Novel Model Organisms for the Study Of Diabetic Complications

August 5, 2010

1. What is the current status of the area in terms of basic, translational and clinical research with regards to all diabetic complications?

2. What is the potential impact of these studies on the field of complications research? Is there sufficient and meaningful significance to the idea/approach to move the field of complications research forward?

3. Are there technical or scientific barriers preventing progress in the area?

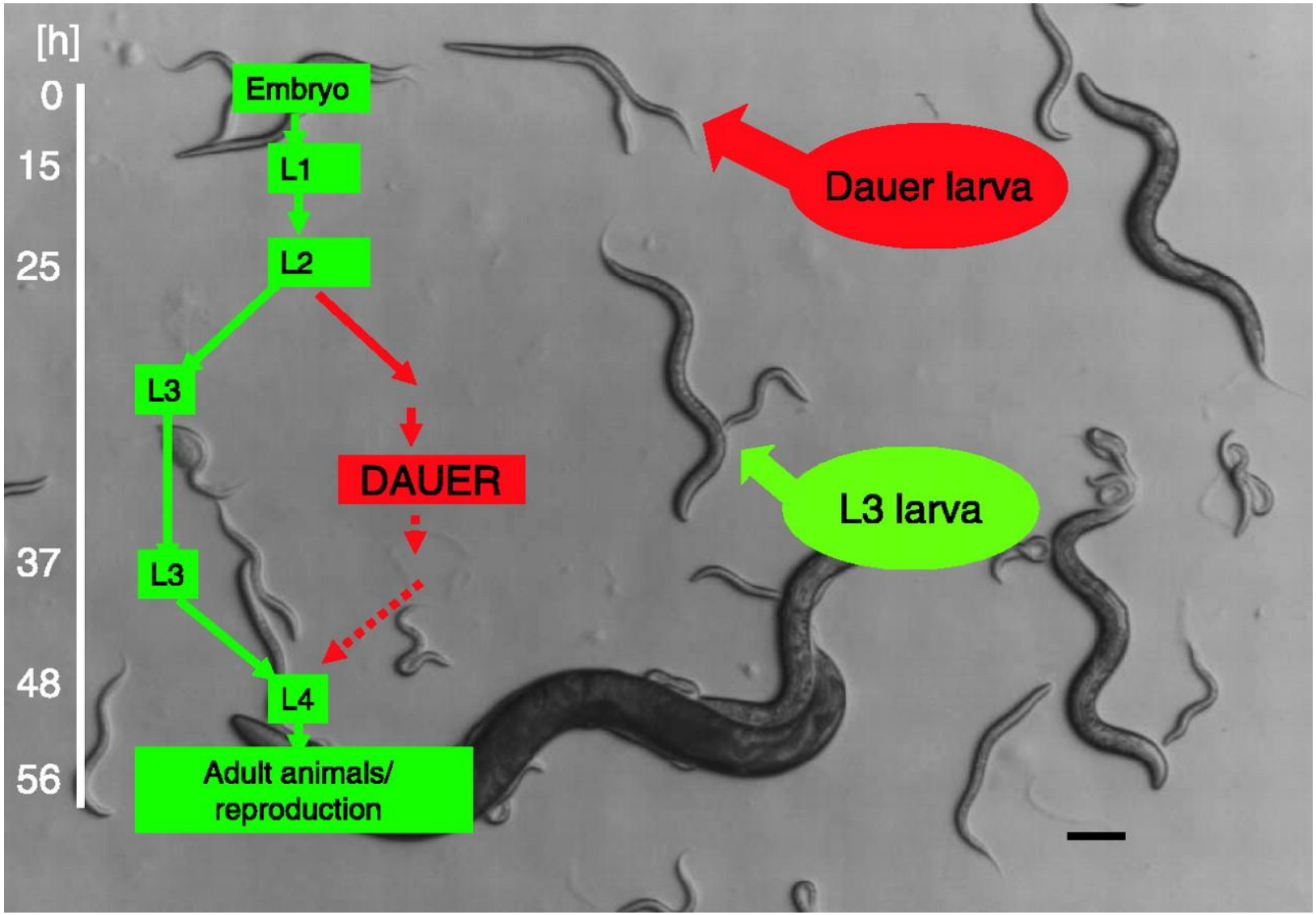
4. Are there clear objectives that can be outlined for this area which, if achieved, will enhance our understanding and treatment of complications? Are there existing human samples or resources with DNA analysis, omics, histology and pathology that could be utilized to help achieve these goals?

