, and switch to the consumption of stored fat when food is scarce (1, 2). In mammals, an overnight fast stimulates fat breakdown for the production of energy, enabling maintenance of glucose supply for CNS function and yielding acetyl-CoA for ketone body synthesis (3, 4). Orchestration of the fasting response is critical, and mutations that disrupt it can cause potentially lethal hypoglycemia and hypoketonemia (4, 5). Indeed, even subtle misregulation of these pathways can bring about diabetes and obesity (1, 6).

A complex network of hormonal signals and regulatory mechanisms governs adaptation to food availability. In mammals, much of the fasting response is transcriptionally regulated (7–9). Increased fat consumption is facilitated by the induction of genes involved in fatty acid β-oxidation, whereas repression of glycolysis genes and activation of gluconeogenic genes maintains glucose supply. Additionally, activation of ketogenic genes enhances conversion of fatty acid β-oxidation products into ketone bodies (3). Despite these general observations, studies of the fasting response have been limited to a select group of genes in only a subset of physiological tissues and settings; consequently, regulatory mechanisms that mediate adaptation to fasting within the context of a whole animal are still poorly understood.

Recent studies have established *Caenorhabditis elegans* as a tractable subject for investigating energy metabolism, revealing, for example, conservation of several fat metabolism pathways (10–13). To investigate regulatory networks that mediate response to nutritional stress in *C. elegans*, we developed a quantitative RT-PCR (QRT-PCR) strategy that measures the expression of a comprehensive set of genes involved in glucose and fat metabolism. Here, we have exploited this strategy to characterize the fasting response in all developmental stages of *C. elegans*. Our central aims were to identify key metabolic genes influenced by food availability and to monitor physiological changes resulting from regulation of these "fasting response genes." Moreover, we also sought to define regulatory factors involved in nutritional response, testing a role for the nuclear hormone receptor NHR-49.

Methods

Assignment of Fatty Acid and Glucose Metabolism Genes. Fatty acid and glucose metabolism genes were identified by using the KEGG pathway database (www.genome.ad.jp/kegg/pathway.html) or by employing BLAST searches designed to find *C. elegans* ORFs highly related to genes that encode mammalian glucose and fat metabolism enzymes or plant glyoxylate cycle proteins. For prediction of subcellular localization, we used TARGETP (server version 1.01; www.cbs.dtu.dk/services/TargetP) (14) and PSORT (http://psort.nibb.ac.jp/form.html).

Fasting Assays. Preparation of gravid adults and bleaching of animals was carried out as described (11). Worms were grown and fasting assays were performed at 22.5°C. To collect L1 fasted animals, 250,000 embryos obtained from bleached adult hermaphrodites were allowed to hatch on NGM-lite plates in the absence of food; after 14–18 h (embryos generally hatched 2–6 h after bleaching), worms were collected, washed, and frozen in liquid nitrogen. For L1 fed animals, 80,000 embryos were hatched on OP50 bacteria and collected after 4–6 h of feeding. For other larval stages, embryos were added to NGM plates containing OP50 bacteria; animals were harvested after 24 h (for L2 animals), 36 h (for L3 animals), 48 h (for L4 animals), and 96 h (for adult animals). For fasted animals, 80,000 embryos were divided onto 10 NGM-lite plates containing OP50 bacteria. When animals had reached the appropriate stage, they were washed extensively with M9 (five to six washes) and placed onto NGM-lite plates containing no bacteria for 12 h. Sterile animals [CF512*fer-15*(*b26*);*fem-1*(*hc17*)] were used to assay the adult stage of *C. elegans* development (15). Sterile animals are advantageous for adult analysis because embryonic RNA contribution is eliminated. Sterile animals were grown and maintained as described (15).

Kinetic Assays. For kinetic assays, 80,000 early L4 animals were washed five to six times with M9 and placed onto NGM-lite plates containing no food. Fasted worms were harvested 1, 2, 4, 8, and 12 h after food withdrawal, washed in M9, and immedi-

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Abbreviations: QRT-PCR, quantitative RT-PCR; PUFA, polyunsaturated fatty acid.

