Aus dem Institut für Humanernährung und Lebensmittelkunde
der Christian-Albrechts-Universität zu Kiel

Sex differences in *Caenorhabditis elegans* in body composition, lipid storage and gene expression under *ad libitum* and dietary restriction conditions

Dissertation
zur Erlangung des Doktorgrades
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

vorgelegt von

M.Sc. oec. troph. Claudia Miersch

aus Bergen auf Rügen

Kiel, 2012

Dekanin: Prof. Dr. Rainer Horn
1. Berichterstatter: Prof. Dr. Frank Döring
2. Berichterstatter: Prof. Dr. Gerald Rimbach
Tag der mündlichen Prüfung: 12.07.2012

Gedruckt mit Genehmigung
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

Wenn einer sucht, dann geschieht es leicht,
dass sein Auge nur noch das Ding sieht, das er sucht,
dass er nichts zu finden, nichts in sich einzulassen vermag,
weil er immer nur an das Gesuchte denkt,
weil er vom Ziel besessen ist.
Suchen heißt: ein Ziel haben.
Finden aber heißt: frei sein, offen stehen, kein Ziel haben.

(Hermann Hesse)

TABLE OF CONTENTS

Preface **I-X**

Table of contents I
List of figures II
List of tables IV
Abbreviations V
Abstract VII
Zusammenfassung IX

1 Introduction **1-8**

1.1 *Caenorhabditis elegans* as a model organism 1
1.2 Sex differences in *C. elegans* 2
1.3 Dietary restriction in *C. elegans* 4
1.4 Influence of paternal nutrition on offspring phenotype 7
1.5 Aims of the study 8

2 Material and Methods **9-13**

3 Results **14-55**

3.1 Analysis of sex differences in *C. elegans* 14
3.2 Response of males and hermaphrodites to dietary restriction 34
3.3 Influence of paternal dietary restriction on progeny fat content 52

4 Discussion **56-68**

4.1 Sex differences under *ad libitum* conditions in body proportions, RNA content, carbohydrate metabolism and gene expression of C-type lectins 56
4.2 Influence of dietary restriction on fat storage, lipid metabolism, developmental growth and reproduction in males and hermaphrodites 62
4.3 Influence of paternal dietary restriction on progeny fat content 66
4.4 Conclusion 67

5 References **69-81**

6 Appendix **82-117**

6.1 Supporting information 82
6.2 Acknowledgments 114

LIST OF FIGURES

figure no.	title	page
figure 1.	Life cycle of *C. elegans* from egg to adulthood.	1
figure 2.	Anatomy of *C. elegans* males (A) and hermaphrodites (B).	3
figure 3.	Morphology (A) and flow cytometry-based separation (B) of adult males and hermaphrodites in different *C. elegans* male mutants.	15
figure 4.	Relationship between time of flight (TOF) and body length (A) and extinction (EXT) and body volume (B).	16
figure 5.	Flow cytometry-based parameters of body proportions of *him-8 GFP* males and hermaphrodites at different developmental stages.	17
figure 6.	Body length (A) and body volume (B) of adult males and hermaphrodites in different *C. elegans* male mutants.	18
figure 7.	Body composition of males and hermaphrodites at different developmental stages.	19
figure 8.	Glucose and trehalose content of males and hermaphrodites at different developmental stages.	21
figure 9.	Total RNA content of young adult and adult *him-8 GFP* males and hermaphrodites.	22
figure 10.	Number of differentially expressed genes between *him-8 GFP* males and hermaphrodites.	24
figure 11.	Differential expression of C-type lectins between *him-8 GFP* males and hermaphrodites at young adulthood (66 h) and adulthood (76 h).	30
figure 12.	Changes of body proportions under DR (OD 1.5) in males and hermaphrodites at different developmental stages.	35
figure 13.	Changes of protein and total RNA content under DR (OD1.5) in *him-8 GFP* males and hermaphrodites at different developmental stages.	37
figure 14.	Changes of body composition under DR (OD1.5) in *him-8 GFP* males and hermaphrodites at different developmental stages.	38
figure 15.	Lipid droplets under *ad libitum* (top) and DR (OD 1.5, bottom)	39

conditions in *him-8* males (A) and hermaphrodites (B).

figure 16. Lipid droplets under *ad libitum* (top) and DR (OD 1.5, bottom) conditions in *him-5* males (A) and hermaphrodites (B). 40

figure 17. Lipid droplets under two DR (OD 1.5 and OD 0.7) conditions in *fog-2* males (A) and females (B). 41

figure 18. Changes of glucose and trehalose content under DR (OD 1.5) in *him-8 GFP* males and hermaphrodites at different developmental stages. 42

figure 19. Functional annotation clustering of DR regulated genes from *him-8 GFP* worms at young adult (66 h) stage. 44

figure 20. Functional annotation clustering of DR regulated genes from *him-8 GFP* worms at adult (76 h) stage. 46

figure 21. Differential expression of sperm-associated genes under DR (OD 1.5) in *him-8 GFP* males and hermaphrodites. 49

figure 22. Temporal gene expression drift of major sperm protein (*msp*) genes under DR (OD 1.5) in *him-8 GFP* hermaphrodites (top) and males (bottom). 50

figure 23. Temporal gene expression drift of sperm-associated genes under DR (OD 1.5) in *him-8 GFP* hermaphrodites (grey) and males (black). 51

figure 24. Experimental design to study the effects of paternal DR on the F1 generation. 52

figure 25. Influence of different DR conditions on P0 male body proportions. 53

figure 26. Influence of different paternal DR conditions on progeny body proportions. 54

figure 27. Influence of different paternal DR conditions on progeny fat content (A) and relative fat content (B). 55

figure 28. Conversion of glucose in males and hermaphrodites. 59

LIST OF TABLES

table no.	title	page
table 1.	Male-specific genes.	25
table 2.	Hermaphrodite-specific genes.	28
table 3.	Sex differences in the gene expression of carbohydrate metabolic genes.	32
table 4.	Number of DR-regulated genes in both sexes at both developmental stages.	43
table 5.	Summary of sex differences under *ad libitum* and DR conditions in *C. elegans*.	56

Supporting information

table S1	Calculation of dietary restriction ranking.	82
table S2	Body composition of males and hermaphrodites at different developmental stages in different male mutants.	83
table S3	Glucose and trehalose content of males and hermaphrodites at different developmental stages in different male mutants.	84
table S4	The entire list of male-specific genes.	85
table S5	The entire list of hermaphrodite-specific genes.	92
table S6	Body composition of *him-8 GFP* males and hermaphrodites under *ad libitum* and DR at different developmental stages.	98
table S7	Glucose and trehalose content of *him-8 GFP* males and hermaphrodites under *ad libitum* and DR at different developmental stages.	99
table S8	Overlapping genes between both sexes and both developmental stages.	100
table S9	Expression level and DR to *ad libitum* ratio of sperm-associated genes in both sexes at young adult (66 h) stage.	108
table S10	Expression level and DR to *ad libitum* ratio of sperm-associated genes in both sexes at adult (76 h) stage.	111

ABBREVIATIONS

acbp	**A**cyl-**C**oenzyme A **b**inding **p**rotein
age	**age**ing alteration
AMPK	5' adenosine monophosphate-activated protein kinase
ANOVA	analysis of variance
ATP	andenosine-5'-triphosphate
AU	arbitrary unit
BCA	bicinchoninic acid
ceh	***C**. **e**legans* **h**omeobox
C. elegans	*Caenorhabditis elegans*
CGC	*Caenorhabditis* Genetics Center
cht	**chit**inase
clec	**C**-type **lec**tin
cyp	**cy**tochrome **P**450 family
daf	abnormal **da**uer **f**ormation
DAVID	Database for Annotation, Visualization and Integrated Discovery
dhs	**d**e**h**ydrogenases, **s**hort chain
D. melanogaster	*Drosophila melanogaster*
dpy	**d**um**py** (shorter than wild type)
DR	dietary restriction
eat	**eat**ing (abnormal pharyngeal pumping)
EXT	extinction
flh	**FLYWCH** zinc finger transcription factor homolog
fog	**f**eminization **o**f **g**ermline
FOX	forkhead box
GFP	green fluorescence protein
gob	**g**ut **ob**structed or defective
gsp	GLC7 (yeast **G**lc **S**even) like **p**hosphatase
gsy	**g**lycogen **sy**nthase
hif	(**h**ypoxia **i**nducible **f**actor) homologa
him	**h**igh **i**ncidence of **m**ales
hsf	**h**eat **s**hock **f**actor
KEGG	Kyoto Encyclopedia of Genes and Genomes
let	**let**hal
lipl	**lip**ase **l**ike
lsy	**l**aterally **sy**mmetric (defective in lateral symmetry)
mab	**m**ale **ab**normal
maoc	**MAO**-**C**-like dehydratase domain
msp	**m**ajor **s**perm **p**rotein
mtl	**m**e**t**a**l**lothionein
mxl	**m**a**x**-**l**ike protein
NAD^+	nicotinamide adenine dinucleotide
NGM	Nematode Growth Medium
nhr	**n**uclear **h**ormone **r**eceptor
nlp	**n**europeptide-**l**ike **p**rotein
NMR	Nuclear Magnetic Resonance
nmur	**n**euro**m**edin **U** **r**eceptor

Nrf	nuclear respiratory factor
pes	**p**atterned **e**xpression **s**ite
pha	defective **pha**ynx development
pkd	**p**olycystic **k**idney **d**isease (related)
pmt	**p**hosphoethanolamine **m**ethyl**t**ransferase
PMT	photomultiplier
RNA	ribonucleic acid
rRNA	ribosomal RNA
sams	**S**-**a**denosyl **m**ethionine **s**ynthetase
scl	**SCP**-**l**ike extracellular protein
SD	standard deviation
sdz	**SKN**-1 **d**ependent **z**ygotic transcript
skn	**Sk**i**n**head
spe	defective **spe**rmatogenesis
ssp	**s**perm-**s**pecific family class **P**
ssq	**s**perm-**s**pecific family class **Q**
sss	**s**perm-**s**pecific family class **S**
sst	**s**perm-**s**pecific **t**ranscript
tbx	**T**-**b**o**x** family
TOF	time of flight
TOR	target of rapamycin
tps	**t**rehalose 6-**p**hosphate **s**ynthase
ugt	**U**DP-**g**lucoronosyl**t**ransferase
ztf	**z**inc finger putative **t**ranscription **f**actor family

ABSTRACT

The nematode *Caenorhabditis elegans* (*C. elegans*) contains two sexes, males and hermaphrodites, which are highly dimorphic in anatomy, nervous system and behavior. However, the physiology of males in comparison to hermaphrodites under normal feeding conditions and in the context of dietary restriction (DR) is largely unexplored. Here, body proportions and composition, certain aspects of carbohydrate metabolism, fat storage and gene expression of males and hermaphrodites were analyzed under *ad libitum* and DR conditions. Furthermore, males were used to determine the influence of different paternal DR regimes on F1 progeny fat content. In this study male-enriched mutant stains were used, one with a sex-specific GFP signal, led to a large number of males being separated from hermaphrodites by flow-cytometry.

Analysis of sex differences under *ad libitum* conditions unraveled that the fat-to-fat-free mass as well as the body volume adjusted fat mass was similar between the sexes, although the body size was over 50 % lower in adult males than in age-matched hermaphrodites. In contrast, males had an about 2-fold lower volume adjusted total RNA content than hermaphrodites. Biochemical and NMR-based analyses revealed higher trehalose and much lower glucose levels in males than in hermaphrodites, resulting in a 5.4-fold higher trehalose to glucose ratio. These sex differences were reflected by gene expression data since genes encoding key enzymes of the glycolysis and trehalose synthesis pathway were higher expressed in males than in hermaphrodites. Notably, the phosphofructokinase gene (C50F4.2) showed a 29-fold higher expression level in males. Comparative analysis of gene expression data identified 285 male-specific and 160 hermaphrodite-specific genes including transcription factors and many C-type lectin genes. More than 35 % of all C-type lectin genes were higher expressed in males and many of them had a more than 100-fold higher expression level.

As response to DR both sexes increased their fat-to-fat-free mass ratio and enlarged their lipid droplets to a similar extent. However, the reduction of body size, protein content and total RNA content was more pronounced in hermaphrodites under DR than in males. Functional enrichment analysis of gene expression data showed a DR-induced down-regulation of several embryogenesis-associated genes in hermaphrodites. Moreover, DR promoted an ongoing expression of sperm-

associated genes from young adulthood to adulthood in hermaphrodites but not in males.

In order to examine the influence of paternal diet on the next generation, *fog-2* mutant strain was used. *fog-2* hermaphrodites do not produce sperm and are therefore termed as females, while *fog-2* males are unaffected. Males undergoing several DR regimes were crossed with *ad libitum* fed females. The corresponding progeny were analyzed according to their fat content with BODIPY^{TM} 493/503 staining. An inverted U-shaped relationship between the extent of paternal DR and the level of progeny fat content was found. The relationship was evident in both progeny sexes, while body proportions did not change.

In conclusion, these findings extend the knowledge of sex differences in *C. elegans* under *ad libitum* and DR conditions. The data identified sex differences in carbohydrate metabolism, which are linked to gene expression under *ad libitum* condition. DR increases the fat stores of both sexes in form of large lipid droplets and prolongs the reproductive program of hermaphrodites. Furthermore, these results establish a connection between paternal food consumption and adaptive developmental plasticity.

ZUSAMMENFASSUNG

Der Fadenwurm *Caenorhabditis elegans* (*C. elegans*) enthält in seiner Population zum größten Teil sich selbst befruchtende Hermaphroditen. Jedoch treten durch Non-Disjunction Ereignisse während der Meiose auch wenige Männchen (ca. 0.3 %) auf. Beide Geschlechter unterscheiden sich hinsichtlich ihrer Anatomie, ihres Nervensystems und ihres Verhaltens. Aufgrund des geringen Vorkommens ist über die Physiologie der Männchen im Vergleich zu den Hermaphroditen, insbesondere im Rahmen einer Nahrungsrestriktion (NR), kaum etwas bekannt. Ziel dieser Arbeit war es daher, die Körperproportionen, die Körperzusammensetzung, verschiedene Aspekte des Kohlenhydrat- und Fettstoffwechsels sowie das Genexpressionsprofil von Männchen und Hermaphroditen unter *ad libitum* und NR Bedingungen zu analysieren. Außerdem wurden nahrungsrestriktiv ernährte Männchen verwendet, um den Einfluss einer paternalen NR auf den Fettgehalt der F1-Nachkommen zu untersuchen. Für alle Analysen wurden Mutanten-Stämme verwendet, die einen höheren Anteil an Männchen in ihrer Population aufweisen. Zusätzlich wurde auf einen Mutanten-Stamm zurückgegriffen, der GFP-markierte Männchen enthält, wodurch mittels Durchflusszytometrie eine optimale Abtrennung von Hermaphroditen erreicht werden konnte.

Unter *ad libitum* Bedingungen zeigten beide Geschlechter ein ähnliches Verhältnis von Fettmasse zur fettfreien Masse, obwohl Männchen über 50 % kleiner sind als Hermaphroditen. Im Gegensatz dazu, wiesen Männchen einen 2-fach geringeren RNA Gehalt auf. Biochemische und NMR-basierte Methoden zeigten weiterhin, dass Männchen höhere Trehalose aber niedrigere Glukose Werte im Vergleich zu Hermaphroditen besitzen. Diese Geschlechtsunterschiede spiegelten sich deutlich auf Genexpressionsebene wieder. Gene von Schlüsselenzymen der Glykolyse und der Trehalosesynthese wurden in Männchen höher exprimiert als in Hermaphroditen. Das Gen der Phosphofruktokinase (C50F4.2) zeigte dabei mit einer 29-fach höheren Expression in Männchen einen deutlichen Unterschied zu Hermaphroditen. Die weitere Analyse der Genexpressionsdaten identifizierte 285 Gene spezifisch für Männchen und 160 Gene spezifisch für Hermaphroditen. Innerhalb dieser Gene fanden sich viele Transkriptionsfaktoren und C-Typ Lektine. Besonders auffällig war, dass von allen C-Typ Lektinen in *C. elegans* über 35 % höher in Männchen exprimiert wurden.

Unter NR konnte in Hermaphroditen wie auch in Männchen ein Anstieg der Fettmasse und eine Vergrößerung der Fetttröpfchen festgestellt werden. Beide Geschlechter reduzierten ihre Körpermasse und ihren Proteingehalt mit wesentlich deutlicheren Effekten in Hermaphroditen. Zusätzlich war der RNA-Gehalt in Hermaphroditen deutlich abgesenkt, während bei den Männchen keinerlei Veränderungen messbar waren. Mit Hilfe einer Anreicherungsanalyse der Genexpressionsdaten konnte gezeigt werden, dass es unter NR zu einer verminderten Expression von Embryogenese-Genen in Hermaphroditen kommt. Außerdem blieb die unter *ad libitum* beobachtete Absenkung der Transkriptspiegel von spermien-spezifischen Genen unter NR Bedingungen aus.

Um den Einfluss der paternalen Ernährung auf die nächste Generation zu untersuchen, wurden fog-2 Hermaphroditen verwendet, die keine Spermien bilden können und daher als Weibchen bezeichnet werden. fog-2 Männchen werden durch diese Mutation nicht beeinflusst. Im Experiment wurden Männchen verschiedenen NR Bedingungen ausgesetzt und anschließend mit *ad libitum* gefütterten Weibchen gekreuzt. Die daraus entstehenden Nachkommen wurden hinsichtlich ihres Fettgehaltes mit einer fixierten BODIPY^{TM} 493/503 Fettfärbung untersucht. In den Nachkommen von nahrungsrestriktiv ernährten Männchen wurde eine inverse U-förmige Beziehung zwischen ihrem Fettgehalt und dem Ausmaß der paternalen NR gefunden. Dieser Zusammenhang wurde in beiden Geschlechtern beobachtet, ohne dass es zu Veränderungen in den Körperproportionen kam.

Diese Arbeit erweitert unser Wissen über Geschlechtsunterschiede beim Fadenwurm *C. elegans* unter normalen und restriktiven Nahrungsbedingungen. Die Daten zeigen unter *ad libitum* Bedingungen eine Verknüpfung zwischen den gefundenen Geschlechtsunterschieden im Kohlenhydratstoffwechsel und der Genexpression. Unter NR Bedingungen erhöhen beide Geschlechter ihre Fettreserven über größere Lipidtröpfchen und die Hermaphroditen setzen verzögert in die Reproduktionsphase ein. Weiterhin eröffnen die Ergebnisse eine Verbindung zwischen dem paternalen Nahrungskonsum und der physiologischen Varianz in der Entwicklung eines Organismus.

1 INTRODUCTION

1.1 *Caenorhabditis elegans* as a model organism

Caenorhabditis elegans (*C. elegans*) is a free-living non-parasitic nematode, which belongs to the family *Rhabditidae*. It feeds on microorganisms and is found in the soil, especially in rotting material, throughout the temperate regions of the world. In the laboratory, worms can easily be maintained on agar plates or in liquid cultures using *Escherichia coli* (*E. coli*) bacteria as food source [1]. The development of *C. elegans* from egg to adult takes about 3 days at 20 °C if sufficient food is available (**figure 1**). After embryogenesis, the worm passes through four larval stages before reaching adulthood. When during development food is rare at a high population density, worms undergo an alternative arrested developmental state, called dauer [2]. In this situation worms do not age and are able to survive long periods without food. This ensures the survival of individuals in a changing environment.

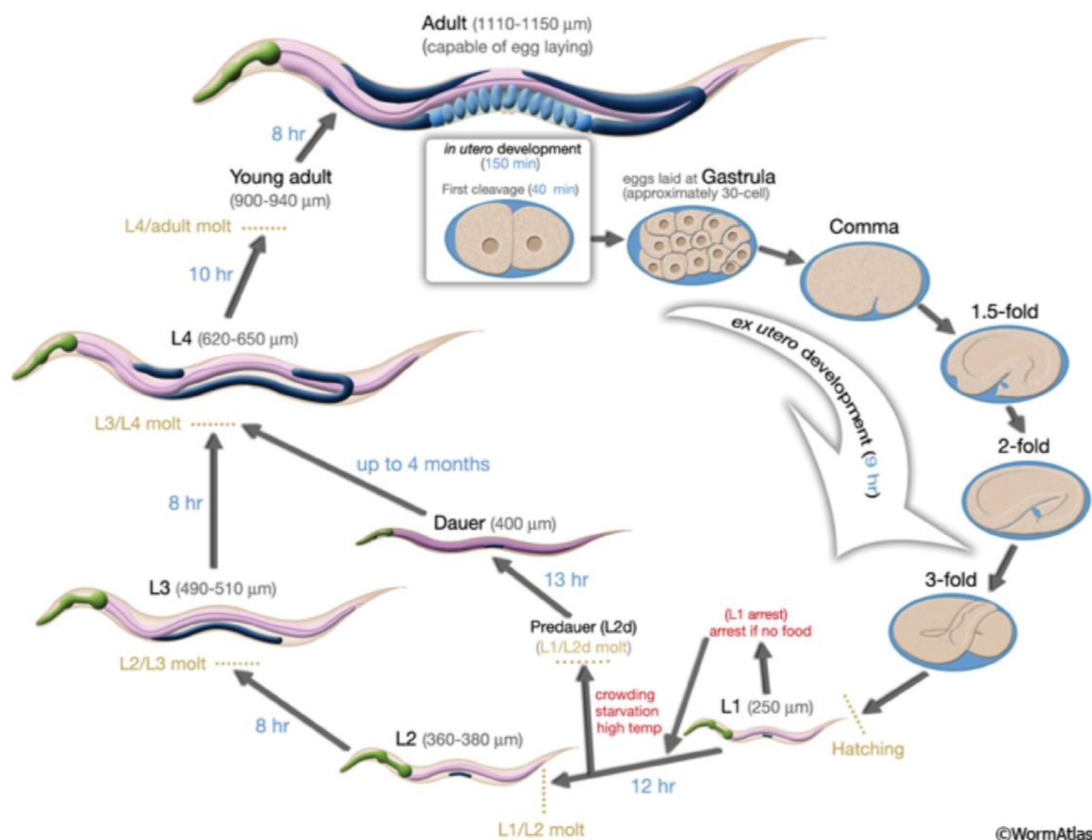


Figure 1. Life cycle of *C. elegans* from egg to adulthood.
The graphical image was taken from WormAtlas (www.wormatlas.org).

Sydney Brenner introduced *C. elegans* as a model system for biological research in the early 1970’s [3] and since that time it has been widely analyzed. Its popularity is based on a series of reasons including its short life cycle, its cheap and ease handling in the laboratory, its small size (about 1 mm), its large brood size (about 300 progeny) and its uniform development. It can simply be studied in the microscope since it is transparent throughout the whole life. By production of glycerol stocks, worms can be long-term stored at a freezing temperature of -80 °C. Additionally, the genome of *C. elegans* was the first sequenced one from a multi-cellular organism [4]. Since the genetically tractable model *C. elegans* offers a complex metabolic regulation, it is also used for nutritional research [5].

1.2 Sex differences in *C. elegans*

C. elegans consists of two sexes - self-fertilizing hermaphrodites (diploid X chromosome) and conventional males (single X chromosome). Hermaphrodites are the dominant sex, however, at a low frequency (about 1 in 500, <0.3 %) males are generated by spontaneous X chromosome non-disjunction events occurring during meiosis [6]. Mating produces a uniform number of males and hermaphrodites because male sperm replace hermaphrodite sperm from fertilization due to bigger sperm resulting in an equal segregation of sex chromosomes [7,8]. Although males are smaller in size than hermaphrodites, they have more somatic cells (1031 in males and 959 in hermaphrodites) [9,10]. Furthermore, the male anatomy (**figure 2 A**) differs from hermaphrodite according to the one gonadal arm, the rounded fan-like tail and some specialized muscles, neurons and sensory cues [11]. Adult hermaphrodites have a peaked tail, a vulva, a uterus and two gonadal arms that produce first a limited number of sperm and then oocytes (**figure 2 B**). The pharynx, the excretory system and the main body muscles do not show sexual dimorphic characteristics [9,10,12]. Most of the physical differences between the sexes arise from a different larval development since males and hermaphrodites are nearly identical at hatching. Under the stereomicroscope, males can be first distinguished from hermaphrodites at the larval stage L4, when the male tail starts to become blunt-ended. The postembryonic development, characterized by forming sex-specific cell lineages and differentiated cells, has been previously examined [9,13].

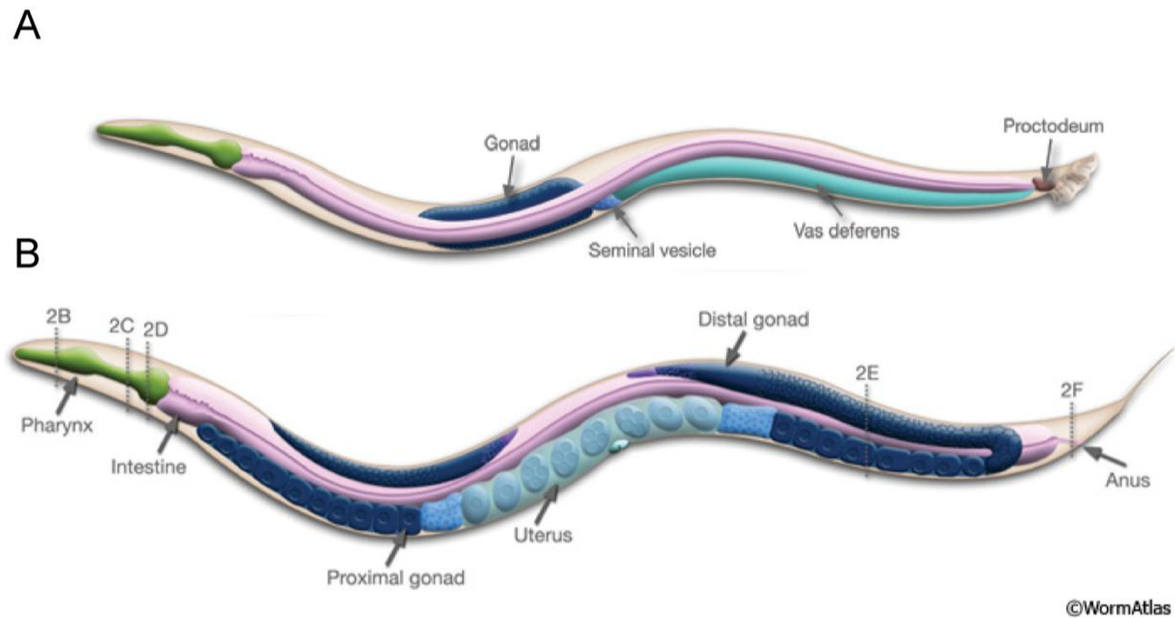


Figure 2. Anatomy of *C. elegans* males (A) and hermaphrodites (B).
Virtual drawings of both sexes were taken from WormAtlas.

Due to sex-specific neurons and sex differences in the core nervous system itself, *C. elegans* exhibits a sexual dimorphic nervous system [14]. As a result of this difference, males and hermaphrodites possess some sex-specific behavior, such as hermaphrodite egg laying behavior and male mating behavior [15-17]. Furthermore, some common behaviors are pronounced differently between the sexes, including the food leaving behavior, locomotion, olfaction, learning and memory capabilities [18-21]. Food leaving behavior occurs in males, when they are cultivated on agar plates with an adequate food source without hermaphrodites, whereas hermaphrodites remain on agar plates when no males are present [19]. The authors conducted that males try to find sexual partners for mating, while hermaphrodites do not need to mate for reproduction.

There are also sex differences in physiological aspects like life span, stress response and immune resistance. In many mammals, including humans, females live longer than males [22] but in *C. elegans* the lifespan of both sexes strongly depends on the culture conditions and social interactions. Cultivating males and hermaphrodites on agar plates at 20 °C result in a median lifespan of 10 and 17 days, respectively [23]. However, when males are cultivated individually, the male median lifespan rise above the hermaphrodite lifespan by 20 %. This increase is possibly due to the lack of male-

male interaction and some other unknown aspects of locomotion behavior. It was also found that mating dramatically reduces the median life span of hermaphrodites from 17 to 8 days [24]. In terms of oxidative stress, males are much more sensitive to hydrogen peroxide and paraquat than hermaphrodites due to the sex-specific transcription factor *mab-3* [25]. In contrast, males are more resistant to the fungal pathogen *Cryptococcus neoformans* than hermaphrodites depending on the *daf-16* transcription factor [26]. Sex differences are also visible on the molecular level, as indicated by gene expression analysis [27-29]. These studies focused on gene expression and sex differences during larval development and cell differentiation.

The average body size of adult males is about threefold lower than that of adult hermaphrodites [30]. Chemically, worms consist largely of water (nearly 85 %) [31] followed by proteins, lipids, carbohydrates, nucleic acids and minerals. Adult hermaphrodites have a triglyceride content that is five- to tenfold lower than the protein content [32-34]. The carbohydrate trehalose is supposed to function in the soluble transport of sugar but its level are lower in comparison to glucose [35-38]. So far, there is essentially no information about body composition and carbohydrate metabolism in *C. elegans* males.

1.3 Dietary restriction in *C. elegans*

Dietary restriction (DR) is a nutritional regime without signs of malnutrition. During DR the intake of all macro- and micronutrients is reduced to about 20-50 % compared to *ad libitum* feeding [39]. In addition to DR, the term caloric restriction is often used synonymously. Here, the term DR is used since a reduction of bacteria is inevitably accompanied by a reduced intake of carbohydrate, protein and/ or fat. DR modulates a wide range of biological processes and particularly impacts ageing and age-related disorders (reviewed in [39]). It has been demonstrated that DR affects lifespan [40], mortality [40], tumor progression [41], neurodegenerative disorders [42,43], insulin sensitivity [44,45], immune defense [46], physical activity [47] and reproduction [48]. Many findings have been found in rodents and especially lifespan extension has been seen in a wide variety of species such as the Baker's yeast *Saccharomyces cerevisiae* [49,50], the nematode *C. elegans* [51], the fruit fly *Drosophila*

melanogaster (*D. melanogaster*) [52,53], grasshoppers [54], spiders [55], hamsters [56], dogs [57] and even primates [58,59].

Since the model organism *C. elegans* has often been used for studying aging, it was initially observed that DR induces lifespan extension [51]. A variety of environmental and genetic methods have been developed to examine DR in this model. At normal feeding conditions, worms are maintained on agar plates or in liquid cultures with an adequate amount of *E. coli* bacteria. To induce DR, one can (1) prepare different bacterial dilutions in liquid culture [51] or on plates [60], (2) produce plates with different dilutions of bactopeptone [61], (3) use axenic culture medium [62,63] or (4) analyze *eat* (abbreviation of eating) mutants, which have a reduced food intake [64]. Furthermore, it is possible to prepare agar plates containing no bacteria to starve worms [65,66].

Despite a long history on DR research, it is not precisely known yet, how DR mediates its beneficial effects. As DR increases lifespan in nearly every animal evaluated, researchers assume the existence of evolutionarily conserved pathways [67]. A reduced activity of the insulin/insulin-like growth factor cascade, a decline in the TOR/AMPK amino acid sensing pathway, an activation of oxidative stress pathways, an altered chromatin silencing by sirtuins and a modulated activity of central transcription factors (reviewed in [68]) are discussed as possible mechanisms. The insulin/insulin-like growth factor signaling pathway is highly conserved analogical to the mammalian insulin and insulin-like growth factor-1 pathways [69]. Worms with a loss-of-function mutation in the insulin/IGF-1 receptor *daf-2* or in genes acting downstream (i.e. *age-1*) show an increased lifespan [70,71]. This effect is mediated via the FOXO (forkhead box O) transcription factor *daf-16* [70,71]. However, some studies suggested that the insulin/IGF-1 signaling is not required for a DR-mediated lifespan extension in *C. elegans* since worms with mutations in this pathway still increase their lifespan under DR [64,72]. The protein kinase TOR is a central factor in a putative amino acid sensing pathway that plays an essential role in cell growth, protein synthesis and metabolic regulation [73,74]. Inhibition of *let-363*, the *C. elegans* ortholog of mammalian TOR, by RNAi extends lifespan [75]. Also an increased expression of the NAD^+-dependent protein deacetylase sirtuin 2.1 results in an increased lifespan [76,77]. However, there are

conflicting data whether lifespan extension caused by DR is sirtuin dependent [78] or not [65]. Several transcriptional factors have also been discussed to mediate DR induced longevity like *pha-4*, *skn-1*, *hif-1* and *hsf-1* [79-82]. *pha-4* is orthologous to the mammalian FOXA (forkhead box A) family of transcription factors, involved in embryonic development and metabolic homeostasis [83,84]. *skn-1* is similar to the mammalian *Nrf1* and *Nrf2* transcription factors, which mediate stress response and induce detoxification enzymes [85,86]. *hif-1* (hypoxia) and *hsf-1* (heat-shock) are also involved in the stress response system of *C. elegans* [87,88]. Whether a pathway is regulated under DR and to what extent depends on the particular DR protocol [89], the mutant strains used and the developmental time period of the DR regime.

While in *C. elegans* the life-prolonging effect of DR and the identification of involved genes and pathways have been in the spotlight during the last years [90], the effects and mechanisms of DR on metabolism have not been studied extensively. Comparative genomic analyses and metabolic studies unraveled that the intermediary metabolism present among eukaryotes can also be found in *C. elegans* (see WormBase, KEGG or [91]). These include anabolism and catabolism reactions of lipids, carbohydrates and proteins like glycolysis, gluconeogenesis, β-oxidation or citric acid cycle [92]. *C. elegans* generally stores energy in form of fat (mainly triglyceride) and carbohydrates (glycogen, trehalose and glucose) [92,93]. The major fat storage compartments are intracellular lipid droplets [94] as also seen in other species [95,96]. These droplets are found in intestinal and skin-like hypodermal cells [93,97]. The key metabolic pathways, especially with regard to lipid metabolism and homeostasis, are highly conserved in *C. elegans* [5,92]. Thus, this model is indeed suitable for analyzing the metabolism. So far, it is known that DR increases resistance to thermal and oxidative stress [65,72,98], elevates activity of stress-defense enzymes (superoxide dismutase, catalase) [62,72], reduces body size [99], decreases ATP concentrations but does not reduce the metabolic rate of *C. elegans* [62,100,101]. Moreover, DR or starvation influences different behavior like locomotion [102], pharyngeal pumping [103], reproduction [51,79] and olfaction [104].

Although the effects of DR are extensively studied in several species, reports about sex differences in the response to DR are rare. In rats, males maintain a higher body weight under DR than females, whereas females increase their activity level,

decrease the gonad size and improve their cognitive performance in comparison to males [105]. Circulating lipids and energy-regulating hormones showed similar responses to DR in both sexes [105]. In *D. melanogaster*, a sex-specific DR effect is found for lifespan [106]. In this study female flies are more sensitive to DR and starvation than males, resulting in a greater lifespan extension. For *C. elegans*, the majority of studies analyzing the effects of DR used hermaphrodites. Some reports have shown that starvation or inedible food leads to changes in mating behavior [107-109], physical activity, oxygen consumption and body composition [107,110] of males. However, it is essentially unknown, how *C. elegans* males respond to DR.

1.4 Influence of paternal nutrition on offspring phenotype

Evidence that the environment has not only an influence on the organism itself but also on its offspring has been reported in many studies but the focus lay often on the maternal side [111]. Several parental factors including stress [112], nutrition [113,114] and chemicals [115,116] have been studied. Maternal undernutrition in rodents causes intrauterine growth retardation, lower birth weight followed by a catch-up growth phase, glucose intolerance, insulin resistance and adipocyte hypertrophy in two generations [117,118]. In many species it is difficult to define the exact mechanism of maternal effects on offspring phenotype since the offspring develop *in utero* and are influenced by the mother postnatally. Many of these aspects disappear in the analysis of paternal effects but there are far fewer results at the moment.

Epidemiological data from human populations indicate that a paternal experience of famine is associated with obesity and cardiovascular disease in the F2 generation [119,120]. Three recent studies in rodents provide first experimental evidence that fasting [121], high-fat diet [122] or low-protein diet [123] of fathers affect offspring adiposity and its related diseases. Beside the nutritional status the paternal exposure to drugs, toxins and chemicals is related to changes in offspring phenotype [124]. Laboratory mice studies of paternal alcoholism revealed a variety of alterations in behavior, development and physiology [125,126]. Effects on the offspring are also found when males are treated with other drugs and toxins [127]. Up to now there is still little information about how parental effects are transferred to the offspring.

Alterations in offspring gene expression, DNA methylation, chromatin packaging and RNA content have been described [122,123], implicating that parental epigenetic marks may be transmitted into the next generation. But examples of true gametic epigenetic inheritance are rare in literature [128]. On the other hand some non-gametic epigenetic inheritance has been discovered like the transmission of maternal virus [129] or behavioral mother-offspring-interactions [112].

Studies using *C. elegans* as model for analyzing parental effects indicated that environmental information can be "communicated" to the progeny [130,131]. Indeed, these reports show that exposure of hermaphrodites to osmotic [130] or olfactory [131] stimuli alter progeny phenotype but paternal influence on progeny was not evaluated. Exciting results have recently shown for the first time in *C. elegans* that longevity can be epigenetically transmitted until three generations via modulation of H3K4me3 regulators [132].

1.5 Aims of the study

The present study focused on the systematic analysis of body proportion and composition parameters, certain aspects of carbohydrate metabolism and fat storage as well as gene expression profile of *C. elegans* males and hermaphrodites under *ad libitum* (**result section 3.1**) and DR (**result section 3.2**) conditions. As a sub-goal of this study, the sex differences in physiology should be linked to gene expression results. Furthermore, it was tested whether a paternal induced non-genetic and intergenerational inheritance is measurable in *C. elegans* and whether it depends on the treatment dose. For this purpose, the relationship between the extent of paternal DR and the progeny fat content at adulthood was examined (**result section 3.3**).

2 MATERIAL AND METHODS

Nematode cultivating and strains

C. elegans worms were maintained on Nematode Growth Medium (NGM) agar plates seeded with *E. coli* OP50 at 20 °C as described by Brenner [3]. The strain Bristol N2 was used as wild type control. Mutants used in this study were *fog-2 (q71)* V, *him-8 (e1489)* IV, *him-5 (e1490)* V and *him-8 (e1489)* IV; *nls128* II [pkd-2::GFP; lin-15 (+)]. All strains were obtained from *Caenorhabditis* Genetics Center (CGC, University of Minnesota, USA), except *him-8 (e1489)* IV; *nls128* II, generously provided by the Horvitz laboratory. All experiments were carried out with synchronized eggs obtained by hypochlorite treatment of gravid worms.

Microscopic imaging and determination of body proportions

Worms were visualized using an Axio Observer D1 inverted microscope (Carl Zeiss AG, Jena, Germany) fitted with a digital camera (Axiocam MRm, Carl Zeiss AG). Body length, width, perimeter and area were determined by bordering the worms in Axio Vision Software (version 4.8, Carl Zeiss AG). Body volume (V) was determined from area (A) and perimeter (P) as described [133,134] using the following equation:

$$V = \frac{\pi \left(P + \sqrt{P^2 - 16A}\right)\left(P - \sqrt{P^2 - 16A}\right)^2}{256}.$$

COPAS flow cytometric analysis

Flow cytometry was used to select a defined number of eggs for equal experimental settings, to analyze worm characteristics and to separate males and hermaphrodites for worm extracts and RNA isolation. Axial length (time of flight, TOF), optical density (extinction, EXT) and fluorescence (green, yellow, red) were automatically measured for each worm. The instrument was equipped as described in Klapper *et al.* [32]. The gain signals for extinction, green, yellow and red were set to 1. The photomultiplier (PMT) value for the green channel was set to 900. The threshold value for signal and TOF minimum were adopted depending on whether eggs or worms were sorted. For all experiments specific gating and sorting criteria were used.

Separation of males using COPAS flow cytometry

The separation procedure for generating pure male samples varied for different strains. The transgenic strain *him-8 (e1489)* IV; *nls128* II expresses GFP under the control of the *pkd-2* promoter only in male-specific neurons. Thus, males were separated from hermaphrodites by a specific green fluorescence signal using flow cytometry. No separation was possible before young adult stage (~64-66 h), due to low signal strength in earlier stages. Based on this mutant strain, it was possible to obtain a nearly 100 % rate of males. In N2 and all other mutants (*fog-2*, *him-8*, *him-5*) separation of males were achieved by choosing an area in green versus yellow fluorescence (auto-fluorescence) dot blot because hermaphrodites exhibit a higher auto-fluorescence than males. Since good separation rates were only attained when worms were one day of adulthood (90 h), this time point was used for all measurements. The rate of males ranged between 87 % and 99 % depending on the strain.

Dietary restriction protocol

The dietary restriction method was a modified protocol from Klass *et al.* [51] and recently developed in our laboratory by D. Palgunow. Worms were kept on NGM plates with (*ad libitum* feeding, control) or without bactopeptone (dietary restriction [DR]). Additionally, NGM plates were seeded with a defined quantity of bacteria (see **supporting information table S1**) and two bacterial dilutions (OD_{600nm} [OD] 1.5 or 0.7) were used for DR-plates to create a dose-dependency. Unless otherwise indicated 600 eggs per plate were utilized and worms were maintained on the same plates until they were harvested.