5. Can this area provide new progress in the quest to identify circulating blood and urine biomarkers?

6. Can existing NIH resources be leveraged to address these questions? Can partnering with existing NIH supported CTSAs, PPGs, Centers or ongoing technology driven cores to better phenotype progressive disease across models provide assistance to this area of research? Would this be useful to advance the field forward?

Can this area of research assist our understanding of all diabetic complications (including other co-morbidities such as non alcoholic fatty liver disease, peritonitis, gastrointestinal complications, peripheral vascular disease, etc)?

The development of C. elegans at 25 {degrees}C.

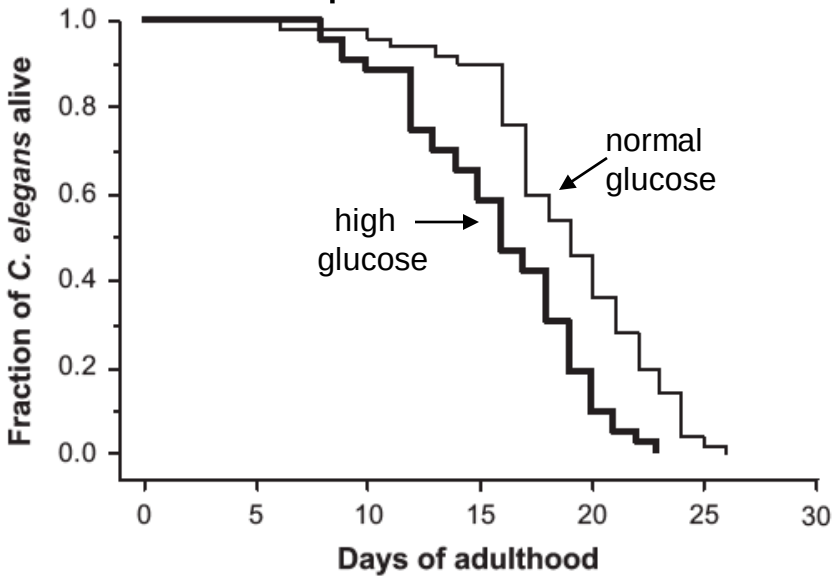


J Endocrinol. 2006 Aug;190(2):191-202

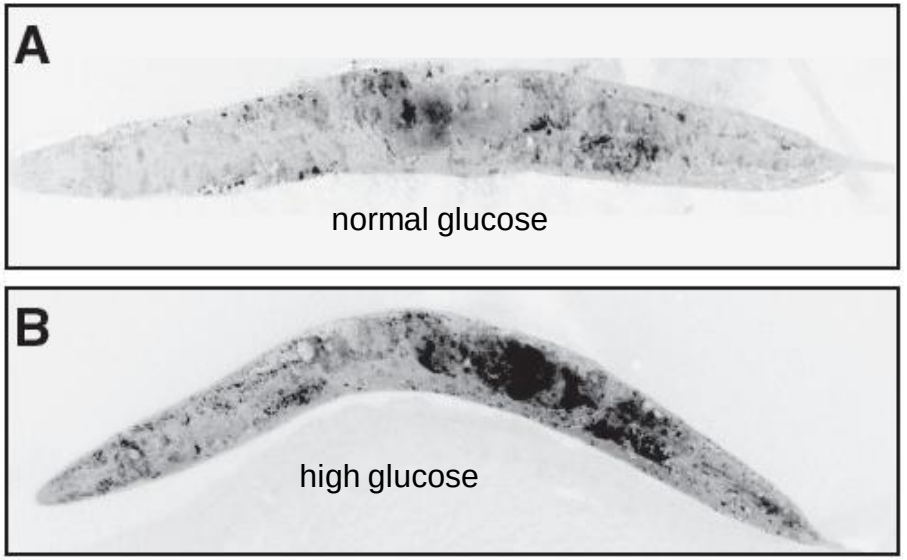
Using *C. elegans* to study effects of hyperglycemia