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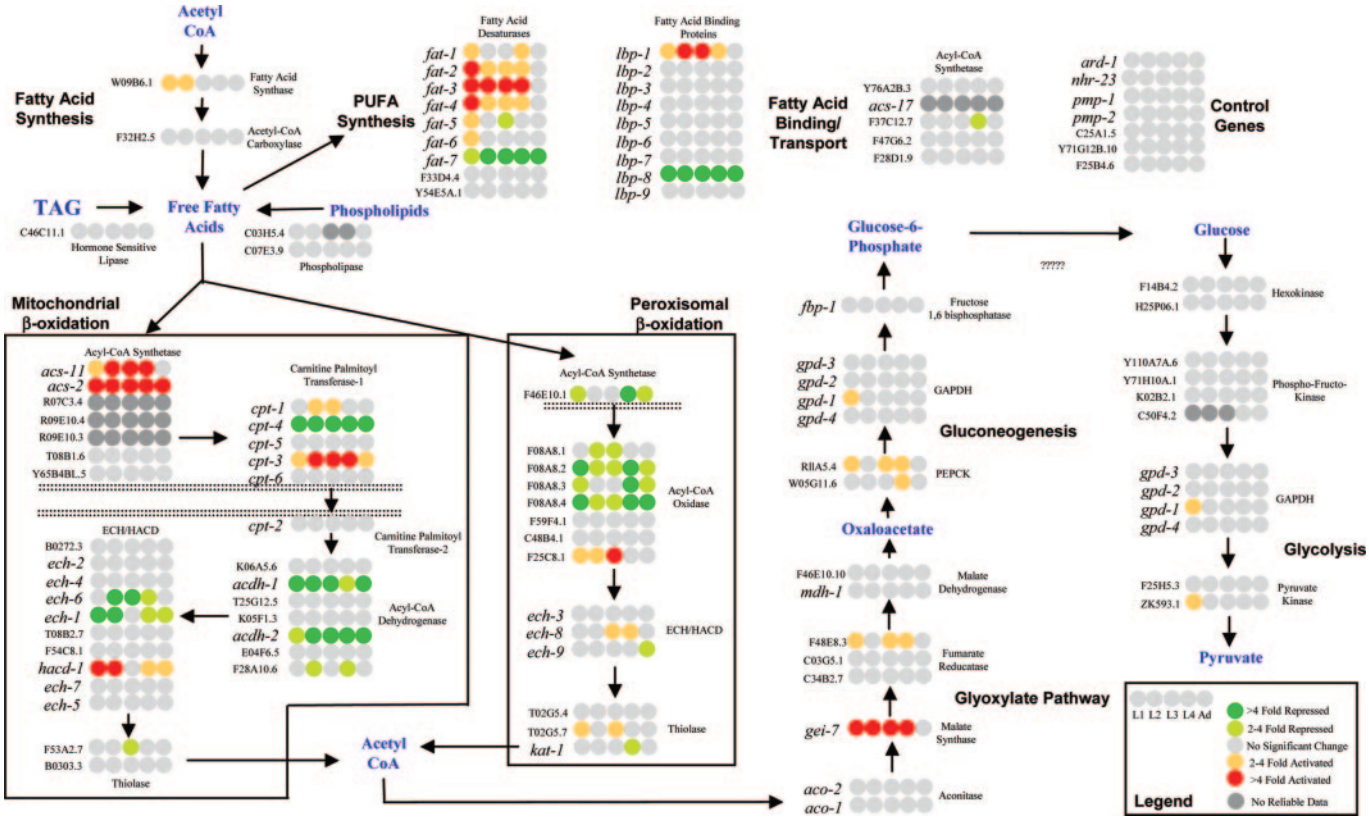

Fig. 1. Changes in energy metabolism gene expression after 12 h of fasting. Putative *C. elegans* fatty acid, glucose, and glyoxylate metabolism pathways are shown here. Each gene is predicted, due to a high degree of sequence homology, to code for the enzyme indicated in the figure (see *Methods*). QRT-PCR was used to measure the expression of these 97 genes in fed and fasted animals. The circles represent fasting dependent changes in the L1, L2, L3, L4, and adult stages (from left to right) of *C. elegans* development. Genes expressed at lower levels in fasted worms are shown in light green (2- to 4-fold) or dark green (>4-fold). Genes expressed at higher levels are shown in light red (2- to 4-fold) or dark red (>4-fold). Several control genes, used to normalize the data, are also displayed in this figure.

ately frozen in liquid nitrogen. Early L4 animals were chosen to ensure that fasted animals did not enter into any of the early programs associated with dauer formation.

QRT-PCR Measurements. QRT-PCR measurements were conducted as described (ref. 11; see *Supporting Text*, which is published as supporting information on the PNAS web site). Primer sequences are available upon request. To determine the relationship between cycle number (C_t) and mRNA levels, primers were calibrated by using serial dilutions of cDNA and genomic DNA. For the initial fasting survey, data for each developmental stage were collected from two to three independent fasting experiments (see Table 3, which is published as supporting information on the PNAS web site). In all cases, data for each experiment were collected in duplicate. For more rigorous analysis of the fasting response genes, two to three additional fasting experiments were performed for each developmental stage. For all of the fasting response genes and for the kinetic analysis, the expression of each gene was determined by using two independent sets of primers. QRT-PCR data were normalized by using the following control genes: *ard-1*, *nhr-23*, *pmp-1*, *pmp-2*, C25A1.5, Y71G12B.10, and F25B4.6.

GFP Reporter Analysis. Construction of the *acs-2*:*gfp* reporter was described (11). Germ-line transformation was performed by injecting 50 ng/λ of *acs*:*2*:*gfp* and 50 ng/λ pRF4 *rol-6(su1006)* into N2 animals. To monitor ACS-2:GFP expression in fed animals, worms containing the *acs-2*:*gfp* transgene were grown on OP50 bacteria and were examined for GFP fluorescence when they had reached the early adult stage of development. For fasted animals, early L4 worms containing the *acs-2*:*gfp* transgene were transferred to agar plates containing no food, and were examined for GFP expression after 12 h of fasting. GFP reporter expression was observed as described (11).

Biochemical Analysis of Fatty Acid Composition. GC/MS analysis of fatty acid abundance was performed as described (16). For GC/MS analysis, fed and fasted animals were grown and collected as described above.

Results and Discussion

Characterization of the *C. elegans* Fasting Response. We implemented a QRT-PCR strategy to measure the response of 97 energy metabolism genes to short-term food withdrawal (fasting) in *C. elegans*. Genes assayed included those expected to function in fatty acid synthesis, lipolysis, β-oxidation, desaturation, and binding/transport; as well as genes predicted to participate in glucose metabolism pathways such as glycolysis, gluconeogenesis, and the glyoxylate pathway (Fig. 1). Fasting-dependent changes in gene expression were measured in all four larval stages, and in sterile adults.