BODIPY^{TM} 493/503 fixative staining and fluorescence imaging

BODIPY^{TM} 493/503 fixative staining was performed as previously described [32]. Worms were harvested, washed in M9 for 2-3 times, anesthetized in sodium azide (2 %) for 2 min, washed again and incubated in paraformaldehyde (4 %) for 15 min at room temperature. After three cycles of freeze and thaw in liquid nitrogen, worms were washed with M9 and incubated in BODIPY^{TM} 493/503 staining solution (1 µg/ml) for 1 h at room temperature. Worms were then washed again with M9 and were used for microscopic analysis. BODIPY-stained worms were imaged with the fluorescence unit of the microscope described above. Worms, which were compared

in analysis with each other, were photographed at a fixed exposure time. The Axio Vision Software (Carl Zeiss AG) was used to quantify the BODIPY fluorescence and to improve the sharpness of all illustrated pictures with unsharp masking algorithm. BODIPY fluorescence was expressed as absolute fat content (BODIPY fluorescence per worm, **figure 27 A**) or as relative fat content (BODIPY fluorescence per worm and body volume, **figure 27 B**).

Worm extracts and biochemical assays

1000-1500 worms were collected in 2 ml tubes using flow cytometry, suspended in 100 µl NET buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA pH 8.0, 0.5 % CHAPS, protease inhibitor cocktail [Roche Diagnostics GmbH, Mannheim, Germany]) and homogenized with a bead-beating homogenizer (Precellys®24, Bertin Technologies, Montigny le Bretonneux, France). Homogenization was made with ceramic beads (1.4 mm) at full speed (6500 rpm) for 20 s as 1 cycle. The unsolvable cell debris was pelletized at full speed for 20 min at 4 °C and then removed. The resulting supernatants were stored at -80 °C until analyzed in biochemical assays. Protein and triglyceride content were measured from worm extracts using BCA Protein Assay kit (Pierce protein assay kit, Thermo Fisher Scientific Inc., Rockford, USA) and an enzymatic triglyceride assay kit (Analyticon, Lichtenfels, Germany) with a separate triacylglycerol standard (BioCat, Heidelberg, Germany), respectively. Trehalose and glucose were quantified simultaneously by trehalose assay kit (Megazyme, Bray, Ireland). Individual worm attributes (TOF and EXT) were measured during worm sorting. To equalize body volume differences between males and hermaphrodites a hermaphrodite to male extinction ratio was calculated, which was used to normalize male body composition and carbohydrate values so that they are comparable to hermaphrodite.

Nuclear magnetic resonance (NMR) spectroscopy

5000 worms per sample were collected using flow cytometry and homogenized in 270 µl distilled water by Precellys®24 with the same conditions as mentioned above. Unsolvable residues were removed by centrifugation and the samples were stored at -80 °C. NMR analysis was carried out at LipoFIT Analytic GmbH (Regensburg, Germany) on a Bruker Avance II + at 600 MHz. The amount of glucose and trehalose were determined by normalizing each integral to the corresponding integral of the

standard solution. Each experiment was performed in triplicate. The same extracts were determined biochemically and compared with NET buffer results.

Isolation of total RNA for gene expression analysis

1200-1800 worms were collected with flow cytometry and suspended in 350 µl RLT buffer (RNeasy mini Kit, Qiagen, Hilden, Germany). Worms were homogenized with Precellys®24 (full speed, 15 s, 2 cycles) and QIAshredder columns (Qiagen). Total RNA was extracted with RNeasy mini Kit (Qiagen) including a DNA digestion according to the manufacturer's instructions. The RNA amount and integrity was assessed spectrophotometrically (BioPhotometer, Eppendorf, Hamburg, Germany) and with a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, USA) using the RNA 6000 Nano Kit (Agilent Technologies). Each sample contained at least 10 µg total RNA per µl.

Gene expression analysis

Microarray processing and normalization of row data was done by Source Bioscience (imaGenes, Berlin, Germany) using a custom-designed Agilent gene expression microarray. The microarray was developed by Source Bioscience/imaGenes (Steffen Hennig) and contained 61643 oligonucleotides, which resulted in 26843 genes. Normalization was done by quantile normalization, which was calculated by R-package [135]. Each experiment was performed in triplicate. For analysis of sex differences the average expression of each gene was calculated with a sex-specific ratio (male- or hermaphrodite-specific). As statistical cut-off, $p<0.01$ (two-tailed Student's t-test) was applied. Gene ontology of male- and hermaphrodite-specific genes was examined with WormBase database (WormBase release WS229, www.wormbase.org). Genes, involved in carbohydrate metabolism, were identified with Kyoto Encyclopedia of Genes and Genomes pathway (KEGG, http://www.genome.jp/kegg/). To determine gene expression changes under DR an average expression ratio of DR to *ad libitum* condition was calculated for each gene. Gene ontology of DR-regulated genes was identified with WormBase. The database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.7, http://david.abcc.ncifcrf.gov/) was used for functional gene annotation to unravel over-represented gene families. Enrichment score and p-value with Benjamini multiple test correction was specified by DAVID.

F1 experiment and dietary restriction ranking

For F1 experiments several DR and starvation conditions were used. Modulation of DR was achieved by two bacterial dilutions (OD 1.5 and 0.7) and sorting of different number of eggs (300-900) per plate. For starvation conditions, worms were dietary restricted for 48 h and then starved for 24 h (OD 0.7-starvation) or starved for 24 h and then dietary restricted for 48 h (starvation-OD 0.7). Mating was performed on normal NGM plates spread with a dot of OP50 bacteria. Males were removed after 4 h in order to limit the time of mating. Females were allowed to lay eggs for 6 to 8 h before they were removed. The resulting F1 generation from all regimes were maintained on *ad libitum* plates until one day of adulthood (~88 h). The available food amount per worm was calculated by considering the quantity of bacteria, the worms per plate and the starvation time point. To quantify bacteria, the optical density on plates was measured after incubation at 37 °C over night. The ranking of each condition was performed according to the calculated food amount (see **supporting information table S1**). The harshest DR was allocated as value 8. The ranks were awarded as follows: 1 (OD 1.5, 300 eggs), 2 (OD 0.7, 300 eggs), 3 (OD 1.5, 600 eggs), 4 (OD 1.5, 900 eggs), 5 (OD 0.7, 600 eggs), 6 (OD 0.7, 900 eggs), 7 (starvation-OD 0.7) and 8 (OD 0.7-starvation).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). To determine statistically significant differences among male strains (**figure 6**) and between DR conditions (**figure 12 C**) one-way analysis of variance (ANOVA) was performed, followed by a Bonferroni multiple comparison test. When inhomogeneity of variance was evident, the Dunnett's T3 test was used as posthoc test. Correlation between TOF and body length and EXT and body volume was conducted (**figure 4**) using linear regression with calculation of coefficient of determination (r^2) and p-value. For evaluation of F1 experiments, regression analysis was conducted using linear or quadratic regression, respectively, depending on, which model fitted best. All other results were analyzed with Student's t-test under consideration of variance homogeneity. Results were considered significant at $p<0.05$. All statistical analyses were performed with SPSS (version 11.0).

3 RESULTS

3.1 Analysis of sex differences in *C. elegans*

N2, fog-2, him-8, him-8 GFP and him-5 male mutants are similar in morphology

To unravel sex differences in *C. elegans*, *him* (**h**igh **i**ncidence of **m**ales) and *fog* (**f**eminization **o**f **g**ermline) mutants, which produce higher proportions of males in comparison to N2 wild type hermaphrodites, were used. N2 males, which were obtained in higher frequency by mating, were also utilized in initial control experiments. Mutations in *him-8* and *him-5* gene result in more than 30 % males due to meiotic X chromosome loss [6,136]. The transgenic mutant strain *him-8 (e1489)* IV; *nls128* II (abbreviatory: *him-8 GFP*) has also a higher incidence of males due to his *him-8* background and expresses a GFP signal in male-specific neurons [137]. *fog-2* hermaphrodites are functional females because they do not produce sperm [138]. Hence, this strain is maintained by mating and nearly 50% of males are produced. To compare the morphology of the selected male mutants *fog-2*, *him-8*, *him-8 GFP* and *him-5* with N2 wild type, adult worms were inspected by microscopy. Differences in behavior between mutants and N2 were not obviously under a dissecting microscope. Both sexes from all analyzed mutants were similar in general anatomy and morphology in comparison to N2 wild type (**figure 3 A**). Thus, the used male mutants proved to be suitable for studying sex differences in *C. elegans* regarding certain physiological aspects.

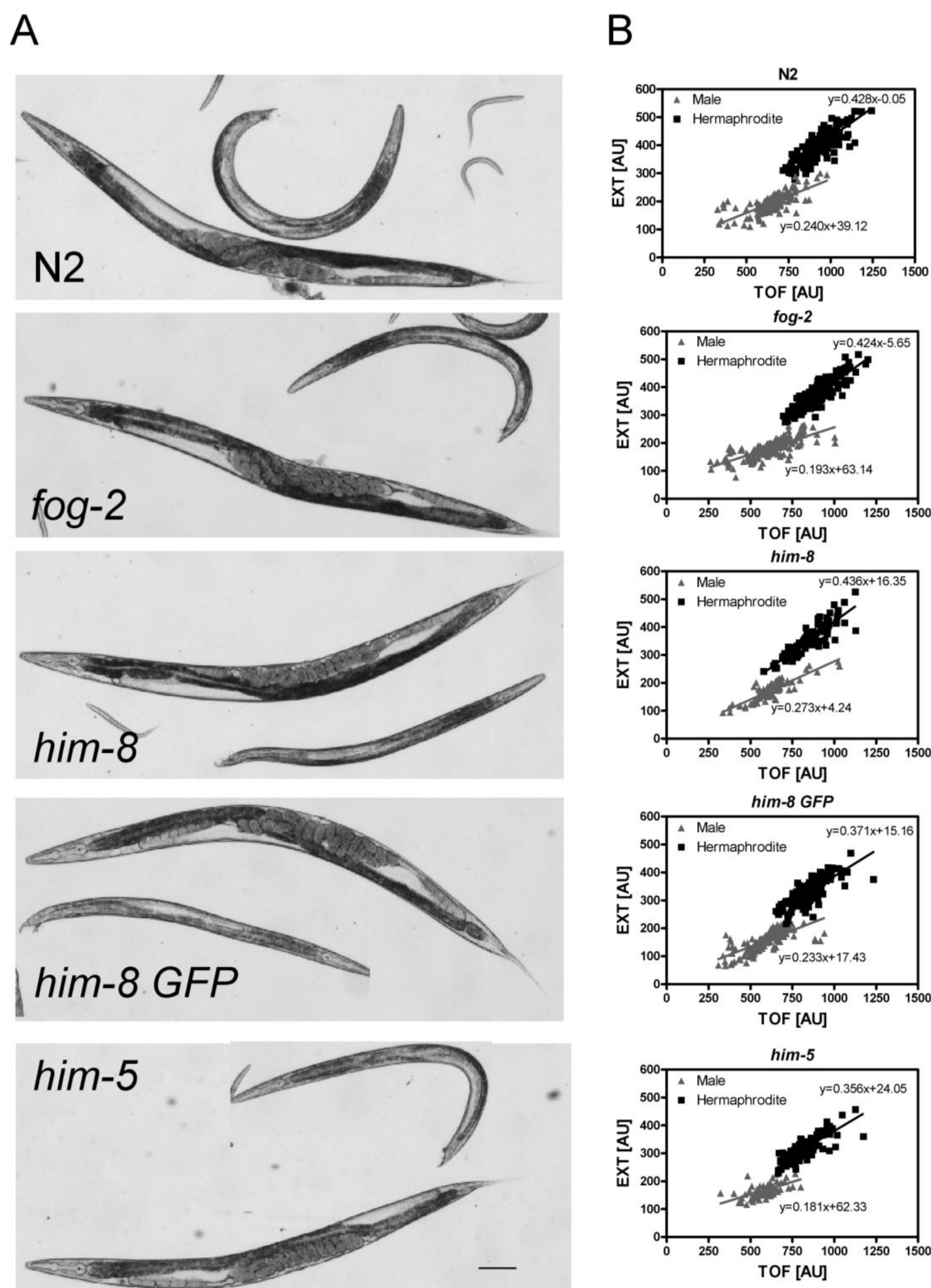


Figure 3. Morphology (A) and flow cytometry-based separation (B) of adult males and hermaphrodites in different *C. elegans* male mutants.
Mutants were synchronized by hypochlorite treatment of gravid worms and were analyzed at one day of adulthood (90 h). (A) Microscopic pictures of wild type (N2) and male mutants. Scale bar: 100 µm. (B) Flow cytometry displays time of flight (TOF) and extinction (EXT) values (expressed as AU, arbitrary unit) for males and hermaphrodites in N2 and different mutants. Each point represents a single worm. Linear regression lines show differences between males and hermaphrodites regarding TOF and EXT. The indicated equations correspond to the regression lines.

The flow cytometry-based TOF and EXT values are proxies for body length and body volume

In this study the flow cytometry-based parameters time of flight (TOF) and extinction (EXT) were often used for basal characterization of males and hermaphrodites. In order to confirm, that the TOF value reflects the axial length of a worm and the EXT value is related to the body volume [32], they were systematically compared with body length and body volume from microscopic images. To cover a wide range of body proportion data, *ad libitum* and DR conditions as well as different male mutants and different developmental stages were included in the analysis. A significant relationship between TOF and body length (r^2=0.87, p<0.001, **figure 4 A**) and EXT and body volume (r^2=0.87, p<0.001, **figure 4 B**) was identified. Moreover, based on TOF and EXT values, adult males and hermaphrodites from different male mutants were clearly resolved from one another and clustered in well-defined clouds (**figure 3 B**). Hence, flow cytometry-based TOF and EXT values are found to be good proxies for body length and body volume and offer a possibility for the separation of males and hermaphrodites.

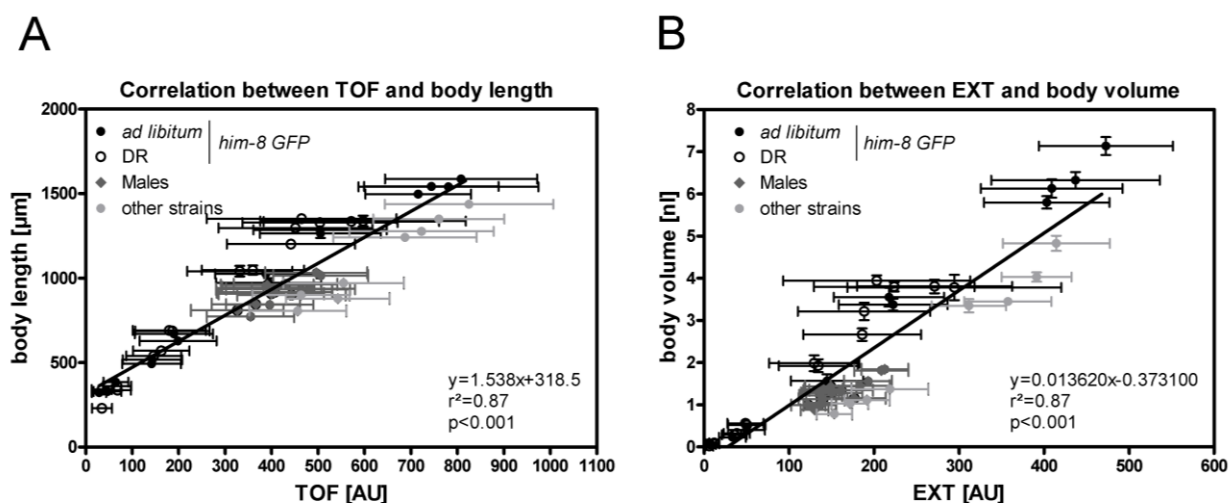


Figure 4. Relationship between time of flight (TOF) and body length (A) and extinction (EXT) and body volume (B).
The data include different feeding conditions (*ad libitum*, DR), both sexes, different strains (N2, *fog-2*, *him-8*, *him-8 GFP* and *him-5*) and different developmental stages. Each data point represents mean ± SD from one experiment. Linear regression equations, p and r^2 values were calculated.

The body length and body volume of males is similar at larval stages but 36 and 53 % lower, respectively, at adulthood in comparison to age-matched hermaphrodites

As mentioned earlier, at hatching males are almost indistinguishable from hermaphrodites, however, during development morphological differences become more and more apparent [11]. Thus, the body length (TOF, **figure 5 A**) and body volume (EXT, **figure 5 B**) of *him-8 GFP* males were compared with *him-8 GFP* hermaphrodites at larval and adult stages.

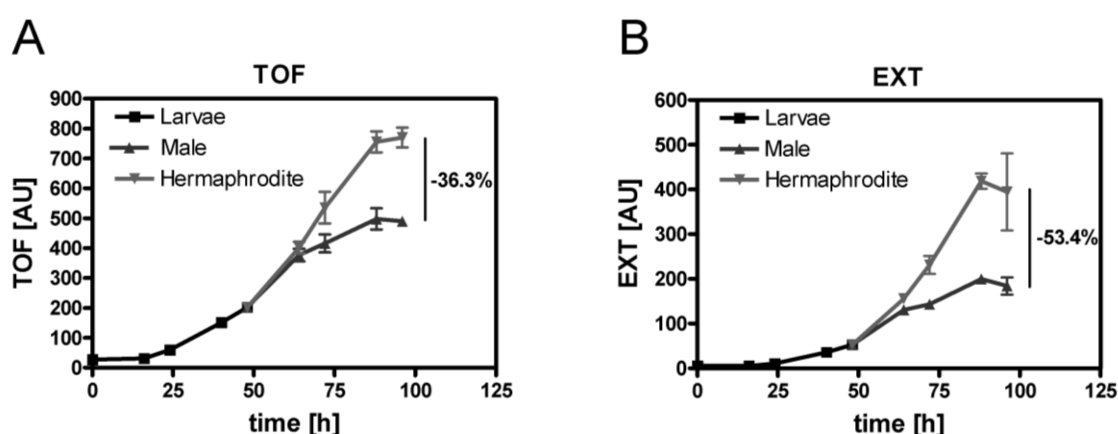


Figure 5. Flow cytometry-based parameters of body proportions of *him-8 GFP* males and hermaphrodites at different developmental stages.
Time of flight (TOF, A) and extinction (EXT, B) values of *him-8 GFP* worms were assessed via flow cytometry at different developmental stages (egg [0 h] till adult [96 h]). *him-8 GFP* males and hermaphrodites could be separated from young adult stage (64 h) based on the GFP signal. Relative differences between males and hermaphrodites are given at 96 h. Results from three independent experiments are shown as mean ± SD.

At young adult stage (64 h) males and hermaphrodites were nearly identical in body size. As expected, sex differences in body size became more and more evident when worms reached adulthood. At one day of adulthood (96 h) *him-8 GFP* males had a 36.3 % lower body length (TOF) and a 53.4 % lower body volume (EXT) than age-matched hermaphrodites. Microscopic data confirmed lower body length (**figure 6 A**) and body volume (**figure 6 B**) of adult males in *him-8 GFP*, N2 and all other male mutants used. ANOVA analysis indicated partially significant differences among male mutant strains ranging between 3.3 to 6.7 % for males and 2.2 to 14.7 % for hermaphrodites in body length (body volume: 13.7 to 28.9 % for males, 10.7 to 35.2

% for hermaphrodites). However, it is quite obvious that substantial differences in body proportions exist between both sexes at adulthood but not at larval stages.

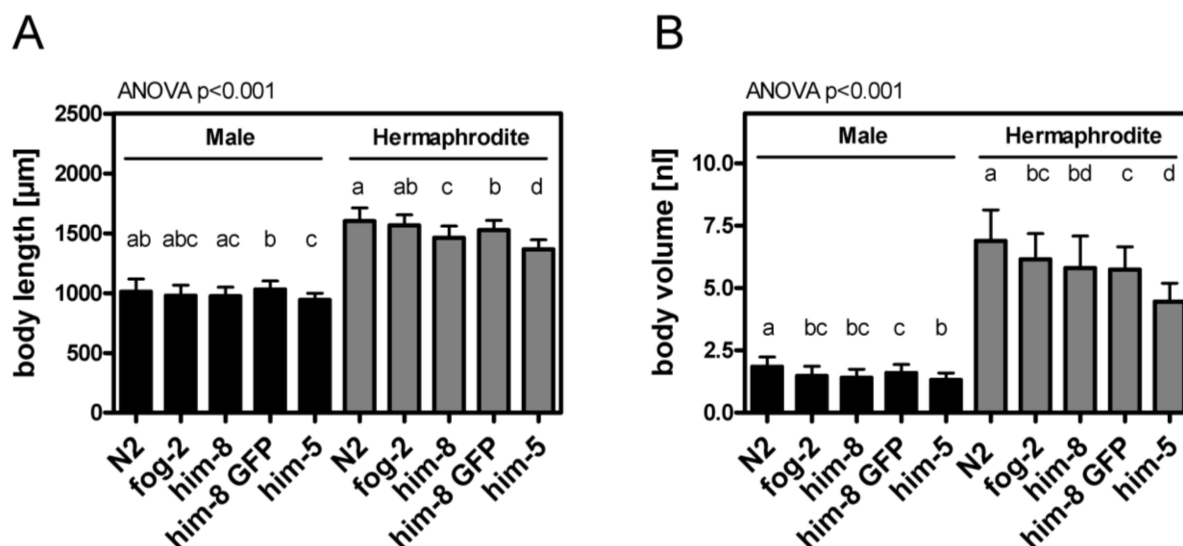


Figure 6. Body length (A) and body volume (B) of adult males and hermaphrodites in different *C. elegans* male mutants.
Mutants were synchronized by hypochlorite treatment of gravid worms and were analyzed at one day of adulthood (90 h). Microscopic images from each mutant strain were used to determine body length and body volume of males and hermaphrodites. Values are expressed as mean ± SD and include two independent experiments. 12-24 worms were scored in each experiment. Bars with different letters are significantly different to other strains ($p<0.05$). To determine statistical differences a one-way ANOVA was made followed by posthoc test (Bonferroni multiple comparison test or Dunnett's T3, when inhomogeneity of variance was evident).

The fat-to-fat-free mass and the volume adjusted fat mass of adult males is similar to adult hermaphrodites

As body proportions of adult worms show sex differences, differences in body composition between sexes could be assumed. To test this hypothesis, triglyceride and protein contents, which represent the fat and fat-free mass of *C. elegans* [32], were determined in *him-8 GFP* males and hermaphrodites at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h). As shown in **figure 7 A and B**, males exhibited a significant lower triglyceride and protein content per worm at all three developmental stages compared to hermaphrodites (see also **supporting information table S2**).

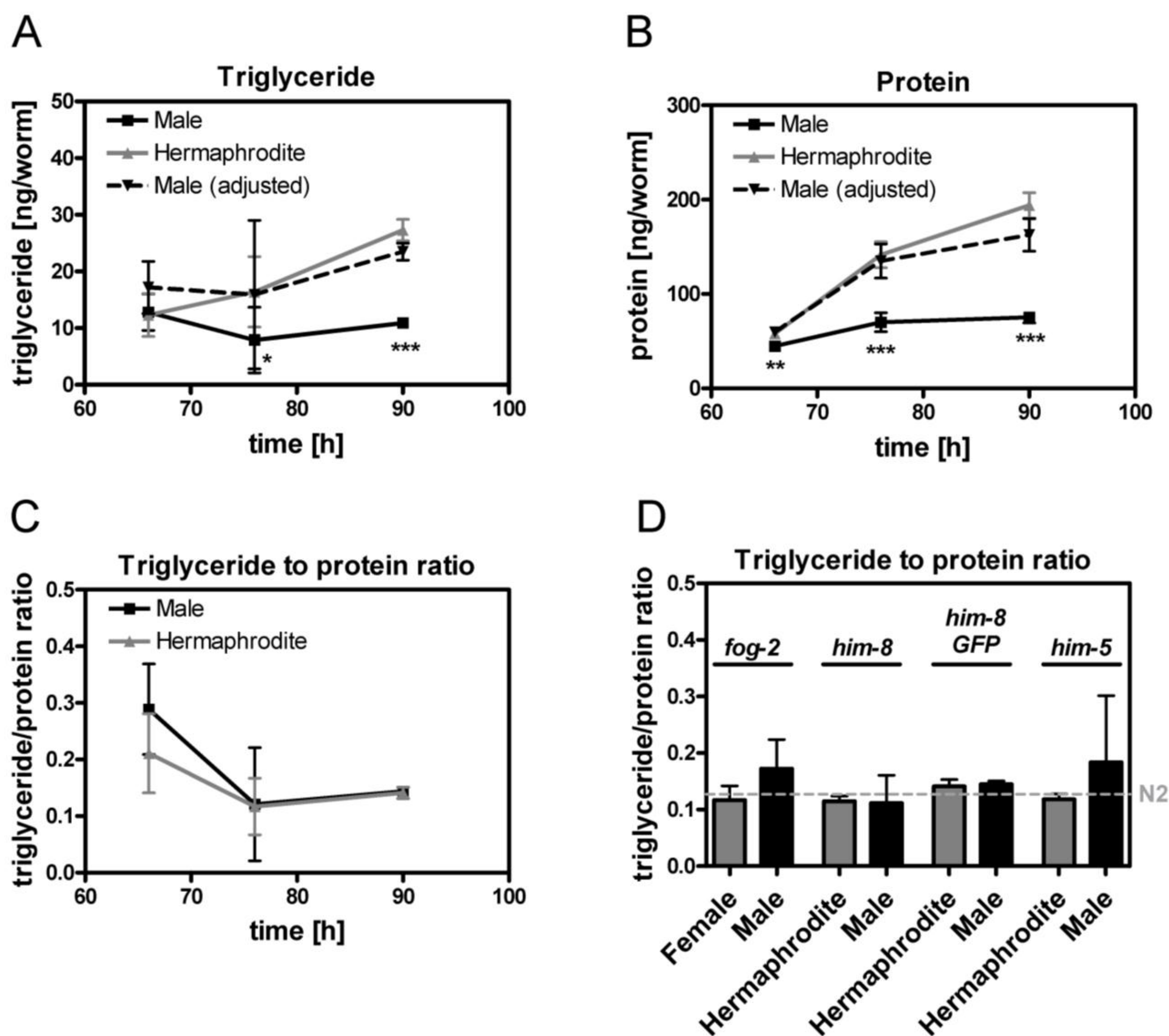


Figure 7. Body composition of males and hermaphrodites at different developmental stages.
Triglyceride content (A), protein content (B) and the resulting triglyceride to protein ratio (C) of *him-8 GFP* males and hermaphrodites at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h). Male body composition parameters were adjusted to the body volume so that they were comparable to hermaphrodite (A, B). Comparison of the triglyceride to protein ratio in *fog-2*, *him-8*, *him-8 GFP* and *him-5* mutants at one day of adulthood (90 h, D). Results of N2 hermaphrodites were used as a reference and included as a dashed line (D). Data represent the mean ± SD from 3-8 independent experiments. Significant differences between males and hermaphrodites are indicated with asterisks (* $p<0.05$, ** $p<0.01$, *** $p<0.001$, t-test).

From a physiological point of view, the proportion of fat and protein in relationship to the body volume as well as the fat-to-fat-free mass is of central importance when evaluating the body composition of an organism [32]. As shown in **figure 7 A and B**, adjusting male triglyceride and protein values to body volume resulted in no apparent differences between both sexes in *him-8 GFP* mutants. This observation was confirmed by the triglyceride to protein ratio demonstrating no significant differences between *him-8 GFP* males and hermaphrodites at all three developmental stages (**figure 7 C**). In agreement, the triglyceride to protein ratio between males and hermaphrodites of *fog-2*, *him-8* and *him-5* mutants was also not significant different at adulthood (90 h, **figure 7 D, supporting information table S2**). The obtained ratios in hermaphrodites are similar to those reported in the literature [32-34]. In summary, *C. elegans* males and hermaphrodites have a similar body composition at three different adult stages.

The trehalose to glucose ratio is 3.9 to 5.4-fold higher in adult males in comparison to adult hermaphrodites

To get insight into metabolic differences between the sexes, the glucose and trehalose content of *him-8 GFP* males and hermaphrodites were analyzed at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h). As shown in **figure 8 A**, adult males had significant lower glucose levels than hermaphrodites, even after adjusting male glucose values to body volume (see also **supporting information table S3**). Adult *him-8 GFP* males exhibited equal trehalose levels (66 h, 76 h) or significant higher levels (90 h) compared to adult hermaphrodites (**figure 8 B**). After adjustment of trehalose content to body volume, *him-8 GFP* males contained significant higher levels than hermaphrodites in two out of three adult stages (**figure 8 B**). The obtained trehalose content in young adult hermaphrodites is similar to reported values but are somewhat lower [139,140]. The contrasting occurrence of glucose and trehalose levels in males and hermaphrodites yielded in a 1.4-(66 h), 3.9- (76 h) and 5.4-fold (90 h) higher trehalose to glucose ratio in adult males (**figure 8 C**). This sex difference was also present in the *fog-2, him-8* and *him-5* mutants (**figure 8 D, supporting information table S3**). Consistent with the biochemical data a significant higher trehalose to glucose ratio was found in males compared to hermaphrodites using NMR spectroscopy (**figure 8 E**).

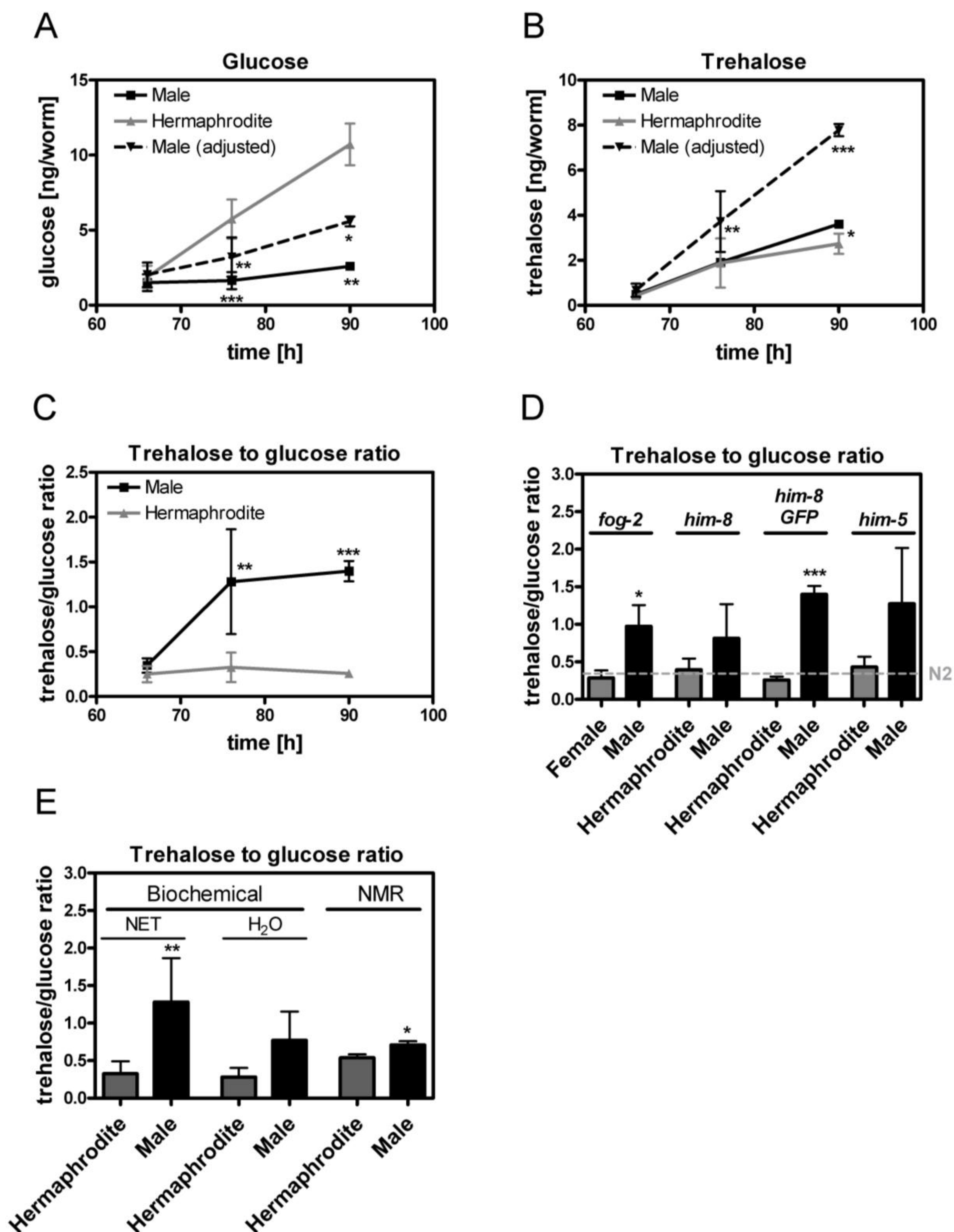


Figure 8. Glucose and trehalose content of males and hermaphrodites at different developmental stages.
Glucose content (A), trehalose content (B) and the resulting trehalose to glucose ratio (C) of *him-8 GFP* males and hermaphrodites at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h). Male glucose and trehalose content were adjusted to the body volume so that they were comparable to hermaphrodite (A, B). Comparison of the trehalose to glucose ratio in *fog-2*, *him-8*, *him-8 GFP* and *him-5* mutants at one day of adulthood (90 h, D). Results of N2 hermaphrodites were used

as a reference and included as a dashed line (D). Trehalose and glucose levels were validated with NMR spectroscopy (E). Trehalose to glucose ratio from biochemical assays (NET buffer or destilled water as solvent) and NMR spectroscopy were presented. Data show the mean ± SD from 3-8 independent experiments. Significant differences between males and hermaphrodites are indicated with asterisks (* p<0.05, ** p<0.01, *** p<0.001, Student's t-test).

Adult males have 2.7 to 4.8-fold lower total RNA content than adult hermaphrodites

As part of the gene expression analysis, total RNA content of *him-8 GFP* males and hermaphrodites were determined when worms reached young adulthood (66 h) and adulthood (76 h). Interestingly, a 2.7 to 4.8-fold lower total RNA content per worm was found in males compared to hermaphrodites (**figure 9**). This significant difference was also evident after adjusting the male's total RNA content for body volume (1.8 to 2.4-fold). Additionally, males did not change their total RNA content from young adult to adult stage, whereas the total RNA content of hermaphrodites increased significantly.

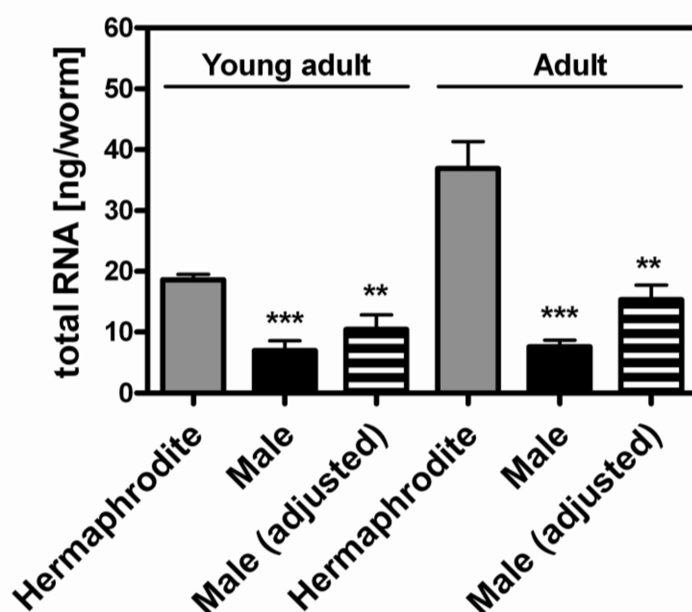


Figure 9. Total RNA content of young adult and adult *him-8 GFP* males and hermaphrodites.
Total RNA were isolated from young adult (66 h) and adult (76 h) *him-8 GFP* males and hermaphrodites. RNA content of males was adjusted to the body volume so that it was comparable to hermaphrodite. Data represent mean ± SD, n=3. Statistical differences between male or male (adjusted) and hermaphrodite were determined with a Student's t-test (**p<0.01, ***p<0.001).

Identification of 285 male-specific and 160 hermaphrodite-specific genes

To identify genes differentially expressed in both sexes, comparative microarray analysis was performed in *him-8 GFP* males and hermaphrodites in young adult (66 h) and adult (76 h) worms. From the 26.843 genes analyzed, 9763 genes showed an expression level that was significantly different (p<0.01) between both sexes at young adult stage (adult stage: 14488 genes, **figure 10 A and B**). Of these, 2827 genes (adult: 4369 genes) had an expression level that was at least 2-fold higher in males than in hermaphrodites (male-enriched genes). 2854 genes (adult: 6203 genes) were considered as hermaphrodite-enriched. Next, it was searched for genes, which were at least 10-fold differently expressed between the sexes, overlapped between both adult stages and showed an expression level of <100 in the other sex. These genes were considered as sex-specific. 285 male-specific and 160 hermaphrodite-specific genes (**figure 10 C and D**) were identified. A large proportion of these genes has no gene name or encodes for proteins of unknown function, illustrating incompleteness of *C. elegans* gene annotation. Therefore, a table only with male- and hermaphrodite-specific genes that could be assigned to a gene class (**table 1 and 2**) were created. Full data showing all genes are given in **supporting information table S4** (male-specific) **and S5** (hermaphrodite-specific).

Within the male- and hermaphrodite-specific genes several transcription factors, which were differentially expressed in both sexes, were found. Hermaphrodites showed a much higher expression of *ceh-49* (adult: 166.0-fold, p<0.001), *ceh-83* (42.6-fold, p<0.001), *tbx-9* (94.7-fold, p<0.001), *tbx-36* (98.8, p<0.001), *flh-3* (97.2-fold, p<0.001), *lsy-27* (189.6-fold, p=0.001), *pes-1* (34.4-fold, p=0.004) and *ztf-25* (239.9-fold, p<0.001) (**table 2**). In contrast, *mab-23* (43.5-fold, p<0.001), *mab-3* (44.0-fold, p=0.003), *ceh-7* (11.2-fold, p<0.001), *nhr-266* (13.9-fold, p=0.008) and *nhr-113* (72.5-fold, p=0.002) were identified as male-specific transcription factors (**table 1**). Moreover, some metabolic genes were detected such as *lips-2*, *nas-17*, *nas-19* and *acl-12* being differentially expressed between males and hermaphrodites.

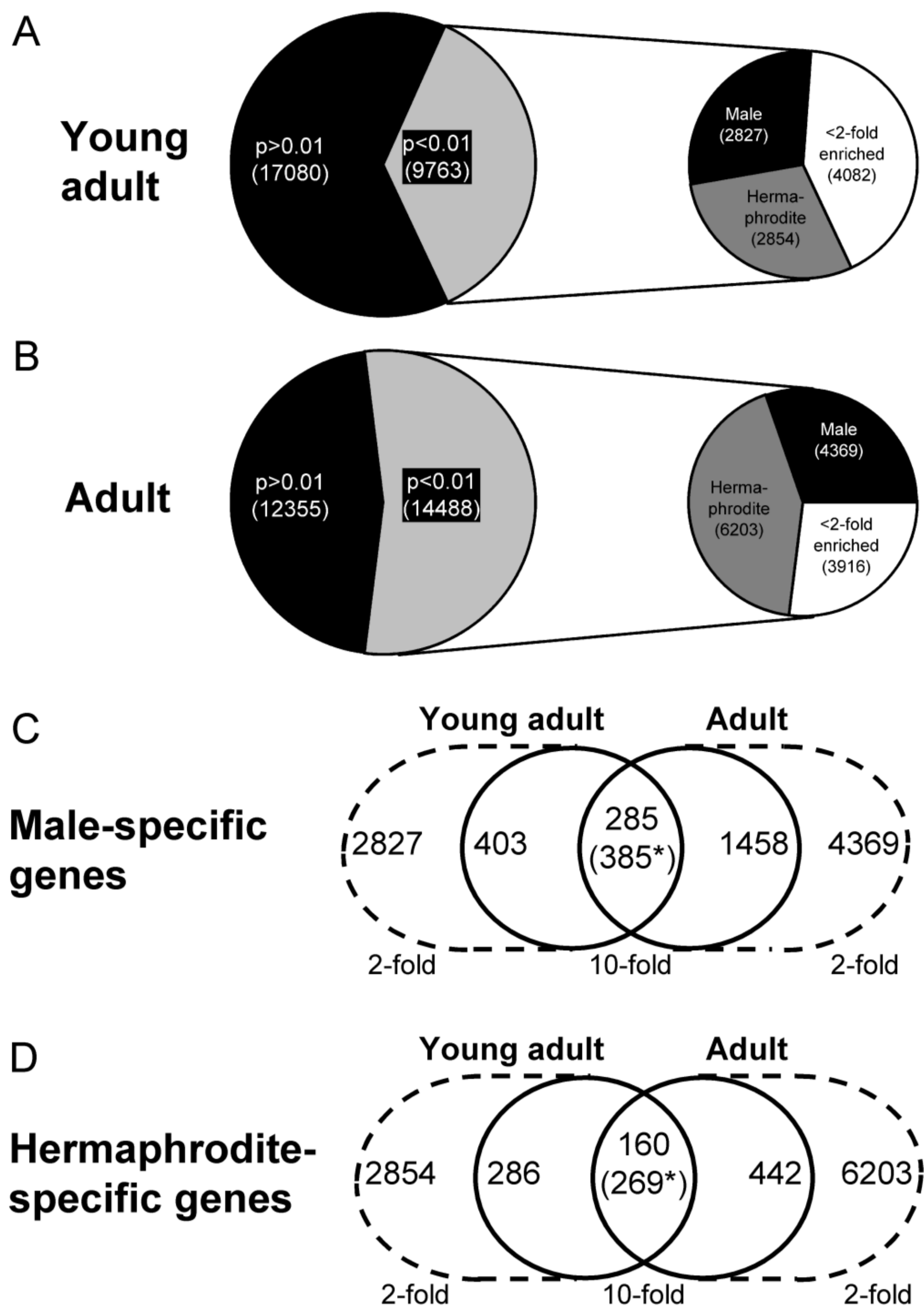


Figure 10. Number of differentially expressed genes between *him-8 GFP* males and hermaphrodites.
The pie charts (A, B) show the number of differentially expressed genes between *him-8 GFP* males and hermaphrodites at young adult (66 h, A) and adult (76 h, B) stage. Genes, which had at least a 2-fold higher level in the corresponding sex, were considered as male-enriched (black) or hermaphrodite-enriched (grey) genes. The number of genes is listed within the brackets. The Venn diagrams (C, D) display the male- (C) or hermaphrodite- (C) specific genes, which result from the overlap between young adulthood (66 h) and adulthood (76 h). To scale down the number of genes the cut-off was increased from 2-fold (dashed cycles) to 10-fold (solid circles). * Genes, which had in the other sex an expression level that was higher than 100, were excluded.