Schlotterer et al Diabetes 2009

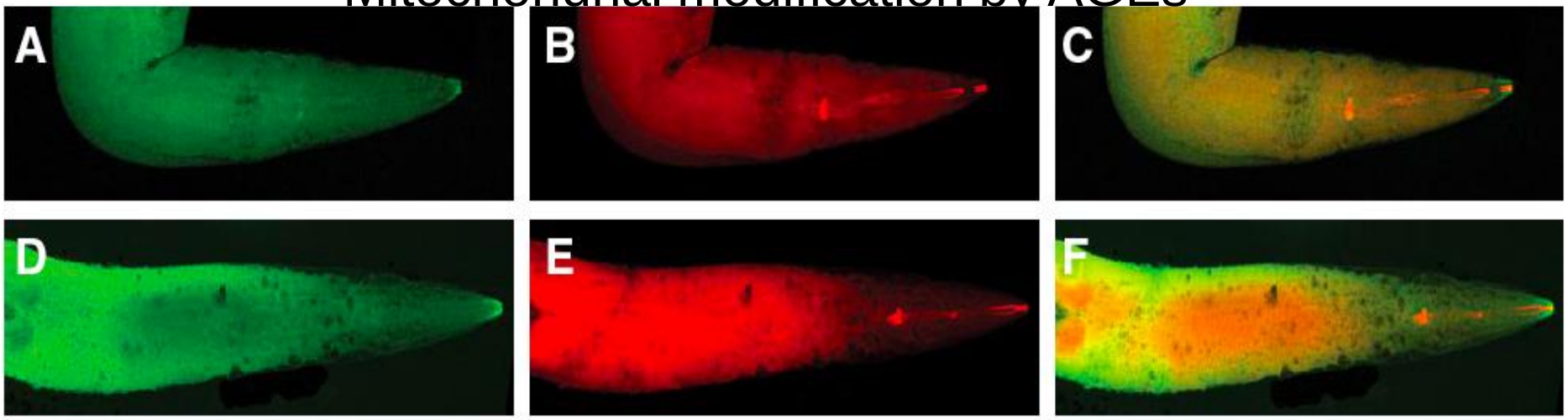
Life span



Reactive oxygen species



Mitochondrial modification by AGEs



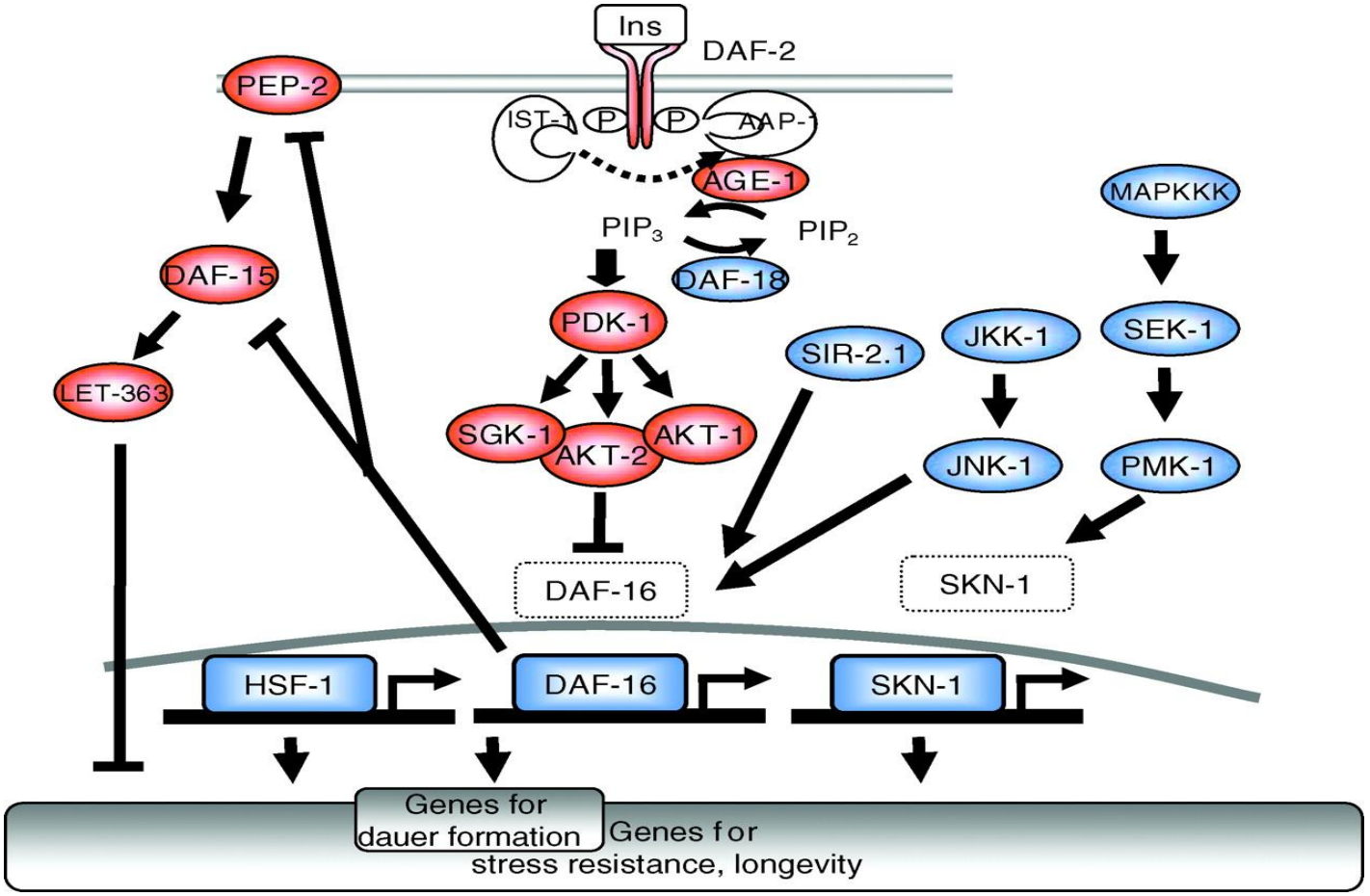
mitotracker

Methylglyoxal-derived hydroimidazolone

Merge

DAF-2 is the *C. Elegans* homologue to the mammalian insulin receptor