In our initial survey, we found that fasting influenced the expression of 39 metabolic genes in at least one developmental stage (Fig. 1 and Table 3). Eighteen of these genes were significantly altered by food withdrawal throughout development; we designated these as "fasting response" genes (Table 1). The fact that these genes are similarly affected in all developmental stages suggests that they are part of a general metabolic response to food deprivation,

Table 1. Summary of fasting response genes

Gene		Predicted enzyme (homology)	Physiological process	Fold activation/repression L1	L2	L3	L4	Adult
Fasting-induced genes								
F28F8.2	*acs-2*	Acyl-CoA synthetase (61%)	Mitochondrial β-oxidation	**114** (64–203)	**18** (11–29)	**7.7** (5.2–11)	**32** (26–40)	**11** (9–13)
Y48G9A.10	*cpt-3*	Carnitine palmitoyl transferase-1 (49%)	Mitochondrial β-oxidation	**6.9** (3.4–14)	**17** (9–34)	**12** (7–22)	**12** (7–18)	**3.7** (2.6–5.3)
W08D2.4	*fat-3*	Fatty acid Δ6-desaturase	PUFA synthesis	**8.6** (7.8–9.5)	**11** (8.2–16)	**10** (7.7–12)	**7** (5.6–8.7)	**1.6** (1.3–2.1)
C05E4.9	*gei-7*	Malate synthase/Isocitrate lyase	Glyoxylate pathway	**13** (8.5–21)	**8.9** (6.8–12)	**4.3** (3.4–5.6)	**15** (11–22)	None
R09B5.6	*hacd-1*	3-hydroxyacyl-CoA dehydrogenase (61%)	Mitochondrial β-oxidation	**67** (52–88)	**5.9** (5.1–6.9)	**1.8** (1.3–2.6)	**2.7** (2.2–3.4)	**2.5** (2.2–2.8)
F41C3.3	*acs-11*	Acyl-CoA synthetase (46%)	Mitochondrial β-oxidation	**4.1** (2.8–6.1)	**10** (6.3–17)	**6.7** (5.6–8.2)	**11** (7–16)	None
F40F4.3	*lbp-1*	Fatty acid binding protein (42%)	Lipid trafficking	**2.8** (2.5–3.2)	**5.4** (4.8–6.2)	**4.5** (3.9–5.1)	**4.2** (3.4–5.1)	**1.3** (1.1–1.4)
T13F2.1	*fat-4*	Fatty acid Δ5-desaturase	PUFA synthesis	**4.8** (4.2–5.5)	**3.4** (2.9–4.1)	**3.5** (3.0–4.1)	**2.5** (2.2–3.0)	**1.3** (1.2–1.5)
W02A2.1	*fat-2*	Fatty acid Δ12-desaturase	PUFA synthesis	**5.4** (4.8–6.1)	**3.7** (2.9–4.8)	**3.6** (3.1–4.2)	**2.2** (1.8–2.8)	None
Fasting-repressed genes								
T22G5.6	*lbp-8*	Fatty acid binding protein (68%)	Lipid trafficking	**36** (28–48)	**50** (36–70)	**114** (87–50)	**75** (55–105)	**20** (17–23)
C17C3.12	*acdh-2*	SC-Acyl-CoA dehydrogenase (64%)	Mitochondrial β-oxidation	**5.6** (3.5–9.1)	**38** (34–41)	**54** (42–70)	**26** (18–38)	**15** (10–21)
F10D2.9	*fat-7*	Stearoyl-CoA desaturase (68%)	Oleic acid synthesis	**2.2** (1.4–3.5)	**15** (12–18)	**26** (21–33)	**54** (35–80)	**10** (7–14)
C55B7.4	*acdh-1*	SC-Acyl-CoA dehydrogenase (77%)	Mitochondrial β-oxidation	**19** (8.4–43)	**8.9** (7.4–11)	**7.1** (6.0–8.4)	**3.0** (2.1–4.2)	**47** (32–70)
K11D12.4	*cpt-4*	Carnitine palmitoyl transferase-1 (51%)	Mitochondrial β-oxidation	**21** (17–28)	**9.3** (6.7–13)	**10** (7–15)	**7.1** (5.5–9.3)	**7.2** (5.6–9.4)
F08A8.2		Acyl-CoA oxidase (58%)	Peroxisomal β-oxidation	**6.3** (4.7–6.4)	**4.2** (3.4–5.2)	**4.4** (3.4–5.8)	**4.3** (4.2–4.4)	**4.4** (3.6–5.5)
F08A8.4		Acyl-CoA oxidase (57%)	Peroxisomal β-oxidation	**6.0** (4.8–7.6)	**2.8** (2.2–3.6)	**2.8** (2.5–3.2)	**5.2** (4.7–5.7)	**5.1** (4.1–6.3)
C29F3.1	*ech-1*	Enoyl-CoA hydratase (67%)	Mitochondrial β-oxidation	**5.3** (4.0–7.2)	**4.5** (4.0–5.2)	**2.8** (2.2–3.6)	**2.0** (1.6–2.5)	**2.6** (2.2–3.1)
T05G5.6	*ech-6*	Enoyl-CoA hydratase (76%)	Mitochondrial β-oxidation	**2.2** (1.8–2.6)	**5.7** (4.5–7.2)	**4.4** (3.7–5.2)	**3.5** (2.7–5.6)	**1.9** (1.6–2.2)

Data are displayed in bold as fold activation or repression, and the numbers in parentheses represent the statistical range (based on standard error). Enzymatic function is predicted based on amino acid homology. The homology of each gene to its mammalian counterpart is displayed in parentheses as % amino acid similarity. SC, short chain; PUFA, polyunsaturated fatty acid.

rather than a response specific to a particular starvation-resistant developmental fate, such as dauer or L1-arrest. Overall, fasting preferentially affected genes involved in mitochondrial and peroxisomal β-oxidation, fatty acid desaturation, and fatty acid binding/transport. In contrast, the effects of fasting on genes involved in lipolysis, fatty acid synthesis, and glucose metabolism were markedly smaller (Fig. 1).