Table 1. Male-specific genes[a].

Gene class	Gene name	Molecular function[b]	Male to hermaphrodite ratio Young adult (Adult)	P-value Young adult (Adult)
C-type lectin	*clec-92*	protein interactions	279.5 (935.9)	<0.001 (0.009)
	clec-93	(some genes from this	43.0 (109.7)	0.002 (<0.001)
	clec-95	class are defense-related)	220.8 (925.5)	0.001 (0.005)
	clec-96		257.5 (898.3)	0.002 (<0.001)
	clec-102		20.6 (62.8)	<0.001 (<0.001)
	clec-107		903.5 (1582.3)	0.003 (<0.001)
	clec-108		145.8 (508.9)	0.002 (0.001)
	clec-109		132.6 (1046.5)	0.002(<0.001)
	clec-110		186.2 (816.1)	0.005 (0.006)
	clec-113		13.9 (12.0)	<0.001 (<0.001)
	clec-116		28.0 (33.3)	0.003 (0.004)
	clec-125		176.6 (404.7)	0.003 (0.002)
	clec-126		121.8 (171.5)	0.004 (<0.001)
	clec-129		496.1 (1421.1)	<0.001 (<0.001)
	clec-130		341.0 (772.0)	0.003 (<0.001)
	clec-132		140.9 (167.2)	<0.001 (0.004)
	clec-133		220.9 (1046.3)	0.006 (<0.001)
	clec-134		1593.0 (1063.3)	0.001 (0.002)
	clec-135		582.3 (1292.6)	0.001 (<0.001)
	clec-136		139.4 (1309.1)	<0.001 (0.002)
	clec-141		185.7 (541.8)	0.001 (<0.001)
	clec-157		356.9 (201.2)	<0.001 (<0.001)
	clec-158		189.4 (925.3)	<0.001 (<0.001)
	clec-159		145.5 (198.7)	0.002 (0.006)
	clec-161		267.2 (952.3)	0.002 (<0.001)
	clec-164		24.4 (35.4)	0.002 (<0.001)
	clec-176		12.2 (10.8)	0.004 (0.004)
	clec-179		23.0 (52.9)	0.006 (0.008)
	clec-181		256.1 (729.2)	<0.001 (0.001)
	clec-183		247.9 (578.6)	<0.001 (<0.001)
	clec-193		57.3 (65.2)	<0.001 (0.002)
	clec-213		34.1 (44.6)	0.003 (<0.001)
	clec-217		316.0 (1447.9)	0.001 (<0.001)
	clec-219		183.1 (1163.3)	<0.001 (0.002)
	clec-232		252 2 (633.3)	0.002 (0.002)
	clec-250		19.8 (15.4)	0.003 (<0.001)
	clec-254		42.9 (21.1)	0.004 (0.002)
	clec-256		19.3 (33.8)	0.002 (0.007)
	clec-257		33.8 (51.9)	0.008 (0.001)
	clec-259		148.9 (238.4)	0.001 (0.006)
	clec-261		232.0 (1092.4)	0.004 (0.006)
	clec-263		320.2 (999.5)	0.002 (0.003)
galactose-binding lectin	F46A8.3	sugar binding	312.0 (855.3)	0.007 (0.002)
	F46A8.5		337.1 (1335.6)	<0.001 (<0.001)
	F46A8.8		366.4 (1046.8)	<0.001 (<0.001)
	F49F1.10		251.4 (1195.6)	0.009 (<0.001)
	F49F1.11		323.8 (1330.1)	0.004 (0.003)
	F49F1.9		460.5 (884.5)	<0.001 (0.001)
fungus-induced protein related	*fipr-16*	antimicrobial activity	307.8 (792.9)	0.005 (<0.001)

SCP-like extracellular protein	*scl-8* *scl-18*	defense-related protein containing SCP domain	538.6 (1034.2) 159.7 (1323.1)	0.001 (0.004) 0.006 (<0.001)
hypersensitive to pore-forming toxin	*hpo-33* *hpo-37*	immune defense	159.8 (1352.6) 462.4 (606.1)	0.002 (<0.001) 0.004 (0.008)
polycystins and coexpressed genes	*cwp-2* *cwp-3* *lov-1* *pkd-2*	neuropeptide signaling/ transmembrane transport	54.5 (339.3) 44.6 (110.4) 55.6 (111.6) 41.1 (120.3)	0.001 (<0.001) 0.002 (0.003) 0.004 (0.003) 0.001 (0.002)
Nematode astacin protease	*nas-17* *nas-19*	astacin-like metalloprotease	598.6 (1335.3) 337.8 (1556.8)	0.002 (<0.001) 0.001 (<0.001)
lipase related	*lips-2*	triacylglycerol lipase	203.9 (638.2)	0.006 (0.002)
insulin related	*ins-25*	hormone activity	17.8 (18.7)	0.002 (0.002)
collagen and related protein	*col-183*	structural constituent of cuticle	77.0 (264.2)	0.001(<0.001)
ribosomal protein	*rpl-11.2*	structural constituent of ribosome	144.4 (1826.8)	<0.001 (<0.001)
SET (trithrax/polycomb) domain containing protein	*set-15*	histone H3 methyltransferase	80.0 (201.9)	0.002 (0.002)
trypsin like protease	*try-5*	endopeptidase activity	41.5 (76.5)	<0.001 (0.004)
transpliced leader sequence	*sls-2.18*	unknown	16.1 (22.9)	<0.001 (<0.001)
innexin	*inx-1*	unknown	18.4 (21.1)	0.004 (0.006)
serpentine receptor, class D, class G, class I, class H, class V, class Z	*srg-1* *sri-25* *srh-287* *srd-1* *srz-93* *srv-11*	receptor like protein olfactory receptor chemosensory receptor pseudogene transmembrane receptor activity	14.4 (16.2) 10.5 (41.3) 13.1 (13.0) 15.7 (14.0) 13.4 (16.1) 10.9 (17.4)	0.008 (0.008) <0.001 (0.005) <0.001 (0.003) 0.004 (0.008) 0.001 (0.002) 0.004 (0.008)
seven TM receptor	*str-55*	olfactory receptor	87.2 (46.2)	0.005 (0.001)

male abnormal	*mab-23* *mab-3*	transcription factor activity	22.7 (43.5) 25.0 (44.0)	<0.001 (<0.001) <0.001 (0.003)
C. elegans homeobox	*ceh-7*	transcription factor activity	10.9 (11.2)	0.003 (<0.001)
dystroglycan	*dgn-3*	unknown	39.6 (55.7)	<0.001 (<0.001)
MX region of TRA-2 related	*xtr-2*	protein-protein interaction, regulating negatively tra-2	14.3 (26.4)	0.002 (<0.001)
FMRF-like peptide	*flp-23*		109.4 (959.2)	0.002 (<0.001)
TNF receptor associated factor (TRAF) homolog	*trf-1*	signal transduction, regulation of apoptosis	28.1 (40.9)	0.003 (0.004)
nuclear hormone receptor family	*nhr-266* *nhr-113*	transcription factor activity	16.4 (13.9) 16.6 (72.5)	0.009 (0.008) <0.001 (0.002)
cytochrome P450 family	*cyp-35B1*	oxidoreductase, electron carrier activity, monooxygenase activity	28.0 (12.9)	0.007 (<0.001)
neuropeptide-like protein	*nlp-22*	unknown	32.4 (60.4)	<0.001 (0.006)
F-box A protein	*fbxa-204*	pseudogene	63.0 (91.0)	<0.001 (<0.001)

[a] supporting information table S4 contains a complete list of all genes (also unknown genes and genes without a gene class)
[b] sometimes the function of a gene is not precisely defined or only described as “predicted” function, information about genes was obtained from Wormbase.

Table 2. Hermaphrodite-specific genes[a].

Gene class	Gene name	Molecular function[b]	Hermaphrodite to male ratio Young adult (Adult)	P-value Young adult (Adult)
F-box B protein	*fbxb-67*	protein-protein interaction (ubiquitin protein degradation pathway)	12.9 (37.3)	<0.001 (<0.001)
F-box C protein	*fbxc-31*		12.8 (57.4)	0.005 (0.002)
	fbxc-32		84.1 (191.9)	0.007 (<0.001)
	fbxc-42		29.7 (151.9)	0.006 (<0.001)
F-box A protein	*fbxa-184*		11.7 (40.4)	0.005 (<0.001)
C-type lectin	*clec-190*	protein-interactions	245.0 (192.8)	0.009 (<0.001)
	clec-223		494.7 (177.8)	0.009 (0.004)
serpentine receptor, class A, class H, class I	*sra-14*	transmembrane receptor	10.1 (16.5)	<0.001 (<0.001)
	srh-85	pseudogene	152.3 (197.9)	0.001 (0.003)
	srh-43	pseudogene	483.6 (104.1)	<0.001 (0.002)
	sri-40	chemoreceptor	30.1 (102.0)	0.004(<0.001)
protein kinase	*kin-34*	protein tyrosine kinase activity	11.6 (219.6)	0.006 (0.003)
C. elegans homeobox	*ceh-49*	transcription factor activity	77.0 (166.0)	0.007 (<0.001)
	ceh-83		17.1 (42.6)	0.006 (<0.001)
T box family	*tbx-9*	transcription factor activity	37.6 (94.7)	0.002 (<0.001)
	tbx-36		91.1 (98.8)	0.004 (<0.001)
regulator of G protein signaling	*rgs-8.1*	signal transducer activity	17.9 (78.2)	0.007 (0.002)
	rgs-9		170.7 (192.2)	0.002 (0.003)
FLYWCH zinc finger transcription factor homolog	*flh-3*	transcription factor activity	35.8 (97.2)	0.004 (<0.001)
laterally symmetric (defective in lateral symmetry)	*lsy-27*	transcription factor activity	114.5 (189.6)	0.007 (0.001)
patterned expression site	*pes-1*	transcription factor activity	10.1 (34.4)	0.004 (0.004)
	pes-23	transmembrane transporter activity	97.2 (55.6)	<0.001 (<0.001)
transthyretin-related family domain	*ttr-14*	unknown	514.1 (144.2)	0.002 (0.001)
	ttr-34		140.3 (67.0)	0.004 (<0.001)
maternal effect germ-cell defective	*meg-1*	P granule segregation	311.7 (159.3)	0.001 (0.001)

collagen	*col-135*	structural constituent of cuticle	779.4 (176.0)	0.003 (<0.001)
paired zinc finger protein	*pzf-1*	meiotic chromosome segregation	206.4 (127.8)	<0.001 (<0.001)
sperm specific family, class P	*ssp-37*	structural molecule activity	1013.1 (170.9)	<0.001 (<0.001)
zinc finger putative transcription factor family	*ztf-25*	transcription factor activity	257.4 (239.9)	0.001 (<0.001)
nanos related	*nos-2*	zinc ion binding	183.8 (161.5)	0.004 (<0.001)
trypsin-like protease	*try-1*	endopeptidase	93.6 (120.9)	0.004 (<0.001)
cell division cycle related	*cdc-25.3*	tyrosine phosphatase, cell cycle regulator	527.2 (173.9)	0.003 (<0.001)
acyltransferase-like	*acl-12*	acyltransferase activity	11.5 (28.2)	0.006 (<0.001)
eph(f)rin	*efn-3*	epidermal organization	33.8 (31.1)	<0.001 (<0.001)
FERM domain (protein4.1-ezrin-radixin-moesin) family	*frm-9*	unknown	52.5 (42.0)	0.002 (0.001)
RhoGAP for Rac-1 and cdc-42	*rrc-1*	signal transduction	58.2 (10.6)	0.006 (0.003)
regulator of fusion	*ref-1*	regulation of transcription	16.5 (28.0)	0.001 (<0.001)

[a] supporting information table S5 contains a complete list of all hermaphrodite-specific genes (also unknown genes and genes without gene class name)
[b] sometimes the function of a gene is not precisely defined or only described as “predicted” function, information about genes was obtained from Wormbase.

More than 35 % of clec genes are higher expressed in males than in hermaphrodites

Within the male-specific genes (**table 1**) many genes encoding for C-type lectins (*clec*) were dramatically higher expressed in males than in hermaphrodites. 42 *clec* genes showed a male-specific gene expression, whereas only 2 *clec* genes were hermaphrodite-specific. Within the entire C-type lectin gene family, there was an over-represented male-enriched expression of *clec* genes at both adult stages (**figure 11**). Fewer than 5 % (12 genes) of *clec* genes were higher expressed (>2-fold, $p<0.05$) in hermaphrodites, while in males over 35 % (95 genes) exhibited higher expression levels. It was particularly striking that many lectins showed a more than 100-fold higher expression in males than in hermaphrodites.

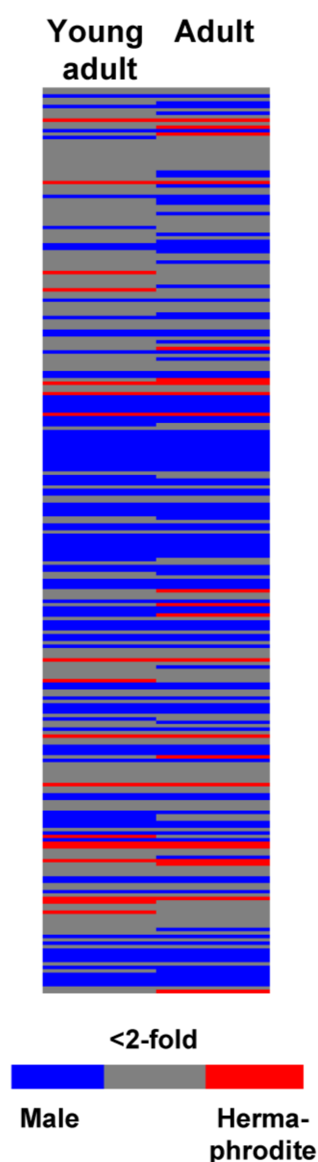


Figure 11. Differential expression of C-type lectins between *him-8 GFP* males and hermaphrodites at young adulthood (66 h) and adulthood (76 h). The expression differences of each C-type lectin gene in *him-8 GFP* males and hermaphrodites is indicated by a color. Red (blue), expression level is at least 2-fold higher ($p<0.05$) in hermaphrodites (males) in comparison to males (hermaphrodites). Grey, no differences in expression level between both sexes. Each row shows one C-type lectin gene from *clec-1* (top) to *clec-266* (bottom).

The phosphofructokinase gene C50F4.2 is 29-fold higher expressed in males than in hermaphrodites

Differences in glucose and trehalose levels between males and hermaphrodites could be caused by gene expression. Thus, the expression of genes, which encode for enzymes being involved in carbohydrate metabolism were analyzed in both sexes (**table 3**). Adult males showed a 2.68-fold ($p<0.001$) higher expression of the trehalose-6-phosphatase synthase (*tps-2*) than adult hermaphrodites confirming the trehalose measurements. Trehalose-6-phosphate phosphatase (*gob-1*) gene expression was also slightly increased in males (1.4/1.6 [two isoforms]). In contrast, adult hermaphrodites had higher mRNA levels of UTP-glucose-1-phosphate uridylyltransferase (2.5, $p<0.01$) and glycogen synthase (*gsy-1*, 2.2, $p<0.01$) than adult males.

Consistent with differences in trehalose and glycogen metabolism, sex differences in the expression of genes encoding for glycolytic enzymes were found. In adult hermaphrodites there was an increased gene expression of hexokinase (0.43/0.28 [two isoforms], $p<0.01$, **table 3**). In contrast, adult males exhibited a higher gene expression of phoshofructokinase and pyruvate kinase. In *C. elegans* two genes encoding a putative phosphofructokinase are known- C50F4.2 and Y71H10A.1. Both genes showed a higher expression in adult males. Remarkably, the expression of C50F4.2 was more than 29-fold ($p=0.004$) increased in males than in hermaphrodites. Additionally, one homologue of the fructose bisphosphate aldolase (*aldo-1*) was also higher expressed in adult males than in adult hermaphrodites (2.3/2.8 [two isoforms], $p<0.01$). Taken together, these data imply that glycolysis and trehalose synthesis may be up-regulated in males, resulting in lower glucose and equal or higher trehalose levels compared to hermaphrodites.

Table 3. Sex differences in the gene expression of carbohydrate metabolic genes[a].

Gene ID	Gene name	Molecular Function[b]	Male to hermaphrodite ratio	
			young adult	adult
Trehalose metabolism				
ZK54.2b.1	*tps-1*	**trehalose-6-phosphatase synthase** (glucose-6-phosphatase+UDP-glucose → trehalose 6-phosphate)	1.25	**1.17***
ZK54.2a	*tps-1*		**1.32***	**1.23***
F19H8.1	*tps-2*		**1.72***	**2.68*****
H13N06.3a	*gob-1*	**trehalose-6-phosphate phosphatase** (trehalose 6-phosphate → trehalose)	1.04	1.36
H13N06.3b	*gob-1*		1.15	**1.61***
F57B10.7	*tre-1*	**trehalase** (trehalose → 2x glucose)	**0.84***	**0.71****
T05A12.2.1	*tre-2*		**1.35***	**1.45****
T05A12.2.2	*tre-2*		**1.53***	**1.46*****
W05E10.4	*tre-3*		1.11	**1.85****
F15A2.2	*tre-4*		**0.96***	0.99
C23H3.7	*tre-5*		1.15	1.18
Glycogen metabolism				
R05F9.6		**phosphotransferase** (glucose 6-phosphate → glucose 1-phosphate)	0.94	**0.68*****
Y43F4B.5a.1			0.98	0.95
Y43F4B.5a.2			0.97	**0.84****
Y43F4B.5b			1.08	0.98
K08E3.5f		**UTP-glucose-1-phosphate uridylyltransferase** (glucose 1-phosphate → UDP-glucose)	**0.66****	**0.39****
K08E3.5a.1			**0.71***	**0.41*****
Y46G5A.31	*gsy-1*	**glycogensynthase** (UDP-glucose → glyogen)	0.87	**0.46****
T22F3.3a		**glycogen phosporylase** (glycogen → glucose 1-phosphate)	0.85	**0.53****
T22F3.3b.2			0.93	**0.51*****
Glycolysis				
F14B4.2a.2		**hexokinase** (glucose → glucose 6-phosphate)	0.79	**0.43****
F14B4.2b			**0.60***	**0.28*****
Y87G2A.8b	*gpi-2*	**glucose 6-phosphate isomerase** (glucose 6-phosphate → fructose 6-phophate)	0.92	**0.78****
Y87G2A.8a.2	*gpi-2*		0.96	0.89
C50F4.2		**6-phosphofructokinase**	**3.33*****	**29.6****

Y71H10A.1a Y71H10A.1b.3 Y71H10A.1b.1		(fructose-6P → fructose -1,6 bisphosphate)	**1.33***** **1.54*** 1.10	**1.61***** **1.83*** 0.98
T05D4.1.2 T05D4.1.1 F01F1.12a F01F1.12b.1	*aldo-1* *aldo-2*	**fructose bisphosphate aldolase** (fructose-1,6 bisphosphate → glyceraldehyd-3 phosphate)	**2.11*** **2.12*** 0.94 0.99	**2.85***** **2.33**** 0.81 0.94
F33H1.2.2 F33H1.2.1 K10B3.7.2 T09F3.3.1 T09F3.3.2	*gpd-4* *gpd-3* *gpd-2*	**glyceraldehyd 3-phosphate dehydrogenase** (glyceraldehyd-3phosphate → glycerate-1,3 bisphosphate)	**0.38**** **0.39*** 1.00 **0.37**** **0.45****	**0.19***** **0.20**** 0.96 **0.19**** **0.23*****
T03F1.3	*pgk-1*	**phosphoglycerate kinase** (glycerate-1,3 bisphosphate → glycerate-3 phosphate)	0.89	**0.69*****
F57B10.3b.2 F57B10.3a		**phosphoglycerate mutase** (glycerate-3 phosphate → glycerate-2 phosphate)	1.03 1.05	0.99 0.97
T21B10.2a.1 T21B10.2b.1	*enol-1*	**enolase** (glycerate-2 phosphate → phosphoenolpyruvate)	**0.90*** 0.92	**0.79**** **0.82***
F25H5.3a F25H5.3e ZK593.1	*pyk-1* *pyk-2*	**pyruvate kinase** (phosphoenolpyruvate → pyruvate)	0.91 1.18 **0.68***	**0.79*** **2.17**** **1.16***

[a] Asterisks indicate statistical significance measured by Student's t-test, *p<0.05, **p<0.01, ***p<0.001, blue (red): significantly expressed in males (hermaphrodites)
[b] Genes, which are involved in carbohydrate metabolism, were identified with KEGG pathway.

3.2 Response of males and hermaphrodites to dietary restriction

Dietary restriction-induced reduction in body proportions, protein content and total RNA content is more pronounced in hermaphrodites than in males

To compare the response of males and hermaphrodites to DR, *him-8 (e1489)* IV; *nls128* II (abbreviatory: *him-8 GFP*) mutant strain was generally used again. This strain allows an accurate separation of males and hermaphrodites using flow cytometry due to its sex-specific GFP signal [137]. Furthermore, it is suitable for studying DR effects in both sexes since it has a higher incidence of males in comparison to N2 wild type and the morphology, body proportions and body composition of *him-8 GFP* males and hermaphrodites is similar to N2 (see **figure 3 and 6**).

It has been shown that the body size of males is 30 to 50 % lower at adulthood in comparison to hermaphrodites (**figure 5 and** [30]). To compare the effects of DR on body proportions between the sexes, flow cytometry-based parameters TOF and EXT were used in order to analyze a large number of worms. It was shown in the previous result section that the TOF and EXT values are proxies for body length and body volume in *C. elegans* (**figure 4 and** [32]). As shown in **figure 12 A and B**, both sexes reduced their body length (TOF) and body volume (EXT) under DR at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h). Interestingly, DR-induced reduction in body proportions was significantly more pronounced in *him-8 GFP* hermaphrodites (e.g. 76 h EXT: -26.2 %) than in males (e.g. 76 h EXT: -7.9 %). This observation was confirmed by body proportion analysis of microscopic images obtained from another male mutant (*fog-2*) subjected to two different DR conditions (**figure 12 C**). Microscopic inspection of dietary restricted *him-8* worms showed that both sexes are thinner than their *ad libitum* fed counterparts with a somewhat higher effect in hermaphrodites (**figure 12 D and E**). Carefully assessment of images revealed that dietary restricted hermaphrodites contained fewer eggs than *ad libitum* fed hermaphrodites.

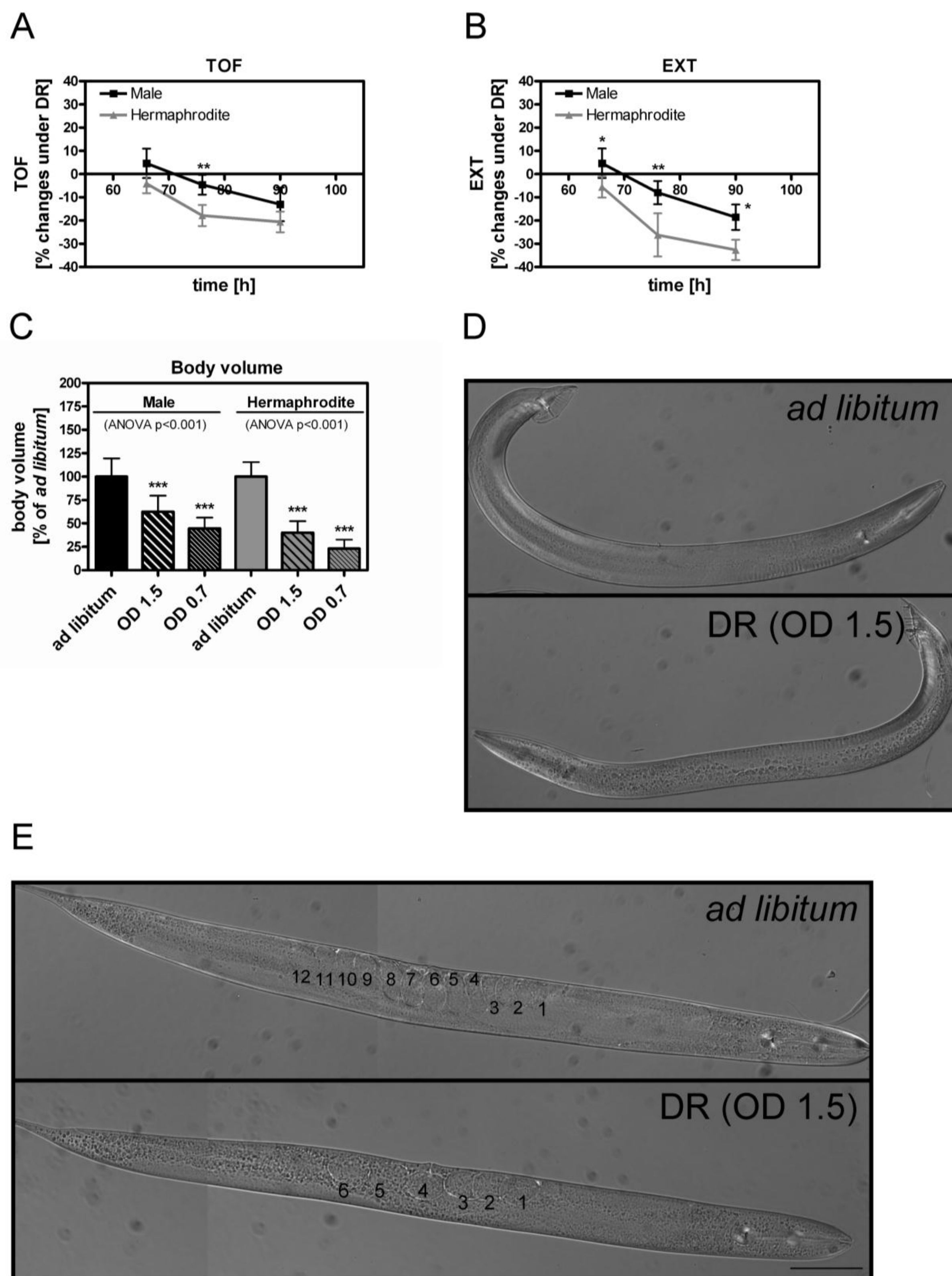


Figure 12. Changes of body proportions under DR (OD 1.5) in males and hermaphrodites at different developmental stages.

Analysis of body proportions using flow-cytometry (A, B) and microscopic images (C-E) from different male mutants. Time of flight (TOF, (A)) and extinction (EXT, ((B)) values of *him-8 GFP* worms were assessed with flow cytometry at young adult (66 h), adult (76 h) and one day of adult (90 h) stage. Relative changes under DR in

comparison to *ad libitum* were calculated. The mean ± SD from 3-5 experiments is presented. Differences between both sexes were statistically analyzed with Student‘s t-test (* $p<0.05$, ** $p<0.01$). *fog-2* worms (C) were analyzed under the microscope when *ad libitum* fed females started to lay eggs (~ 72 h). Body volume was calculated from area and perimeter with Axio Vision Software. Two DR conditions (OD 1.5 and OD 0.7) were used. Mean ± SD from 3 experiments is given. To determine statistical significant differences of both DR conditions to *ad libitum*, a one-way ANOVA was made with a followed posthoc test (***$p<0.001$). Microscopic images of *him-8* males (D) and hermaphrodites (E) under *ad libitum* and DR (OD 1.5) condition are presented. Worms were photographed when *ad libitum* fed hermaphrodites started to lay eggs. Eggs were numbered to allow counting. Scale bar: 100 µm, magnification: 100 x.

Since body size may correlate with protein and total RNA content, these read-outs were compared between males and hermaphrodites in response to DR. As shown in **figure 13 A**, the protein content per worm was reduced under DR in both sexes but this response was significantly higher in hermaphrodites (e.g. 90 h: -47.2 %) than in males (e.g. 90 h: -36.5 %, **supporting information table S6**). Moreover, hermaphrodites reduced their total RNA content under DR at both developmental stages by a factor of 1.9 (66 h) and 3.0 (76 h), respectively, whereas males exhibited no changes (**figure 13 B and C**). Together, measurements of body length, body volume, protein and RNA content under DR indicate that hermaphrodites respond more pronounced than males to a DR treatment.

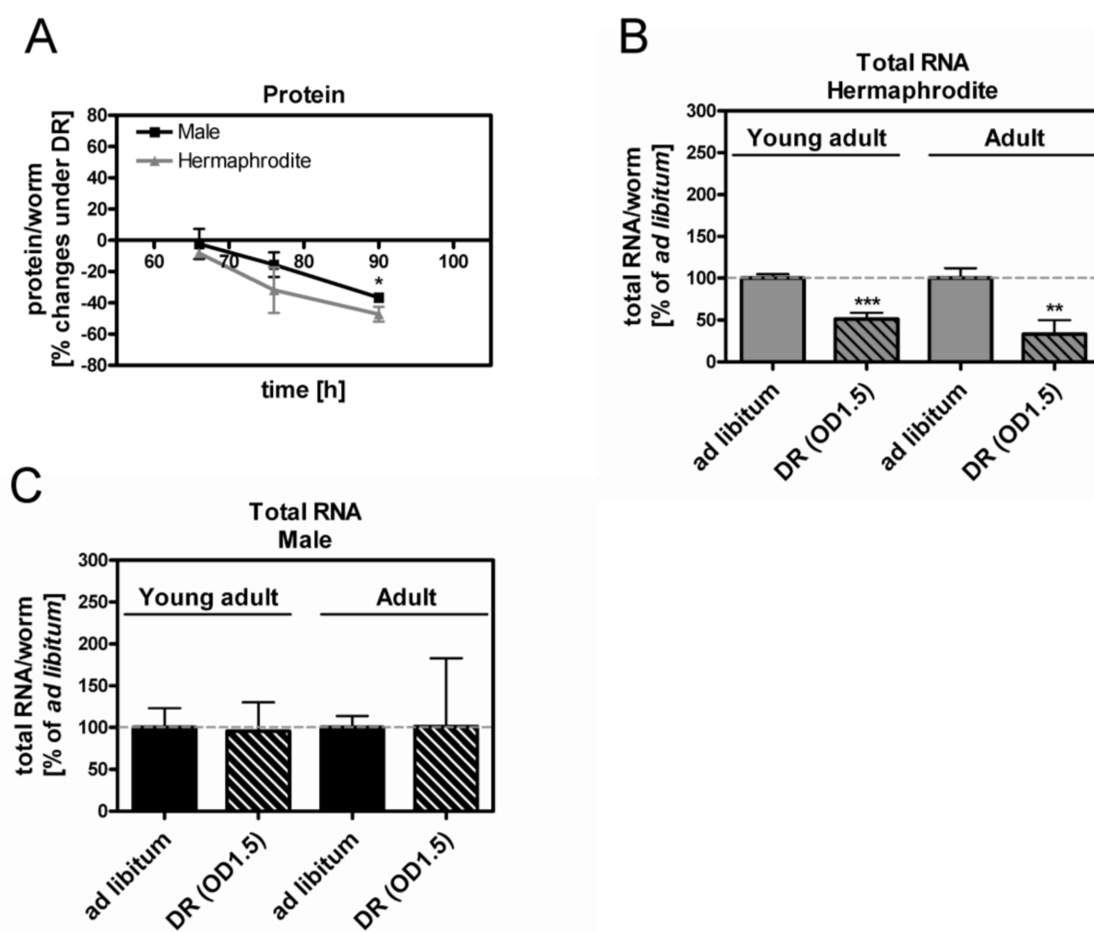


Figure 13. Changes of protein and total RNA content under DR (OD1.5) in *him-8 GFP* males and hermaphrodites at different developmental stages.
Changes of protein content (A) under DR of *him-8 GFP* males and hermaphrodites at young adult (66 h), adulthood (76 h) and one day of adulthood (90 h) were assessed. Relative changes of protein under DR in comparison to *ad libitum* feeding were calculated and differences between both sexes were statistically analyzed with Student‘s t-test (*p<0.05). Total RNA were isolated from young adult (66 h) and adult (76 h) *him-8 GFP* worms. The relative RNA content, as % of *ad libitum*, from hermaphrodites (B) and males (C) is presented. Significant differences between *ad libitum* and DR worms are indicated with asterisks (**p<0.01, ***p<0.001, Student‘s t-test). The mean ± SD from 3-5 experiments is given.

Dietary restriction enhances the fat-to-fat-free mass in both sexes to a similar extent

Next, the triglyceride content per worm and the triglyceride to protein ratio was determined in *him-8 GFP* males and hermaphrodites under *ad libitum* and DR conditions at young adult (66 h), adult (76 h) and one day adult (90 h) stage. These body composition parameters represent the fat mass and the fat-to-fat-free mass of *C. elegans* [32]. In both sexes, the triglyceride content per worm as well as the

calculated triglyceride to protein ratio was increased under DR in all adult stages (**figure 14 A and B, supporting information table S6**). The extent of this effect was similar between the sexes.

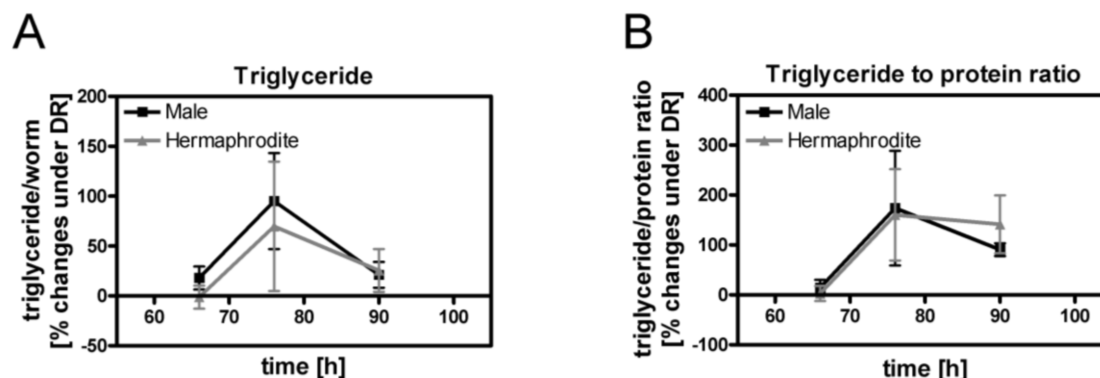


Figure 14. Changes of body composition under DR (OD1.5) in *him-8 GFP* males and hermaphrodites at different developmental stages.
Changes of triglyceride content (A) and the triglyceride to protein ratio (B) under DR of *him-8 GFP* males and hermaphrodites at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h) were assessed. Relative changes under DR in comparison to *ad libitum* feeding were calculated. The mean ± SD from 3-5 experiments is presented. Differences in body composition changes between both sexes were statistically analyzed with Student‘s t-test.

Dietary restriction enlarges lipid droplets in both sexes

Recently, it was discovered in our laboratory that DR enlarges lipid droplets in N2 hermaphrodites (D. Palgunow, unpublished data). Therefore, the effect of DR on lipid droplet size and distribution was compared between hermaphrodites and males. For this purpose, both sexes of *him-8* mutant worms were cultivated under *ad libitum* and DR conditions until adulthood and were stained fixatively with the BODIPY^{TM} 493/503 dye to visualize lipid droplets. As recently described, the accuracy of this staining method was confirmed by co-localization studies with other dyes, staining of lysosome related organelles, TAG measurements as well as by CARS and Raman spectroscopy [32]. *Ad libitum* fed adult *him-8* males and hermaphrodites had finely distributed lipid droplets of nearly the same size (**figure 15 A and B**). In contrast, dietary restricted males and hermaphrodites exhibited larger lipid droplets with a lower number of small sized droplets (**figure 15 A and B**). In both sexes, the enlarged lipid droplets were visible throughout the body in intestinal and hypodermal regions.

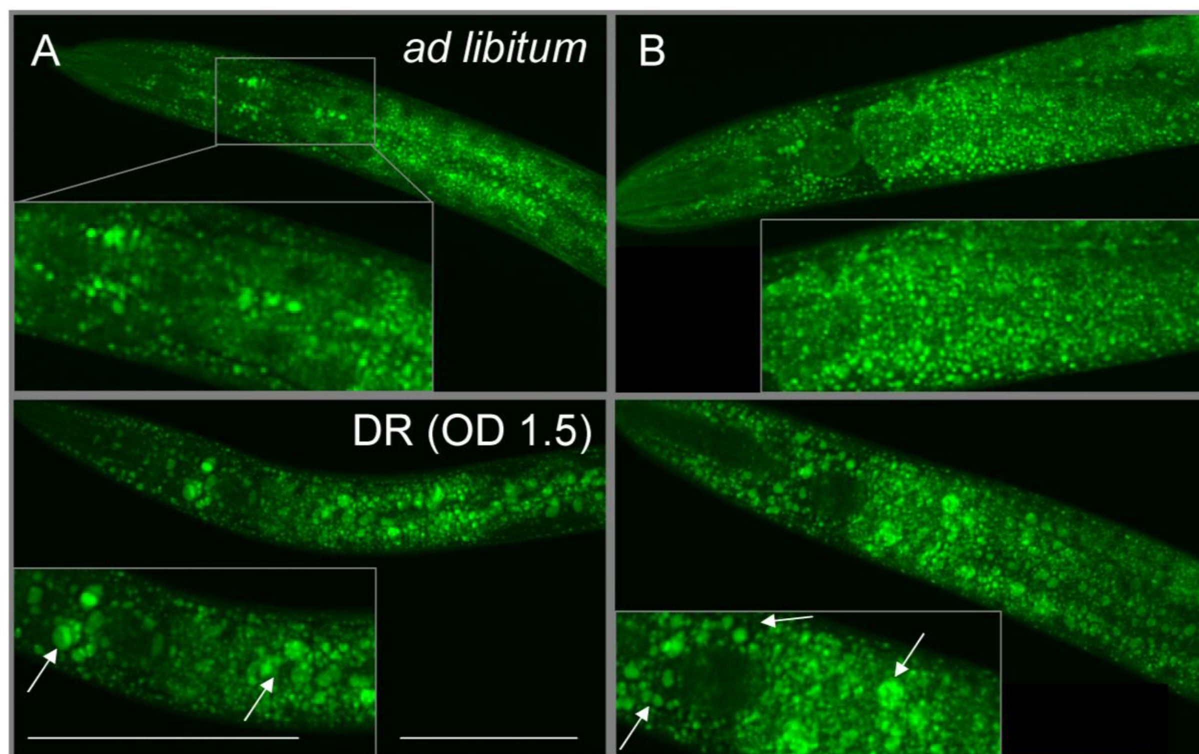


Figure 15. Lipid droplets under *ad libitum* (top) and DR (OD 1.5, bottom) conditions in *him-8* males (A) and hermaphrodites (B).
him-8 worms were cultivated at the corresponding condition until *ad libitum* fed hermaphrodites started to lay eggs. BODIPY-fixative staining was performed as described in Material and Methods. As an exception, worms were transferred after 56 h on fresh DR plates to avoid very harsh food conditions. Pictures were photographed at the same exposure time and improved according to their sharpness with unsharp masking algorithm (Axio Vision). From each condition the area behind the pharynx was zoomed out (white rectangle). Large lipid droplets are indicated with arrows. Scale bar: 100 µm.

To confirm this observation in other male mutants, *him-5* and *fog-2* were additionally analyzed. As shown in **figure 16 and 17** *him-5* and *fog-2* worms contained also larger lipid droplets under DR in both sexes. Under more drastic DR conditions (OD 0.7), the enlargement of lipid droplets seemed to be more enhanced in *fog-2* males than in *fog-2* females (**figure 17**). Overall, DR increases lipid droplet size in intestinal and hypodermal cells in both sexes. This effect seems to be more pronounced under harsh DR condition, especially in males.