Although there is only one receptor, there are 38 insulin-like peptides

Model of the DAF-2 insulin/IGF-signaling pathways and their modulators

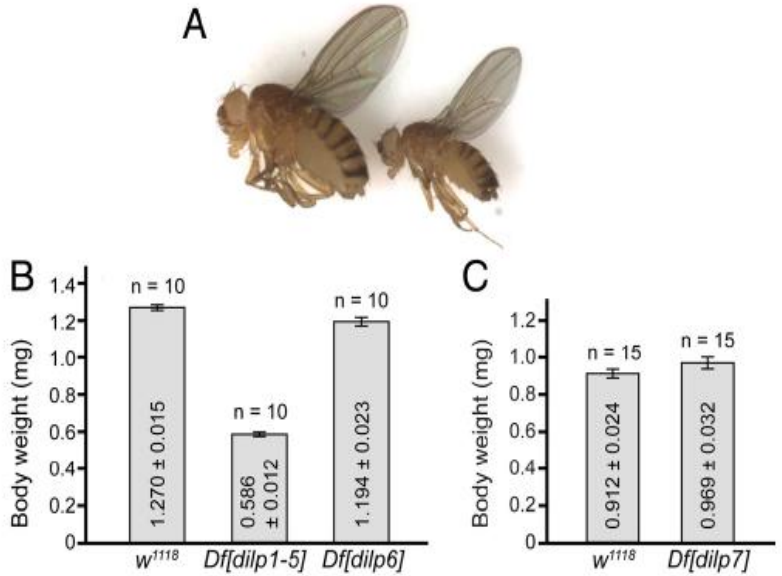


J Endocrinol. 2006 Aug;190(2):191-202

Loss of Insulin-like Peptides DILPS1-5 Produces a Diabetic-like Condition in *Drosophilia*