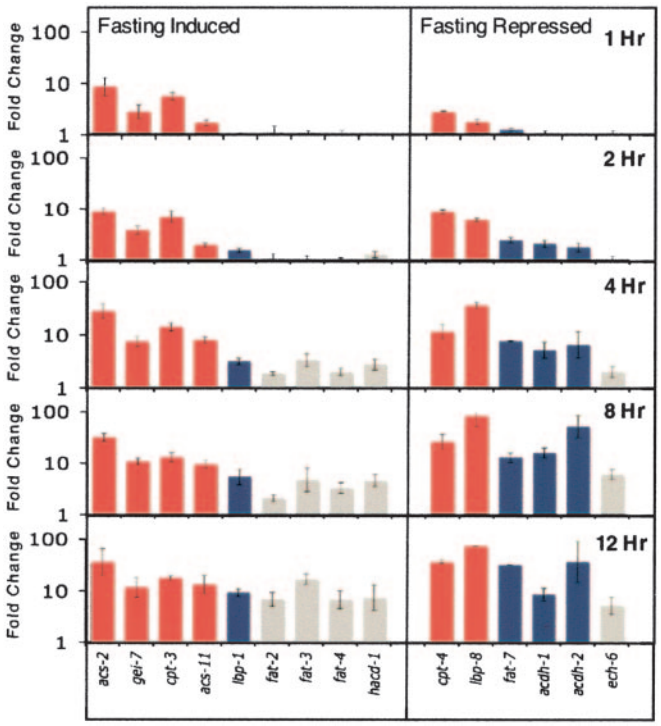

Fig. 2. Kinetics of the fasting response. The expression level of each fasting response gene was measured in early L4 fed animals 1, 2, 4, 8, and 12 h after food withdrawal. Data are reported as fold activation or repression. Genes were divided into three categories: early response genes are indicated in red, delayed response genes are indicated in blue, and late response genes are shown in gray. Error bars represent standard error of measurement.

Kinetic Analysis of Fasting Response Genes. To determine how quickly gene expression reacted to fasting, we examined the fasting response genes 1, 2, 4, 8, and 12 h after food was removed from animals in the early L4 stage of development. We found that the response was multiphasic, with distinct early, delayed, and late genes (Fig. 2). Early genes responded without apparent lag, displaying significant induction or repression just 1 h after food was removed. In contrast, delayed genes showed no detectable change in expression until 2 h after food removal, and late genes were not measurably altered until 4 h after the initiation of fasting.

The fact that the fasting response genes displayed different kinetic profiles suggests that they may be regulated by distinct nutritional signaling mechanisms. Because the early genes respond with no apparent lag, they are likely reacting to a rapid signaling mechanism that directly indicates the absence of food to gene regulatory machinery. In contrast, delayed and late genes may respond to secondary signals or may be indirect targets of food deprivation; e.g., reacting to changes in metabolic conditions exerted by early response genes. Alternatively, delayed genes may simply take longer to receive and process primary food deprivation signals. It will be interesting to determine whether genes with similar kinetic profiles are modulated by common regulatory mechanisms, and whether they function together to carry out their roles in nutritional adaptation.

Fasting Effects on Mitochondrial Fatty Acid β-Oxidation. Nine of the 18 *C. elegans* fasting response genes appear to encode enzymes involved in mitochondrial fatty acid β-oxidation (Table 1). Four were strongly induced: two acyl-CoA synthetase (ACS) genes (*acs-2* and *acs-11*), one carnitine palmitoyl transferase-1 (CPT-I) gene (*cpt-3*), and one 3′-OH acyl-CoA dehydrogenase (HAD) gene (*hacd-1*). Mitochondrial ACS enzymes activate fatty acids for transport into the mitochondria via CPT-I and the carnitine-shuttle system (17). Thus, fasting-dependent induction of *acs-2*, *acs-11*, and *cpt-3* likely enhances the flow of fatty acids into the mitochondrial matrix for β-oxidation. These findings are similar

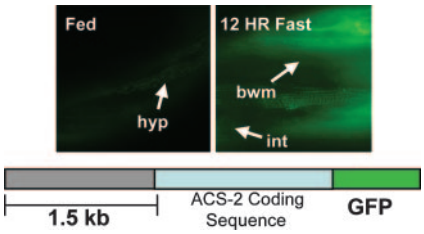

Fig. 3. Induction and localization of ACS-2:GFP protein upon fasting. The two images display *acs-2*:*gfp* injected worms in the early adult stage of development. The animal in *Left* is well fed, whereas the two worms in *Right* have been fasted for 12 h. Arrows point to GFP expression in the hypodermis (hyp), body wall muscle (bwm), and intestine (int). The ACS-2::GFP construct is also diagramed in this figure.

to those in fasted mammals, in which increased expression of ACS and CPT-I genes is also thought to stimulate fat breakdown upon fasting (9, 18).

Surprisingly, fasting of *C. elegans* also led to the repression of mitochondrial β-oxidation genes, including a separate CPT-1 isoform (*cpt-4*), two short-chain acyl-CoA dehydrogenase genes (*acdh-1* and *acdh-2*), and two enoyl-CoA hydratase genes (*ech-1* and *ech-6*) (Table 1). Thus, food deprivation does not produce a global increase in mitochondrial β-oxidation gene expression, but selectively stimulates and represses different isoforms of these genes (see Fig. 1). We speculate that ordered activation and repression of β-oxidation genes might reflect tissue-specific regulation, differential control of enzymes with specific fatty acid chain length preferences, or an organized diversion of fatty acids into alternative β-oxidation enzyme complexes assembled to channel products into distinct metabolic fates. Although the effects were not nearly as dramatic, fasting also resulted in both activation and repression of genes involved in peroxisomal fatty acid β-oxidation (Fig. 1).