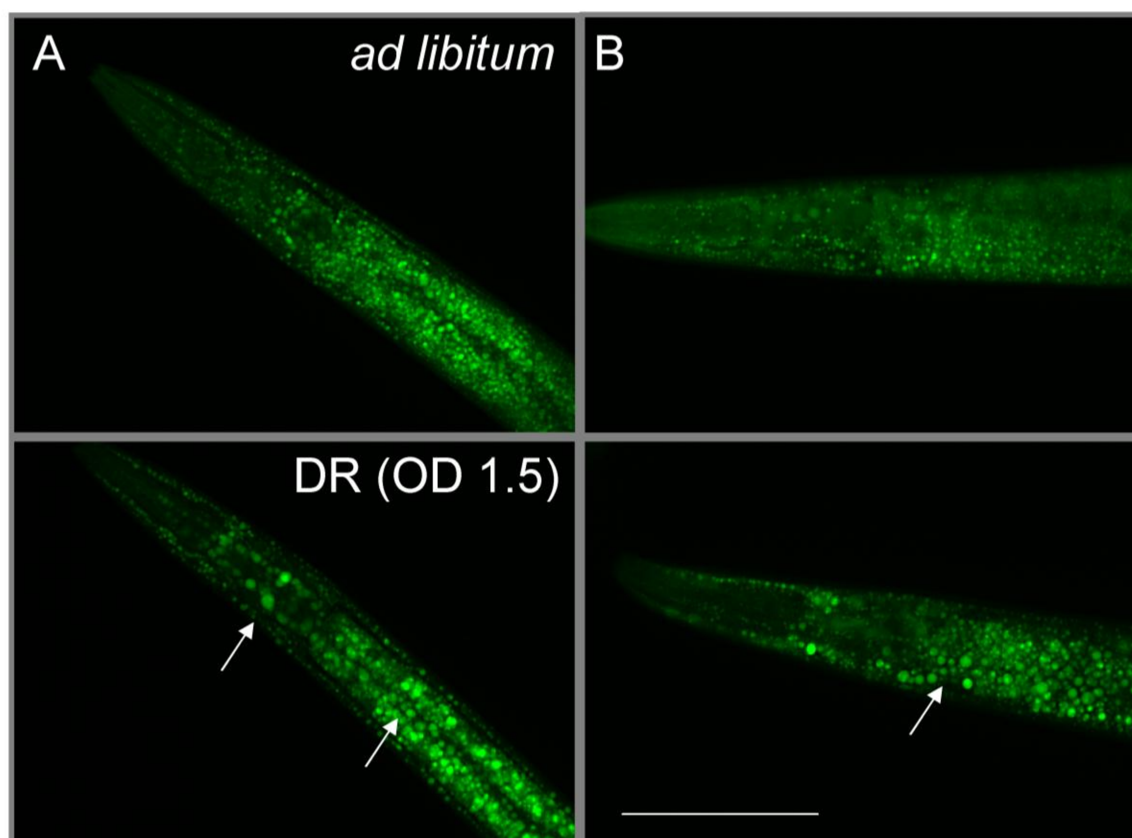


Figure 16. Lipid droplets under *ad libitum* (top) and DR (OD 1.5, bottom) conditions in *him-5* males (A) and hermaphrodites (B).
him-5 worms were cultivated at the corresponding condition until *ad libitum* fed hermaphrodites started to lay eggs. BODIPY-fixative staining was performed as described in Material and Methods. As an exception, worms were transferred after 56 h on fresh DR plates to avoid very harsh food conditions. Pictures were photographed at the same exposure time and improved according to their sharpness with unsharp masking algorithm (Axio Vision). Large lipid droplets are indicated with arrows. Scale bar: 100 µm.

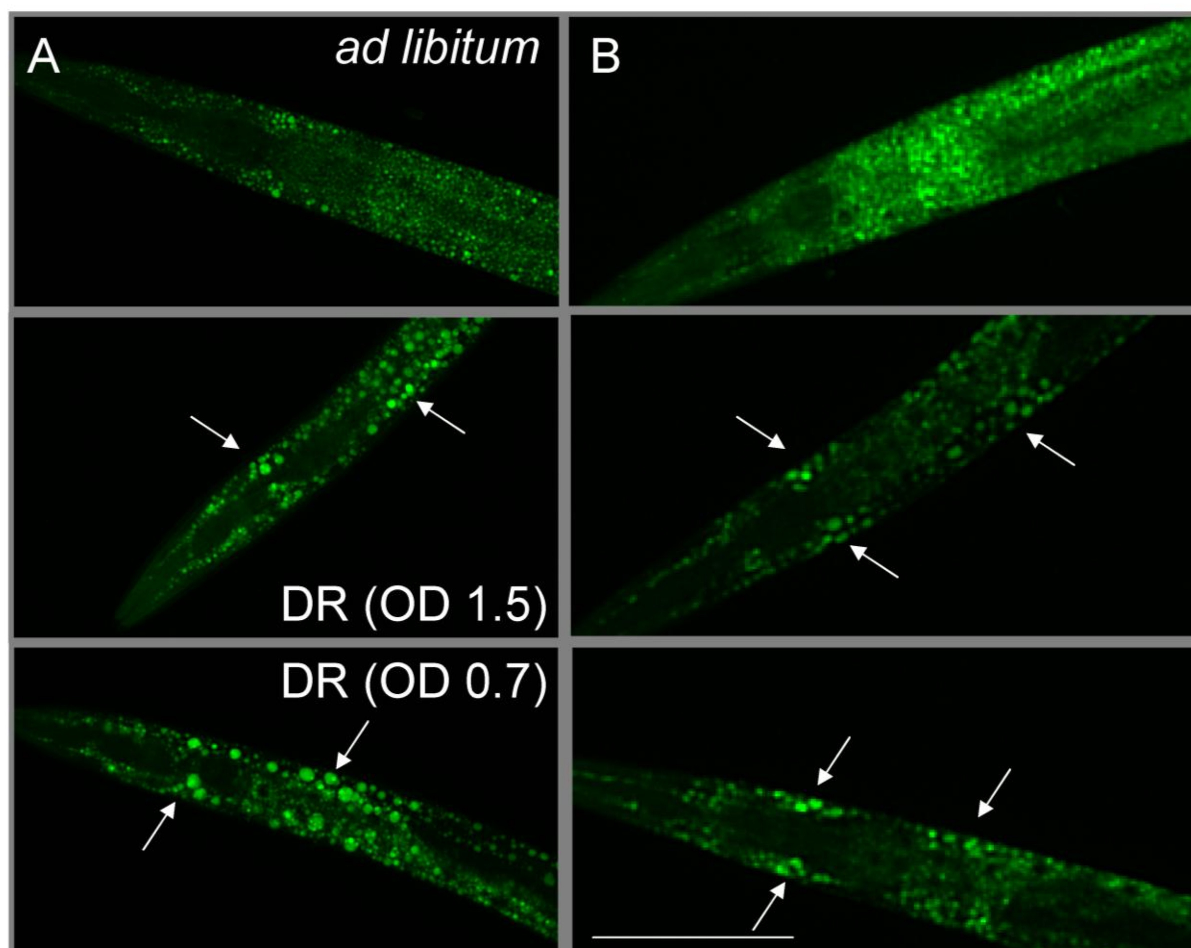


Figure 17. Lipid droplets under two DR (OD 1.5 and OD 0.7) conditions in *fog-2* males (A) and females (B).
fog-2 males and females were maintained at *ad libitum* and two DR conditions until *ad libitum* fed females started to lay eggs and then fixative stained with BODIPY. Pictures from the same sex were photographed at the same exposure time and improved according to their sharpness with unsharp masking algorithm (Axio Vision). Large lipid droplets are indicated with arrows. Scale bar: 100 µm.

Dietary restriction reduces glucose and trehalose content in both sexes to a similar extent

In the first result section a 3.9-5.4-fold higher trehalose to glucose ratio was unraveled in adult males in comparison to hermaphrodites under *ad libitum* condition (**figure 8**). Thus, the trehalose and glucose content of both sexes in response to DR was determined in *him-8 GFP* worms at young adulthood (66 h), adulthood (76 h) and one day of adulthood (90 h). As shown in **figure 18 A and B**, the glucose and trehalose content per worm were decreased under DR in both sexes to a similar extent (see also **supporting information table S7**). The resulting trehalose to

glucose ratios under DR were similar to those found under *ad libitum* conditions (**figure 18 C**). Thus, DR reduces glucose and trehalose content in both sexes to a similar extent.

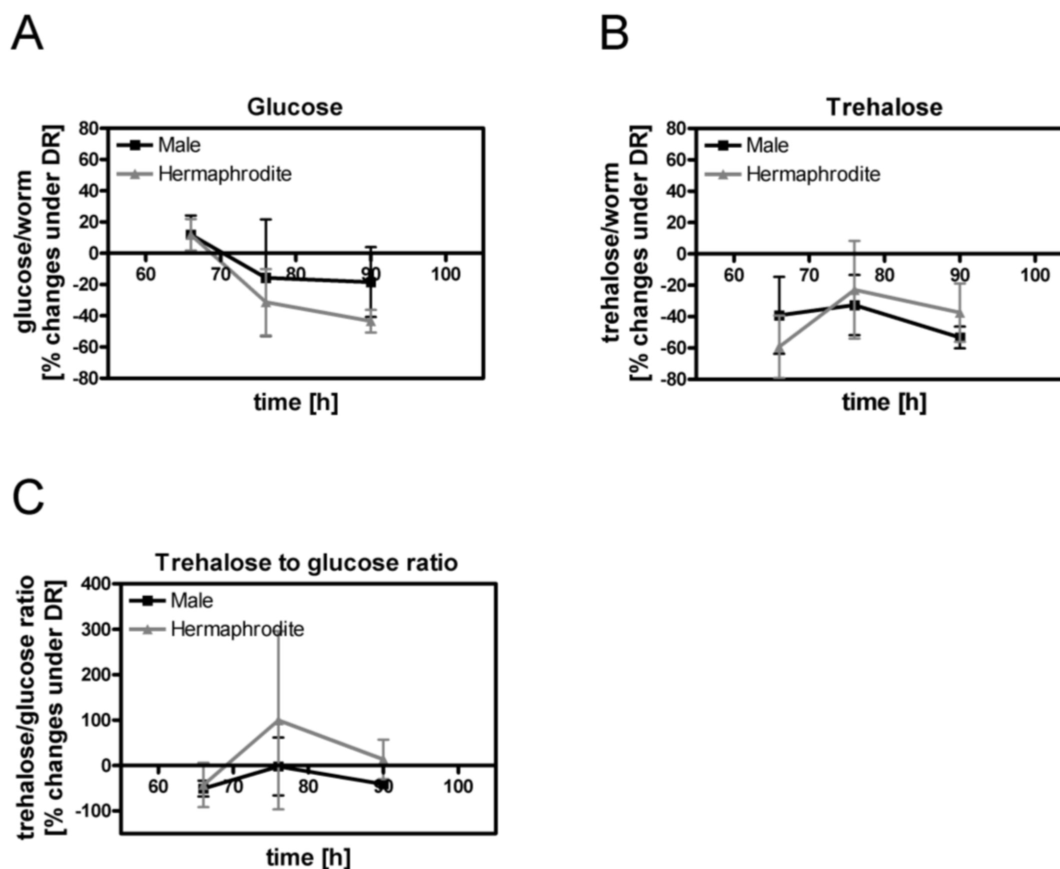


Figure 18. Changes of glucose and trehalose content under DR (OD 1.5) in *him-8 GFP* males and hermaphrodites at different developmental stages.
Changes of glucose content (A), trehalose content (B) and the resulting trehalose to glucose ratio (C) under DR in *him-8 GFP* males and hermaphrodites at young adult (66 h), adulthood (76 h) and one day of adulthood (90 h) were assessed. Relative changes under DR in comparison to *ad libitum* feeding were calculated. The mean ± SD from 3-5 experiments is presented. Differences in carbohydrate changes between both sexes were statistically analyzed with Student's t-test.

Dietary restriction up-regulates collagen genes and down-regulates UDP-glucuronosyltransferases and cytochrome P450 genes in both sexes

To determine the response of hermaphrodites and males to DR on gene expression, a microarray-based gene expression profiling was performed. Isolated RNA from *ad libitum* fed and dietary restricted (OD 1.5) *him-8 GFP* males and hermaphrodites at young adulthood (66 h) and adulthood (76 h) was used. To reduce the complexity of

large data sets, genes regulated in both sexes (see **table 4** for cut-off criteria) and developmental stages were overlapped (**table 4, supporting information table S8**). This overlap analysis resulted in 5 and 23 genes, which were consistently up-regulated and down-regulated, respectively (**table 4**). It should be noted that the metallothionein 2 (*mtl-2*) gene was identified as up-regulated by 4.6 to 9.9-fold in both sexes at both stages (**supporting information table S8**). This gene functions in metal homeostasis and stress response [141] and is responsive to DR in a variety of mice mammalian tissues [142]. Genes identified as consistently down-regulated under DR are involved in ammonium transport, defense response, lipid metabolic process, proteolysis inhibition, cell adhesion and enzyme activity. Interestingly, lipase like 5 (*lipl-5*) possessed 5.5 to 16.6-fold lower mRNA levels in both sexes at both adult stages (see **supporting information table S8**).

Table 4. Number of DR-regulated genes in both sexes at both developmental stages.

Criteria	Overlap between male and hermaphrodite		
	Young adult (66 h)	Adult (76 h)	Overlap between both stages
Down-regulated in both sexes (ratio <0.5, p<0.05)	38	155	23
Up-regulated in both sexes (ratio > 2, p<0.05)	77	94	5
Differential regulated between both sexes (ratio >1.5 or <0.66, p<0.05)	8	31	-
Regulated in male but not in hermaphrodite (Male: ratio >2 or <0.5, Hermaphrodite: ratio between 0.66-1.5)	133	216	11
Regulated in hermaphrodite but not in male (Hermaphrodite: ratio >2 or <0.5, Male: ratio between 0.66-1.5)	399	997	73

To identify DR enriched gene families, functional annotation clustering analysis was performed using all genes being at least 2-fold (p<0.05) regulated under DR in both

sexes and at both adult stages (**figure 19 A and 20 A**). This approach identified three gene families being regulated under DR in both sexes simultaneously. Genes from the collagen family were enriched and showed an up-regulation in response to DR in males (**figure 19 B**) and hermaphrodites (**figure 19 C**) at young adulthood. Collagens are ubiquitous structural proteins, which are the major component of the nematode cuticle [143].

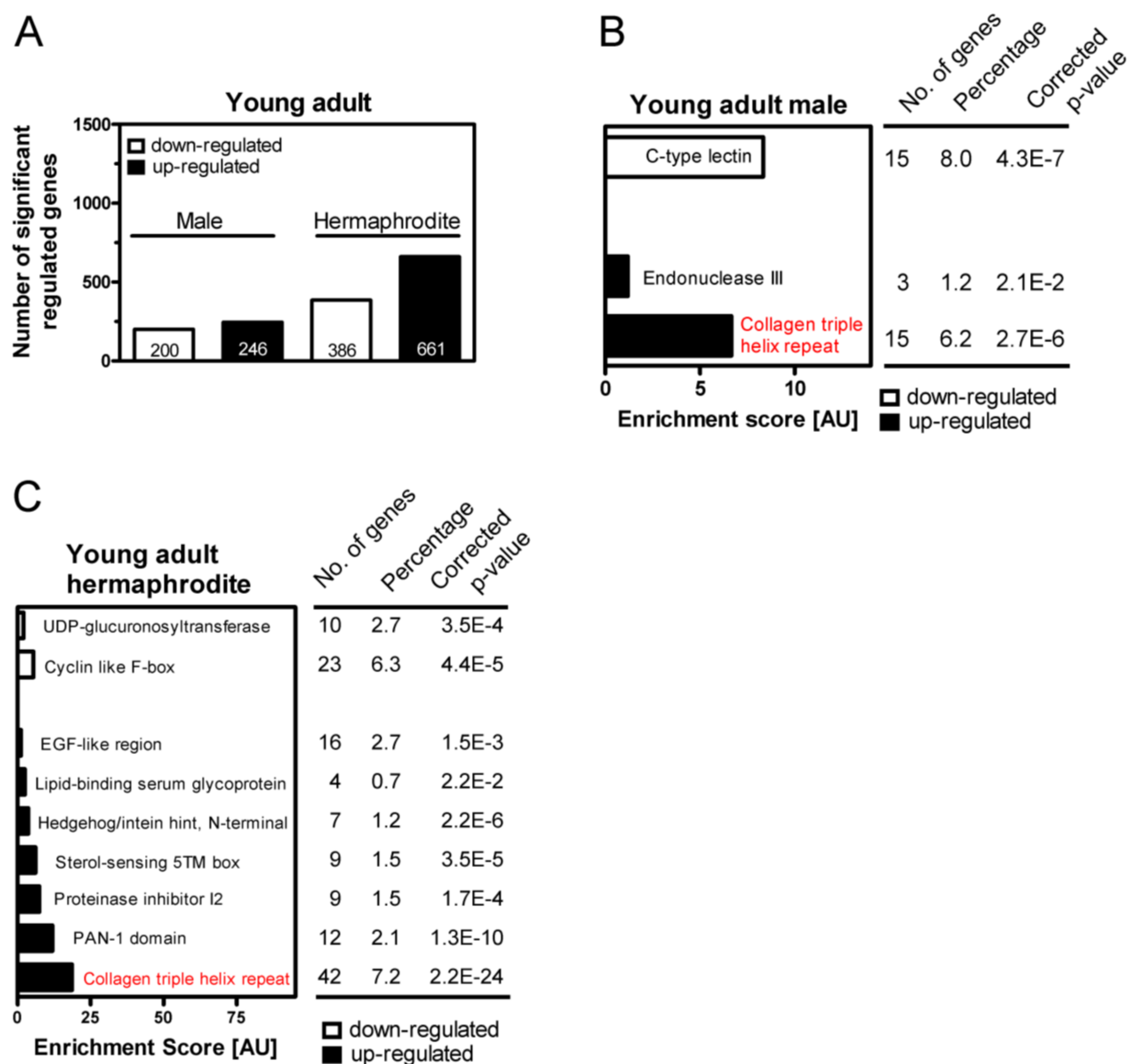


Figure 19. Functional annotation clustering of DR regulated genes from *him-8 GFP* worms at young adult (66 h) stage.
(A) Data show the number of significant ($p<0.05$) regulated genes (at least 2-fold) under DR in both sexes at young adulthood, which were imported in DAVID database (version 6.7, http://david.abcc.ncifcrf.gov/) for functional annotation analysis. (B, C) DR regulated genes in males (B) and hermaphrodites (C), which were over-represented in their respective group (down- or up-regulated), are clustered

according to their biological significance (enrichment score). From functional annotation analysis only protein families, domains and functional sites (Interpro term) were extracted and represented. Number of genes in the gene family, percentage (involved genes/total genes) and corrected p-value (Benjamini correction) are also illustrated. Gene families, which were written in red, were enriched in both sexes.

Furthermore, genes belonging to the UDP-glucuronosyltransferases (*ugt*) and cytochrome P450 (*cyp*) family were down-regulated under DR in both sexes at adulthood (**figure 20 B and C**). Members of these gene families are important for the detoxification machinery of *C. elegans* [144]. Overall, DR modulates genes in males and hermaphrodites, which are linked to detoxification and cuticle assembly.

Dietary restriction regulates C-type lectin genes, lipase (class 3) and saposin B genes in males but not in hermaphrodites

Based on our overlap analysis of genes responsive to DR (**table 4**) 11 genes (e.g. chitinase [*cht-1*], neuromedin U receptor [*nmur-3*], neuropeptide-like protein [*nlp-4*]) were identified, which were regulated under DR in males but not in hermaphrodites (**supporting information table S8**). Moreover, the functional annotation clustering analysis revealed that several members of the C-type lectin gene family (*clec*) were affected by DR only in males (**figure 19 B and 20 B**). 15 and 17 *clec* genes were up-regulated under DR in males at young adulthood and adulthood, respectively. Additionally, 13 *clec* genes were down-regulated in response to DR in adult males. Some members of the *clec* gene family play an essential role in defense-mediated reactions [145]. Furthermore, members of the lipase (class 3) and saposin B gene family were only down-regulated in adult males but not in hermaphrodites. Lipases hydrolyze ester linkages of triglyceride, which is an important step in fat catabolism. Saposins are small lysosomal proteins, which remove lipid substrates from membranes and thus degradation enzymes can act [146]. Taken together, DR induces a male-specific gene expression response, which indicates an altered immune defense and a reduced fat catabolism.

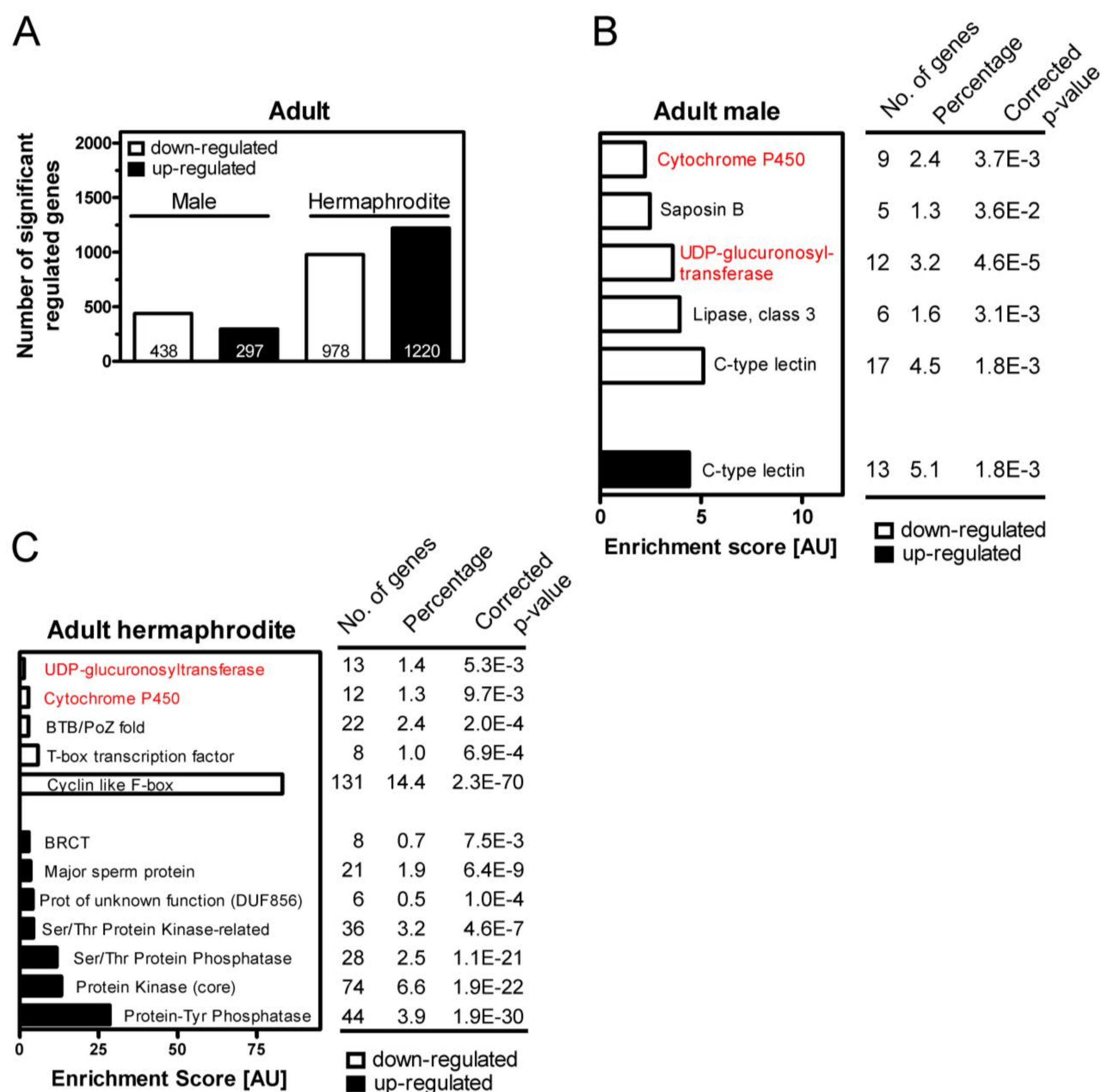


Figure 20. Functional annotation clustering of DR regulated genes from *him-8 GFP* worms at adult (76 h) stage.

(A) Data show the number of significant ($p<0.05$) regulated genes (at least 2-fold) under DR in both sexes at adulthood, which were imported in DAVID database for functional annotation analysis. (B, C) DR regulated genes in males (B) and hermaphrodites (C), which were over-represented in their respective group (down- or up-regulated), are clustered according to their biological significance (enrichment score). Functional annotation analysis was performed as mentioned in **figure 19**. Gene families, which were written in red, were enriched in both sexes.

Dietary restriction alters the expression of genes enriched in several gene families including the major sperm protein family in hermaphrodites but not in males

The overlap analysis of gene expression data identified 73 genes, which were modulated in their gene expression under DR only in hermaphrodites (**table 4**). Many of these genes (**supporting information table S8**) are part of the SKN-1 dependent zygotic transcript (*sdz*) and F-box protein gene family, which are important for embryogenesis [147]. The latter group of genes was also identified by our functional annotation clustering analysis. This approach revealed a hermaphrodite-specific response to DR at young adulthood and adulthood for 8 and 10 gene families, respectively (**figure 19 C and 20 C**). Young adult hermaphrodites showed an up-regulation of genes belonging to the sterol sensing domain and hedgehog gene family (**figure 19 C**). Both gene families are required for normal cuticle assembly, growth and molting process [148-150]. Nine protease inhibitor genes were also up-regulated under DR in hermaphrodites but not in males at young adult stage. The majority of these genes belonging to the proteinase inhibitor I2 gene family inhibit proteases of the S1 family to prevent physiological unwanted proteolysis [151,152].

In addition to cyclin like F-box, T-box transcription factor and BTB/Poz fold protein domain gene families showed a decreased expression under DR only in adult hermaphrodites (**figure 20 C**). All three gene families are involved in embryonic development [147]. Cyclin like F-box proteins are associated with SKP1 in a protein complex, function as an E3 ligase in the ubiquitin protein degradation pathway [153]. Furthermore, it is known that BTB proteins merge the functional properties of SKP1 and F-box proteins [154]. Members of the T-box family are transcription factors, which are required for early cell-fate decisions [155]. Furthermore, many protein kinases and phosphatases encoding genes belonging to four families were up-regulated under DR in adult hermaphrodites but not in males (**figure 20 C**). Genes encoding for the major sperm protein (*msp*) family were also identified as up-regulated under DR only in adult hermaphrodites. *Msp* genes function in sperm motility and influence oocyte maturation and sheath cell contraction [156]. A detailed analysis of all *msp* genes revealed that these genes were either unchanged or down-regulated in adult males (**figure 21**). Thus, nearly all genes of the *msp* gene family seem to be up-regulated under DR in adult hermaphrodites but not in males.

Dietary restriction promotes an ongoing expression of sperm-associated genes from young adulthood to adulthood in hermaphrodites but not in males

Next, it was examined whether other genes associated with sperm function were also modulated in response to DR, especially in hermaphrodites. Genes belonging to the sperm-specific family class P (*ssp*), class Q (*ssq*), class S (*sss*), sperm-specific transcript (*sst*), defective spermatogenesis genes (*spe*) and further genes encoding a major sperm protein domain had significantly higher expression levels under DR in adult hermaphrodites, whereas males exhibited almost no changes (**figure 21, supporting information table S10**). At young adulthood almost all sperm-associated genes were not regulated under DR in both sexes (**figure 21, supporting information table S9**). In hermaphrodites, the expression of *msp* genes dropped down moderately (group 1) or considerably (group 2) from young adult to adult stage under *ad libitum* conditions (**figure 22**). This decline was not present under DR, where the levels remained constant (group 2) or increased slightly (group 1) from young adult to adult stage. In males, mRNA steady-state levels of *msp* genes increased from young adult to adult stage under *ad libitum* as well as under DR conditions.

Expression pattern of all other sperm-associated genes showed similar sex differences as the *msp* genes (**figure 23**). Thus, DR promotes an ongoing expression of sperm-associated genes in hermaphrodites but not in males from young adult to adult stage.

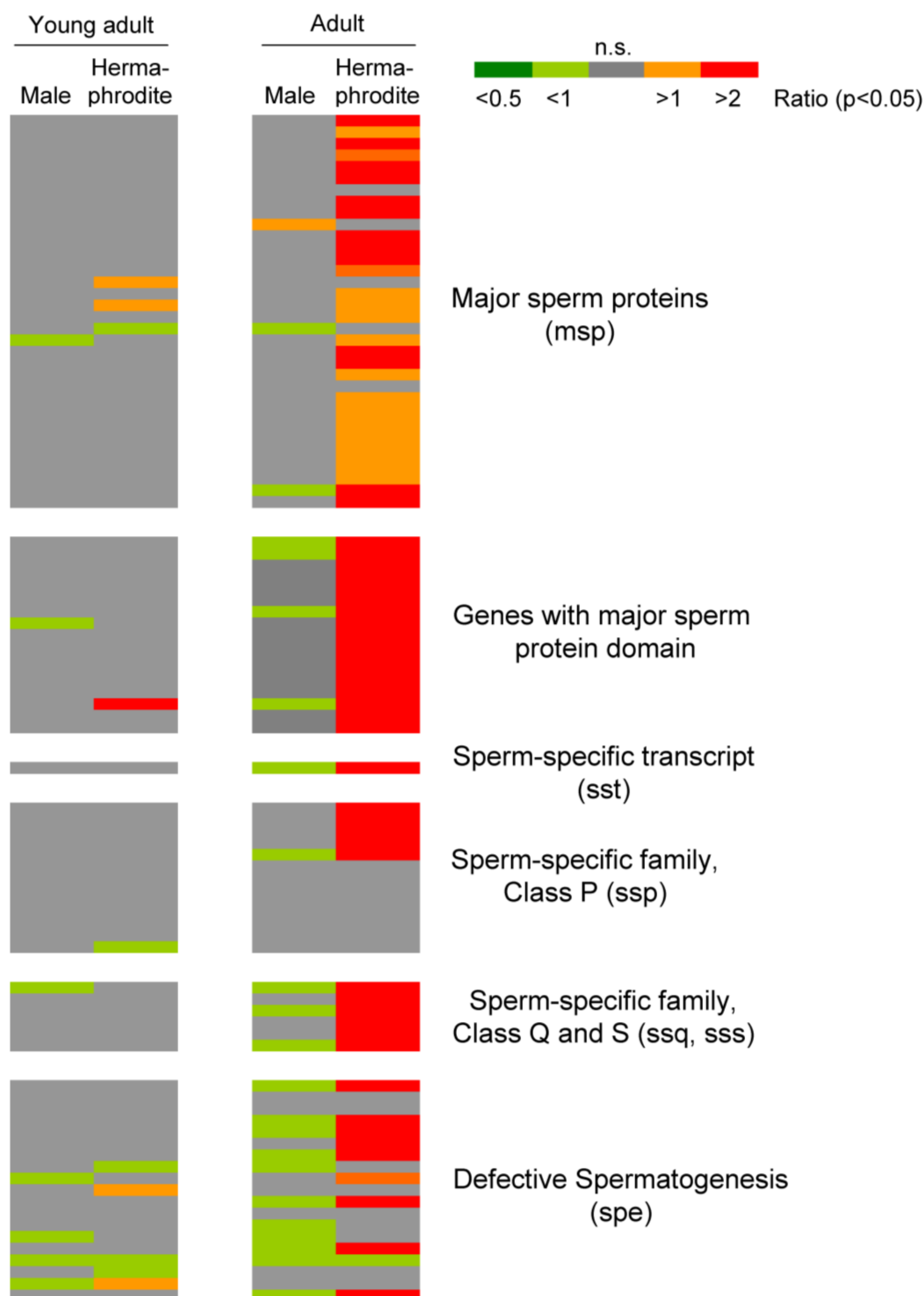


Figure 21. Differential expression of sperm-associated genes under DR (OD 1.5) in *him-8 GFP* males and hermaphrodites.
Data from both sexes at young adulthood (66 h, left side) and adulthood (76 h, right side) are illustrated. Each row represents one gene with the corresponding average ratio (DR/*ad libitum*), which is replaced by a color. Orange (ratio between 1 and 2) and red (ratio>2) represent an increased gene expression under DR, while light green (ratio between 0.5 and 1) and dark green (ratio<0.5) represent a decreased gene expression under DR. Genes, which were not significantly altered were marked as grey. See **supporting information table S9 and S10** for full data showing genes and the expression values.

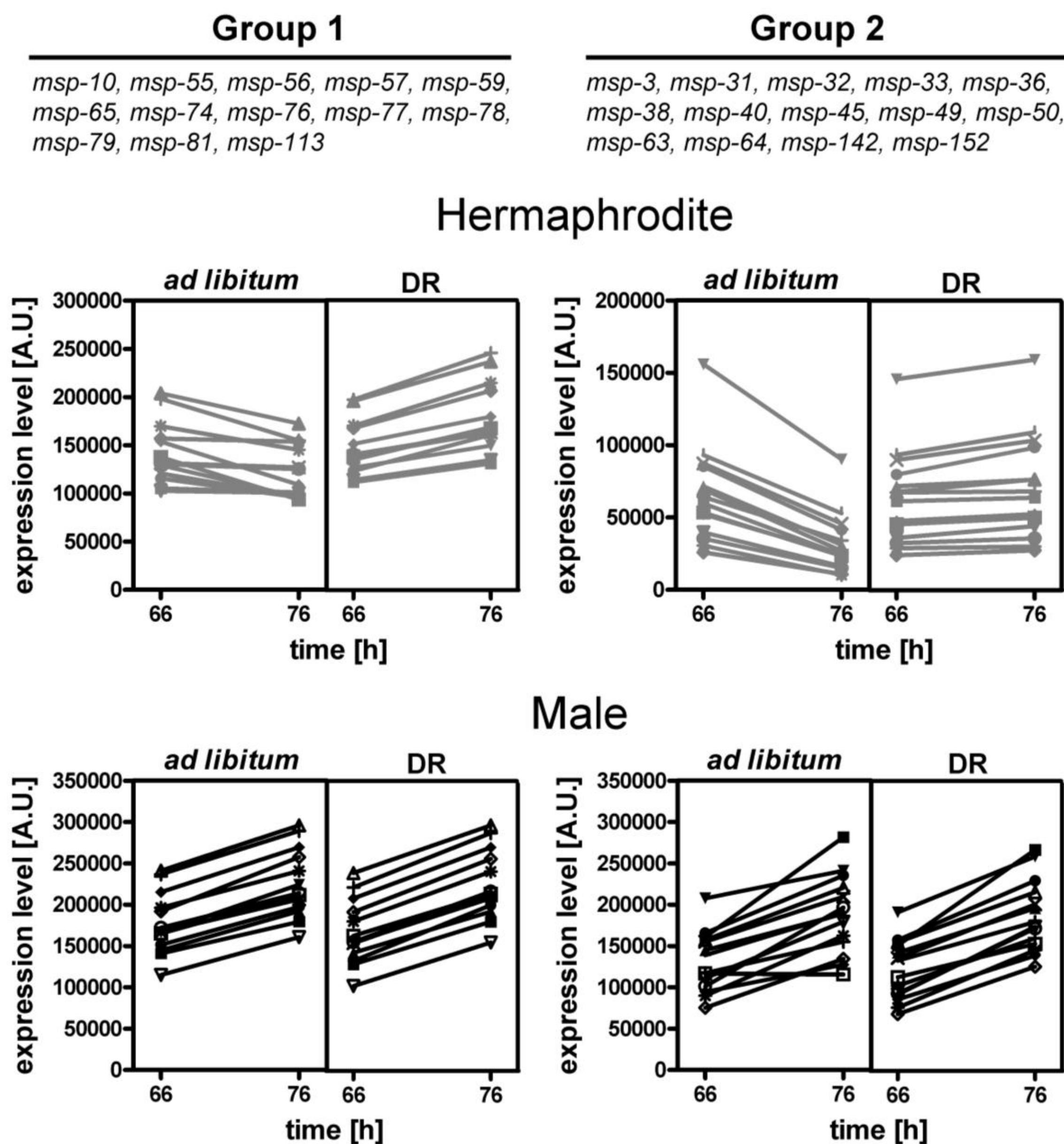
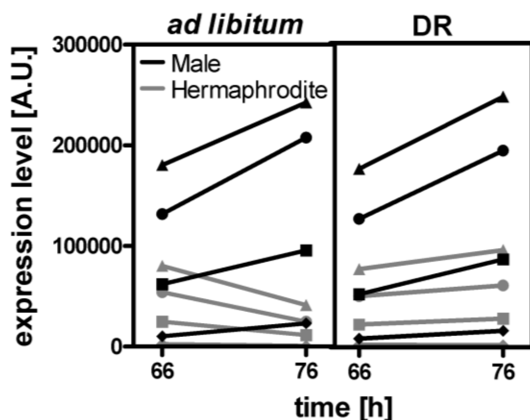
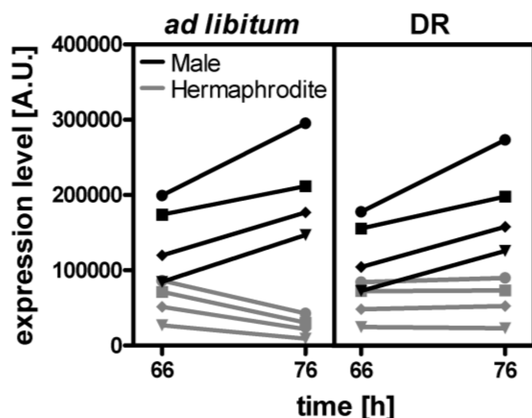


Figure 22. Temporal gene expression drift of major sperm protein (*msp*) genes under DR (OD 1.5) in *him-8 GFP* hermaphrodites (top) and males (bottom).
Changes in gene expression levels of *msp* from young adulthood (66 h) to adulthood (76 h) in hermaphrodites (grey) and males (black) under *ad libitum* and DR (OD 1.5) are shown. *Msp* genes are divided into two groups according to their expression pattern in hermaphrodites.

A Sperm-specific family, class P
(e.g. *ssp-9*, *ssp-10*, *ssp-11*, *ssp-16*)



B Sperm-specific family, class Q
(e.g. *ssq-1*, *ssq-2*, *ssq-3*, *ssq-4*)



C defective spermatogenesis
(e.g. *spe-9*, *spe-10*, *spe-11*, *spe-15*, *spe-19*, *spe-38*)

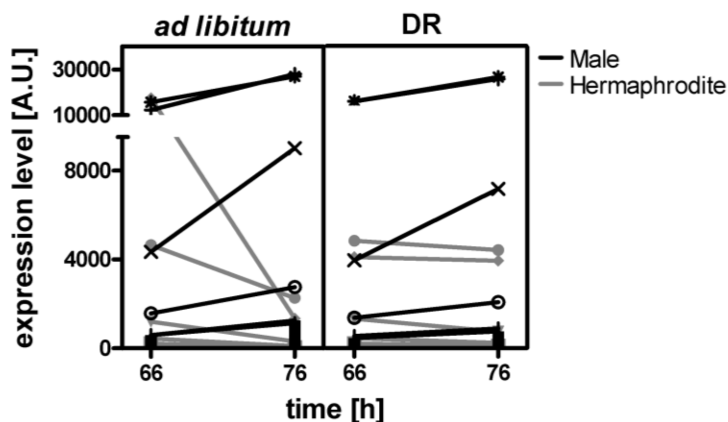


Figure 23. Temporal gene expression drift of sperm-associated genes under DR (OD 1.5) in *him-8 GFP* hermaphrodites (grey) and males (black).
Some genes from sperm-specific family, class P (A), class Q (B) and defective spermatogenesis genes (C) were exemplarily analyzed. Changes in gene expression levels from young adulthood (66 h) to adulthood (76 h) in males and hermaphrodites under *ad libitum* and DR (OD 1.5) are shown.

3.3 Influence of paternal dietary restriction on progeny fat content

To measure the influence of a paternal DR on the next generation, *fog-2 (q71)* mutant strain was used. This strain is suitable for studying paternal effects in *C. elegans* since *fog-2* hermaphrodites are functional females. They do not produce sperm, while *fog-2* males are unaffected [138]. Males (P0) developed at eight different DR regimes and were then mated with unfertilized females (P0) consuming an *ad libitum* diet (**figure 24**). As control, *ad libitum* fed males were crossed with *ad libitum* fed females. The resulting F1 progeny grew up under *ad libitum* condition until adulthood and were then analyzed.

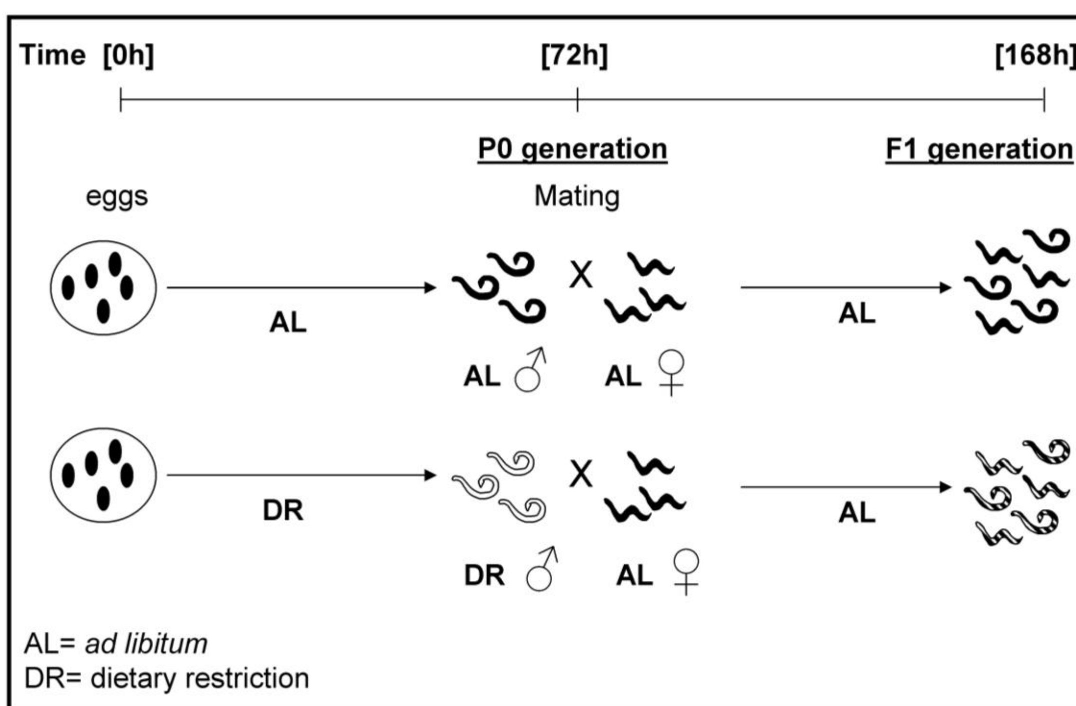


Figure 24. Experimental design to study the effects of paternal DR on the F1 generation.
fog-2 males were dietary restricted from egg (0 h) to adulthood (~72 h) and subsequently mated with *ad libitum* fed unfertilized *fog-2* females. As control, *ad libitum* fed males were crossed with *ad libitum* fed females. The resulting F1 generation was maintained under *ad libitum* conditions until one day of adulthood (72 h-168 h).