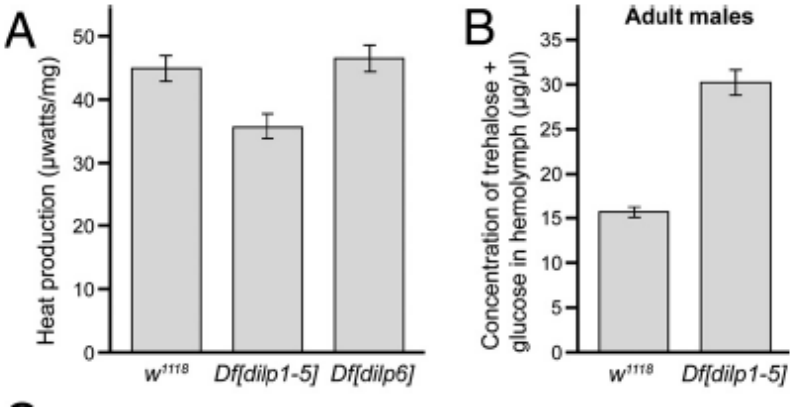
Zhang et al PNAS 2009

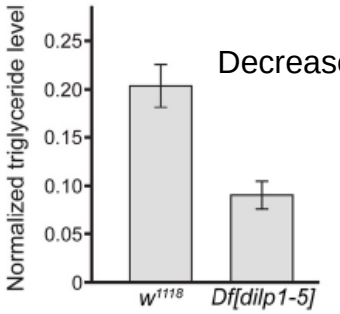
Impaired growth



Decreased metabolic rate

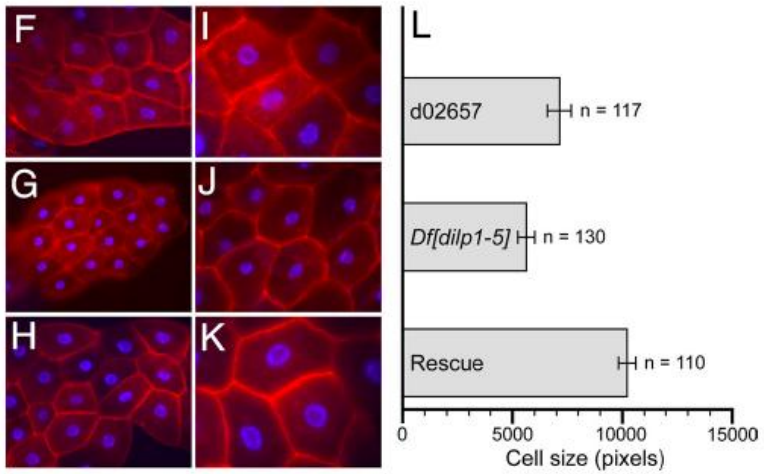
Increased circulating sugars



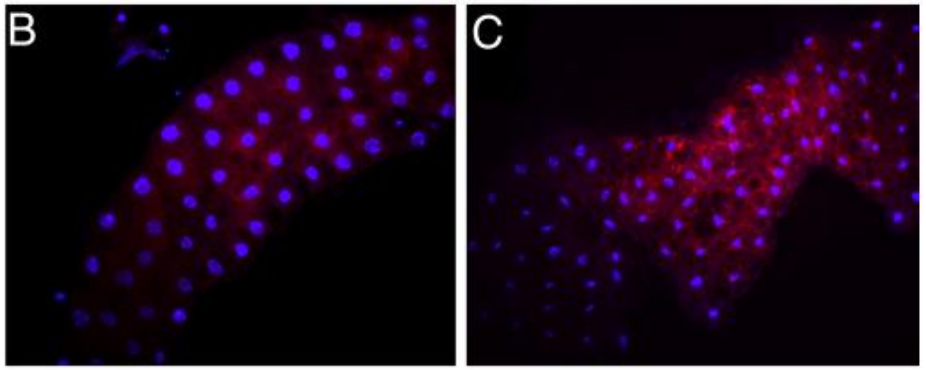


Decreased triglycerides

Decreased larval fat cell size



Fat cell autophagy



Drosophila genes affecting lipometabolism and their human orthologues.