Fasting Activates a Rapid and Coordinated Energy Mobilization System. In all larval stages, fasting strongly enhanced expression of the malate-synthase/isocitrate-lyase gene *gei-7* (Table 1). GEI-7 is a key enzyme in the glyoxylate pathway, which helps to convert fatty acid β-oxidation products into sugars. Our results are consistent with previous studies demonstrating that *gei-7* is induced by fasting in all developmental stages (19, 20). It is likely that increased *gei-7* expression under these conditions is instrumental in converting energy stored as fat into sugars. The glyoxylate pathway is analogous to the ketogenic pathway in mammals, which exploits acetyl-CoA generated by fatty acid β-oxidation to produce ketone bodies, a sugar substitute in times of fasting. Accordingly, fasting in mammals results in the induction of ketogenic enzymes (3).

Expression of the *gei-7* gene increased dramatically within 1 h of food removal, with a kinetic profile similar to that of the fasting-induced mitochondrial β-oxidation genes *acs-2* and *cpt-3* (Fig. 2). We suggest that these genes function together as a primary fasting response system, *acs-2* and *cpt-3* helping to mobilize fat stores for energy production by enhancing flow of fatty acids into mitochondria for β-oxidation, and *gei-7* helping to convert β-oxidation products into a carbohydrate energy source.

Fasting-Dependent Induction of ACS-2 in Intestine and Muscle. Several genes that affect overall fat storage, such as *nhr-49*, *fat-7*, and *daf-2*, modulate the expression of *acs-2*, which also controls fat storage (10, 11, 21). These studies, in combination with our finding that *acs-2* is one of strongest fasting response genes, suggest that *acs-2* is a key target for controlling fat expenditure via mitochondrial β-oxidation. To determine how fasting affected the expression and localization of ACS-2 protein *in vivo*, we analyzed an ACS-2:GFP reporter construct in fed and fasted animals. In fed animals, ACS-2:GFP protein is expressed in many tissues, including hypodermis, pharyngeal muscle, intestine, and neurons (Fig. 3 and data not shown). Consistent with the QRT-PCR data, we observed a dramatic increase in ACS-2::GFP after 12 h of fasting, especially in body-wall muscle and intestine (Fig. 3). As predicted, the ACS-2::GFP protein localized in a pattern consistent with that of mitochondrial proteins (22).

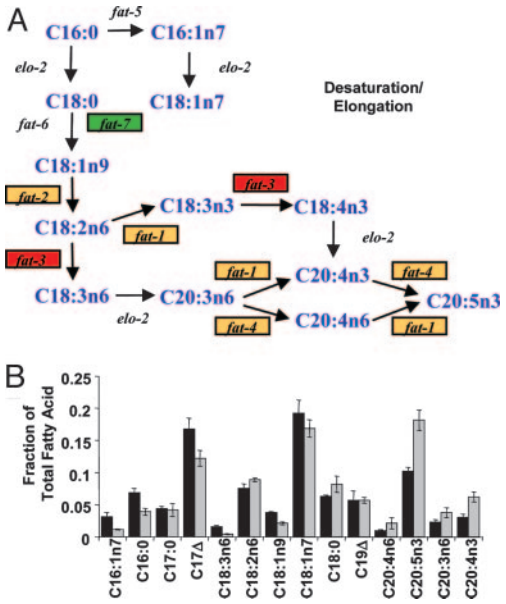

Fig. 4. Fasting effects on fatty acid composition. (*A*) The fatty acid desaturation/elongation pathway (16, 25). The genes shown in green are strongly repressed by fasting, the genes shown in light red are modestly induced, and the genes shown in dark red are strongly induced (see Table 1). (*B*) Long-chain fatty acid abundance in early L4 fed animals (black bars) and early L4 animals fasted for 12 h (light gray bars). Total fatty acids were extracted from whole animals and quantified by GC/MS. The relative abundance of each fatty acid species is expressed as fraction of total measured fatty acid. The data shown here were obtained from three independent experiments, and the error bars represent standard error.

Previous studies have demonstrated that GEI-7, like ACS-2, is induced in the intestine and body wall muscle upon fasting (20). Thus, the coinduction of GEI-7 and ACS-2 in these two tissues further supports the hypothesis that these two proteins cooperate as a system to mobilize fats for conversion into sugars.

Fasting Affects Expression of Genes that Encode Fatty Acid-Binding Proteins (FABPs). Two fasting response genes, *lbp-1* and *lbp-8*, share significant homology with mammalian FABPs (Table 1). FABPs associate with various lipids and are typically involved in the trafficking of fatty acids (23, 24). In fact, it has been proposed that FABPs can inhibit β-oxidation by sequestering fatty acids (23). Interestingly, *lbp-8* expression was repressed after food removal, with kinetics similar to that observed for induction of the fat mobilization genes *acs-2*, *gei-7*, and *cpt-3* (see Fig. 2). It is tempting to speculate that repression of *lbp-8* may help free up fatty acids for β-oxidation, thus contributing to the primary nutrient response. In any case, it is likely that regulation of these two FABPs helps to mobilize and distribute fatty acids under conditions of fasting.