As expected, increasing the extent of DR led to a decrease in body length (up to 59.7 % of control), body width (66.4 %) and body volume (34.7 %) of P0 males demonstrating dose-dependency of DR regimes (**figure 25**).

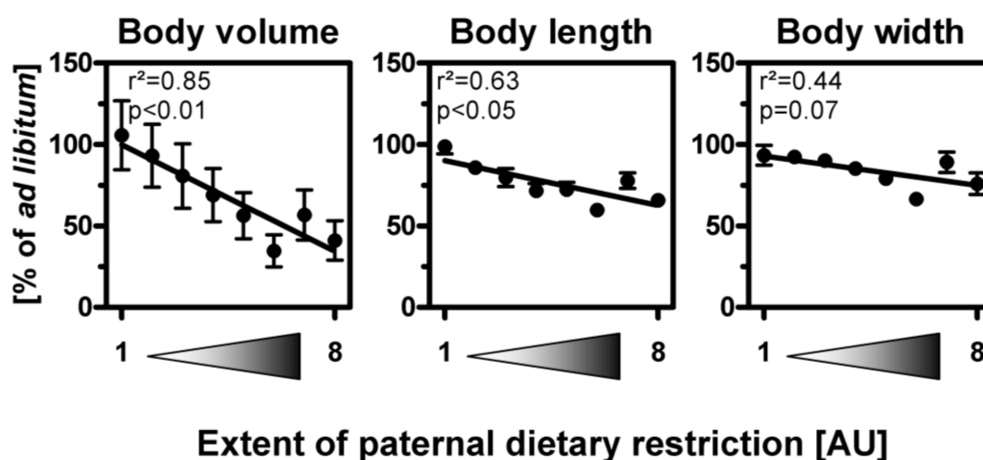


Figure 25. Influence of different DR conditions on P0 male body proportions. The extent of DR was ranked between 1 (moderate restriction) and 8 (strong restriction). *Ad libitum* fed P0 males served as control. Data represents mean ± SD of 2-4 independent experiments. At least 6 to 32 worms per DR condition were utilized in each experiment.

In contrast, the extent of paternal DR did not influence the body volume of F1 males as well as F1 females (**figure 26 A**). Body length and body width of the progeny were also unaffected (**figure 26 B and C**). Thus, body proportions as an important confounding factor of fat content was not influenced in the progeny of dietary restricted males. Next, the fat content of food restricted male progeny was quantified using a fixative BODIPY^{TM} 493/503 staining procedure. The accuracy of this fat staining method was confirmed by co-localisation studies using CARS and Raman microscopy, imaging of lysosome related organelles and biochemical measurement [32]. An inverted U-shaped relationship between the extent of paternal DR and the progeny fat content (**figure 27 A**) was found. Increasing the extent of paternal DR led to a successive increase in fat content of progeny until reaching a maximum of about 160 % of *ad libitum*. Further reduction of paternal food to very low levels decreased fat content of progeny to those levels found in the control experiment. Calculation of fat content per body volume confirmed our finding that paternal DR affects the level of fat in the F1 progeny (**figure 27 B**). This relationship occurs in males and females (**figure 27 A and B**).

Figure 26. Influence of different paternal DR conditions on progeny body proportions.
Body volume (A), body length (B) and body width (C) of F1 progeny are presented. The extent of DR was ranked between 1 (moderate restriction) and 8 (strong restriction). Progeny from *ad libitum* fed P0 males served as control. Data represents mean ± SD of 2-4 independent experiments. At least 6 to 32 worms per DR condition were utilized in each experiment.

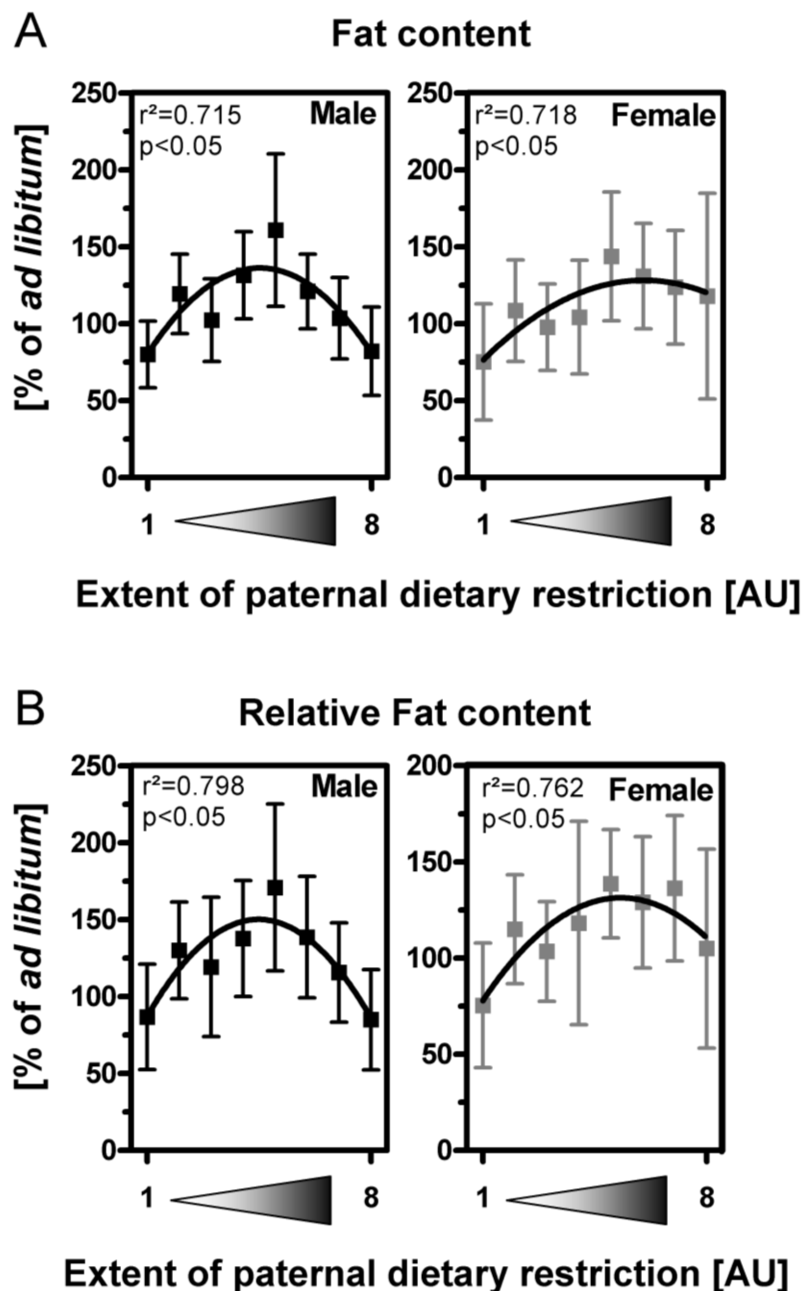


Figure 27. Influence of different paternal DR conditions on progeny fat content (A) and relative fat content (B).
The extent of DR was ranked between 1 (moderate restriction) and 8 (strong restriction). Fat content per worm was determined in F1 adulthood using BODIPY™ 493/503 based fixative staining procedure. The relative fat content was adjusted to body volume. Progeny from *ad libitum* fed P0 males served as control. Data represents mean ± SD of 2-4 independent experiments. At least 6 to 32 worms per DR condition were utilized in each experiment. Regression analysis showed a significant fit to a quadratic model. In figure 27 A the quadratic equations are $y= -4.5967x^2+41.12x+44.756$ (male) and $y= -2.03x^2+12.17x+109.79$ (female). In figure 27 B the equations are $y=-5.17x^2+46.43x+45.81$ (male) and $y= -2.78x^2+20.18x+95.31$ (female).

4 DISCUSSION

4.1 Sex differences under *ad libitum* conditions in body proportions, RNA content, carbohydrate metabolism and gene expression of C-typ lectins

The nematode *C. elegans* became one of the most widely used model organism in modern biology. During the last years, *C. elegans* was also established as a model in order to examine physiological aspects at molecular and cellular level in an intact organism [5]. So far, sex differences in physiology and metabolism were not studied extensively in *C. elegans* because it is laborious to separately analyze a high number of males and hermaphrodites. To circumvent this methodical problem, male enriched mutants were used in this study, one with a sex-specific GFP signal, which grew up on the same plate and were separated for analysis by flow cytometry. Using this approach, adult males exhibited smaller body proportions, nearly equal triglyceride and protein levels than hermaphrodites when values were adjusted for body volume, lower total RNA content, higher trehalose levels and lower glucose levels (see **table 5**). In addition, a number of genes, which were considered as sex-specific and may explain some physiological differences between adult male and hermaphrodites in *C. elegans* were identified.

Table 5. Summary of sex differences under *ad libitum* and DR conditions in *C. elegans*.

Parameter	*Ad libitum*	Changes under DR
<u>Body proportions</u>		
Body length	⚥ > ♂	⚥ > ♂
Body volume	⚥ >>> ♂	⚥ > ♂
<u>Body composition</u>		
Protein (adjusted)	⚥ > ♂ (⚥ = ♂)	⚥ > ♂
Triglyceride (adjusted)	⚥ > ♂ (⚥ = ♂)	⚥ = ♂
Triglyceride to protein ratio	⚥ = ♂	⚥ = ♂
Glucose (adjusted)	⚥ >> ♂ (⚥ > ♂)	⚥ >= ♂

Trehalose (adjusted)	⚥ =< ♂ (⚥ < ♂)	⚥ = ♂
Trehalose to glucose ratio	⚥ << ♂	⚥ >= ♂
<u>Lipid droplet</u>		
Size	⚥ = ♂	⚥ < ♂
Number	⚥ = ♂	⚥ < ♂
<u>Gene expression</u>		
Generell findings	**Sex-specific:** transcription factors, *clec* genes **Differences:** carbohydrate metabolic genes	**Regulated in both sexes:** *lipl-5*, collagens, UDP-glucoronosyl-transferases, cytochrome P450 **Regulated only in hermaphrodite:** embryogenesis and sperm-associated genes **Regulated only in male:** *clec*, saposin B, lipase (class 3)

Adult males are smaller and contain lower RNA but have similar body composition in comparison to hermaphrodites

Before and at young adult stage (66 h) there were only minor differences between males and hermaphrodites inbody proportion and composition parameters. These results are in accordance with findings from Thoemke *et al.* who found hardly any differences at the transcriptional level between males and hermaphrodites up to larval stage L4 [29]. At adult stage, males are over 50 % smaller than hermaphrodites. This may be explained by sex differences in the RNA content. Males did not change their total RNA content from young adult to adult stage. In contrast, total RNA content of hermaphrodites increased considerably during this period. Total RNA is mainly composed of ribosomal RNA (rRNA, visible in RNA electrophoretic analysis) and the repression of rRNA synthesis inhibits ribosome biosynthesis and cell growth [157]. Recent work by Honjoh *et al.* demonstrated that an inhibition of the translation machinery in both sexes reduces the body length to a greater extent in hermaphrodites than in males (personal communication, S. Honjoh, [158]). The authors assumed that males have a lower translation rate. This corresponds to the

lower levels of total RNA in males in our experiments, suggesting a lower expression of rRNA genes, resulting in a lower ribosome biosynthesis, lower translation rate and lower growth rate from the young adult to adult stage in males than in hermaphrodites. The triglyceride to protein ratio as well as the volume adjusted triglyceride and protein values were similar between males and hermaphrodites. Hence, sex differences in body composition, as described in other organisms [159-162], are not obvious in *C. elegans*. Notably, the determined protein and triglyceride values for adult hermaphrodites are in line with data from the literature [32-34]. Thus, there exist sex differences in body proportions and RNA content in *C. elegans* but not in body composition.

The carbohydrate metabolism differs between C. elegans sexes at the metabolite and gene expression level

Trehalose is a soluble, non-reducing disaccharide of two molecules glucose, which naturally occurs in many species like bacteria, yeast and invertebrates [35,36]. It is synthesized in a two-step pathway from glucose 6-phosphate and UDP-glucose by trehalose 6-phosphate synthase and trehalose 6-phosphate phosphatase [35]. Two trehalose phosphate synthases (*tps-1* and *tps-2*) and one trehalose phosphate phosphatase (*gob-1*) genes have been characterized in *C. elegans* [163,164]. Both trehalose synthesis substrates are also needed for glycogen synthesis. According to our results it can be conducted that males utilize glucose (UDP-glucose, glucose 6-phosphate) for producing rather trehalose than glycogen whereas it may be opposite in hermaphrodites (**figure 28**). This hypothesis is reflected in gene expression data with higher expression levels of trehalose synthesis genes and lower expression of glycogen synthesis genes in males than in hermaphrodites. Also in line with the hypothesis, a higher trehalose level was found in males. However, the measured ratios between glucose and trehalose is contrary to the findings of Hanover *et al.* where lower glucose than trehalose levels were observed in hermaphrodites [37]. A reason for this discrepancy could lie in the different methods used. In our study the biochemical results were validated with NMR spectroscopy and similar levels could be measured in different mutants. Additionally, trehalose is reported to be present in much higher concentrations than free glucose in other nematodes [35].

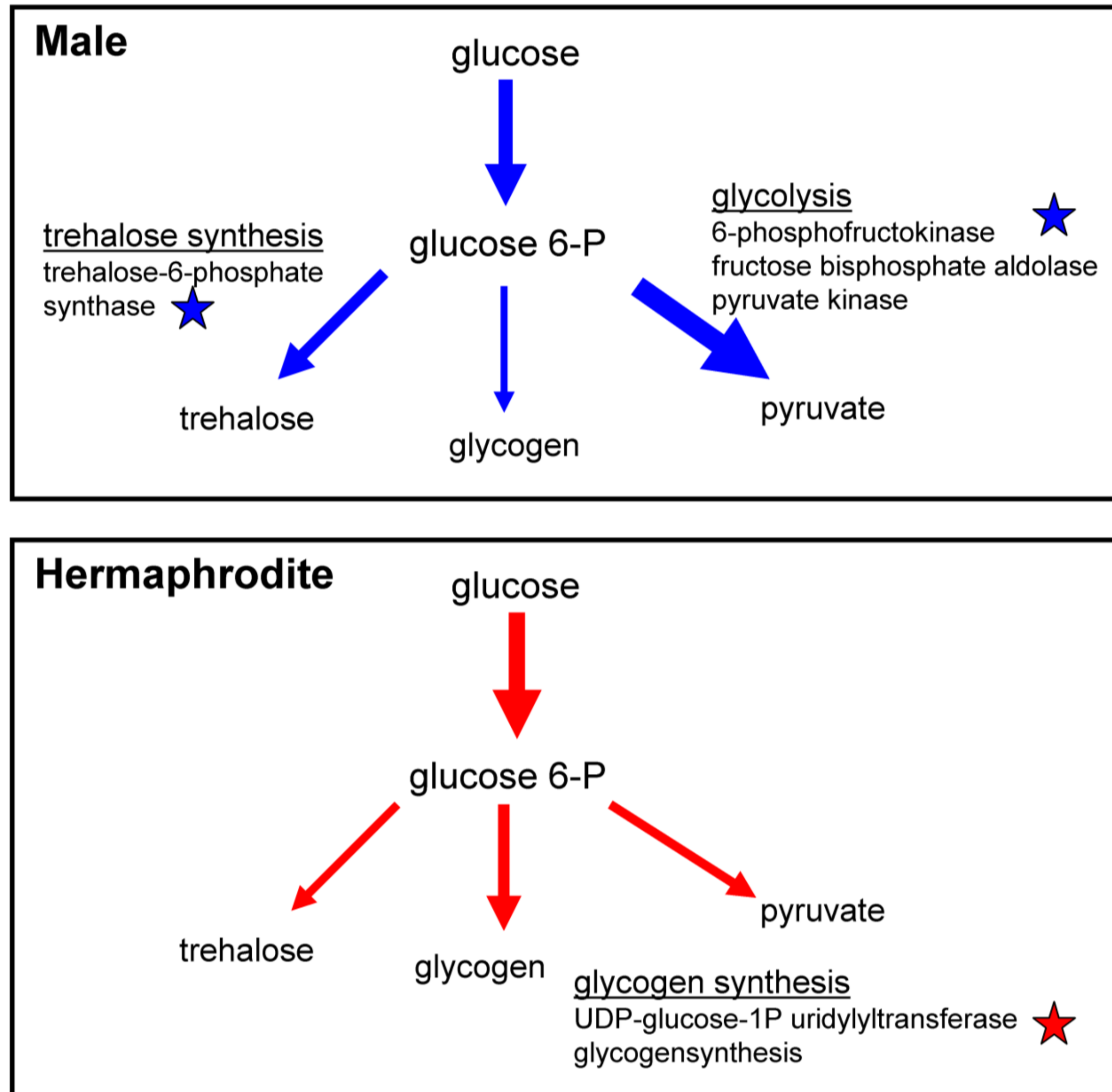


Figure 28. Conversion of glucose in males and hermaphrodites.
Glucose can be catabolized to pyruvate in glycolysis, converted to trehalose or synthesized to glycogen. How much each pathway is possibly pronounced in each sex is marked by the thickness of the arrows. Asterisks mark significant regulated genes.

Evidence from several studies indicate that trehalose have various functions in the organism [36]. In *C. elegans* it is believed that trehalose serves as a sugar transporter between the cell types because a functional glucose-6-phophatase is lacking [35,36,38]. It is also predicted that trehalose protects *C. elegans* and other nematodes against environmental stresses including desiccation, heat and hypertonia [35,140,165] by guarding lipid membranes and stabilising proteins

structures [166,167]. Eggs and dauer larvae, which are highly resistant against environmental stresses, contain high levels of trehalose [35,164]. Higher levels of trehalose in males could mean that males are more resistant against desiccation than hermaphrodites. Whether that is the case, has to be clarified in further studies.

Furthermore, a much lower glucose level was found in males than in hermaphrodites. This finding was supported by gene expression data because genes encoding for glycolytic enzymes were higher expressed in males in comparison to hermaphrodites. Importantly, the key enzyme of glycolysis, the phosphofructokinase, was 29-fold higher expressed in males. These data suggest that males have a higher flux through glycolysis than hermaphrodites (**figure 28**). It is known from mammals that glycolysis encoding genes are extensively modified during spermatogenesis [168] because sperm require large amounts of ATP for motility [169-171]. Furthermore, spermiogenesis can be blocked by mitochondrial inhibitors in *C. elegans* indicating that aerobic metabolism and therefore glycolysis may be essential for sperm production [172]. All these findings suggest that *C. elegans* males have higher glycolysis rates due to spermatogenesis and spermiogenesis. This hypothesis is further supported by the fact that the phosphofructokinase (C50F4.2) transcripts are enriched during spermatogenesis [28].

Lectin genes are differentially expressed between the sexes in C. elegans

In microarray experiments male- and hermaphrodite-specific genes were identified. Of note, hermaphrodites and males grew up under the same conditions on the same plate. So far, all studies analyzing sex-specific gene expression did not consider these criteria [27-29]. Nevertheless, there is a high degree of agreement between the published [27-29] and the data presented here. 76.2 % of male-specific genes have also been declared in one of these studies as a male-specific gene. 43.9 % have been even mentioned in at least two of these studies. For hermaphrodite-specific genes, a similar agreement was found with 80.3 % and 43.4 %, respectively. Surprisingly, only few metabolic genes were detected showing a sex-specific pattern (*lips-2*, *nas-17*, *nas-19* and *acl-12*). It can be concluded that males and hermaphrodites do not possess fundamentally different pathways in intermediary metabolism. Rather, quantitative sex differences in metabolism, as found for the carbohydrate metabolism, can be expected.

Among the sex-specific genes many transcription factors were differentially expressed in both sexes. All hermaphrodite-specific transcription factors identified function in embryogenesis [147,173-175] and hence no reproduction-independent transcription factor was found. Male-specific transcription factors are either involved in the development of the tail, act in male-specific neurons [176-179] or have a unknown function. All male-specific transcription factors identified have previously been described as male enriched [27]. Thus, no new transcription factor involved in sex differentiation in *C. elegans* was discovered.

Many C-type lectins were dramatically higher expressed in males than in hermaphrodites. C-type lectins are a diverse group of proteins, which currently contains 265 genes. They occur in plants, bacteria, viruses and animals as a class of proteins being involved in different protein-interactions [180]. Some members of this gene family seem to be involved in innate immunity because they are up-regulated after pathogen exposure [145]. However, the functional role of C-type lectins in invertebrates is essentially not known. The sex-specific expression of C-type lectins could be explained by two models. The first model suggests a sexual dimorphic immune system. This is confirmed by the fact that other defense-related genes than C-type lectins were also differentially expressed in males compared to hermaphrodites. In rodents, sex-specific survival after pathogenic infections is known [181]. The second model assumes that C-type lectins fulfil sex-specific tasks in males. In line with this hypothesis, only three out of 42 male-specific C-type lectins (clec-217, clec-256, clec-263) were up-regulated after pathogen infection [145]. In contrast, several *clec* genes (*clec-197*, *clec-99*, *clec-92*, *clec-157*, *clec-208*, *clec-207*) and *scl-8*, which were identified as male-specific or male-enriched in our analysis, are associated with the male sperm chromatin [182], indicating its possible roles in spermatogenesis. Furthermore, a study showed that C-type lectins are regulated by *mxl-2* (max-like protein, bHLHZip transcription factor), which is involved in the process of cell migration [183]. Further investigation, especially on the natural function of this gene family, is needed to confirm that C-type lectins fulfil immune-independent roles in *C. elegans* males.

4.2 Influence of dietary restriction on fat storage, lipid metabolism, developmental growth and reproduction in males and hermaphrodites

A limited energy availability has lasting consequences for an organism, because it requires energy for basal metabolism, physical activity, growth and reproduction [184]. In the present study it was shown that DR during development from egg to adulthood a) enhances the fat-to-fat-free mass ratio, b) enlarges lipid droplets, c) reduces glucose and trehalose content, d) up-regulates collagen genes and e) down-regulates UDP-glucuronosyltransferases and cytochrome P450 genes in both sexes of *C. elegans* (**table 5**). Measurements of body length, body volume, protein content and RNA content indicated that hermaphrodites respond more pronounced to a DR treatment than males. In hermaphrodites, DR leads to a down-regulation of embryogenesis-associated genes and to an up-regulation of sperm-associated genes. Based on these findings, the sex-independent and sex-specific response to DR in *C. elegans* will be discussed in the context of fat storage, lipid metabolism, developmental growth and reproduction.

Influence of dietary restriction on fat storage and lipid metabolism in hermaphrodites and males

The results showed that hermaphrodites as well as males respond to DR during development with a remarkable and similar adaptation in their fat metabolism and storage, thereby both sexes increased their fat content and fat-to-fat-free mass ratio. In line with this, it was found that inedible bacteria increase fat staining in *C. elegans* [107]. In contrast, in fasting worms, fat mobilization is induced resulting in a decreased fat content and a reduced number of lipid droplets [185,186]. Thus, the fat metabolism seems to be regulated oppositely in *C. elegans* depending on whether it responds to DR or fasting. Additionally, it is known that lipid stores are essential for metabolic adaptations under scarce food conditions in *C. elegans* [110]. Therefore, worms may increase their fat stores under DR to survive a long term food restriction. Also, gene expression analysis revealed that the lipase like 5 (*lipl-5*) gene was dramatically reduced (5.5-16.6-fold) under DR in both sexes at both adult stages, indicating that triglyceride degradation may be reduced. It should be noted that gene expression analysis detected no other genes of lipid metabolism, which were consistently regulated under DR in both sexes and stages. Thus, *lipl-5* may have a

specific role in mediating DR induced increase in fat storage in both sexes of *C. elegans*.

An increase of the fat-to-fat-free mass in response to DR indicated a reduced metabolic rate of the worms. In fact, this kind of adaptation has also been reported in DR treated mammals [187,188], in the offspring of undernourished female rats during pregnancy [189,190] and in growth retarded human children during development [191-193]. Furthermore, an increase of the fat-to-fat-free mass ratio in combination with a reduced metabolic rate was observed during recovery from DR (semi-starvation) in growing rats [194,195] and adult humans [196-199]. Therefore, we propose that this classical adaptation phenomenon in response to DR, also known as catch-up fat phenomenon [200], was recapitulated in dietary restricted *C. elegans* hermaphrodites and males.

Another sex-independent response to DR is the down-regulation of several UDP-glucoronosyltransferases and cytochrome P450 gene family members. This might reflect an energy saving adaptation to DR, because the enzymes of these genes function in energy consuming reactions of the metabolism of xenobiotics [38,201] and lipophilic compounds [202,203]. A similar response was found in rodents, where protein energy malnutrition leads to a decrease in cytochrome P450 protein expression and enzyme activity [204-207]. Opposite regulation of these genes were found in dauer larvae and *daf-2* mutants [38,208]. Together, the DR induced down-regulation of genes, which are linked to detoxification and energy consumption, in both sexes of *C. elegans* is a further indicator for a reduced metabolic rate.

As also observed in both sexes of *C. elegans*, the increased triglyceride content under DR is associated with an enlargement of intestinal and hypodermal lipid droplets. This alteration of lipid droplet size was initially described only in hermaphrodites (D. Palgunow, unpublished). Several studies in *C. elegans* have identified genetic pathways that seem to be essential for lipid homeostasis and lipid droplet size in *C. elegans*. Genes from peroxisomal ß-oxidation (*maoc-1*, *dhs-28*, *daf-22*), intracellular fatty acid transport (*acbp-1*) and phospholipid synthesis (*sams-1*, *pmt-1*) have been shown to function in lipid droplet formation [94,209-212]. None of these genes were noticed in this gene expression analysis, indicating that other

genes might be involved in DR induced enlargement of lipid droplets. As the surface-to-volume ratio is reduced in larger lipid droplets, their degradation via lipases might be slowed down. Thus, the enlargement of lipid droplets and the reduced expression of *lipl-5* gene under DR might be an adaptation phenomenon of both *C. elegans* sexes in order to survive longer periods of reduced food availability. Supporting this hypothesis, enlarged lipid droplets in *C. elegans* were also observed during the dauer stage [213].

Influence of dietary restriction on developmental growth and reproduction in hermaphrodites and males

During development, much energy is needed for growth. When energy is limited, a developmental delay and/or a reduced growth was found in many species including worms growing in chemical defined media [214] and rodents subjected to food restriction [215]. This adaptation was also observed in this study, where DR during development led to a reduced body size in hermaphrodites as well as in males. In line with this, DR up-regulates several collagen encoding genes in both sexes and hedgehog genes as well as sterol sensing domain genes [148-150] only in hermaphrodites. Since these genes are involved in molting, which occurs at the end of each larval stage [216], the reduced body size in response to DR might be caused by an altered molting process, which might be more definite in hermaphrodites than in males.

With respect to sex differences, the body size reducing effect of DR as well as the decrease in protein and total RNA content were more pronounced in hermaphrodites than in males. One reason for these sex differences could be that adult hermaphrodites are much bigger in size than males. Thus, hermaphrodites need much more energy for growth and protein synthesis than males. From a physiological point of view, sex differences in response to DR might be associated with different reproductive roles. Hermaphrodites have to invest much more energy resources for producing progeny than males, as also known from mammals [217,218]. The oocyte maturation, ovulation and fertilization as well as the synthesis of oocyte metabolites (e.g. yolk proteins) are energetically very expensive. In line with this, adult dietary restricted hermaphrodites have much fewer eggs than their age-matched *ad libitum* fed counterparts. This observation is linked to a down-regulation of many

embryogenesis-associated genes: cyclin like F-box, T-box transcription factor and BTB/Poz fold protein domain genes. It is known that DR reduces the fecundity of *C. elegans* [51,79] and delays the reproductive maturity in rats [219]. Our phenomenon differs from the adult reproductive diapause, described by Angelo and Van Gilst [220], since more than two embryos were observed in dietary restricted hermaphrodites.

Furthermore, an ongoing expression of sperm-associated genes was observed in dietary restricted hermaphrodites but not in males. Since males continuously produce sperm through adult life, no differences between *ad libitum* fed and dietary restricted males were possibly seen. It is known that *msp* and other sperm-associated genes are expressed solely in spermatocytes [221-224], which are the last cells running protein biosynthesis in the process of spermatogenesis [225]. Normally spermatogenesis, simultaneous with spermiogenesis, is proceeding in hermaphrodites from 51 h to 62-65 h after hatching at 20 °C [8]. Whether more spermatozytes are present at adult stage in dietary restricted hermaphrodites needs to be further investigated. Beside sperm-associated genes, an increase in the gene expression of several protein kinases and phosphatases encoding genes was found in hermaphrodites but not in males. Data from Reinke *et al.* [226] showed that among sperm-enriched genes, genes encoding for protein kinases and phosphatases were over-represented. They postulated that these enzymes are necessary to modulate protein activity during spermiogenesis, which proceeds without additional protein biosynthesis. A recent report showed that the protein phosphatases GSP-3 and GSP-4 are required for sperm development and motility in *C. elegans* [227]. These two genes were also higher expressed in dietary restricted hermaphrodites (data not shown). Taken together, it can be assumed that spermatogenesis is prolonged in dietary restricted hermaphrodites, which may result in delayed embryogenesis as indicated by the down-regulation of embryogenesis-associated genes. As reproduction requires a lot of energy, dietary restricted hermaphrodites may delay their developmental progression to adulthood and suspend the onset of reproduction to save resources.

4.3 Influence of paternal dietary restriction on progeny fat content

This study unraveled that a paternal DR affects F1 progeny fat content in an inverse U-shaped manner. Increasing the extent of paternal DR led to an increase in F1 progeny fat content, but further reduction of paternal food decreased progeny fat content to control levels. Similar results were found in both sexes. In rodents, the effects of paternal fasting or low-protein diet on progeny were also found in both sexes [121,123] . In contrast, a paternal high-fat diet affects progeny males but not females [122].

In rodents, mother-offspring-interactions as a possible reason for maternal effects on offspring phenotype are known [111,112]. In our experiments, males were removed from the fertilized females. Thus, an interaction between dietary restricted males and their progeny can be excluded. In literature, it is also taken into account that males can induce maternal effects [124]. An association between male quality and maternal reproductive investment was postulated. It was observed that female zebra finches, which were mated with attractive-made males laid heavier eggs and the offspring had faster growth rates than females, which were mated with unattractive-made males [228]. Thus, it can not be excluded that well fed females adjust their reproductive investment to their offspring due to bad nourished mating partners.

Our experimental design implicated that males were food deprived from egg to adulthood. Thus, whole male spermatogenesis was proceeded during reduced food availability, which may change epigenetic marks in germ cells. In mammals, global cytosine methylation is not altered in sperm by paternal nutrition but changes in RNA content occur [123]. The methylation of DNA can definitively be excluded in our experiment, since worms do not methylate their DNA [229]. In recent years, small non-coding RNAs are attributed a special role in paternal epigenetic inheritance [111]. A study in mice strengthened the evidence showing that microRNA from sperm, which is injected into normal fertilized eggs could transmit tail colour variation to the next generation [230]. Another possible mechanism to explain paternal effects is histone modification [231]. It was recently demonstrated in *C. elegans* that histone modifications are maintained in parental germ lines [232,233], carried into the progeny [232] and can persist over generations [132]. Whether the nutritional stress

being induced in males altered epigenetic markers in sperm and whether these modifications are retained in the next generation, remains to be elucidated.

Our results may have far-reaching implications in the context of the “Thrifty Phenotype“ [234] or “Predictive Adaptive Response” [235] hypotheses. The latter proposes that the degree of mismatch between the pre- und postnatal environments influences the metabolic phenotype of an organism. Under the assumption that developmental programming could be mediated paternally, males may transmit information of the harsh nutritional environment to their progeny via epigenetic reprogramming events in sperm [122,123]. Thus, *ad libitum* condition in the offspring may lead to a discrepancy between expected and received environment, resulting in higher progeny fat content of adulthood. This seems to be true until a certain set-point, whereas a further increase of food restriction in males abolishes the effect on progeny fat content.

4.4 Conclusion

Taken together, this study combines the use of a male mutant expressing a sex-specific GFP signal with flow cytometry in order to analyze a high number of *C. elegans* males and hermaphrodites under standardized conditions. In comparison to adult hermaphrodites, males are smaller in size and contain lower RNA content but have similar fat-to-fat-free mass. Males contain higher trehalose levels and lower glucose levels than hermaphrodites. These sex differences are linked to expression levels of genes encoding key enzymes of the carbohydrate metabolism. Moreover, certain lectin genes differentially expressed in *C. elegans* sexes were identified. The physiological relevance of the determined sex differences, particularly in the context of sex-specific reproduction roles, has to be tested in further studies.

Under restrictive food conditions males seem to response less sensitive than hermaphrodites, however, both sexes show large-scale changes in their body size, body composition and gene expression. When food becomes limited during development, almost all organisms redistribute their energy resources between body maintenance, growth and reproduction in order to ensure survival. This kind of environmentally induced phenotypic plasticity is often accompanied by the

establishment of an alternative “life history trait”, a term introduced by evolutionary biologists [236]. In *C. elegans*, well known alternative life-history traits under limited food are the dauer stage, the egg retention with internal hatch and the adult reproductive diapause [220,237,238]. Our results indicate that DR during development may also lead to an alternative life-history trait characterized by build-up of energy resources in form of large and possibly lipolysis resistant lipid droplets in both sexes and a slowed reproductive program in hermaphrodites.

Moreover, our experiments suggest that a paternal induced intergenerational inheritance might be possible in *C. elegans*. This findings, together with previous studies [121-123], extent the concept of developmental and adaptive plasticity to include the extent of paternal food consumption in the origin of phenotypic alterations.

5 REFERENCES

[1] Stiernagle T (2006) Maintenance of C. elegans. WormBook: 1-11.

[2] Hu PJ (2007) Dauer. WormBook: 1-19.

[3] Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77: 71-94.

[4] Consortium CeS (1998) Genome sequence of the nematode C. elegans: a platform for investigating biology. Science 282: 2012-2018.

[5] Jones KT, Ashrafi K (2009) Caenorhabditis elegans as an emerging model for studying the basic biology of obesity. Dis Model Mech 2: 224-229.

[6] Hodgkin J, Horvitz HR, Brenner S (1979) Nondisjunction Mutants of the Nematode CAENORHABDITIS ELEGANS. Genetics 91: 67-94.

[7] LaMunyon CW, Ward S (1998) Larger sperm outcompete smaller sperm in the nematode Caenorhabditis elegans. Proc Biol Sci 265: 1997-2002.

[8] Ward S, Carrel JS (1979) Fertilization and sperm competition in the nematode Caenorhabditis elegans. Dev Biol 73: 304-321.

[9] Sulston JE, Albertson DG, Thomson JN (1980) The Caenorhabditis elegans male: postembryonic development of nongonadal structures. Dev Biol 78: 542-576.

[10] Sulston JE, Horvitz HR (1977) Post-embryonic cell lineages of the nematode, Caenorhabditis elegans. Dev Biol 56: 110-156.

[11] Emmons SW, Sternberg PW (1997) Male Development and Mating Behavior. in C. elegans II: Chapter 12, pp 295-334.

[12] Hodgkin J (1988) Sexual dimorphism and sex determination. In the nematode C elegans (ed WB Wood) Chap. 9: pp 243-279.

[13] Kimble J, Hirsh D (1979) The postembryonic cell lineages of the hermaphrodite and male gonads in Caenorhabditis elegans. Dev Biol 70: 396-417.

[14] Portman DS (2007) Genetic control of sex differences in C. elegans neurobiology and behavior. Adv Genet 59: 1-37.

[15] Hardaker LA, Singer E, Kerr R, Zhou G, Schafer WR (2001) Serotonin modulates locomotory behavior and coordinates egg-laying and movement in Caenorhabditis elegans. J Neurobiol 49: 303-313.

[16] Liu KS, Sternberg PW (1995) Sensory regulation of male mating behavior in Caenorhabditis elegans. Neuron 14: 79-89.

[17] Loer CM, Kenyon CJ (1993) Serotonin-deficient mutants and male mating behavior in the nematode Caenorhabditis elegans. J Neurosci 13: 5407-5417.

[18] Lee K, Portman DS (2007) Neural sex modifies the function of a C. elegans sensory circuit. Curr Biol 17: 1858-1863.

[19] Lipton J, Kleemann G, Ghosh R, Lints R, Emmons SW (2004) Mate searching in Caenorhabditis elegans: a genetic model for sex drive in a simple invertebrate. J Neurosci 24: 7427-7434.

[20] Mah KB, Rankin CH (1992) An analysis of behavioral plasticity in male Caenorhabditis elegans. Behav Neural Biol 58: 211-221.

[21] Vellai T, McCulloch D, Gems D, Kovacs AL (2006) Effects of sex and insulin/insulin-like growth factor-1 signaling on performance in an associative learning paradigm in Caenorhabditis elegans. Genetics 174: 309-316.

[22] Smith DW (1989) Is greater female longevity a general finding among animals? Biol Rev Camb Philos Soc 64: 1-12.

[23] Gems D, Riddle DL (2000) Genetic, behavioral and environmental determinants of male longevity in Caenorhabditis elegans. Genetics 154: 1597-1610.
[24] Gems D, Riddle DL (1996) Longevity in Caenorhabditis elegans reduced by mating but not gamete production. Nature 379: 723-725.
[25] Inoue H, Nishida E (2010) The DM domain transcription factor MAB-3 regulates male hypersensitivity to oxidative stress in Caenorhabditis elegans. Mol Cell Biol 30: 3453-3459.
[26] van den Berg MC, Woerlee JZ, Ma H, May RC (2006) Sex-dependent resistance to the pathogenic fungus Cryptococcus neoformans. Genetics 173: 677-683.
[27] Jiang M, Ryu J, Kiraly M, Duke K, Reinke V, et al. (2001) Genome-wide analysis of developmental and sex-regulated gene expression profiles in Caenorhabditis elegans. Proc Natl Acad Sci U S A 98: 218-223.
[28] Reinke V, Gil IS, Ward S, Kazmer K (2004) Genome-wide germline-enriched and sex-biased expression profiles in Caenorhabditis elegans. Development 131: 311-323.
[29] Thoemke K, Yi W, Ross JM, Kim S, Reinke V, et al. (2005) Genome-wide analysis of sex-enriched gene expression during C. elegans larval development. Dev Biol 284: 500-508.
[30] Hirose T, Nakano Y, Nagamatsu Y, Misumi T, Ohta H, et al. (2003) Cyclic GMP-dependent protein kinase EGL-4 controls body size and lifespan in C elegans. Development 130: 1089-1099.
[31] Jonassen T, Marbois BN, Faull KF, Clarke CF, Larsen PL (2002) Development and fertility in Caenorhabditis elegans clk-1 mutants depend upon transport of dietary coenzyme Q8 to mitochondria. J Biol Chem 277: 45020-45027.
[32] Klapper M, Ehmke M, Palgunow D, Bohme M, Matthaus C, et al. (2011) Fluorescence-based fixative and vital staining of lipid droplets in Caenorhabditis elegans reveal fat stores using microscopy and flow cytometry approaches. J Lipid Res 52: 1281-1293.
[33] Takahashi K, Yoshina S, Masashi M, Ito W, Inoue T, et al. (2009) Nematode homologue of PQBP1, a mental retardation causative gene, is involved in lipid metabolism. PLoS One 4: e4104.
[34] Zarse K, Ristow M (2008) Antidepressants of the serotonin-antagonist type increase body fat and decrease lifespan of adult Caenorhabditis elegans. PLoS One 3: e4062.
[35] Behm CA (1997) The role of trehalose in the physiology of nematodes. Int J Parasitol 27: 215-229.
[36] Elbein AD, Pan YT, Pastuszak I, Carroll D (2003) New insights on trehalose: a multifunctional molecule. Glycobiology 13: 17R-27R.
[37] Hanover JA, Forsythe ME, Hennessey PT, Brodigan TM, Love DC, et al. (2005) A Caenorhabditis elegans model of insulin resistance: altered macronutrient storage and dauer formation in an OGT-1 knockout. Proc Natl Acad Sci U S A 102: 11266-11271.
[38] McElwee JJ, Schuster E, Blanc E, Thomas JH, Gems D (2004) Shared transcriptional signature in Caenorhabditis elegans Dauer larvae and long-lived daf-2 mutants implicates detoxification system in longevity assurance. J Biol Chem 279: 44533-44543.
[39] Speakman JR, Mitchell SE (2011) Caloric restriction. Mol Aspects Med 32: 159-221.