Food Deprivation Influences Fatty Acid Saturation. Fasting significantly altered the mRNA levels of several *C. elegans fat* genes, which encode fatty acid desaturases involved in polyunsaturated

DEVELOPMENTAL BIOLOGY

fatty acid (PUFA) biosynthesis and regulation of fatty acid metabolism (Table 1 and Fig. 4*A*). To determine whether fasting-dependent changes in *fat* gene expression influenced fatty acid desaturation levels *in vivo*, we used GC/MS to measure the abundance of saturated and unsaturated fatty acids in fed and fasted worms.

Our GC/MS analysis revealed several changes in fatty acid composition, most notably, a pronounced increase in the relative levels of C20 PUFAs. This rise in PUFA abundance likely reflects, at least in part, conversion of shorter chain fatty acids into PUFAs. Indeed, we found that the relative levels of PUFA precursors such as C16:0, C18:1n9, and C18:3n6 are reduced upon fasting (see Fig. 4). Although we cannot yet rule out indirect causes, we believe that the fasting-dependent increase in PUFA abundance may directly result from the enhanced expression of fatty acid desaturases. How fasted animals may benefit from increased PUFA synthesis remains to be determined.

Although the levels of many PUFA precursors are diminished upon fasting, we observed an increase in the relative level of another PUFA precursor, saturated C18:0 (stearic acid) (Fig. 4*B*). The fact that the stearic acid level is maintained under conditions of food deprivation, whereas other species are converted into PUFAs, may be attributable to strong fasting-dependent repression of the *fat-7* gene, which encodes a stearoyl-CoA desaturase (SCD) (Table 1 and Fig. 4*A*). By converting stearic acid to oleic acid, the FAT-7 SCD plays a key role in controlling the relative abundance of saturated and monounsaturated fatty acids in *C. elegans* (11, 25). During fasting, saturated fatty acid input is likely reduced in two ways: first, worms no longer absorb saturated fats from food, and second, repression of fatty acid synthesis may inhibit production of new saturated fatty acids. Consequently, repression of *fat-7* may help to preserve stearic acid levels under fasting conditions by preventing their conversion into unsaturated species.

Conversely, when animals are well fed and saturated fats are actively ingested and synthesized, *fat-7* is induced to ensure that the relative levels of saturated fat do not become too high. Taken together, these findings suggest a key role for *fat-7* in maintaining appropriate levels of saturated and unsaturated fats in response to nutritional intake. Finally, because the *fat-7* SCD inhibits fatty acid β-oxidation (11), we also expect that reduced *fat-7* expression would lead to increased fat consumption via β-oxidation.

NHR-49 Is a Key Component of the Fasting Response. Several of the fasting response genes, including *acs-2, fat-7, lbp-8*, and *gei-7*, are regulatory targets of the *C. elegans* nuclear hormone receptor *nhr-49* in fed worms (11). To determine whether *nhr-49* also participated in adaptation to food deprivation, we examined the fasting response genes in an *nhr-49* deletion mutant [*nhr-49(nr2041)*]. In L4 animals, we found that the nutrient-dependent regulation of 7 of the 18 fasting response genes was impaired by *nhr-49* deletion (Fig. 5*A*). Fasting response genes displayed a similar *nhr-49* dependence in other developmental stages (data not shown). Notably, fasting did not appreciably alter *nhr-49* expression in any developmental stage (data not shown).

Fasting-dependent induction of three mitochondrial fatty acid β-oxidation genes, *acs-2*, *acs-11*, and *hacd-1*, was virtually eliminated by *nhr-49* deletion, and stimulation of the glyoxylate pathway gene *gei-7* was partially abrogated (Fig. 5*A*). Two *nhr-49*-regulated genes, *acs-2* and *gei-7*, depended on *nhr-49* for both basal (fed state) and induced (fasting-dependent) expression, whereas *hacd-1* and *acs-11* did not require *nhr-49* for expression in the fed state, but did require *nhr-49* for induction during fasting (Table 2). Interestingly, *hacd-1* was repressed by fasting in *nhr-49(nr2041)* worms.

Three of the fasting-repressed genes, *cpt-4*, *fat-7*, and *lbp-8*, were also affected by *nhr-49* knockout (Fig. 5*A*). *fat-7* expression is nearly eliminated in both fed and fasted *nhr-49(nr2041)*

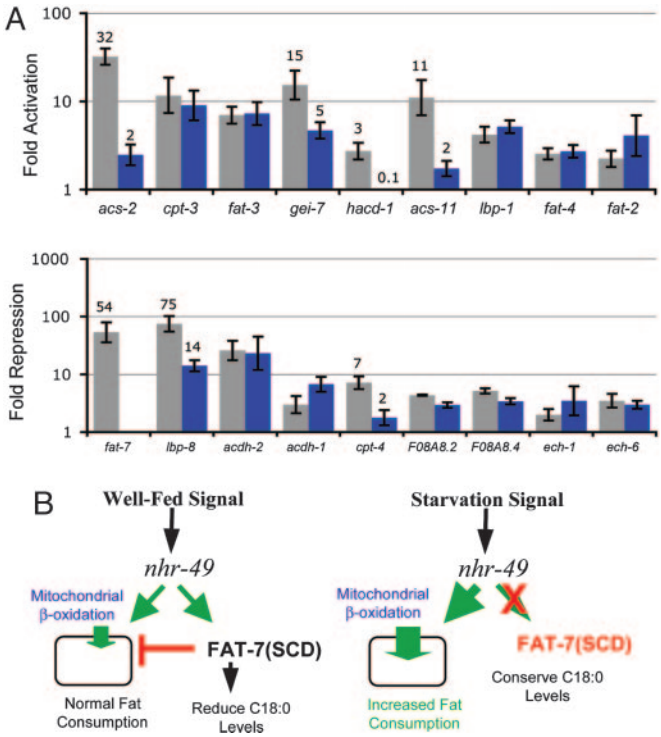

Fig. 5. The effect of *nhr-49* deletion on the fasting response. (*A*) Fasting dependent activation or repression of each fasting response gene in L4 WT (gray bars) and L4 *nhr-49*(*nr2041*) (blue bars). Error bars represent standard error of measurement. For *nhr-49*-dependent genes, the fold-activation or repression values are displayed in the graph, above the corresponding bars. (*B*) Model demonstrating the putative role of *nhr-49* in regulating fat expenditure and composition in response to changing food availability.