[40] Weindruch R (1996) The retardation of aging by caloric restriction: studies in rodents and primates. Toxicol Pathol 24: 742-745.

[41] Weindruch R (1992) Effect of caloric restriction on age-associated cancers. Exp Gerontol 27: 575-581.

[42] Adams MM, Shi L, Linville MC, Forbes ME, Long AB, et al. (2008) Caloric restriction and age affect synaptic proteins in hippocampal CA3 and spatial learning ability. Exp Neurol 211: 141-149.

[43] Martin B, Mattson MP, Maudsley S (2006) Caloric restriction and intermittent fasting: two potential diets for successful brain aging. Ageing Res Rev 5: 332-353.

[44] Barzilai N, Banerjee S, Hawkins M, Chen W, Rossetti L (1998) Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. J Clin Invest 101: 1353-1361.

[45] Bowman TA, Ramakrishnan SK, Kaw M, Lee SJ, Patel PR, et al. (2010) Caloric restriction reverses hepatic insulin resistance and steatosis in rats with low aerobic capacity. Endocrinology 151: 5157-5164.

[46] Sun D, Muthukumar AR, Lawrence RA, Fernandes G (2001) Effects of calorie restriction on polymicrobial peritonitis induced by cecum ligation and puncture in young C57BL/6 mice. Clin Diagn Lab Immunol 8: 1003-1011.

[47] Duffy PH, Leakey JE, Pipkin JL, Turturro A, Hart RW (1997) The physiologic, neurologic, and behavioral effects of caloric restriction related to aging, disease, and environmental factors. Environ Res 73: 242-248.

[48] Brecchia G, Bonanno A, Galeati G, Federici C, Maranesi M, et al. (2006) Hormonal and metabolic adaptation to fasting: effects on the hypothalamic-pituitary-ovarian axis and reproductive performance of rabbit does. Domest Anim Endocrinol 31: 105-122.

[49] Jiang JC, Jaruga E, Repnevskaya MV, Jazwinski SM (2000) An intervention resembling caloric restriction prolongs life span and retards aging in yeast. Faseb J 14: 2135-2137.

[50] Lin SJ, Defossez PA, Guarente L (2000) Requirement of NAD and SIR2 for life-span extension by calorie restriction in Saccharomyces cerevisiae. Science 289: 2126-2128.

[51] Klass MR (1977) Aging in the nematode Caenorhabditis elegans: major biological and environmental factors influencing life span. Mech Ageing Dev 6: 413-429.

[52] Chapman T, Partridge L (1996) Female fitness in Drosophila melanogaster: an interaction between the effect of nutrition and of encounter rate with males. Proc Biol Sci 263: 755-759.

[53] Clancy DJ, Gems D, Hafen E, Leevers SJ, Partridge L (2002) Dietary restriction in long-lived dwarf flies. Science 296: 319.

[54] Hatle JD, Wells SM, Fuller LE, Allen IC, Gordy LJ, et al. (2006) Calorie restriction and late-onset calorie restriction extend lifespan but do not alter protein storage in female grasshoppers. Mech Ageing Dev 127: 883-891.

[55] Austad SN (1989) Life extension by dietary restriction in the bowl and doily spider, Frontinella pyramitela. Exp Gerontol 24: 83-92.

[56] Stuchlikova E, Juricova-Horakova M, Deyl Z (1975) New aspects of the dietary effect of life prolongation in rodents. What is the role of obesity in aging? Exp Gerontol 10: 141-144.

[57] Kealy RD, Lawler DF, Ballam JM, Mantz SL, Biery DN, et al. (2002) Effects of diet restriction on life span and age-related changes in dogs. J Am Vet Med Assoc 220: 1315-1320.

[58] Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, et al. (2009) Caloric restriction delays disease onset and mortality in rhesus monkeys. Science 325: 201-204.

[59] Ramsey JJ, Colman RJ, Binkley NC, Christensen JD, Gresl TA, et al. (2000) Dietary restriction and aging in rhesus monkeys: the University of Wisconsin study. Exp Gerontol 35: 1131-1149.

[60] Greer EL, Dowlatshahi D, Banko MR, Villen J, Hoang K, et al. (2007) An AMPK-FOXO pathway mediates longevity induced by a novel method of dietary restriction in C. elegans. Curr Biol 17: 1646-1656.

[61] Hosono R, Nishimoto S, Kuno S (1989) Alterations of life span in the nematode Caenorhabditis elegans under monoxenic culture conditions. Exp Gerontol 24: 251-264.

[62] Houthoofd K, Braeckman BP, Lenaerts I, Brys K, De Vreese A, et al. (2002) Axenic growth up-regulates mass-specific metabolic rate, stress resistance, and extends life span in Caenorhabditis elegans. Exp Gerontol 37: 1371-1378.

[63] Vanfleteren JR, Braeckman BP (1999) Mechanisms of life span determination in Caenorhabditis elegans. Neurobiol Aging 20: 487-502.

[64] Lakowski B, Hekimi S (1998) The genetics of caloric restriction in Caenorhabditis elegans. Proc Natl Acad Sci U S A 95: 13091-13096.

[65] Kaeberlein TL, Smith ED, Tsuchiya M, Welton KL, Thomas JH, et al. (2006) Lifespan extension in Caenorhabditis elegans by complete removal of food. Aging Cell 5: 487-494.

[66] Lee GD, Wilson MA, Zhu M, Wolkow CA, de Cabo R, et al. (2006) Dietary deprivation extends lifespan in Caenorhabditis elegans. Aging Cell 5: 515-524.

[67] Fontana L, Partridge L, Longo VD (2010) Extending healthy life span--from yeast to humans. Science 328: 321-326.

[68] Mair W, Dillin A (2008) Aging and survival: the genetics of life span extension by dietary restriction. Annu Rev Biochem 77: 727-754.

[69] Barbieri M, Bonafe M, Franceschi C, Paolisso G (2003) Insulin/IGF-I-signaling pathway: an evolutionarily conserved mechanism of longevity from yeast to humans. Am J Physiol Endocrinol Metab 285: E1064-1071.

[70] Dorman JB, Albinder B, Shroyer T, Kenyon C (1995) The age-1 and daf-2 genes function in a common pathway to control the lifespan of Caenorhabditis elegans. Genetics 141: 1399-1406.

[71] Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R (1993) A C. elegans mutant that lives twice as long as wild type. Nature 366: 461-464.

[72] Houthoofd K, Braeckman BP, Johnson TE, Vanfleteren JR (2003) Life extension via dietary restriction is independent of the Ins/IGF-1 signalling pathway in Caenorhabditis elegans. Exp Gerontol 38: 947-954.

[73] Hay N, Sonenberg N (2004) Upstream and downstream of mTOR. Genes Dev 18: 1926-1945.

[74] Schmelzle T, Hall MN (2000) TOR, a central controller of cell growth. Cell 103: 253-262.

[75] Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, et al. (2003) Genetics: influence of TOR kinase on lifespan in C. elegans. Nature 426: 620.

[76] Guarente L (2005) Calorie restriction and SIR2 genes--towards a mechanism. Mech Ageing Dev 126: 923-928.

[77] Tissenbaum HA, Guarente L (2001) Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. Nature 410: 227-230.

[78] Wang Y, Tissenbaum HA (2006) Overlapping and distinct functions for a Caenorhabditis elegans SIR2 and DAF-16/FOXO. Mech Ageing Dev 127: 48-56.

[79] Bishop NA, Guarente L (2007) Two neurons mediate diet-restriction-induced longevity in C. elegans. Nature 447: 545-549.

[80] Chen D, Thomas EL, Kapahi P (2009) HIF-1 modulates dietary restriction-mediated lifespan extension via IRE-1 in Caenorhabditis elegans. PLoS Genet 5: e1000486.

[81] Panowski SH, Wolff S, Aguilaniu H, Durieux J, Dillin A (2007) PHA-4/Foxa mediates diet-restriction-induced longevity of C. elegans. Nature 447: 550-555.

[82] Steinkraus KA, Smith ED, Davis C, Carr D, Pendergrass WR, et al. (2008) Dietary restriction suppresses proteotoxicity and enhances longevity by an hsf-1-dependent mechanism in Caenorhabditis elegans. Aging Cell 7: 394-404.

[83] Friedman JR, Kaestner KH (2006) The Foxa family of transcription factors in development and metabolism. Cell Mol Life Sci 63: 2317-2328.

[84] Kalb JM, Lau KK, Goszczynski B, Fukushige T, Moons D, et al. (1998) pha-4 is Ce-fkh-1, a fork head/HNF-3alpha,beta,gamma homolog that functions in organogenesis of the C. elegans pharynx. Development 125: 2171-2180.

[85] An JH, Blackwell TK (2003) SKN-1 links C. elegans mesendodermal specification to a conserved oxidative stress response. Genes Dev 17: 1882-1893.

[86] Tullet JM, Hertweck M, An JH, Baker J, Hwang JY, et al. (2008) Direct inhibition of the longevity-promoting factor SKN-1 by insulin-like signaling in C. elegans. Cell 132: 1025-1038.

[87] Hajdu-Cronin YM, Chen WJ, Sternberg PW (2004) The L-type cyclin CYL-1 and the heat-shock-factor HSF-1 are required for heat-shock-induced protein expression in Caenorhabditis elegans. Genetics 168: 1937-1949.

[88] Jiang H, Guo R, Powell-Coffman JA (2001) The Caenorhabditis elegans hif-1 gene encodes a bHLH-PAS protein that is required for adaptation to hypoxia. Proc Natl Acad Sci U S A 98: 7916-7921.

[89] Greer EL, Brunet A (2009) Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in C. elegans. Aging Cell 8: 113-127.

[90] Kenyon CJ (2010) The genetics of ageing. Nature 464: 504-512.

[91] Vastrik I, D'Eustachio P, Schmidt E, Gopinath G, Croft D, et al. (2007) Reactome: a knowledge base of biologic pathways and processes. Genome Biol 8: R39.

[92] Braeckman BP, Houthoofd K, Vanfleteren JR (2009) Intermediary metabolism. WormBook: 1-24.

[93] Ashrafi K (2007) Obesity and the regulation of fat metabolism. WormBook: 1-20.

[94] Zhang SO, Box AC, Xu N, Le Men J, Yu J, et al. (2010) Genetic and dietary regulation of lipid droplet expansion in Caenorhabditis elegans. Proc Natl Acad Sci U S A 107: 4640-4645.

[95] Goodman JM (2008) The gregarious lipid droplet. J Biol Chem 283: 28005-28009.

[96] Martin S, Parton RG (2006) Lipid droplets: a unified view of a dynamic organelle. Nat Rev Mol Cell Biol 7: 373-378.
[97] Hellerer T, Axang C, Brackmann C, Hillertz P, Pilon M, et al. (2007) Monitoring of lipid storage in Caenorhabditis elegans using coherent anti-Stokes Raman scattering (CARS) microscopy. Proc Natl Acad Sci U S A 104: 14658-14663.
[98] Lithgow GJ, White TM, Melov S, Johnson TE (1995) Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress. Proc Natl Acad Sci U S A 92: 7540-7544.
[99] Tain LS, Lozano E, Saez AG, Leroi AM (2008) Dietary regulation of hypodermal polyploidization in C. elegans. BMC Dev Biol 8: 28.
[100] Houthoofd K, Braeckman BP, Lenaerts I, Brys K, De Vreese A, et al. (2002) No reduction of metabolic rate in food restricted Caenorhabditis elegans. Exp Gerontol 37: 1359-1369.
[101] Houthoofd K, Braeckman BP, Lenaerts I, Brys K, Matthijssens F, et al. (2005) DAF-2 pathway mutations and food restriction in aging Caenorhabditis elegans differentially affect metabolism. Neurobiol Aging 26: 689-696.
[102] Sawin ER, Ranganathan R, Horvitz HR (2000) C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron 26: 619-631.
[103] Avery L, Horvitz HR (1990) Effects of starvation and neuroactive drugs on feeding in Caenorhabditis elegans. J Exp Zool 253: 263-270.
[104] Colbert HA, Bargmann CI (1997) Environmental signals modulate olfactory acuity, discrimination, and memory in Caenorhabditis elegans. Learn Mem 4: 179-191.
[105] Martin B, Pearson M, Kebejian L, Golden E, Keselman A, et al. (2007) Sex-dependent metabolic, neuroendocrine, and cognitive responses to dietary energy restriction and excess. Endocrinology 148: 4318-4333.
[106] Magwere T, Chapman T, Partridge L (2004) Sex differences in the effect of dietary restriction on life span and mortality rates in female and male Drosophila melanogaster. J Gerontol A Biol Sci Med Sci 59: 3-9.
[107] Gruninger TR, Gualberto DG, Garcia LR (2008) Sensory perception of food and insulin-like signals influence seizure susceptibility. PLoS Genet 4: e1000117.
[108] Gruninger TR, Gualberto DG, LeBoeuf B, Garcia LR (2006) Integration of male mating and feeding behaviors in Caenorhabditis elegans. J Neurosci 26: 169-179.
[109] LeBoeuf B, Guo X, Garcia LR (2011) The effects of transient starvation persist through direct interactions between CaMKII and ether-a-go-go K+ channels in C. elegans males. Neuroscience 175: 1-17.
[110] Tan KT, Luo SC, Ho WZ, Lee YH (2011) Insulin/IGF-1 receptor signaling enhances biosynthetic activity and fat mobilization in the initial phase of starvation in adult male C. elegans. Cell Metab 14: 390-402.
[111] Youngson NA, Whitelaw E (2008) Transgenerational epigenetic effects. Annu Rev Genomics Hum Genet 9: 233-257.
[112] Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, et al. (2004) Epigenetic programming by maternal behavior. Nat Neurosci 7: 847-854.
[113] Wolff GL, Kodell RL, Moore SR, Cooney CA (1998) Maternal epigenetics and methyl supplements affect agouti gene expression in Avy/a mice. Faseb J 12: 949-957.

[114] Zambrano E, Martinez-Samayoa PM, Bautista CJ, Deas M, Guillen L, et al. (2005) Sex differences in transgenerational alterations of growth and metabolism in progeny (F2) of female offspring (F1) of rats fed a low protein diet during pregnancy and lactation. J Physiol 566: 225-236.
[115] Anway MD, Cupp AS, Uzumcu M, Skinner MK (2005) Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science 308: 1466-1469.
[116] Vandegehuchte MB, Janssen CR (2011) Epigenetics and its implications for ecotoxicology. Ecotoxicology 20: 607-624.
[117] Jimenez-Chillaron JC, Isganaitis E, Charalambous M, Gesta S, Pentinat-Pelegrin T, et al. (2009) Intergenerational transmission of glucose intolerance and obesity by in utero undernutrition in mice. Diabetes 58: 460-468.
[118] Peixoto-Silva N, Frantz ED, Mandarim-de-Lacerda CA, Pinheiro-Mulder A (2011) Maternal protein restriction in mice causes adverse metabolic and hypothalamic effects in the F1 and F2 generations. Br J Nutr 106: 1364-1373.
[119] Kaati G, Bygren LO, Edvinsson S (2002) Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet 10: 682-688.
[120] Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, et al. (2006) Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet 14: 159-166.
[121] Anderson LM, Riffle L, Wilson R, Travlos GS, Lubomirski MS, et al. (2006) Preconceptional fasting of fathers alters serum glucose in offspring of mice. Nutrition 22: 327-331.
[122] Ng SF, Lin RC, Laybutt DR, Barres R, Owens JA, et al. (2010) Chronic high-fat diet in fathers programs beta-cell dysfunction in female rat offspring. Nature 467: 963-966.
[123] Carone BR, Fauquier L, Habib N, Shea JM, Hart CE, et al. (2010) Paternally induced transgenerational environmental reprogramming of metabolic gene expression in mammals. Cell 143: 1084-1096.
[124] Curley JP, Mashoodh R, Champagne FA (2011) Epigenetics and the origins of paternal effects. Horm Behav 59: 306-314.
[125] Abel EL, Tan SE (1988) Effects of paternal alcohol consumption on pregnancy outcome in rats. Neurotoxicol Teratol 10: 187-192.
[126] Ledig M, Misslin R, Vogel E, Holownia A, Copin JC, et al. (1998) Paternal alcohol exposure: developmental and behavioral effects on the offspring of rats. Neuropharmacology 37: 57-66.
[127] Hales BF, Robaire B (2001) Paternal exposure to drugs and environmental chemicals: effects on progeny outcome. J Androl 22: 927-936.
[128] Whitelaw NC, Whitelaw E (2008) Transgenerational epigenetic inheritance in health and disease. Curr Opin Genet Dev 18: 273-279.
[129] Morse HC, 3rd, Yetter RA, Stimpfling JH, Pitts OM, Fredrickson TN, et al. (1985) Greying with age in mice: relation to expression of murine leukemia viruses. Cell 41: 439-448.
[130] Remy JJ (2010) Stable inheritance of an acquired behavior in Caenorhabditis elegans. Curr Biol 20: R877-878.
[131] Frazier HN, 3rd, Roth MB (2009) Adaptive sugar provisioning controls survival of C. elegans embryos in adverse environments. Curr Biol 19: 859-863.

[132] Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, et al. (2011) Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans. Nature 479: 365-371.

[133] Azevedo RB, Keightley PD, Lauren-Maatta C, Vassilieva LL, Lynch M, et al. (2002) Spontaneous mutational variation for body size in Caenorhabditis elegans. Genetics 162: 755-765.

[134] Salomon MP, Ostrow D, Phillips N, Blanton D, Bour W, et al. (2009) Comparing mutational and standing genetic variability for fitness and size in Caenorhabditis briggsae and C. elegans. Genetics 183: 685-692, 681SI-619SI.

[135] Bolstad BM, Irizarry RA, Astrand M, Speed TP (2003) A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. Bioinformatics 19: 185-193.

[136] Phillips CM, Wong C, Bhalla N, Carlton PM, Weiser P, et al. (2005) HIM-8 binds to the X chromosome pairing center and mediates chromosome-specific meiotic synapsis. Cell 123: 1051-1063.

[137] Barr MM, Sternberg PW (1999) A polycystic kidney-disease gene homologue required for male mating behaviour in C. elegans. Nature 401: 386-389.

[138] Schedl T, Kimble J (1988) fog-2, a germ-line-specific sex determination gene required for hermaphrodite spermatogenesis in Caenorhabditis elegans. Genetics 119: 43-61.

[139] Honda Y, Tanaka M, Honda S (2010) Trehalose extends longevity in the nematode Caenorhabditis elegans. Aging Cell 9: 558-569.

[140] Lamitina ST, Strange K (2005) Transcriptional targets of DAF-16 insulin signaling pathway protect C. elegans from extreme hypertonic stress. Am J Physiol Cell Physiol 288: C467-474.

[141] Hockner M, Dallinger R, Sturzenbaum SR (2011) Nematode and snail metallothioneins. J Biol Inorg Chem 16: 1057-1065.

[142] Swindell WR (2011) Metallothionein and the biology of aging. Ageing Res Rev 10: 132-145.

[143] Page AP, Johnstone IL (2007) The cuticle. WormBook: 1-15.

[144] Lindblom TH, Dodd AK (2006) Xenobiotic detoxification in the nematode Caenorhabditis elegans. J Exp Zool A Comp Exp Biol 305: 720-730.

[145] Schulenburg H, Hoeppner MP, Weiner J, 3rd, Bornberg-Bauer E (2008) Specificity of the innate immune system and diversity of C-type lectin domain (CTLD) proteins in the nematode Caenorhabditis elegans. Immunobiology 213: 237-250.

[146] Munford RS, Sheppard PO, O'Hara PJ (1995) Saposin-like proteins (SAPLIP) carry out diverse functions on a common backbone structure. J Lipid Res 36: 1653-1663.

[147] Baugh LR, Hill AA, Slonim DK, Brown EL, Hunter CP (2003) Composition and dynamics of the Caenorhabditis elegans early embryonic transcriptome. Development 130: 889-900.

[148] Burglin TR, Kuwabara PE (2006) Homologs of the Hh signalling network in C. elegans. WormBook: 1-14.

[149] Frand AR, Russel S, Ruvkun G (2005) Functional genomic analysis of C. elegans molting. PLoS Biol 3: e312.

[150] Zugasti O, Rajan J, Kuwabara PE (2005) The function and expansion of the Patched- and Hedgehog-related homologs in C. elegans. Genome Res 15: 1402-1410.

[151] Laskowski M, Jr., Kato I (1980) Protein inhibitors of proteinases. Annu Rev Biochem 49: 593-626.
[152] Rawlings ND, Tolle DP, Barrett AJ (2004) Evolutionary families of peptidase inhibitors. Biochem J 378: 705-716.
[153] Patton EE, Willems AR, Tyers M (1998) Combinatorial control in ubiquitin-dependent proteolysis: don't Skp the F-box hypothesis. Trends Genet 14: 236-243.
[154] Xu L, Wei Y, Reboul J, Vaglio P, Shin TH, et al. (2003) BTB proteins are substrate-specific adaptors in an SCF-like modular ubiquitin ligase containing CUL-3. Nature 425: 316-321.
[155] Pocock R, Ahringer J, Mitsch M, Maxwell S, Woollard A (2004) A regulatory network of T-box genes and the even-skipped homologue vab-7 controls patterning and morphogenesis in C. elegans. Development 131: 2373-2385.
[156] Miller MA, Nguyen VQ, Lee MH, Kosinski M, Schedl T, et al. (2001) A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. Science 291: 2144-2147.
[157] Frank DJ, Roth MB (1998) ncl-1 is required for the regulation of cell size and ribosomal RNA synthesis in Caenorhabditis elegans. J Cell Biol 140: 1321-1329.
[158] Honjoh S, Kajiwara Y, Nishida E (2011) The sexual dimorphic response to dietary restriction. C elegans meeting 2011 Abstract volume, Abstract number 256B: 78.
[159] Bonnet X, Shine R, Naulleau G, Vacher-Vallas M (1998) Sexual dimorphism in snakes: different reproductive roles favour different body plans. Proc R Soc Lond B 265: 179-183.
[160] Boos M, Zorn T, Le Maho Y, Groscolas R, Robin JP (2002) Sex differences in body composition of wintering Mallards (Anas platyrhynchos): possible implications for survival and reproductive performance. Bird Study 49: 212-218.
[161] Kirchengast S (2010) Gender Differences in body composition from childhood to old age: an evolutionary point of view. J Life Sci 2: 1-10.
[162] Schulte-Hostedde AI, Millar JS, Hickling GJ (2001) Sexual dimorphism in body composition of small mammals. Can J Zool 79: 1016-1020.
[163] Kormish JD, McGhee JD (2005) The C. elegans lethal gut-obstructed gob-1 gene is trehalose-6-phosphate phosphatase. Dev Biol 287: 35-47.
[164] Pellerone FI, Archer SK, Behm CA, Grant WN, Lacey MJ, et al. (2003) Trehalose metabolism genes in Caenorhabditis elegans and filarial nematodes. Int J Parasitol 33: 1195-1206.
[165] Jagdale GB, Grewal PS, Salminen SO (2005) Both heat-shock and cold-shock influence trehalose metabolism in an entomopathogenic nematode. J Parasitol 91: 988-994.
[166] Crowe JH, Crowe LM (2000) Preservation of mammalian cells-learning nature's tricks. Nat Biotechnol 18: 145-146.
[167] Jain NK, Roy I (2009) Effect of trehalose on protein structure. Protein Sci 18: 24-36.
[168] Vemuganti SA, de Villena FP, O'Brien DA (2010) Frequent and recent retrotransposition of orthologous genes plays a role in the evolution of sperm glycolytic enzymes. BMC Genomics 11: 285.
[169] Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, et al. (2004) Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic

enzyme, is required for sperm motility and male fertility. Proc Natl Acad Sci U S A 101: 16501-16506.
[170] Mukai C, Okuno M (2004) Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. Biol Reprod 71: 540-547.
[171] Peterson RN, Freund M (1969) Glycolysis by washed suspensions of human spermatozoa. Effect of substrate, substrate concentration, and changes in medium composition on the rate of glycolysis. Biol Reprod 1: 238-246.
[172] Ward S, Hogan E, Nelson GA (1983) The initiation of spermiogenesis in the nematode Caenorhabditis elegans. Dev Biol 98: 70-79.
[173] Andachi Y (2004) Caenorhabditis elegans T-box genes tbx-9 and tbx-8 are required for formation of hypodermis and body-wall muscle in embryogenesis. Genes Cells 9: 331-344.
[174] Hope IA (1994) PES-1 is expressed during early embryogenesis in Caenorhabditis elegans and has homology to the fork head family of transcription factors. Development 120: 505-514.
[175] Zhang F, O'Meara MM, Hobert O (2011) A left/right asymmetric neuronal differentiation program is controlled by the Caenorhabditis elegans lsy-27 zinc-finger transcription factor. Genetics 188: 753-759.
[176] Kagoshima H, Cassata G, Burglin TR (1999) A Caenorhabditis elegans homeobox gene expressed in the male tail, a link between pattern formation and sexual dimorphism? Dev Genes Evol 209: 59-62.
[177] Lints R, Emmons SW (2002) Regulation of sex-specific differentiation and mating behavior in C. elegans by a new member of the DM domain transcription factor family. Genes Dev 16: 2390-2402.
[178] Raymond CS, Shamu CE, Shen MM, Seifert KJ, Hirsch B, et al. (1998) Evidence for evolutionary conservation of sex-determining genes. Nature 391: 691-695.
[179] Shen MM, Hodgkin J (1988) mab-3, a gene required for sex-specific yolk protein expression and a male-specific lineage in C. elegans. Cell 54: 1019-1031.
[180] Kilpatrick DC (2002) Animal lectins: a historical introduction and overview. Biochim Biophys Acta 1572: 187-197.
[181] Pasche B, Kalaydjiev S, Franz TJ, Kremmer E, Gailus-Durner V, et al. (2005) Sex-dependent susceptibility to Listeria monocytogenes infection is mediated by differential interleukin-10 production. Infect Immun 73: 5952-5960.
[182] Chu DS, Liu H, Nix P, Wu TF, Ralston EJ, et al. (2006) Sperm chromatin proteomics identifies evolutionarily conserved fertility factors. Nature 443: 101-105.
[183] Pickett CL, Breen KT, Ayer DE (2007) A C. elegans Myc-like network cooperates with semaphorin and Wnt signaling pathways to control cell migration. Dev Biol 310: 226-239.
[184] Wang T, Hung CC, Randall DJ (2006) The comparative physiology of food deprivation: from feast to famine. Annu Rev Physiol 68: 223-251.
[185] Jo H, Shim J, Lee JH, Lee J, Kim JB (2009) IRE-1 and HSP-4 contribute to energy homeostasis via fasting-induced lipases in C. elegans. Cell Metab 9: 440-448.
[186] McKay RM, McKay JP, Avery L, Graff JM (2003) C elegans: a model for exploring the genetics of fat storage. Dev Cell 4: 131-142.

[187] Bevilacqua L, Ramsey JJ, Hagopian K, Weindruch R, Harper ME (2005) Long-term caloric restriction increases UCP3 content but decreases proton leak and reactive oxygen species production in rat skeletal muscle mitochondria. Am J Physiol Endocrinol Metab 289: E429-438.
[188] Blanc S, Schoeller D, Kemnitz J, Weindruch R, Colman R, et al. (2003) Energy expenditure of rhesus monkeys subjected to 11 years of dietary restriction. J Clin Endocrinol Metab 88: 16-23.
[189] Anguita RM, Sigulem DM, Sawaya AL (1993) Intrauterine food restriction is associated with obesity in young rats. J Nutr 123: 1421-1428.
[190] Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD (2000) Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol Endocrinol Metab 279: E83-87.
[191] Hoffman DJ, Martins PA, Roberts SB, Sawaya AL (2007) Body fat distribution in stunted compared with normal-height children from the shantytowns of Sao Paulo, Brazil. Nutrition 23: 640-646.
[192] Martins PA, Hoffman DJ, Fernandes MT, Nascimento CR, Roberts SB, et al. (2004) Stunted children gain less lean body mass and more fat mass than their non-stunted counterparts: a prospective study. Br J Nutr 92: 819-825.
[193] Martins VJ, Toledo Florencio TM, Grillo LP, do Carmo PFM, Martins PA, et al. (2011) Long-lasting effects of undernutrition. Int J Environ Res Public Health 8: 1817-1846.
[194] Dulloo AG, Girardier L (1993) Adaptive role of energy expenditure in modulating body fat and protein deposition during catch-up growth after early undernutrition. Am J Clin Nutr 58: 614-621.
[195] Dulloo AG, Girardier L (1993) 24 hour energy expenditure several months after weight loss in the underfed rat: evidence for a chronic increase in whole-body metabolic efficiency. Int J Obes Relat Metab Disord 17: 115-123.
[196] Dulloo AG, Jacquet J (1998) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. Am J Clin Nutr 68: 599-606.
[197] Dulloo AG, Jacquet J, Girardier L (1996) Autoregulation of body composition during weight recovery in human: the Minnesota Experiment revisited. Int J Obes Relat Metab Disord 20: 393-405.
[198] Dulloo AG, Jacquet J, Girardier L (1997) Poststarvation hyperphagia and body fat overshooting in humans: a role for feedback signals from lean and fat tissues. Am J Clin Nutr 65: 717-723.
[199] Keys A, Brozek J, Henschel A, Mickelsen O, Taylor HL (1950) The biology of human starvation. University of Minnesota press.
[200] Dulloo AG, Jacquet J, Montani JP (2002) Pathways from weight fluctuations to metabolic diseases: focus on maladaptive thermogenesis during catch-up fat. Int J Obes Relat Metab Disord 26 Suppl 2: S46-57.
[201] Gems D, McElwee JJ (2005) Broad spectrum detoxification: the major longevity assurance process regulated by insulin/IGF-1 signaling? Mech Ageing Dev 126: 381-387.
[202] Mansuy D (1998) The great diversity of reactions catalyzed by cytochromes P450. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 121: 5-14.
[203] Tukey RH, Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. Annu Rev Pharmacol Toxicol 40: 581-616.

[204] Cancino-Badias L, Reyes RE, Nosti R, Perez I, Dorado V, et al. (2003) Modulation of rat liver cytochrome P450 by protein restriction assessed by biochemical and bacterial mutagenicity methods [corrected]. Mutagenesis 18: 95-100.

[205] Cho MK, Kim YG, Lee MG, Kim SG (1999) Suppression of rat hepatic cytochrome P450s by protein-calorie malnutrition: complete or partial restoration by cysteine or methionine supplementation. Arch Biochem Biophys 372: 150-158.

[206] Lee PC, Struve MF, Bezerra JA, Duncan B (1997) Effects of protein malnutrition on liver cytochrome P450s. Nutrition Research 17: 1577-1587.

[207] Zhang W, Parentau H, Greenly RL, Metz CA, Aggarwal S, et al. (1999) Effect of protein-calorie malnutrition on cytochromes P450 and glutathione S-transferase. Eur J Drug Metab Pharmacokinet 24: 141-147.

[208] Wang J, Kim SK (2003) Global analysis of dauer gene expression in Caenorhabditis elegans. Development 130: 1621-1634.

[209] Elle IC, Simonsen KT, Olsen LC, Birck PK, Ehmsen S, et al. (2011) Tissue- and paralogue-specific functions of acyl-CoA-binding proteins in lipid metabolism in Caenorhabditis elegans. Biochem J 437: 231-241.

[210] Joo HJ, Yim YH, Jeong PY, Jin YX, Lee JE, et al. (2009) Caenorhabditis elegans utilizes dauer pheromone biosynthesis to dispose of toxic peroxisomal fatty acids for cellular homoeostasis. Biochem J 422: 61-71.

[211] Li Y, Na K, Lee HJ, Lee EY, Paik YK (2011) Contribution of sams-1 and pmt-1 to lipid homoeostasis in adult Caenorhabditis elegans. J Biochem 149: 529-538.

[212] Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, et al. (2011) A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell 147: 840-852.

[213] Narbonne P, Roy R (2009) Caenorhabditis elegans dauers need LKB1/AMPK to ration lipid reserves and ensure long-term survival. Nature 457: 210-214.

[214] Szewczyk NJ, Udranszky IA, Kozak E, Sunga J, Kim SK, et al. (2006) Delayed development and lifespan extension as features of metabolic lifestyle alteration in C. elegans under dietary restriction. J Exp Biol 209: 4129-4139.

[215] Picarel-Blanchot F, Alvarez C, Bailbe D, Pascual-Leone AM, Portha B (1995) Changes in insulin action and insulin secretion in the rat after dietary restriction early in life: influence of food restriction versus low-protein food restriction. Metabolism 44: 1519-1526.

[216] Singh RN, Sulston JE (1978) Some observations on moulting in Caenorhabditis elegans. Nematologica 24: 63-71.

[217] Wade GN, Schneider JE (1992) Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev 16: 235-272.

[218] Wade GN, Schneider JE, Li HY (1996) Control of fertility by metabolic cues. Am J Physiol 270: E1-19.

[219] Merry BJ, Holehan AM (1979) Onset of puberty and duration of fertility in rats fed a restricted diet. J Reprod Fertil 57: 253-259.

[220] Angelo G, Van Gilst MR (2009) Starvation protects germline stem cells and extends reproductive longevity in C. elegans. Science 326: 954-958.

[221] Arduengo PM, Appleberry OK, Chuang P, L'Hernault SW (1998) The presenilin protein family member SPE-4 localizes to an ER/Golgi derived organelle and is required for proper cytoplasmic partitioning during Caenorhabditis elegans spermatogenesis. J Cell Sci 111 (Pt 24): 3645-3654.

[222] Pavelec DM, Lachowiec J, Duchaine TF, Smith HE, Kennedy S (2009) Requirement for the ERI/DICER complex in endogenous RNA interference and sperm development in Caenorhabditis elegans. Genetics 183: 1283-1295.
[223] Ward S, Burke DJ, Sulston JE, Coulson AR, Albertson DG, et al. (1988) Genomic organization of major sperm protein genes and pseudogenes in the nematode Caenorhabditis elegans. J Mol Biol 199: 1-13.
[224] Zannoni S, L'Hernault SW, Singson AW (2003) Dynamic localization of SPE-9 in sperm: a protein required for sperm-oocyte interactions in Caenorhabditis elegans. BMC Dev Biol 3: 10.
[225] L'Hernault SW (2006) Spermatogenesis. WormBook: 1-14.
[226] Reinke V, Smith HE, Nance J, Wang J, Van Doren C, et al. (2000) A global profile of germline gene expression in C. elegans. Mol Cell 6: 605-616.
[227] Wu JC, Go AC, Samson M, Cintra T, Mirsoian S, et al. (2012) Sperm development and motility are regulated by PP1 phosphatases in Caenorhabditis elegans. Genetics 190: 143-157.
[228] Gilbert L, Williamson KA, Hazon N, Graves JA (2006) Maternal effects due to male attractiveness affect offspring development in the zebra finch. Proc Biol Sci 273: 1765-1771.
[229] Simpson VJ, Johnson TE, Hammen RF (1986) Caenorhabditis elegans DNA does not contain 5-methylcytosine at any time during development or aging. Nucleic Acids Res 14: 6711-6719.
[230] Rassoulzadegan M, Grandjean V, Gounon P, Vincent S, Gillot I, et al. (2006) RNA-mediated non-mendelian inheritance of an epigenetic change in the mouse. Nature 441: 469-474.
[231] Wenzel D, Palladino F, Jedrusik-Bode M (2011) Epigenetics in C. elegans: facts and challenges. Genesis 49: 647-661.
[232] Arico JK, Katz DJ, van der Vlag J, Kelly WG (2011) Epigenetic patterns maintained in early Caenorhabditis elegans embryos can be established by gene activity in the parental germ cells. PLoS Genet 7: e1001391.
[233] Katz DJ, Edwards TM, Reinke V, Kelly WG (2009) A C. elegans LSD1 demethylase contributes to germline immortality by reprogramming epigenetic memory. Cell 137: 308-320.
[234] Hales CN, Barker DJ (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia 35: 595-601.
[235] Gluckman PD, Hanson MA (2004) Living with the past: evolution, development, and patterns of disease. Science 305: 1733-1736.
[236] Stearns SC (2000) Life history evolution: successes, limitations, and prospects. Naturwissenschaften 87: 476-486.
[237] Chen J, Caswell-Chen EP (2004) Facultative Vivipary is a Life-History Trait in Caenorhabditis elegans. J Nematol 36: 107-113.
[238] Fielenbach N, Antebi A (2008) C. elegans dauer formation and the molecular basis of plasticity. Genes Dev 22: 2149-2165.

6 APPENDIX

6. 1 Supporting information

Supporting information table S1. Calculation of dietary restriction ranking.

Condition	Total bacteria per plate	Bacteria per worm	Ranking
Ad libitum	2.66E+10	4.44E+7	
OD1.5 300 eggs		1.93E+6	1
OD1.5 600 eggs	5.79E+8	9.65E+5	3
OD1.5 900 eggs		6.43E+5	4
OD0.7 300 eggs		1.08E+6	2
OD0.7 600 eggs		5.38E+5	5
OD0.7 900 eggs	3.23E+8	3.59E+5	6
Starvation-OD0.7		3.59E+5*	7
OD0.7-starvation		3.59E+5*	8

*Starvation during the greatest growth phase (L4-adult) has more impact on the worms than starvation during egg and L1. Thus, we assigned starvation-OD 0.7 with rank 7 and OD 0.7-starvation with rank 8.

Supporting information table S2. Body composition of males and hermaphrodites at different developmental stages in different male mutants[a].

Mutant	Stage	Sex	Protein [ng/worm]	Triglyceride [ng/worm]	Triglyceride to protein ratio
him-8 GFP	Young adult (66 h)	Hermaphrodite	58.1 (±4.7)	12.3 (± 3.8)	0.21 (± 0.07)
		Male	44.9 (± 4.6)	12.8 (± 3.2)	0.28 (± 0.08)
		Male (adjusted)	59.5 (± 3.3)	17.2 (± 4.6)	
	Adult (76h)	Hermaphrodite	141.6 (± 13.8)	16.4 (± 6.2)	0.12 (± 0.05)
		Male	70.1 (± 9.9)	7.9 (± 5.8)	0.12 (± 0.10)
		Male (adjusted)	135.0 (± 18.1)	15.9 (± 13.1)	
	One day adult (90 h)	Hermaphrodite	194.1 (± 13.4)	27.3 (± 1.9)	0.14 (± 0.01)
		Male	75.3 (± 5.9)	10.9 (± 0.5)	0.14 (± 0.006)
		Male (adjusted)	162.7 (± 17.3)	23.5 (± 1.5)	
fog-2	One day adult (90 h)	Female	211.3 (± 31.9)	25.0 (± 7.7)	0.12 (± 0.02)
		Male	92.7 (± 8.3)	15.9 (± 4.5)	0.17 (± 0.05)
		Male (adjusted)	200.2 (± 22.1)	34.5 (± 10.4)	
him-8	One day adult (90 h)	Hermaphrodite	203.7 (± 15.0)	23.4 (± 3.4)	0.11 (± 0.009)
		Male	88.3 (± 3.1)	9.8 (± 3.9)	0.11 (± 0.05)
		Male (adjusted)	184.8 (± 7.4)	20.4 (± 8.2)	
him-5	One day adult (90 h)	Hermaphrodite	156.9 (± 32.1)	18.6 (± 4.0)	0.12 (± 0.01)
		Male	69.5 (± 8.1)	13.0 (± 8.4)	0.18 (± 0.12)
		Male (adjusted)	135.3 (± 18.0)	25.8 (± 17.3)	
N2	One day adult (90 h)	Hermaphrodite	249.7 (± 9.9)	31.3 (± 3.5)	0.13 (± 0.02)

[a] Data is presented as mean (± SD) from 3-8 experiments. Male body composition parameters were adjusted to body volume so that they were comparable with hermaphrodite.

Supporting information table S3. Glucose and trehalose content of males and hermaphrodites at different developmental stages in different male mutants[a].

Mutant	Stage	Sex	Trehalose [ng/worm]	Glucose [ng/worm]	Trehalose to glucose ratio
him-8 GFP	Young adult (66 h)	Hermaphrodite	0.43 (± 0.17)	1.87 (± 0.75)	0.25 (± 0.09)
		Male	0.49 (± 0.18)	1.50 (± 0.56)	0.35 (± 0.08)
		Male (adjusted)	0.67 (± 0.30)	2.03 (± 0.82)	
	Adult (76 h)	Hermaphrodite	1.88 (± 1.09)	5.74 (± 1.30)	0.32 (± 0.16)
		Male	1.91 (± 0.66)	1.64 (± 0.57)	1.28 (± 0.58)
		Male (adjusted)	3.72 (± 1.35)	3.21 (± 1.31)	
	One day adult (90 h)	Hermaphrodite	2.74 (± 0.45)	10.7 (± 1.39)	0.26 (± 0.05)
		Male	3.61 (± 0.21)	2.59 (± 0.24)	1.40 (± 0.11)
		Male (adjusted)	7.78 (± 0.27)	5.58 (± 0.34)	
fog-2	One day adult (90 h)	Female	2.84 (± 1.44)	9.45 (± 2.21)	0.29 (± 0.10)
		Male	3.26 (± 0.88)	3.37 (± 0.10)	0.97 (± 0.28)
		Male (adjusted)	7.08 (± 2.08)	7.27 (± 0.11)	
him-8	One day adult (90 h)	Hermaphrodite	3.66 (± 1.83)	8.86 (± 1.93)	0.39 (± 0.15)
		Male	1.98 (± 0.92)	2.50 (± 0.34)	0.81 (± 0.46)
		Male (adjusted)	4.18 (± 2.09)	5.22 (± 0.66)	
him-5	One day adult (90 h)	Hermaphrodite	2.83 (± 1.57)	6.17 (± 2.17)	0.43 (± 0.14)
		Male	1.57 (± 1.06)	1.62 (± 1.11)	1.27 (± 0.74)
		Male (adjusted)	3.06 (± 2.04)	3.20 (± 2.23)	
N2	One day adult (90 h)	Hermaphrodite	4.23 (± 0.46)	12.5 (± 1.60)	0.34 (± 0.03)

[a] Data is presented as mean (± SD) for 3-8 experiments. Male trehalose and glucose level were adjusted to body volume so that they were comparable to hermaphrodite.

Supporting information table S4. The entire list of male-specific genes[a].

Gene ID	Gene name	Male to hermaphrodite ratio		Gene description
		Young adult	Adult	
B0280.16	*sls-2.18*	16.1	22.9	transpliced leader sequence
C04B4.3	*lips-2*	204.0	638.3	triacylglycerol lipase
C06E1.6	*fipr-16*	307.8	793.0	fungus-induced protein
C07G3.10		85.3	114.3	lipid binding (molecular function)
C10G8.2		79.5	144.8	serine-type endopeptidase inhibitor activity
C14E2.4		81.6	313.5	oxidoreductase activity (predicted)
C16A11.8	*clec-135*	582.3	1292.7	c-type lectin, function unknown
C16E9.4b	*inx-1*	18.4	21.1	predicted member of the innexin family
C18F10.4	*srg-1*	14.4	16.2	receptor-like protein, transmembrane receptor activity (predicted)
C28H8.7		28.5	83.7	predicted secreted small molecules methylase
C29F9.5		37.3	54.8	CREB binding protein/P300 and related TAZ Zn-finger proteins, histone acetyltransferase activity (predicted)
C32C4.5a	*mab-23*	25.8	27.5	transcription factor of the DM (DOUBLESEX/MAB-3) class
C32C4.5b	*mab-23*	22.7	43.5	transcription factor of the DM (DOUBLESEX/MAB-3) class
C32E8.6a		76.1	152.2	acyl-CoA synthetase
C34C6.8	*ceh-7*	10.8	11.2	a homeodomain transcription factor
C35C5.2		12.1	14.3	peptidase activity (predicted)
C37H5.11	*cwp-2*	54.5	339.3	coexpressed with polycystins, encodes a nematode-specific protein that is coexpressed with lov-1 and pkd-2
C37H5.4	*cwp-3*	44.6	110.4	coexpressed with polycystins, encodes an unfamiliar protein predicted to be secreted and alleged to be coexpressed with polycystins
C39E9.6	*scl-8*	538.6	1034.2	encodes a predicted extracellular protein that is a member of the C. elegans family of SCP/TAPS domain-containing proteins, defense-related protein containing SCP domain
C41G6.10	*sri-25*	10.5	41.3	predicted olfactory G-protein coupled receptor
C42D4.11	*clec-179*	23.0	52.9	binding
C45G9.10b		103.1	475.3	positive regulation of growth rate (phenotype)
C47A10.9	*srh-287*	13.1	13.0	serpentine receptor, class H
C47G2.6		24.3	20.6	Pseudogene
C50E3.15		11.1	15.1	locomotion (phenotype)
C50E3.3	*clec-213*	34.1	44.6	c-type lectin, function unknown
E03H12.3	*clec-176*	12.2	10.8	binding
EEED8.11	*clec-141*	185.7	541.9	binding
F02D10.1	*col-183*	77.0	264.2	collagens (type IV and type XIII) and related proteins, structural constituent of cuticle
F02H6.6		44.3	66.6	zinc metalloprotein

F07D10.1.1	*rpl-11.2*	144.4	1826.8	encodes a large ribosomal subunit L11 protein; is predicted to function in protein biosynthesis
F07D10.1.2	*rpl-11.2*	142.3	1349.1	encodes a large ribosomal subunit L11 protein; is predicted to function in protein biosynthesis
F07G6.1	*dgn-3*	39.6	55.7	dystroglycan
F10A3.11		28.4	32.2	lipid storage (phenotype)
F11D11.14	*clec-250*	19.8	15.4	c-type lectin, binding (molecular function)
F11D11.5	*clec-254*	42.9	21.1	c-type lectin, binding (molecular function)
F13E9.10		258.2	698.9	predicted alpha-helical protein
F13E9.4		112.8	368.3	predicted alpha-helical protein
F16G10.6		91.0	11.6	extracellular protein with cysteine rich structures
F17A2.1	*xtr-2*	14.3	26.4	encodes a protein with similarity to TRA-2A and TRA-2B in a region known as the MX region, hypothesized to be a protein-protein interaction domain involved in negatively regulating tra-2 activity in the germ line
F17B5.3	*clec-109*	132.6	1046.5	c-type lectin, binding (molecular function)
F17B5.5	*clec-110*	186.2	816.1	c-type lectin, function unknown
F22B7.2	*flp-23*	109.4	959.2	FMRF-like peptide
F26A1.12	*clec-157*	356.9	201.2	c-type lectin, binding (molecular function)
F26D11.9	*clec-217*	316.0	1448.0	c-type lectin, function unknown
F26D2.7	*str-55*	87.2	46.2	7-transmembrane olfactory receptor
F26F2.6	*clec-263*	320.2	999.5	c-type lectin, function unknown
F28B1.1	*hpo-33*	159.8	1352.6	hypersensitive to pore-forming toxin
F32B4.6		746.7	1450.9	predicted alpha/beta hydrolase, acting on ester bonds
F33E2.3	*clec-102*	20.6	62.8	c-type lectin, binding (molecular function)
F33E2.6		42.9	157.8	collagens (type IV and type XIII) and related proteins
F33H1.5	*srd-1*	15.7	14.0	encodes a seven transmembrane chemosensory receptor
F35C5.3		10.9	397.4	reproduction (phenotype)
F35D11.7	*clec-136*	139.4	1309.1	c-type lectin, function unknown
F36G9.11	*clec-232*	252.3	633.3	c-type lectin, binding (molecular function)
F40H6.5		21.8	22.7	sugar binding
F43C11.1		311.6	1759.9	extracellular protein with cysteine rich structures
F43C11.12		179.8	1512.7	extracellular protein with cysteine rich structures
F43C11.2		134.9	924.5	extracellular protein with cysteine rich structures
F45C12.9		87.6	994.7	extracellular protein with cysteine rich structures
F45G2.6	*trf-1*	28.1	40.9	encodes a protein with a meprin-associated Traf homology (MATH) domain that may be involved in apoptosis
F46A8.3		312.0	855.3	Galectin, galactose-binding lectin, sugar binding
F46A8.5		337.1	1335.7	Galectin, galactose-binding lectin, sugar binding

F46A8.8		366.4	1046.8	sugar binding, Galectin
F47G4.1	*clec-113*	13.9	12.0	c-type lectin, function unknown
F49C5.10		74.6	379.7	involved in purine ribonucleoside monophosphate biosynthetic process, deaminase activity
F49F1.10		251.4	1195.6	Galectin, galactose-binding lectin, sugar binding
F49F1.11		323.8	1330.1	Galectin, galactose-binding lectin, sugar binding
F49F1.9		460.5	884.5	Galectin, galactose-binding lectin, sugar binding
F49H6.10	*srz-93*	13.4	16.1	Serpentine Receptor, class Z, pseudogene
F56H1.2	*nhr-266*	16.4	13.9	nuclear hormone receptor family,transcription factor activity
F57E7.5		10.1	32.1	pseudogene
F58A4.5	*clec-161*	267.2	952.3	c-type lectin, binding (molecular function)
F58F9.6		538.5	1390.4	zinc metalloprotein
H25K10.3	*srv-11*	10.9	17.4	serpentine receptor, class V, transmembrane receptor activity
K02E7.t1		62.1	121.0	transfer-RNA
K03B8.2	*nas-17*	598.6	1335.3	encodes an astacin-like metalloprotease
K03B8.5	*nas-19*	337.8	1556.8	encodes an astacin-like metalloprotease
K04H8.1	*clec-116*	28.0	33.3	binding
K07B1.1	*try-5*	41.5	76.5	trypsin-like protease
K07C6.4	*cyp-35B1*	28.0	12.9	cytochrome P450 family, oxidoreductase activity
M162.1	*clec-259*	148.9	238.4	binding
M7.10		391.6	1062.1	predicted alpha-helical protein
R03H10.4		133.9	1162.3	extracellular protein with cysteine rich structures, lipid storage (phenotype)
R03H10.5		19.6	19.0	extracellular protein with cysteine rich structures
R05A10.5		15.1	48.5	zinc metalloprotein
R05H10.7		413.9	425.8	metallocarboxypeptidase activity
R11E3.4	*set-15*	80.0	202.0	encodes a SET domain-containing protein required for normally short lifespan; Histone H3 (Lys9) methyltransferase SUV39H1/Clr4, required for transcriptional silencing, protein binding
R13F6.2	*clec-159*	145.5	198.7	c-type lectin, unknown function
R13F6.8	*clec-158*	189.4	925.3	c-type lectin, unknown function
T05H4.8		115.9	1610.0	pseudogene
T12A2.5		511.9	2092.9	lipid storage (moleculare function)
T12A7.3	*scl-18*	159.7	1323.1	CAP domain, cysteine-rich secretory protein family
T19B10.12		470.8	1744.2	TonB box, conserved site (protein domain)
T20D3.1	*clec-183*	247.9	578.6	c-type lectin, binding
T21C9.11		11.3	10.8	lipid storage (phenotype)
T22G5.1		500.5	1274.2	glycerophosphoryl diester phosphodiesterase activity
T23F2.3		140.7	989.4	Stress responsive protein
T23G4.5		165.8	1137.9	predicted alpha-helical protein

T24D8.3	*nlp-22*	32.5	60.4	neuropeptide-like protein
T28A8.2		24.4	48.5	predicted galactoside 2-alpha-L-fucosyltransferase activity
W02B3.5		65.1	381.6	fukutin-related (protein domain)
W02B3.7		85.8	122.0	fukutin-related (protein domain)
W02D7.12	*clec-219*	183.1	1163.3	c-type lectin, unknown function
W04A4.3		109.1	166.4	gamma-interferon inducible lysosomal thiol reductase
W09G10.5	*clec-126*	121.8	171.5	c-type lectin, binding
W09G10.6	*clec-125*	176.6	404.7	c-type lectin, binding
W10G11.11	*clec-134*	1593.0	1063.3	c-type lectin, unknown function
W10G11.12	*clec-133*	220.9	1046.3	c-type lectin, unknown function
W10G11.13	*clec-132*	140.9	167.2	c-type lectin, binding
W10G11.14	*clec-130*	341.0	772.0	c-type lectin, binding
W10G11.15	*clec-129*	496.1	1421.1	c-type lectin, binding
Y102A5C.10	*fbxa-204*	63.0	91.0	F-box A protein, pseudogene
Y116A8A.3	*clec-193*	57.3	65.2	c-type lectin, unknown function
Y17D7B.6	*clec-256*	19.3	33.8	c-type lectin, unknown function
Y17D7B.8	*clec-257*	33.8	51.9	c-type lectin, unknown function
Y26D4A.4.1	*clec-107*	903.5	1582.3	c-type lectin, unknown function
Y26D4A.4.2	*clec-107*	860.8	1138.6	c-type lectin, unknown function
Y26D4A.6	*clec-108*	145.8	508.9	c-type lectin, binding
Y38E10A.2		46.7	45.5	unknown, lipid storage (phenotype)
Y39B6A.t4		140.2	79.4	t-RNA
Y43F8B.19		84.2	261.0	oxidoreductase activity
Y46D2A.3		86.6	325.2	zinc metalloprotein
Y47D7A.10	*hpo-37*	462.4	606.1	hypersensitive to pore-forming toxin
Y51A2D.1		90.5	398.5	cysteine-type peptidase activity
Y52B11A.5	*clec-92*	279.5	935.9	c-type lectin, binding
Y53C12B.5a	*mab-3*	25.0	44.0	encodes a DM (Doublesex and MAB-3) domain-containing protein, transcription factor activity
Y55F3C.5	*clec-164*	24.5	35.4	c-type lectin, unknown function
Y59H11AR.5	*clec-181*	256.1	729.2	c-type lectin, binding
Y71A12B.6	*clec-112*	14.3	17.4	c-type lectin, unknown function
Y73F8A.1	*pkd-2*	41.2	120.3	encodes an ortholog of human PKD2 (mutated in autosomal dominant polycystic kidney disease), calcium ion binding-ion channel activity
Y82E9BL.12		16.1	71.6	predicted secreted small molecules methylase
ZC15.6	*clec-261*	232.0	1092.4	c-type lectin, binding
ZC334.8	*ins-25*	17.8	18.7	insulin related
ZK1025.9	*nhr-113*	16.6	72.5	nuclear hormone receptor family, transcription factor activity
ZK1248.1		98.7	327.5	metalloendopeptidase activity
ZK1290.1		425.6	1124.4	lipid storage (phenotype)
ZK39.2	*clec-95*	220.8	925.5	c-type lectin, binding
ZK39.4	*clec-93*	43.0	109.7	c-type lectin, binding
ZK39.5	*clec-96*	257.5	898.3	c-type lectin, binding
ZK945.9	*lov-1*	55.6	111.6	encodes an ortholog of human PKD1 (mutated in autosomal dominant polycystic kidney disease) protein binding
B0207.5		144.4	537.4	unknown

B0228.8	76.6	441.2	unknown
C01G10.18	202.7	88.2	unknown
C03B1.4	14.2	14.6	unknown
C04B4.6	43.2	57.2	unknown
C06A12.8	74.0	403.1	unknown
C06E2.9	171.2	546.7	unknown
C08E3.14	72.0	167.6	unknown
C14C6.13	17.4	72.4	unknown
C16C10.9	16.0	10.7	unknown
C16C8.10	232.3	2100.0	unknown
C16C8.17	157.1	1473.5	unknown
C16C8.7	195.7	1175.4	unknown
C16C8.8	201.8	1832.1	unknown
C17H12.11.1	73.0	173.7	unknown
C24A3.9	57.3	48.3	unknown
C25F9.8	101.3	853.3	unknown
C26E1.1	23.8	71.4	unknown
C27C7.5	15.3	12.8	unknown
C35E7.7	104.5	601.5	unknown
C44B12.9	140.4	591.9	unknown
C46E10.3	23.6	45.8	unknown
C48B4.12a	483.8	898.4	unknown
C49C8.6	51.3	320.1	unknown
C50H11.8	12.6	15.8	unknown
C55C3.7	36.6	35.6	unknown
D1022.2	141.0	921.2	unknown
E02H9.1	129.8	374.2	unknown
F07G6.8	192.2	1181.7	unknown
F13A2.5	43.9	190.8	unknown
F13E9.9	51.7	334.0	unknown
F16G10.10	281.8	1703.5	unknown
F16G10.2	51.3	442.3	unknown
F17E9.15	129.3	480.4	unknown
F18E9.7	445.6	1848.1	unknown
F18G5.5	20.8	45.8	unknown
F19H6.5	115.9	480.6	unknown
F19H6.6	109.2	469.0	unknown
F25B3.2	26.0	305.6	unknown
F25D7.5	30.4	78.6	unknown
F26C11.3b	62.6	232.9	unknown
F26C11.4	149.2	988.1	unknown
F28B1.2	91.2	777.7	unknown
F28B1.3	101.1	196.3	unknown
F28B1.9	220.7	948.8	unknown
F33D11.6	46.0	55.1	unknown
F35C5.4	32.8	48.5	unknown
F38E1.10	131.9	1528.1	unknown
F38E11.11	127.1	694.1	unknown
F40E12.1	19.7	16.0	unknown
F40G9.7.1	174.4	1446.0	unknown
F42A6.2	458.9	632.0	unknown
F44A2.7	66.6	24.4	unknown
F46B3.9	23.3	564.1	unknown
F47C12.11	64.0	74.7	unknown

F47C12.6	110.3	902.2	unknown
F47C12.7	137.8	452.5	unknown
F47G9.6	18.5	15.6	unknown
F49C5.7	10.6	37.2	unknown
F55G11.10	33.1	103.9	unknown
F56C4.2	12.5	32.4	unknown
F58F9.8	300.0	454.8	unknown
F59A1.16	411.5	1451.2	unknown
F59A1.6	125.3	1092.5	unknown
F59A6.3	69.0	74.6	unknown
H23L24.1	47.0	64.6	unknown
K03B8.11	499.1	396.8	unknown
K03B8.14	342.4	734.6	unknown
K04F1.8	64.1	234.2	unknown
K08C9.6	339.3	247.9	unknown
K08C9.9	57.4	93.0	unknown
K09C8.2	110.5	1395.8	unknown
K12H6.5	803.7	2061.8	unknown
K12H6.8	593.6	1154.1	unknown
M02D8.7	19.5	36.4	unknown
M04D8.5	14.2	19.3	unknown
R04A9.1	11.7	13.0	unknown
R09E10.8	123.2	1381.5	unknown
R11G10.4	233.7	1192.2	unknown
R13A1.1	13.2	111.0	unknown
R160.6	20.1	17.0	unknown
R53.8	161.0	948.1	unknown
T02D1.7	534.6	1159.4	unknown
T04A6.2	16.7	30.1	unknown
T05A8.7	45.6	70.3	unknown
T10D4.15	112.0	523.5	unknown
T10D4.7	149.6	429.2	unknown
T12A7.9	13.4	69.8	unknown
T20F5.8	25.4	21.1	unknown
T21E8.7	44.1	40.2	unknown
T22C1.12	111.5	1030.9	unknown
T24E12.12	17.2	55.9	unknown
T26E3.6	235.2	1465.7	unknown
T28B4.2	10.1	15.0	unknown
W03G9.7	10.3	15.3	unknown
W04G5.7	13.7	14.5	unknown
W06D12.6	63.8	61.8	unknown
W06G6.12	12.6	12.8	unknown
W07G1.1	13.6	13.3	unknown
W07G1.7	186.7	614.5	unknown
W07G4.8	14.7	12.1	unknown
Y105C5A.9b	80.7	38.4	unknown
Y110A2AL.6	129.7	1641.0	unknown
Y116F11A.3	304.2	1404.9	unknown
Y17G7B.23	50.4	81.9	unknown
Y18D10A.2	132.9	853.3	unknown
Y25C1A.2	145.3	526.1	unknown
Y37F4.3	110.5	890.6	unknown
Y38H6C.18	44.9	51.7	unknown

Y41D4A.1	25.3	86.1	unknown
Y43F8B.10	22.7	35.2	unknown
Y43F8C.15	58.2	236.7	unknown
Y43F8C.16	179.6	1412.1	unknown
Y43F8C.23	77.2	266.9	unknown
Y46G5A.23	333.2	1237.7	unknown
Y47D7A.11	694.9	732.8	unknown
Y48G8AR.3	48.6	105.5	unknown
Y48G9A.6	59.9	165.5	unknown
Y49F6B.13	228.8	1652.3	unknown
Y49F6B.6	743.1	1546.7	unknown
Y53G8AL.3	40.0	72.9	unknown
Y59E1B.1	12.6	14.1	unknown
Y62H9A.2	45.0	57.1	unknown
Y62H9A.8	35.7	167.1	unknown
Y64G10A.2	218.5	1293.1	unknown
Y67A10A.11	46.8	146.1	unknown
Y67A10A.2	29.3	36.1	unknown
Y67D8C.7	244.6	121.5	unknown
Y68A4A.13	25.9	113.2	unknown
Y6G8.14	590.7	1130.4	unknown
Y6G8.16	280.6	890.6	unknown
Y71G12B.5	18.1	10.1	unknown
Y73F8A.10	181.8	1589.1	unknown
Y75B12B.13	242.2	689.0	unknown
Y7A5A.11	23.3	31.0	unknown
Y7A5A.9	14.8	17.1	unknown
Y82E9BL.1	27.4	24.6	unknown
Y82E9BR.7	57.2	46.1	unknown
Y82E9BR.9	41.5	43.7	unknown
ZC178.2	131.4	898.7	unknown
ZC204.17	233.5	890.0	unknown
ZC328.5	281.9	244.2	unknown
ZK1290.11	723.9	985.9	unknown
ZK39.9	901.8	1489.4	unknown

[a] Information about genes was obtained from Wormbase.

Supporting information table S5. The entire list of hermaphrodite-specific genes[a].

Gene ID	Gene name	Hermaphrodite to male ratio		Description
		Young adult	Adult	
C01C10.3	*acl-12*	11.5	28.2	acyltransferase activity
C03E10.5	*clec-223*	494.6	177.8	c-type lectin, binding(molecular function)
C18D11.7		56.4	13.4	protein binding (molecular function)
C27C12.3		25.2	80.3	encodes a novel protein that is conserved in C. elegans; is expressed in the proximal germline
C29G2.1		42.6	161.5	C2H2-type Zn-finger
C30F2.2		26.0	12.4	similar to ARD GTP-binding proteins
C32B5.10	*fbxc-32*	84.1	191.9	F-box C protein
C32B5.6		23.2	71.7	expression is seen only in adults in two symmetrical neurons, whose cell bodies are located on the ventral side of the terminal bulb
C36C9.1		16.4	65.1	expression is seen in pairs of nuclei around the isthmus of the pharynx, nuclei are probably neuronal, in older larvae and adults weak expression is seen in the pharynx
C44C3.8	*srh-85*	152.3	197.9	serpentine receptor, class H, pseudogene
C46E10.8		21.5	69.5	zinc ion binding
C46E10.9		54.0	118.0	expressed in unidentified cells running parallel to the pharynx, zinc ion binding
C47F8.1		24.4	211.1	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, protein binding (molecular function)
C50E3.11		57.8	144.3	folic acid binding, reduced folate carrier activity
C50E3.12		173.1	179.2	locomotion (phenotype)
C53B7.7		120.0	80.1	encodes a neprilysin, neprilysins are thermolysin-like zinc metallopeptidases
E01G6.3		54.7	42.1	hydrolase activity
E02H4.6		120.3	162.0	protein kinase activity, protein serine/threonine kinase activity
F08A8.3		33.2	44.6	acyl-CoA dehydrogenase activity
F08F3.6		110.9	163.2	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins
F09A5.9	*ttr-34*	140.3	67.0	transthyretin-related family domain
F14B8.3	*pes-23*	97.2	55.6	patterned expression site
F14D7.2		138.9	146.7	morphogenesis of an epithelium (biologic function
F15A2.5	*efn-3*	33.8	31.1	Eph(f)rin, encodes a potential GPI-modified ephrin that is, with EFN-2, is required for normal epidermal organization in the male tail
F15E6.6		239.3	65.3	oxidoreductase activity

F16H11.3		259.3	244.1	nucleoside transmembrane transporter activity
F17A9.6	*ceh-49*	77.0	166.0	C. elegans homeobox, encodes a divergent ONECUT class CUT homeobox protein with a single N-terminal cut domain; specific DNA binding-transcription factor activity
F22G12.t1		34.5	35.6	t-RNA
F25H5.9		85.7	72.7	only transcript, no protein
F29G9.7		21.8	50.8	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins
F31B12.3f	*frm-9*	52.5	42.0	FERM domain (protein4.1-ezrin-radixin-moesin) family
F31F6.1		42.3	29.0	encodes a novel protein that is conserved in C. elegans
F31F6.2		26.6	103.8	encodes a novel protein that is conserved in C. elegans, loss of function via large-scale RNAi results in increased fat content
F35C5.2	*sra-14*	10.1	16.5	serpentine receptor, class A, transmembrane receptor activity
F39F10.3		24.2	82.8	protein kinase activity
F45C12.15.1	*ceh-83*	17.1	42.6	C. elegans homeobox, transcription factor activity
F45C12.15.3	*ceh-83*	14.0	42.5	C. elegans homeobox, transcription factor activity
F45C12.8	*fbxa-184*	11.7	40.4	F-box A protein
F45D11.9	*fbxc-42*	29.7	151.9	F-box C protein
F47A4.3a	*rrc-1*	58.2	10.6	RhoGAP for Rac-1 and Cdc-42, is expressed in all life stages with high expression in L1 to L4 larvae stages
F47A4.3c	*rrc-1*	63.9	11.0	RhoGAP for Rac-1 and Cdc-42, is expressed in all life stages with high expression in L1 to L4 larvae stages
F47H4.1	*lsy-27*	114.5	189.6	laterally symmetric (defective in lateral asymmetry)), encodes a nematode specific, fast evolving family of C2H2 zinc finger transcription factor
F49B2.2	*fbxb-67*	12.9	37.3	F-box B protein
F52D2.2	*rgs-8.1*	17.9	78.2	regulator of G protein signaling, signal transducer activity
F54D10.7		245.3	123.1	protein binding, ubiquitin family protein domain
F55B11.5		1406.2	181.9	determination of adult life span (phenotype)
K02B9.1	*meg-1*	311.7	159.3	maternal effect germ-cell defective, encodes a novel protein that localizes exclusively to P granules; is required for germline development and normal levels of fertility
K04C1.5		216.0	235.9	protein kinase activity, protein serine/threonine kinase activity

K07A1.6		769.1	178.8	encodes a putative secreted TIL-domain protease inhibitor paralogous to SWM-1, ISL-1, and the products of 11 other C. elegans genes
M01G12.8		14.8	114.4	pseudogene
M199.4	*clec-190*	245.0	192.8	c-type lectin, unknown function
M199.5	*col-135*	779.4	176.0	collagen, collagen triple helix repeat protein domain
R02C2.2	*kin-34*	11.6	219.6	protein kinase activity-protein serine/threonine kinase activity
R03G8.6		382.9	177.9	metallopeptidase activity and zinc ion binding
R07C3.9	*fbxc-31*	12.8	57.4	F-box C protein
T01E8.2	*ref-1*	16.5	28.0	regulator of fusion, encodes a protein with two basic helix-loop-helix (bHLH) domains that is distantly related to the hairy/Enhancer of split subfamily of bHLH transcription factors, regulation of transcription
T02G6.5		135.6	151.6	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins
T04D3.1		45.4	125.2	uncharacterized coiled-coil containing protein
T05A10.3	*ttr-14*	514.1	144.2	transthyretin-related family domain, cell migration, gonad development, receptor-mediated endocytosis (phenotype)
T05E7.1		96.5	83.6	peroxisomal long chain acyl-CoA thioesterase I/predicted bile acid-CoA-amino acid N-acyltransferase
T05G11.1	*pzf-1*	206.4	127.8	paired zinc finger protein, encodes a protein with three pairs of C2H2 zinc fingers
T07C4.6	*tbx-9*	37.6	94.7	T box family, RNA polymerase II transcription factor activity
T10C6.10a		29.3	133.5	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, lipid storage (phenotype)
T10C6.10b		157.3	156.9	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, lipid storage (phenotype)
T10C6.7		10.1	29.7	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins
T13B5.9		56.4	56.5	metalloendopeptidase activity and zinc ion binding
T28H11.4	*pes-1*	10.1	34.5	patterned expression site, transcription factor activity

W02D9.5	*ssp-37*	1013.1	170.9	sperm specific family, class P, contains major sperm protein (MSP) domain, structural molecule activity
Y105E8B.10		34.2	44.2	pseudogene
Y116A8C.22		14.0	133.1	AT hook-like protein domain, DNA, protein and zinc ion binding
Y11D7A.13	*flh-3*	35.8	97.2	FLYWCH zinc finger transcription factor homolog, transcription factor activity
Y27F2A.3a	*sri-40*	30.1	102.0	serpentine receptor, class I, predicted olfactory G-protein coupled receptor
Y27F2A.3b	*sri-40*	28.4	97.5	serpentine receptor, class I, predicted olfactory G-protein coupled receptor
Y54G11A.16	*srh-43*	483.6	104.1	serpentine receptor, class H, pseudogene
Y6G8.3	*ztf-25*	257.4	239.9	zinc finger putative transcription factor family, Ubiquitous strong expression is seen in early and comma stage embryos
ZC53.7	*rgs-9*	170.7	192.2	regulator of G protein signaling, signal transducer activity
ZK1127.1	*nos-2*	183.8	161.5	nanos related, encodes one of three genes in C. elegans that contains a putative zinc-binding domain similar to the one found in Drosophila nanos
ZK546.15	*try-1*	93.6	120.9	trypsin-like protease), serine-type endopeptidase activity
ZK637.11	*cdc-25.3*	527.2	173.9	cell division cycle related, encodes a tyrosine phosphatase that is a member of the cell division cycle 25 (CDC25) family of cell cycle regulators
ZK829.5	*tbx-36*	91.1	98.8	T box family, transcription factor activity
B0281.4		36.5	129.0	unknown
B0545.4		70.0	73.0	unknown
C08A9.10		1754.5	191.3	unknown
C09G9.8b		14.6	22.2	unknown
C10A4.4		17.7	10.8	unknown
C14F11.4a		333.8	71.5	unknown
C14F11.4b		317.0	73.5	unknown
C17E7.12		345.6	171.7	unknown
C17E7.4		391.8	184.8	unknown
C17E7.9b		128.6	144.2	unknown
C17G1.2		340.3	173.8	unknown
C30G7.4.2		50.4	42.5	unknown
C45H4.14a		32.8	172.5	unknown
C46C2.5		79.0	58.0	unknown
C50E3.13		138.1	193.2	unknown
D1086.10a		1392.1	160.7	unknown
D1086.10c		993.2	231.7	unknown
D1086.11a		145.9	96.3	unknown
D1086.7		1592.1	188.9	unknown
E02H9.7		356.4	164.9	unknown
F02E8.4		207.1	170.8	unknown
F02H6.1		10.2	46.8	unknown
F02H6.2		133.7	219.3	unknown
F02H6.3a.1		109.9	174.0	unknown
F07G6.10		97.0	38.6	unknown

F14H3.3	90.6	176.9	unknown
F22E5.17	76.4	90.5	unknown
F22F4.5	238.2	200.7	unknown
F25H5.8	31.4	13.6	unknown
F26G5.1b	177.2	194.9	unknown
F30A10.13b.2	128.2	94.5	unknown
F36A4.5	277.3	88.9	unknown
F39G3.2	27.0	14.5	unknown
F40G12.11	226.2	157.2	unknown
F52D1.2	36.9	148.6	unknown
F54D11.3	33.8	59.5	unknown
F54D5.5a	23.1	81.9	unknown
F54D5.5b	22.0	80.8	unknown
F54F7.9	17.8	13.9	unknown
F58E1.13	17.5	47.8	unknown
H11L12.1	11.1	19.0	unknown
K01G12.3	21.5	99.7	unknown
K08H2.3	29.7	75.3	unknown
K09A9.8	18.5	17.9	unknown
K09D9.12	45.8	102.3	unknown
K09E3.7	12.5	74.8	unknown
M01G12.14	74.1	26.2	unknown
R04D3.3.1	44.6	126.6	unknown
R04D3.3.2	58.7	141.2	unknown
R04D3.4	165.7	188.1	unknown
R05G9.3	77.3	213.6	unknown
R09A8.1a	55.1	249.6	unknown
R09A8.1b	22.9	74.9	unknown
R09F10.8.1	34.5	107.2	unknown
R09F10.8.2	35.2	112.7	unknown
R10E4.6	25.5	39.9	unknown
T06D4.1a	283.9	167.1	unknown
T06D4.1b.3	450.8	226.5	unknown
T11F8.2.1	112.1	101.3	unknown
T12B5.14	928.3	272.2	unknown
T12B5.15	2541.7	193.3	unknown
T16G12.8	14.2	43.3	unknown
T19H5.7	72.7	111.7	unknown
W04E12.2	22.5	122.7	unknown
W04G3.13	19.3	12.7	unknown
W06D11.2	22.9	24.6	unknown
W07A12.8	10.3	11.8	unknown
Y116F11B.17	10.2	106.5	unknown
Y37H2A.13	31.1	63.4	unknown
Y39B6A.10	19.5	77.5	unknown
Y48E1B.8	272.9	135.2	unknown
Y48G1C.13	23.9	25.8	unknown
Y49F6C.8	281.0	196.7	unknown
Y66C5A.1	96.6	85.2	unknown
Y79H2A.2a	78.4	38.2	unknown
Y79H2A.2b.1	72.9	37.8	unknown
ZC266.2	16.2	17.7	unknown
ZK177.1	45.9	169.8	unknown
C18D11.10	27.5	15.2	unknown

K03B8.4	11.5	15.1	unknown

[a] Information about genes was obtained from Wormbase.

Supporting information table S6. Body composition of *him-8 GFP* males and hermaphrodites under *ad libitum* and DR conditions at different developmental stages[a].

		Young adult (66 h)		Adult (76 h)		One day adult (90 h)	
		Male	Hermaphrodite	Male	Hermaphrodite	Male	Hermaphrodite
Protein [ng/worm]	*Ad libitum*	43.3 (± 4.8)	58.7 (± 4.7)	68.5 (± 10.9)	138.4 (± 12.8)	75.3 (± 5.9)	194.1 (± 13.4)
	DR	42.0 (± 2.3)	53.9 (± 4.5)	54.9 (± 8.8)	84.5 (± 19.6)	47.8 (± 5.4)	102.6 (± 14.6)
	Change [%]	-2.3 (± 9.6)	-8.1 (± 2.2)	-15.5 (± 7.8)	-31.8 (± 14.6)	-36.5 (± 2.6)	-47.2 (± 4.7)
Triglyceride [ng/worm]	*Ad libitum*	14.7 (± 1.7)	14.6 (± 1.3)	8.4 (± 6.7)	16.4 (± 7.0)	10.9 (± 0.5)	27.3 (± 1.9)
	DR	17.2 (± 0.7)	14.3 (± 0.8)	21.8 (± 16.5)	27.3 (± 23.6)	13.2 (± 1.6)	34.0 (± 3.7)
	Change [%]	17.9 (± 11.5)	-1.2 (± 11.5)	95.0 (± 48.1)	69.8 (± 64.8)	21.1 (± 12.9)	25.5 (± 21.5)
Triglyceride to protein ratio	*Ad libitum*	0.34 (± 0.01)	0.25 (± 0.04)	0.13 (± 0.12)	0.12 (± 0.06)	0.14 (± 0.01)	0.14 (± 0.01)
	DR	0.41 (± 0.03)	0.27 (± 0.01)	0.32 (± 0.23)	0.28 (± 0.06)	0.27 (± 0.01)	0.34 (± 0.07)
	Change [%]	20.9 (± 6.5)	7.5 (± 12.4)	129.4 (± 41.1)	155.9 (± 101.7)	90.6 (± 12.9)	141.3 (± 58.1)

[a] Data is presented as mean (± SD) and include 3-5 experiments. Male body composition parameters were adjusted to body volume as described in Material and methods.

Supporting information table S7. Glucose and Trehalose content of *him-8 GFP* males and hermaphrodites under normal feeding conditions and DR at different developmental stages[a].

		Young adult (66 h)		Adult (76 h)		One day adult (90 h)	
		Male	Herma-phrodite	Male	Herma-phrodite	Male	Herma-phrodite
Trehalose [ng/worm]	*Ad libitum*	0.58 (± 0.15)	0.48 (± 0.18)	1.91 (± 0.80)	1.72 (± 1.26)	3.61 (± 0.21)	2.74 (± 0.45)
	DR	0.36 (± 0.18)	0.19 (± 0.14)	1.07 (± 0.24)	1.13 (± 0.42)	1.67 (± 0.14)	1.72 (± 0.60)
	Change [%]	-39.1 (± 24.5)	-59.2 (± 20.0)	-32.6 (± 19.2)	-22.8 (± 31.1)	-53.2 (± 6.9)	-37.4 (± 4.7)
Glucose [ng/worm]	*Ad libitum*	1.84 (± 0.12)	2.34 (± 0.26)	1.82 (± 0.54)	5.45 (± 0.81)	2.59 (± 0.24)	10.7 (± 1.39)
	DR	2.07 (± 0.33)	2.63 (± 0.52)	1.40 (± 1.10)	3.25 (± 2.02)	2.09 (± 0.44)	6.05 (± 1.03)
	Change [%]	11.9 (± 12.3)	11.8 (± 10.0)	-15.7 (± 37.4)	-31.3 (± 21.3)	-18.4 (± 22.4)	-43.4 (± 7.22)
Trehalose to glucose ratio	*Ad libitum*	0.31 (± 0.07)	0.20 (± 0.06)	1.11 (± 0.63)	0.30 (± 0.18)	1.40 (± 0.11)	0.26 (± 0.05)
	DR	0.17 (± 0.06)	0.07 (± 0.04)	1.09 (± 0.58)	0.55 (± 0.51)	0.82 (± 0.09)	0.28 (± 0.07)
	Change [%]	-46.7 (± 16.9)	-63.6 (± 17.5)	1.9 (± 70.3)	21.8 (± 55.2)	-41.3 (± 8.6)	13.4 (± 43.4)

[a] Data is presented as mean (± SD) and include 3-5 experiments. Male body composition parameters were adjusted to body volume as described in Material and methods.

Supporting information table S8. Overlapping genes between both sexes and both developmental stages[a].