animals (Table 2). Thus, it appears that *nhr-49* is required for inducing *fat-7* expression in the fed state and that *nhr-49* activation of *fat-7* is relieved or actively inhibited in fasted animals. In the case of *lbp-8*, expression was compromised ≈4-fold in fed *nhr-49(nr2041)* animals, but in fasted *nhr-49(nr2041)* worms *lbp-8* is repressed to levels close to that of fasted wild type (Table 2). In contrast, *nhr-49* deletion did not affect *cpt-4* expression in fed animals but was essential for *cpt-4* repression in fasted animals (Table 2).

Thus, we propose that *nhr-49* is part of a mechanism that regulates multiple aspects of nutritional response (Fig. 5*B*). When food is being ingested, *nhr-49* modestly stimulates β-oxidation genes and *gei-7*, a system that promotes fat breakdown and conversion to sugars. Furthermore, *nhr-49*-dependent activation of *fat-7* enhances

Table 2. NHR-49-dependent fasting response genes

	Wild type		*nhr-49(nr2041)*	
	Fed	Fasted	Fed	Fasted
acs-2	100 ± 28	2,565 ± 1,050	2.5 ± 0.6	6.2 ± 1.5
gei-7	100 ± 24	1,280 ± 160	42 ± 10	198 ± 36
hacd-1	100 ± 28	260 ± 54	80 ± 4	10 ± 2.4
acs-11	100 ± 24	1,270 ± 254	198 ± 41	340 ± 112
lbp-8	100 ± 5	1.3 ± 0.4	27 ± 6.4	1.9 ± 0.5
fat-7	100 ± 25	1.3 ± 0.4	0.2 ± 0.06	0.3 ± 0.1
cpt-4	100 ± 6.4	8.7 ± 0.8	75 ± 6.2	43 ± 9.7

Comparison of the expression level of each gene (relative mRNA abundance) in wild-type and *nhr-49(nr2041)* worms fed and fasted as L4 larvae. Data are normalized so that the relative abundance of each gene in fed wild-type L4 worms is equal to 100.

the conversion of ingested and synthesized stearic acid into oleic acid, thereby preventing excess accumulation of saturated fats. Under conditions of fasting, *nhr-49* is essential for strong induction of mitochondrial β-oxidation genes and *gei-7*, thereby increasing the mobilization of fat for energy and conversion to sugars. Moreover, because conservation of stearic acid may be necessary under conditions of food deprivation, activation of *fat-7* by *nhr-49* is somehow abrogated.

It is tempting to speculate that *nhr-49* may be controlling these activities by sensing intracellular lipid composition and directly regulating transcription of fasting response genes. Interestingly, the ligand-binding domain of NHR-49 is structurally similar to that of the mammalian HNF4 receptor, which can directly bind fatty acids, including both stearic and oleic acid (26, 27). Despite its homology to HNF4, the activities of *nhr-49* are most reminiscent of the mammalian "lipid sensing" nuclear receptor PPARα. PPARα plays a similar role in the mammalian fasting response by stimulating the expression of genes involved in mitochondrial β-oxidation and ketogenesis (28–31). Thus, our findings reveal a conserved role for nuclear receptors in the adaptation of energy metabolism to changing nutrient availability.

Comparison of "Fasting Response" Genes to Other Nutritional Response Genes. Several fasting response genes identified in this study were previously implicated in another type of nutritional response, the response of starved L1-arrested animals to feeding (13). Indeed, eight of the nine fasting-induced genes, and four of the nine fasting-repressed genes, displayed similar nutritional dependence when food was added to starved L1 animals. However, many of the key fasting-dependent genes identified here, including *cpt-3*, *lbp-8*, *fat-7*, and *acdh-1*, were not implicated in this previous study. The differences between our "fasting response" genes and the "feeding response" genes of Wang *et al.* (13) may reflect the distinction between "starved" animals, i.e., arrested worms that had been held without food for a long period (>30 h), and "fasted" animals; i.e., worms subjected to short-term (<12 h) food withdrawal.

Concluding Remarks. Here we characterized the short-term adaptation of metabolic gene expression to fasting in *C. elegans*. We chose a "biased" set of 97 genes predicted to be involved in fat and glucose metabolism. Although it is likely that many genes not included in this set are also affected by food deprivation, the focus of our study was not to identify all of the genes involved in nutritional response, but rather to study, with a high degree of resolution, the effects of food availability on the regulation of metabolic pathways known to be important for processing dietary intake.

Consistent with studies in mammals, we found that fasting mediated the induction of genes involved in converting fat stores into energy. In addition, we also observed many changes in metabolic gene expression not previously associated with a fasting response, including regulation of fatty acid desaturases, repression of mitochondrial β-oxidation genes, and activation and repression of genes that encode fatty acid-binding proteins. It is likely that many of these observations will be relevant to nutritional response in other metazoans.

It will be interesting to define the signaling networks that activate the *C. elegans* "fasting response system," including the ligands, receptors, and other regulatory factors that interpret nutritional signals to produce modulated gene expression. In this study, we found that *nhr-49* is essential for the fasting-dependent induction of genes predicted to facilitate the mobilization of fat for energy generation, as well as for the feeding-dependent induction of a stearoyl-CoA desaturase. However, we also identified fasting response genes that are independent of *nhr-49*. Thus, the fasting response system likely reflects the integration of multiple regulatory networks.

The biological significance of the fasting response is yet to be determined; i.e., how do these regulatory events help worms to survive food deprivation? Nevertheless, our study highlights several important metabolic and physiological changes that occur upon fasting, and serves as a guide for understanding adaptation to changing food availability. Continued investigation into the gene regulatory events and physiology of the fasting response in *C. elegans* will likely advance our knowledge of energy homeostasis in all metazoans.

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1. Spiegelman, B. M. & Flier, J. S. (2001) *Cell* **104,** 531–543.
2. van den Berghe, G. (1991) *J. Inherit. Metab. Dis.* **14,** 407–420.
3. Cullingford, T. E. (2004) *Prostaglandins Leukotrienes Essent. Fatty Acids* **70,** 253–264.
4. Kelly, D. P., Hale, D. E., Rutledge, S. L., Ogden, M. L., Whelan, A. J., Zhang, Z. & Strauss, A. W. (1992) *J. Inherit. Metab. Dis.* **15,** 171–180.
5. Kelly, D. P. & Strauss, A. W. (1994) *N. Engl. J. Med.* **330,** 913–919.
6. Lewis, G. F., Carpentier, A., Adeli, K. & Giacca, A. (2002) *Endocr. Rev.* **23,** 201–229.
7. Hildebrandt, A. L. & Neufer, P. D. (2000) *Am. J. Physiol.* **278,** E1078–E1086.
8. Jagoe, R. T., Lecker, S. H., Gomes, M. & Goldberg, A. L. (2002) *FASEB. J.* **16,** 1697–1712.
9. Nagao, M., Parimoo, B. & Tanaka, K. (1993) *J. Biol. Chem.* **268,** 24114–24124.
10. Ashrafi, K., Chang, F. Y., Watts, J. L., Fraser, A. G., Kamath, R. S., Ahringer, J. & Ruvkun, G. (2003) *Nature* **421,** 268–272.
11. Van Gilst, M. R., Hadjivassiliou, H., Jolly, A. & Yamamoto, K. R. (2005) *PLoS Biol.* **3,** e53.
12. McKay, R. M., McKay, J. P., Avery, L. & Graff, J. M. (2003) *Dev. Cell* **4,** 131–142.
13. Wang, J. & Kim, S. K. (2003) *Development (Cambridge, U.K.)* **130,** 1621–1634.
14. Emanuelsson, O., Nielsen, H., Brunak, S. & von Heijne, G. (2000) *J. Mol. Biol.* **300,** 1005–1016.
15. Garigan, D., Hsu, A. L., Fraser, A. G., Kamath, R. S., Ahringer, J. & Kenyon, C. (2002) *Genetics* **161,** 1101–1112.
16. Watts, J. L. & Browse, J. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 5854–5859.
17. Coleman, R. A., Lewin, T. M., Van Horn, C. G. & Gonzalez-Baro, M. R. (2002) *J. Nutr.* **132,** 2123–2126.
18. Ryu, M. H., Sohn, H. S., Heo, Y. R., Moustaid-Moussa, N. & Cha, Y. S. (2005) *Nutrition* **21,** 543–552.
19. Liu, F., Thatcher, J. D., Barral, J. M. & Epstein, H. F. (1995) *Dev. Biol.* **169,** 399–414.
20. Liu, F., Thatcher, J. D. & Epstein, H. F. (1997) *Biochemistry* **36,** 255–260.
21. Murphy, C. T., McCarroll, S. A., Bargmann, C. I., Fraser, A., Kamath, R. S., Ahringer, J., Li, H. & Kenyon, C. (2003) *Nature* **424,** 277–283.
22. Kayser, E. B., Morgan, P. G., Hoppel, C. L. & Sedensky, M. M. (2001) *J. Biol. Chem.* **276,** 20551–20558.
23. Hertzel, A. V. & Bernlohr, D. A. (2000) *Trends Endocrinol. Metab.* **11,** 175–180.
24. Erol, E., Kumar, L. S., Cline, G. W., Shulman, G. I., Kelly, D. P. & Binas, B. (2004) *FASEB J.* **18,** 347–349.
25. Watts, J. L. & Browse, J. (2000) *Biochem. Biophys. Res. Commun.* **272,** 263–269.
26. Dhe-Paganon, S., Duda, K., Iwamoto, M., Chi, Y. I. & Shoelson, S. E. (2002) *J. Biol. Chem.* **277,** 37973–37976.
27. Wisely, G. B., Miller, A. B., Davis, R. G., Thornquest, A. D., Jr., Johnson, R., Spitzer, T., Sefler, A., Shearer, B., Moore, J. T., Willson, T. M. & Williams, S. P. (2002) *Structure (Cambridge, U.K.)* **10,** 1225–1234.
28. Le May, C., Pineau, T., Bigot, K., Kohl, C., Girard, J. & Pegorier, J. P. (2000) *FEBS Lett.* **475,** 163–166.
29. Djouadi, F., Weinheimer, C. J. & Kelly, D. P. (1999) *Adv. Exp. Med. Biol.* **466,** 211–220.
30. Leone, T. C., Weinheimer, C. J. & Kelly, D. P. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 7473–7478.
31. Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B. & Wahli, W. (1999) *J. Clin. Invest.* **103,** 1489–1498.