	Young adult (66 h)				Adult (76 h)				
	Hermaphrodite		Male		Hermaphrodite		Male		
Gene ID	Ratio DR/*ad libitum*	P-value	Ratio DR/*ad libitum*	P-value	Ratio DR/*ad libitum*	p-value	Ratio DR/*ad libitum*	p-value	Gene description
Downregulated (p<0.05, ratio<0.5) in both sexes at both developmental stages									
C05E11.5	0.50	0.027	0.45	0.016	0.22	0.000	0.47	0.046	*amt-4* - (*AMmonium Transporter homolog*), *amt-4* encodes a member of the ammonium transporter protein family
F08G5.6	0.39	0.018	0.30	0.047	0.28	0.011	0.10	0.000	CUB-like domain, defense response
F19H8.5	0.27	0.001	0.30	0.024	0.32	0.007	0.38	0.012	*mltn-10* - (*MLt-TeN (mlt-10) related*)
F38A1.14	0.47	0.032	0.42	0.023	0.38	0.025	0.27	0.001	*clec-169* - (*C-type LECtin*)
R08E5.1.1	0.36	0.029	0.43	0.011	0.20	0.001	0.28	0.022	lipid biosynthetic process, metabolic process, tRNA modification (biological process), methyltransferase activity, tRNA (guanine-N7-)-methyltransferase activity
Y49G5A.1	0.33	0.008	0.24	0.001	0.09	0.000	0.35	0.045	Serine proteinase inhibitor (KU family), serine-type endopeptidase inhibitor activity
C01B10.7	0.46	0.005	0.48	0.002	0.22	0.002	0.37	0.007	transferase activity
C39E9.4	0.29	0.002	0.35	0.020	0.13	0.001	0.18	0.006	*scl-6* - (*SCP-Like extracellular protein*), *scl-6* encodes a predicted extracellular protein that is a member of the C. elegans family of SCP/TAPS domain-containing proteins, defense-related
F14H3.10	0.15	0.005	0.22	0.008	0.10	0.001	0.09	0.000	*cyp-35D1* - (*CYtochrome P450 family*), electron carrier activity, heme binding, iron ion binding, monooxygenase activity
F49F1.1	0.09	0.023	0.07	0.041	0.04	0.001	0.02	0.004	secreted surface protein
F49F1.7	0.27	0.011	0.13	0.037	0.30	0.001	0.04	0.005	secreted surface protein
K10F12.1	0.33	0.001	0.29	0.007	0.16	0.000	0.16	0.005	pseudogene
R06B10.3	0.43	0.001	0.34	0.001	0.44	0.002	0.38	0.000	*clec-150* - (*C-type LECtin*)
R07B1.3	0.39	0.021	0.29	0.000	0.21	0.001	0.34	0.001	*scav-5* - (*SCAVenger receptor (CD36 family) related*), Plasma membrane glycoprotein CD36 and related membrane receptors, cell adhesion

T24B8.5	0.20	0.038	0.08	0.031	0.07	0.000	0.01	0.000	encodes an ShK-like toxin peptide containing a domain rich in cysteine residues found in the sea anemone potassium channel inhibitor ShK; T24B8.5 expression is regulated by the PMK-1/p38 MAPK signaling pathway and the ATF-7 transcription factor, which is a downstream target of PMK-1
W04E12.8	0.35	0.001	0.28	0.009	0.34	0.002	0.22	0.005	*clec-50* - (*C-type LECtin*)
Y73B6BL.24.3	0.50	0.049	0.50	0.034	0.40	0.009	0.36	0.003	*acp-6* - (ACid Phosphatase family), Lysosomal & prostatic acid phosphatases
ZK6.7b	0.18	0.001	0.08	0.001	0.14	0.000	0.06	0.000	*lipl-5* - (*LIPase Like*), Triglyceride lipase-cholesterol esterase, lipid metabolic process
F22H10.6	0.47	0.020	0.40	0.005	0.16	0.000	0.17	0.039	unknown
C17F4.7	0.22	0.009	0.17	0.001	0.09	0.000	0.19	0.000	unknown
F28A10.5	0.32	0.002	0.37	0.001	0.13	0.000	0.23	0.002	unknown
F29C4.t1	0.38	0.003	0.29	0.006	0.15	0.000	0.17	0.006	unknown
F30H5.5	0.35	0.001	0.50	0.013	0.33	0.001	0.26	0.000	unknown
Up-regulated (p<0.05, ratio>2) in both sexes at both developmental stages									
T08G5.10	9.86	0.017	7.41	0.000	4.94	0.011	4.57	0.011	*mtl-2* - (*MeTaLlothionein*), *mtl-2* encodes one of two C. elegans metallothioneins, small, cysteine-rich, metal-binding proteins; MTL-2 functions in metal detoxification and homeostasis and stress adaptation
C44H9.1	10.51	0.007	7.70	0.004	7.87	0.001	7.78	0.001	*ugt-15* - (*UDP-GlucuronosylTransferase*), carbohydrate binding, transferase activity, transferring hexosyl groups
F15B9.1	10.99	0.030	3.27	0.044	6.68	0.006	2.33	0.004	*far-3* - (*Fatty Acid/Retinol binding protein*), lipid binding
F35E8.13	7.89	0.006	4.96	0.004	7.02	0.000	2.80	0.000	secreted surface protein, ShK domain-like
T16G1.7	4.03	0.006	2.43	0.035	3.10	0.002	3.00	0.002	is orthologous to the human gene ALIAS DLC1~CANDIDATE TUMOR SUPPRESSOR GENE (DLEC1; OMIM:604050), which when mutated leads

								to disease, CHK kinase-like
Regulated in Hermaphrodites (p<0.05, ratio>2 or <0.5) but not in males (p>0.05, Ratio between 0.66-1.5)								
C08A9.7	0.27	0.049	0.84	0.530	0.30	0.005	1.42	0.576	*sdz-2* - (*SKN-1 Dependent Zygotic transcript*)
C24G7.1	0.30	0.030	0.86	0.615	0.17	0.002	0.76	0.230	Amiloride-Sensitive cation channel 5, non voltage-gated ion channels, sodium channel activity
C38D9.8	0.29	0.012	1.43	0.552	0.28	0.001	1.26	0.167	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins
F15A4.2	0.14	0.031	1.44	0.187	0.37	0.002	1.39	0.205	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, protein binding
F23D12.2	0.27	0.020	1.37	0.177	0.41	0.005	1.01	0.991	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins; this gene's encoded protein also contains an FTH/DUF38 motif, which may also mediate protein-protein interaction
F23F12.11	0.39	0.020	0.90	0.714	0.40	0.050	0.82	0.369	*srb-10* - (*Serpentine Receptor, class B (beta)*), pseudogene
F27D4.t1	0.28	0.009	1.28	0.056	0.29	0.001	0.90	0.157	t-RNA
F27D9.7	0.43	0.021	1.25	0.497	0.33	0.001	1.21	0.365	metalloendopeptidase activity
F45B8.1	0.26	0.010	1.01	0.831	0.47	0.003	0.83	0.594	*rgs-11* - (*Regulator of G protein Signaling*), inhibits signal transduction by increasing the GTPase activity of G protein alpha subunits thereby driving them into their inactive GDP-bound form Binds to G(i)-alpha and G-alpha, but not to G-alpha. Plays an important role in the phototransduction cascade by regulating the lifetime and effective concentration of activated transducin alpha, signal transducer

								activity
F45C12.2	0.26	0.020	1.19	0.620	0.40	0.000	1.48	0.310	*ceh-82* - (*C. Elegans Homeobox*)
F45C12.7	0.22	0.029	1.27	0.517	0.45	0.008	1.07	0.934	*btb-6* - (*BTB (Broad/complex/Tramtrack/Bric a brac) domain protein*), protein binding
F54H12.5	0.41	0.017	1.17	0.499	0.49	0.000	1.07	0.791	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, protein binding
K10G4.7	0.36	0.029	0.70	0.307	0.27	0.027	0.96	0.767	*srh-262* - (*Serpentine Receptor, class H*), pseudogene
R02C2.2	0.18	0.006	1.31	0.532	0.36	0.002	1.21	0.633	*kin-34* - (*protein KINase*), Required for checkpoint mediated cell cycle arrest in response to DNA damage or the presence of unreplicated DNA. May also negatively regulate cell cycle progression during unperturbed cell cycles protein, protein tyrosine kinase activity
R07C3.9	0.27	0.003	0.94	0.467	0.47	0.015	0.72	0.466	*fbxc-31* - (*F-box C protein*)
T22C8.4	0.39	0.002	1.08	0.473	0.46	0.000	0.87	0.751	zinc ion binding
T26C11.7	0.47	0.026	1.28	0.602	0.44	0.002	0.88	0.572	*ceh-39* - (C. Elegans Homeobox), encodes a ONECUT class CUT homeobox protein with a single N-terminal cut domain; CEH-39 acts as an X-signal element (XSE) to affect sex determination; the cut domain may be a compact DNA-binding domain composed of alpha helices; is one of three nematode-specific ONECUT genes in a cluster with *ceh-21* and *ceh-41*, transcription factor activity
T27A8.2	0.44	0.009	1.09	0.298	0.49	0.000	0.73	0.115	transcription factor activity
T28A11.22	0.18	0.009	1.04	0.785	0.41	0.009	1.19	0.248	protein binding

W04A8.5	0.19	0.017	0.99	0.983	0.36	0.008	0.70	0.316	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins
Y116A8C.22	0.22	0.002	0.80	0.233	0.36	0.002	1.13	0.760	*athp-3* - (*AT Hook plus PHD finger transcription factor*), Chromatin remodeling protein, contains PHD Zn-finger, DNA binding, protein binding, zinc ion binding
Y11D7A.13	0.22	0.001	0.93	0.693	0.48	0.009	0.75	0.142	*flh-3* - (*FLYWCH zinc finger transcription factor homolog*), transcription factor activity
Y27F2A.3a	0.40	0.003	1.22	0.614	0.49	0.005	0.90	0.805	*sri-40* - (*Serpentine Receptor, class I*), Predicted olfactory G-protein coupled receptor
Y46G5A.8	0.28	0.007	0.94	0.807	0.42	0.000	1.24	0.147	encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, protein binding
Y50D4B.6	0.46	0.014	1.08	0.715	0.28	0.003	0.66	0.336	fibroblast/platelet-derived growth factor receptor and related receptor tyrosine kinases, protein serine/threonine kinase activity
Y54G2A.t4	0.44	0.016	1.04	0.907	0.49	0.018	0.98	0.863	t-RNA
Y56A3A.15	0.40	0.014	1.06	0.847	0.37	0.005	1.01	0.978	*fbxb-24* - (*F-box B protein*), encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins, Predicted transposase, protein binding
Y57G11C.25	0.20	0.025	0.86	0.434	0.44	0.005	0.70	0.302	*ccch-5* - (*CCCH-type zinc finger putative transcription factor*)
Y57G7A.2	0.38	0.044	0.97	0.832	0.32	0.001	1.07	0.810	encodes a CCCH tandem zinc finger (TZF) protein; based upon its sequence similarity to C. elegans POS-1, the product of Y57G11C.25 is predicted to function as an RNA-binding protein, zinc ion binding, nucleic acid binding
Y73B6BL.14	0.45	0.003	1.07	0.633	0.48	0.001	0.82	0.601	ATP-dependent DNA ligase III, DNA ligase (ATP) activity
Y73F8A.22	0.39	0.002	1.23	0.351	0.40	0.002	0.81	0.647	cysteine rich domain (CW domain) probably distantly related to the C-type lectin

ZC204.8	0.34	0.000	1.10	0.784	0.40	0.005	1.34	0.089	*fbxb-21* - (*F-box B protein*), fbxb-21 encodes a protein containing an F-box, a motif predicted to mediate protein-protein interactions either with homologs of yeast Skp-1p or with other proteins; FBXB-21 also contains a C-terminal type 2 F-box-associated domain of presently unknown function
ZK1251.7	0.27	0.049	1.10	0.365	0.42	0.000	1.02	0.927	*sdz-36* - (*SKN-1 Dependent Zygotic transcript*)
ZK6.3	0.23	0.026	1.07	0.790	0.41	0.001	1.07	0.561	*scl-27* - (*SCP-Like extracellular protein*), Defense-related protein containing SCP domain
ZK673.10	0.18	0.037	0.92	0.661	0.33	0.001	0.88	0.659	*sdz-37* - (*SKN-1 Dependent Zygotic transcript*)
C39B5.2.3	0.25	0.042	1.25	0.223	0.49	0.046	1.04	0.850	unknown
C45H4.14a	0.20	0.001	0.69	0.196	0.41	0.001	0.70	0.310	unknown
C49F5.3	0.34	0.016	1.18	0.334	0.48	0.000	0.76	0.070	unknown
F02H6.4	0.34	0.010	1.12	0.419	0.42	0.005	1.00	0.984	unknown
F14H3.3	0.28	0.011	1.24	0.557	0.48	0.006	0.84	0.772	unknown
F17E9.2	0.40	0.043	0.89	0.579	0.37	0.006	1.08	0.713	unknown
F23D12.10	0.37	0.027	1.05	0.855	0.48	0.002	0.91	0.762	unknown
F29G9.1	0.40	0.012	0.81	0.546	0.44	0.008	1.22	0.414	unknown
F31B9.3	0.30	0.002	0.88	0.634	0.50	0.000	0.87	0.161	unknown
F35G2.3	0.28	0.011	1.01	0.961	0.47	0.002	0.94	0.702	unknown
F36H1.12	0.38	0.033	1.39	0.110	0.48	0.002	0.96	0.605	unknown
F46C3.7	0.46	0.005	1.01	0.972	0.50	0.021	1.08	0.458	unknown
F54F7.6.1	0.34	0.034	1.31	0.465	0.37	0.001	1.05	0.672	unknown
F56D5.5	0.16	0.029	1.49	0.544	0.33	0.001	0.95	0.810	unknown
F58D2.4	0.38	0.030	0.91	0.734	0.44	0.006	1.04	0.805	unknown
K01G12.3	0.22	0.002	0.99	0.886	0.38	0.002	1.08	0.454	unknown
K09E3.5	0.41	0.033	1.13	0.415	0.36	0.001	0.74	0.333	unknown
K09E3.7	0.31	0.002	1.37	0.566	0.46	0.000	0.82	0.310	unknown
K10G4.12	0.46	0.012	0.84	0.551	0.49	0.003	1.09	0.739	unknown
R05G9.3	0.30	0.012	1.21	0.287	0.43	0.002	0.80	0.405	unknown

T05E12.10	0.41	0.017	0.91	0.692	0.36	0.028	1.19	0.585	unknown
T11B7.1	0.42	0.032	1.26	0.526	0.40	0.005	0.68	0.293	unknown
T19H5.7	0.47	0.000	1.33	0.462	0.39	0.001	0.95	0.832	unknown
T24F1.5	2.61	0.047	1.37	0.134	2.92	0.022	1.43	0.157	unknown
T27E9.6	0.43	0.040	1.36	0.507	0.36	0.010	1.28	0.661	unknown
W01H2.2	0.26	0.025	1.01	0.914	0.43	0.000	1.05	0.535	unknown
W04E12.2	0.25	0.002	1.16	0.616	0.45	0.000	0.97	0.901	unknown
W06D11.1	0.39	0.001	1.12	0.199	0.48	0.000	0.99	0.836	unknown
Y110A2AL.1	0.30	0.001	1.01	0.937	0.40	0.001	1.03	0.824	unknown
Y116A8B.1	0.29	0.011	0.92	0.606	0.35	0.002	0.80	0.340	unknown
Y13C8A.1	0.45	0.017	0.76	0.279	0.43	0.002	0.88	0.691	unknown
Y24F12A.3	0.47	0.008	0.80	0.207	0.46	0.006	0.68	0.053	unknown
Y37H2A.13	0.38	0.007	1.03	0.626	0.33	0.004	1.31	0.471	unknown
Y46H3C.5	0.47	0.008	0.85	0.102	0.48	0.002	1.17	0.265	unknown
Y46H3C.7	0.48	0.020	1.17	0.709	0.41	0.001	0.89	0.672	unknown
Y54G2A.3b	0.50	0.016	0.99	0.931	0.48	0.003	0.98	0.884	unknown
Y71F9AL.6	0.24	0.004	1.19	0.413	0.44	0.029	1.40	0.328	unknown
ZC53.1	0.31	0.001	1.26	0.295	0.32	0.000	1.22	0.636	unknown
Regulated in males (p<0.05, ratio>2 or <0.5) but not in hermaphrodites (p>0.05, ratio between 0.66-1.5)									
C04F6.3.1	1.28	0.492	3.14	0.031	0.83	0.558	4.26	0.037	*cht-1* - (*CHiTinase*), encodes a chitinase orthologous to human chitinase-1 (OMIM:600031, mutations are associated with chitotriosidase deficiency); CHT-1 is predicted to function as an extracellular O-glycosyl hydrolase that hydrolyzes the glycosidic bond between two or more carbohydrates; in C. elegans, CHT-1 may play a role in embryogenesis, and may also be required for cuticle degradation during molting and degradation of chitin-containing pathogens as part of a host defense mechanism, hydrolase activity, hydrolyzing O-glycosyl compounds, carbohydrate metabolic process
C04F6.3.2	1.27	0.480	2.69	0.041	0.96	0.905	4.64	0.048	see C04F6.3.1

C05B5.10	1.11	0.822	0.31	0.000	0.77	0.385	0.22	0.004	pseudogene
F01D5.3	0.85	0.610	0.28	0.043	0.88	0.875	0.20	0.015	secreted surface protein, binding
F02E8.2b	0.88	0.313	0.49	0.044	0.89	0.209	0.41	0.005	*nmur-3* - (NMUR (NeuroMedin U Receptor) homolog), FOG: 7 transmembrane receptor, G-protein coupled receptor protein signaling pathway
F59C6.6	1.36	0.195	0.28	0.001	0.93	0.638	0.15	0.001	*nlp-4* - (*Neuropeptide-Like Protein*), nlp-4 encodes three predicted neuropeptide-like proteins; nlp-4 has no none homologs in other nematode species, and its expression pattern has not been determined; in addition, as loss of nlp-4 activity via large-scale RNAi screens does not result in any obvious abnormalities, the precise role of nlp-4-encoded peptides in development and/or behavior is not yet known
Y53F4B.16	0.89	0.681	5.37	0.006	0.98	0.907	3.56	0.021	unknown
Y71H2AM.25	0.92	0.558	2.13	0.018	1.17	0.327	0.48	0.031	cysteine proteinase Cathepsin L, metallocarboxypeptidase activity
D1046.7	0.75	0.454	0.48	0.044	0.77	0.348	0.30	0.021	unknown
F35E12.6.1	0.72	0.129	0.49	0.032	1.08	0.816	0.39	0.003	unknown
F57G8.7	1.13	0.817	0.20	0.026	0.77	0.646	0.07	0.005	unknown

[a] Information about genes was obtained from Wormbase.

Supporting information table S9. Expression level and DR to ad libitum ratio of sperm-associated genes in both sexes at young adult (66 h) stage.

Young adult (66 h)		Expression level hermaphrodite				Expression level male			
Gene name	Gene ID	DR	*ad libitum*	Ratio DR/*ad libitum*	P-value	DR	*ad libitum*	Ratio DR/*ad libitum*	P-value
Major sperm proteins (*msp*)									
msp-3	F26G1.7	61551.3	59653.2	1.03	0.703	152788.8	161129.3	0.95	0.578
msp-10	K07F5.2	112542.5	105367.9	1.07	0.669	129867.6	140875.8	0.92	0.372
msp-31	R05F9.13	72092.2	71503.0	1.01	0.944	143852.5	144933.8	0.99	0.876
msp-32	R05F9.3	146086.7	156001.2	0.94	0.361	193195.1	207503.5	0.93	0.183
msp-33	R05F9.8	47846.4	52126.9	0.92	0.464	87107.0	94479.4	0.92	0.241
msp-36	C04G2.4	80011.0	85158.2	0.94	0.560	159211.5	165560.8	0.96	0.572
msp-37	K08F4.10	2.9	2.6	1.08	0.323	4.4	4.6	0.97	0.879
msp-38	K08F4.8	45557.5	53398.9	0.85	0.293	114016.8	116680.4	0.98	0.823
msp-40	C33F10.9	68133.3	69300.0	0.98	0.879	156501.5	157350.2	0.99	0.885
msp-42	F26G1.8	12.2	12.0	1.02	0.905	44.5	41.3	1.08	0.672
msp-45	F58A6.8	36180.6	39717.1	0.91	0.410	93172.1	111040.4	0.84	0.190
msp-49	C34F11.6	24181.8	25812.2	0.94	0.519	69195.4	75206.8	0.92	0.497
msp-50	C34F11.4	32604.2	35337.9	0.92	0.500	94206.3	101224.8	0.93	0.245
msp-51	ZK354.5	158435.4	160946.5	0.98	0.758	187355.1	197002.5	0.95	0.222
msp-52	F36H12.6	229.8	192.6	1.19	0.041	472.3	472.9	1.00	0.990
msp-55	C09B9.6	136045.4	129234.1	1.05	0.207	143938.9	144792.4	0.99	0.912
msp-56	K07F5.3	127883.9	120773.1	1.06	0.037	156215.6	166980.8	0.94	0.092
msp-57	R13H9.2	152431.6	153724.8	0.99	0.890	209829.2	215193.5	0.98	0.447
msp-58	ZK354.10	785.1	1466.1	0.54	0.011	497.1	833.3	0.60	0.181
msp-59	ZK354.11	124156.1	115558.4	1.07	0.386	133954.4	151301.6	0.89	0.040
msp-63	K05F1.7	90149.9	87285.7	1.03	0.515	137263.3	155097.7	0.89	0.156
msp-64	ZK1248.6	67763.2	64253.5	1.05	0.686	103673.3	117225.8	0.88	0.075
msp-65	ZK354.1	138584.2	137795.4	1.01	0.848	164377.8	165207.9	0.99	0.906
msp-71	F59A6.8	1068.5	1393.7	0.77	0.067	1048.5	875.9	1.20	0.154
msp-74	F09C12.7	197199.1	204055.9	0.97	0.657	240911.8	241232.4	1.00	0.964
msp-76	ZK1251.6	114933.9	102667.8	1.12	0.429	103204.2	114864.2	0.90	0.122
msp-77	F32B6.6	169048.1	156944.2	1.08	0.232	193248.1	191806.5	1.01	0.914
msp-78	T13F2.11	141854.7	130393.3	1.09	0.356	156790.3	172176.6	0.91	0.096

msp-79	T13F2.10	139816.8	131616.1	1.06	0.392	155425.9	166880.1	0.93	0.130
msp-81	K07F5.1	198621.6	198206.2	1.00	0.876	223388.3	236789.1	0.94	0.314
msp-113	ZK354.4.1	171536.9	169669.5	1.01	0.531	182125.0	196402.9	0.93	0.064
	ZK354.4.2	209070.2	207478.5	1.01	0.918	203634.6	232479.5	0.88	0.127
msp-142	K05F1.2	29062.2	30546.6	0.95	0.564	77177.2	89869.1	0.86	0.352
msp-152	ZK546.6	93659.0	93127.4	1.01	0.917	134283.5	138788.9	0.97	0.304
Major sperm protein domain									
C35A5.4	C35A5.4	428.7	437.4	0.98	0.860	1629.2	1575.6	1.03	0.632
C35E7.9	C35E7.9	2154.6	1998.8	1.08	0.488	7201.5	6473.1	1.11	0.425
C36H8.1	C36H8.1	12180.2	10414.2	1.17	0.075	32426.3	32214.4	1.01	0.931
F21H7.5	F21H7.5	8155.5	9469.3	0.86	0.306	21381.9	25612.8	0.83	0.168
F36D3.4	F36D3.4	5886.7	6567.8	0.90	0.354	16819.3	19206.7	0.88	0.283
F36H12.3	F36H12.3	2490.6	2428.3	1.03	0.819	8988.6	7897.9	1.14	0.212
F42A9.7	F42A9.7	2133.9	2301.1	0.93	0.197	6176.5	6949.8	0.89	0.206
msd-1	F44D12.3	37125.1	40637.3	0.91	0.293	100449.6	118963.9	0.84	0.050
msd-2	F44D12.5	47308.1	47942.3	0.99	0.877	111378.2	115506.0	0.96	0.673
msd-3	F44D12.7	51959.0	52414.2	0.99	0.903	129199.7	146126.8	0.88	0.116
msd-4	C35D10.11	18796.3	18031.5	1.04	0.821	53311.8	54897.9	0.97	0.564
F53B6.4	F53B6.4	19246.0	20240.8	0.95	0.583	52764.7	61093.6	0.86	0.095
K08E3.2	K08E3.2	9.8	11.9	0.82	0.654	17.4	13.6	1.28	0.274
W03D8.5	W03D8.5	754.8	918.0	0.82	0.114	2700.5	2318.7	1.16	0.189
Y23H5A.4	Y23H5A.4	2403.8	1971.9	1.22	0.005	5061.8	6842.0	0.74	0.089
Y53F4B.19	Y53F4B.19	1270.6	1563.6	0.81	0.239	5534.7	4544.1	1.22	0.097
ZK1248.17	ZK1248.17	107234.5	107195.9	1.00	0.995	143186.7	153791.4	0.93	0.194
Sperm-specific family, class P (*ssp*)									
ssp-9	T28H11.6	22502.7	24705.2	0.91	0.412	52737.7	61991.4	0.85	0.054
ssp-10	K07F5.9.1	77491.0	80315.2	0.96	0.767	176943.8	180301.7	0.98	0.722
	K07F5.9.2	23898.9	26851.2	0.89	0.310	76619.2	86649.6	0.88	0.186
ssp-11	E03H12.10	50627.4	54060.1	0.94	0.581	127377.9	131812.0	0.97	0.642
ssp-16	T27A3.3	2544.1	2530.9	1.01	0.940	8635.9	10338.4	0.84	0.178
ssp-19	C55C2.2	1439.2	1873.2	0.77	0.110	7573.6	9316.9	0.81	0.180
ssp-31	ZK1225.6	4106.4	4477.9	0.92	0.213	13585.5	16567.8	0.82	0.153
ssp-32	F32B6.7	810.6	778.7	1.04	0.440	2419.7	3032.8	0.80	0.083
ssp-33	R08A2.3	4528.5	4706.4	0.96	0.557	15749.3	18574.7	0.85	0.097

ssp-34	ZC168.6	8082.7	9703.1	0.83	0.256	27179.5	30526.9	0.89	0.348
ssp-35	F55C5.1	2148.7	1923.2	1.12	0.173	5816.8	6251.1	0.93	0.259
ssp-36	T13F2.12	1509.7	1353.7	1.12	0.184	4304.5	4822.1	0.89	0.328
ssp-37	W02D9.5	1437.6	2253.9	0.64	0.001	5.5	3.6	1.53	0.068
Sper-specific family, class Q (*ssq*)									
ssq-1	K07F5.11	72230.4	71375.6	1.01	0.851	176806.5	199616.8	0.89	0.026
ssq-2	T28H11.5	84127.5	86289.3	0.97	0.864	155011.6	174113.8	0.89	0.206
ssq-3	ZC477.1	24572.5	26779.1	0.92	0.277	72328.9	84781.0	0.85	0.226
ssq-4	T28H11.1	48103.7	51342.4	0.94	0.555	103900.6	119711.8	0.87	0.095
Sperm-specific family, class S (*sss*)									
sss-1	F32B6.5	9057.7	9957.3	0.91	0.266	28825.2	31030.1	0.93	0.427
sss-2	F47B8.11	13569.7	15158.4	0.90	0.347	29195.4	35774.3	0.82	0.050
Sperm-specific transcript (*sst*)									
sst-20	F54C1.9	7239.2	6029.7	1.20	0.230	19512.0	21003.3	0.93	0.407
Spermatogenesis-defective (*spe*)									
spe-4	ZK524.1	5109.2	4121.0	1.24	0.105	13242.0	14915.3	0.89	0.165
spe-6	Y66D12A.20	2098.8	1956.9	1.07	0.518	5691.6	6674.1	0.85	0.086
spe-8	F53G12.6	428.8	489.9	0.88	0.089	1169.8	1881.4	0.62	0.088
spe-9	C17D12.6a	442.8	445.7	0.99	0.962	1358.9	1570.6	0.87	0.064
	C17D12.6b	227.8	237.2	0.96	0.878	684.6	925.4	0.74	0.058
spe-10	AC3.10	1324.2	1203.6	1.10	0.315	3949.7	4342.5	0.91	0.218
spe-11	F48C1.7	4091.7	17519.8	0.23	0.421	12253.3	12239.5	1.00	0.986
spe-12	T02E1.1	362.5	490.7	0.74	0.034	1584.4	1668.5	0.95	0.424
spe-15	F47G6.4	4820.1	4647.7	1.04	0.472	12351.3	15653.4	0.79	0.040
spe-17	ZK617.3	3756.1	2341.2	1.60	0.010	7297.8	7726.2	0.94	0.400
spe-19	Y113G7A.10	203.4	216.4	0.94	0.583	538.2	601.5	0.89	0.275
spe-26	R10H10.2	851.4	951.4	0.89	0.067	2593.8	3104.9	0.84	0.089
spe-27	C06E7.6	523.2	545.7	0.96	0.464	1132.9	1524.9	0.74	0.058
spe-29	F25H8.7	77.6	90.9	0.85	0.323	197.9	301.8	0.66	0.018
spe-38	Y52B11A.1	206.0	198.6	1.04	0.756	450.9	594.6	0.76	0.090
spe-39	ZC404.3a	1229.2	1658.0	0.74	0.003	1066.6	1339.1	0.80	0.004
	ZC404.3b	876.5	1294.1	0.68	0.019	822.7	1022.2	0.80	0.074
spe-41	K01A11.4	2831.3	2157.8	1.31	0.004	5575.6	7336.0	0.76	0.035
spe-42	B0240.2	284.4	257.7	1.10	0.618	842.0	978.6	0.86	0.133

Supporting information table S10. Expression level and DR to *ad libitum* ratio of sperm-associated genes in both sexes at adult (76 h) stage.

Adult (76 h)		Expression level hermaphrodite				Expression level male			
Gene name	Gene ID	DR	*ad libitum*	Ratio DR/*ad libitum*	P-value	DR	*ad libitum*	Ratio DR/*ad libitum*	P-value
Major sperm proteins (*msp*)									
msp-3	F26G1.7	64040.2	24192.3	2.65	0.030	269090.4	281646.4	0.96	0.413
msp-10	K07F5.2	131901.0	100977.8	1.31	0.019	181704.1	179300.8	1.01	0.761
msp-31	R05F9.13	76473.7	31095.6	2.46	0.009	198026.3	188882.9	1.05	0.560
msp-32	R05F9.3	159575.7	90204.0	1.77	0.003	260934.0	241219.4	1.08	0.143
msp-33	R05F9.8	52674.9	24440.3	2.16	0.014	140419.8	127511.9	1.10	0.364
msp-36	C04G2.4	98584.8	41346.3	2.38	0.008	231641.0	235186.2	0.98	0.701
msp-37	K08F4.10	4.6	7.3	0.62	0.355	12.2	9.8	1.25	0.353
msp-38	K08F4.8	50236.8	23454.7	2.14	0.029	154705.8	115685.3	1.34	0.200
msp-40	C33F10.9	76706.9	26911.0	2.85	0.023	217670.9	220502.1	0.99	0.755
msp-42	F26G1.8	17.1	15.6	1.10	0.544	85.0	61.9	1.37	0.007
msp-45	F58A6.8	44299.6	16207.3	2.73	0.023	175583.5	179508.3	0.98	0.640
msp-49	C34F11.6	27557.1	10859.2	2.54	0.015	126916.8	134368.5	0.94	0.544
msp-50	C34F11.4	35899.8	15106.4	2.38	0.031	173640.6	196666.3	0.88	0.129
msp-51	ZK354.5	188374.4	126612.5	1.49	0.003	244240.2	237424.8	1.03	0.657
msp-52	F36H12.6	245.0	242.5	1.01	0.754	639.9	856.3	0.75	0.056
msp-55	C09B9.6	169863.7	96328.7	1.76	0.010	194046.9	192246.9	1.01	0.908
msp-56	K07F5.3	150632.4	96330.9	1.56	0.008	210404.2	224257.5	0.94	0.194
msp-57	R13H9.2	180923.2	109099.2	1.66	0.000	272123.9	269380.6	1.01	0.873
msp-58	ZK354.10	2713.7	3836.4	0.71	0.096	499.0	857.6	0.58	0.023
msp-59	ZK354.11	162052.6	96011.0	1.69	0.005	201666.4	197430.3	1.02	0.728
msp-63	K05F1.7	103693.3	45648.6	2.27	0.008	202541.4	209660.2	0.97	0.455
msp-64	ZK1248.6	68366.4	34055.7	2.01	0.017	161582.9	155256.2	1.04	0.343
msp-65	ZK354.1	168745.6	93636.2	1.80	0.005	215367.3	211428.2	1.02	0.778
msp-71	F59A6.8	2292.2	2319.2	0.99	0.848	1236.2	1399.0	0.88	0.180
msp-74	F09C12.7	238402.0	172402.3	1.38	0.007	298772.3	295894.1	1.01	0.809
msp-76	ZK1251.6	135863.5	100568.8	1.35	0.004	156338.0	160702.1	0.97	0.704
msp-77	F32B6.6	208059.8	153791.9	1.35	0.002	257993.5	257393.9	1.00	0.937
msp-78	T13F2.11	164865.7	125301.7	1.32	0.014	218299.3	214166.4	1.02	0.644

msp-79	T13F2.10	164473.2	127122.6	1.29	0.009	213420.3	206474.7	1.03	0.643
msp-81	K07F5.1	247529.1	154881.5	1.60	0.003	289411.3	289319.6	1.00	0.992
msp-113	ZK354.4.1	216107.2	145328.5	1.49	0.003	242702.2	240856.1	1.01	0.900
	ZK354.4.2	263671.4	174058.5	1.51	0.012	268219.5	291720.9	0.92	0.296
msp-142	K05F1.2	30202.4	10268.1	2.94	0.039	146905.3	162602.3	0.90	0.025
msp-152	ZK546.6	109381.7	52932.2	2.07	0.001	181413.5	189468.5	0.96	0.240
Major sperm protein domain									
C35A5.4	C35A5.4	239.6	75.6	3.17	0.032	2872.6	3642.0	0.79	0.020
C35E7.9	C35E7.9	1467.9	538.8	2.72	0.035	10999.6	14537.1	0.76	0.019
C36H8.1	C36H8.1	7813.2	2184.1	3.58	0.044	59432.4	67133.6	0.89	0.090
F21H7.5	F21H7.5	9683.6	3152.5	3.07	0.039	44848.0	48075.4	0.93	0.204
F36D3.4	F36D3.4	6879.1	2249.4	3.06	0.034	36590.0	38705.6	0.95	0.372
F36H12.3	F36H12.3	1899.8	651.6	2.92	0.027	14447.3	17514.7	0.82	0.114
F42A9.7	F42A9.7	1794.9	661.2	2.71	0.032	13241.1	15647.5	0.85	0.048
msd-1	F44D12.3	44853.5	18310.8	2.45	0.011	193077.6	201018.8	0.96	0.502
msd-2	F44D12.5	61811.2	24634.5	2.51	0.029	176754.1	184927.1	0.96	0.434
msd-3	F44D12.7	62419.9	23528.2	2.65	0.020	222757.1	232168.3	0.96	0.149
msd-4	C35D10.11	21016.1	8524.7	2.47	0.031	97337.0	78089.3	1.25	0.334
F53B6.4	F53B6.4	22076.3	7578.2	2.91	0.047	105336.1	118704.2	0.89	0.181
K08E3.2	K08E3.2	30.7	11.7	2.62	0.020	45.6	37.6	1.21	0.415
W03D8.5	W03D8.5	763.3	255.4	2.99	0.032	4835.3	5151.3	0.94	0.489
Y23H5A.4	Y23H5A.4	1357.0	563.4	2.41	0.007	11122.4	13919.8	0.80	0.009
Y53F4B.19	Y53F4B.19	1061.4	506.1	2.10	0.049	7861.0	8274.7	0.95	0.615
ZK1248.17	ZK1248.17	124332.3	61337.7	2.03	0.003	211886.6	198518.3	1.07	0.166
Sperm-specific family, class P (*ssp*)									
ssp-9	T28H11.6	28359.8	11371.8	2.49	0.020	87114.5	95735.0	0.91	0.481
ssp-10	K07F5.9.1	96351.1	41206.2	2.34	0.013	248697.2	242602.2	1.03	0.748
	K07F5.9.2	30648.2	13600.3	2.25	0.024	147414.8	164010.7	0.90	0.061
ssp-11	E03H12.10	61393.4	24873.8	2.47	0.022	195174.4	207611.5	0.94	0.192
ssp-16	T27A3.3	1999.2	810.0	2.47	0.032	16380.4	23073.9	0.71	0.024
ssp-19	C55C2.2	2965.0	1380.1	2.15	0.060	21844.8	30668.5	0.71	0.062
ssp-31	ZK1225.6	5053.0	2001.0	2.53	0.082	30552.0	37599.0	0.81	0.139
ssp-32	F32B6.7	648.5	265.3	2.44	0.072	5989.0	6671.7	0.90	0.416
ssp-33	R08A2.3	5142.8	2031.7	2.53	0.102	34522.7	42195.4	0.82	0.086

ssp-34	ZC168.6	8182.3	3481.5	2.35	0.061	57018.9	45694.2	1.25	0.394
ssp-35	F55C5.11	106.6	104.9	1.02	0.932	309.0	268.5	1.15	0.284
ssp-36	T13F2.12	1217.9	514.0	2.37	0.085	10126.5	11540.6	0.88	0.258
ssp-37	W02D9.5	3933.4	4155.8	0.95	0.575	41.9	35.3	1.19	0.849
Sper-specific family, class Q (*ssq*)									
ssq-1	K07F5.11	72991.9	30459.0	2.40	0.005	272247.7	295281.7	0.92	0.046
ssq-2	T28H11.5	89513.0	42590.6	2.10	0.015	197145.5	211884.2	0.93	0.428
ssq-3	ZC477.1	23214.6	9193.0	2.53	0.025	125364.0	146897.2	0.85	0.014
ssq-4	T28H11.1	52417.5	21897.7	2.39	0.020	157185.6	176852.0	0.89	0.185
Sperm-specific family, class S (*sss*)									
sss-1	F32B6.5	10268.5	3412.5	3.01	0.023	52673.0	54892.5	0.96	0.571
sss-2	F47B8.11	13325.8	3562.5	3.74	0.025	58471.6	65486.7	0.89	0.048
Sperm-specific transcript (*sst*)									
sst-20	F54C1.9	4473.6	1378.5	3.25	0.044	40863.2	46517.0	0.88	0.038
Spermatogenesis-defective (*spe*)									
spe-4	ZK524.1	2691.6	1038.0	2.59	0.040	23463.9	28670.3	0.82	0.010
spe-6	Y66D12A.20	1285.4	798.3	1.61	0.071	9909.1	12501.0	0.79	0.055
spe-8	F53G12.6	284.9	109.1	2.61	0.074	2729.9	3055.6	0.89	0.278
spe-9	C17D12.6a	239.2	99.7	2.40	0.004	2063.3	2749.9	0.75	0.001
	C17D12.6b	134.4	51.7	2.60	0.033	1129.3	1653.0	0.68	0.008
spe-10	AC3.10	743.9	312.3	2.38	0.042	7155.5	9002.9	0.79	0.064
spe-11	F48C1.7	3919.9	1339.7	2.93	0.019	23477.1	27992.2	0.84	0.011
spe-12	T02E1.1	451.3	218.1	2.07	0.057	2690.8	3643.5	0.74	0.004
spe-15	F47G6.4	4403.4	2259.7	1.95	0.024	24509.6	26981.0	0.91	0.192
spe-17	ZK617.3	1410.8	460.7	3.06	0.079	13874.5	16209.8	0.86	0.179
spe-19	Y113G7A.10	88.0	34.2	2.57	0.033	884.2	1137.6	0.78	0.010
spe-26	R10H10.2	625.2	251.9	2.48	0.053	5256.7	5649.2	0.93	0.628
spe-27	C06E7.6	428.4	158.8	2.70	0.052	2682.2	3647.4	0.74	0.037
spe-29	F25H8.7	58.9	19.2	3.06	0.062	404.5	622.3	0.65	0.014
spe-38	Y52B11A.1	112.2	43.5	2.58	0.018	750.8	1248.5	0.60	0.040
spe-39	ZC404.3a	1229.2	1658.0	0.74	0.003	1066.6	1339.1	0.80	0.004
	ZC404.3b	2460.1	2915.0	0.84	0.221	1231.6	1534.7	0.80	0.069
spe-41	K01A11.4	1590.1	729.6	2.18	0.054	11489.9	13461.3	0.85	0.092
spe-42	B0240.2	109.0	41.9	2.60	0.050	1327.8	1869.8	0.71	0.011

6.2 Acknowledgments

List of publications

Parts of this work have been accepted or submitted for publication:

Paternal dietary restriction affects progeny fat content in *Caenorhabditis elegans.*
Claudia Miersch, Frank Döring
IUBMB life, accepted 30-Mar-2012

Sex differences in carbohydrate metabolism are linked to gene expression in *Caenorhabditis elegans.*
Claudia Miersch, Frank Döring
PLoS one, submitted 13-Apr-2012

DANKSAGUNG

Dear Dr. Dougherty,
I am planning to start work on a small metazoan and, from the work you have done with free-living nematodes, it seems that these might be good organisms for me to start with. I am therefore writing to ask you for a culture of *Caenorhabditis elegans*, Bristol strain…Since I have never done anything in this field I hope you will not mind if, from time to time, I have to write to you for advice. One point that puzzles me at the moment is how one gets males, and if one has them, is it possible to propagate them by crosses with the hermaphrodites?

Yours sincerely
Sydney Brenner
(E.C. Friedberg, “Sydney Brenner A Biography”, 2010, Cold Spring Harbor Laboratory Press)

Diese Worte von Nobelpreisträger Sydney Brenner erinnern mich an meinen persönlichen Einstieg mit dem „Wurm“ und machen deutlich, dass ohne Unterstützung viele gute Arbeiten gar nicht erst entstehen können. Während meiner Promotion wurde auch ich in vielerlei Hinsicht von einigen Menschen unterstützt. Daher möchte ich diese Gelegenheit nutzen Danke zusagen.

An erster Stelle möchte ich mich bei meinem Doktorvater Professor Dr. Frank Döring für die Bereitstellung des faszinierenden Themas sowie für die zahlreichen Anregungen, Ratschläge und Ideen bedanken. Besonders geholfen hat mir der verständnisvolle Umgang, das entgegengebrachte Vertrauen und die Freiheit bei der Ausgestaltung der Promotion.

Ein herzlicher Dank gilt Frau Dr. Alexandra Fischer für die wertvolle sachkundige Unterstützung in wissenschaftlichen Fragestellungen und für ein immer offenes Ohr in „nicht-wissenschaftlichen“ Angelegenheiten.

Allen gegenwärtigen und ehemaligen Mitarbeitern danke ich für drei tolle Jahre in der Molpräv-„WG“ mit interessanten und konstruktiven Gesprächen sowie humorvollen und abwechslungsreichen Pausen.

Ein weiterer Dank gilt meinen Freunden Laura Haffert und Gareth Crutchley für das fleißige Korrekturlesen.

Von ganzem Herzen möchte ich meiner Familie danken, die immer für mich da ist und mir in allen Lebenslagen den Rücken stärkt.

Einen ganz besonderen Dank möchte ich meinem Mann Matthias aussprechen, der mich bei meiner beruflichen und persönlichen Entwicklung, auf seine ganz eigene wohlmeinende Art und Weise, unterstützt und mir stets mit seiner Liebe zur Seite steht.

Lebenslauf

Persönliche Daten

Geburtsname	*Kürbitz*
Geburtstag	*14.09.1983*
Geburtsort	*Bergen auf Rügen, Mecklenburg Vorpommern*
Staatsangehörigkeit	*deutsch*
Familienstand	*verheiratet*

Promotion

seid 04/2009	*Promotion in der Abteilung Molekulare Prävention bei Prof. Dr. F. Döring, Institut für Humanernährung und Lebensmittelkunde, Universität Kiel*

Studium

03/2009	*Abschluss Master of Science in Ökotrophologie mit Auszeichnung, Schwerpunkt Ernährungswissenschaften, Universität Kiel* ***Master-Arbeit:** „Therapeutic potential of green tea catechins in pancreatic adenocarcinoma treatment“, Molekulare Onkology, Uniklinikum Schleswig-Holstein*
10/2003-02/2007	*Abschluss Bachelor of Science in Ökotrophologie, Schwerpunkt Ernährungswissenschaften, Universität Kiel* ***Bachelor-Arbeit:** „Association between polymorphisms in PPARγ-cofactor genes and endurance performance in the Genathlete study“, Molekulare Ernährung,*
10/2006-03/2007	*Praktikum in der Abteilung Molekulare Ernährung bei Prof. Dr. F. Döring, Institut für Humanernährung und Lebensmittelkunde*

Schulische Bildung

06/2003	*Allgemeine Hochschulreife*
08/1994-06/2003	*Ernst-Moritz-Arndt-Gymnasium, Bergen auf Rügen*
08/1990-07/1994	*Grundschule Sellin, Ostseebad Sellin*

Eidesstattliche Erklärungen

M.Sc. oec. troph. Claudia Miersch

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel „Sex differences in *Caenorhabditis elegans* in body composition, lipid storage and gene expression under *ad libitum* and dietary restriction conditions“ selbständig und ohne unerlaubte Hilfe angefertigt habe und dass ich die Arbeit noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt habe.

Kiel, den 11. Mai 2012

Hiermit erkläre ich, dass gegen mich kein strafrechtliches Ermittlungsverfahren schwebt.

Kiel, den 11. Mai 2012

Hiermit erkläre ich, dass die Arbeit den Grundsätzen der guten wissenschaftlichen Praxis, wie sie von der DFG definiert worden sind, entspricht.

Kiel, den 11. Mai 2012