Drosophila gene (abbreviation)	Human orthologue
Lipid droplets	
[Perilipin1/Lipid storage droplet-1], Perilipin2/Lipid storage droplet-2 ([plin1/Lsd-1], plin2/Lsd-2)[a]	*PERILIPINs (PLIN1-5)*
ADP ribosylation factor 79F (Arf79F)	*ARF1*
gartenzwerg (garz)	*GBF1*
COPI subunits	*COPI* subunits
CG7580	*UQCRQ*
brahma (brm)	*SMARCA4*
Lipoanabolism	
midway (mdy)	*DGAT1*
dSREBP/ Helix loop helix protein 106 (HLH106)	*SREBPs*
SCAP	*SCAP*
desat1	*SCD*
Lipocatabolism	
brummer (bmm)	*PNPLA2/LAL*
[Lipase 4 (Lip4)]	*LIPA/LAL*
swiss cheese (sws)	*PNPLA6/NTE*
Enigma (Egm)	*acyl-CoA dehydrogenases*
Hepatocyte nuclear factor 4 (Hnf4)	*HNF4A,G*
Cholesterol metabolism	
Niemann-Pick type C-1a (Npc1a)	NPC1
Niemann-Pick type C-1b (Npc1b)	NPC1L1
Phospholipid metabolism	
Tafazzin (Taz)	TAZ
CTP:phosphocholine cytidylyltransferase 1 and 2 (Cct1, Cct2)	PCYT1
Sphingosine-1-phosphate lyase (Sply)	SPGL1
GlcT-1	UGCG
Energy homeostasis signalling	
Insulin-like peptide 2,3,5 (Ilp2,3,5)	*INS and IGF isoforms*
Insulin-like receptor (InR)	*INSR*
chico	*IRS1-4*
Akt1	*AKT isoforms*
Lnk	*SH2B3*
widerborst (wbd)	*PPP2R5E*
forkhead box sub-group O (foxo)	*FOXO isoforms*
Adipokinetic hormone (Akh)	*GCG* (Glucagon)*
Adipokinetic hormone receptor/Gonadotropin-releasing hormone receptor (Akhr/GRHR)	
Target of rapamycin (Tor)	*MTOR*
Thor/4E-BP	*EIF4EBP*
melted (melt)	*VEPH1*
miR-278	
expanded (ex)	FERM domain proteins
Lk6	*MKNK1, 2*
Neural Lazarillo (NLaz)	APOD
Others	
adipose (adp)	*WDTC1*
bride of sevenless (boss)	*GPRC5B*
Bmcp/dUCP5	*UCP5/SCL25A14*
bubblegum (bgm)	*ACSBG1*
lethal (2) 44DEa (l(2)44DEa/ dAcsl)	*ACSL3, 4*
miR-14	
Activating transcription factor-2 (Atf-2)	*NPDC-1*
Cytochrome P450-4g1 (Cyp4g1)	lipid ω-hydrolases
Invadolysin	*LMLN*
cubitus interruptus (ci)	*GLI* isoforms
patched (ptc)	*PTCH1, 2*

The insect nephrocyte is a podocyte-like cell with a filtration slit diaphragm

Helen Weavers[1]*, Silvia Prieto-Sánchez[2]*, Ferdinand Grawe[3], Amparo Garcia-López[4], Ruben Artero[4], Michaela Wilsch-Bräuninger[5], Mar Ruiz-Gómez[2], Helen Skaer[1] & Barry Denholm[1]

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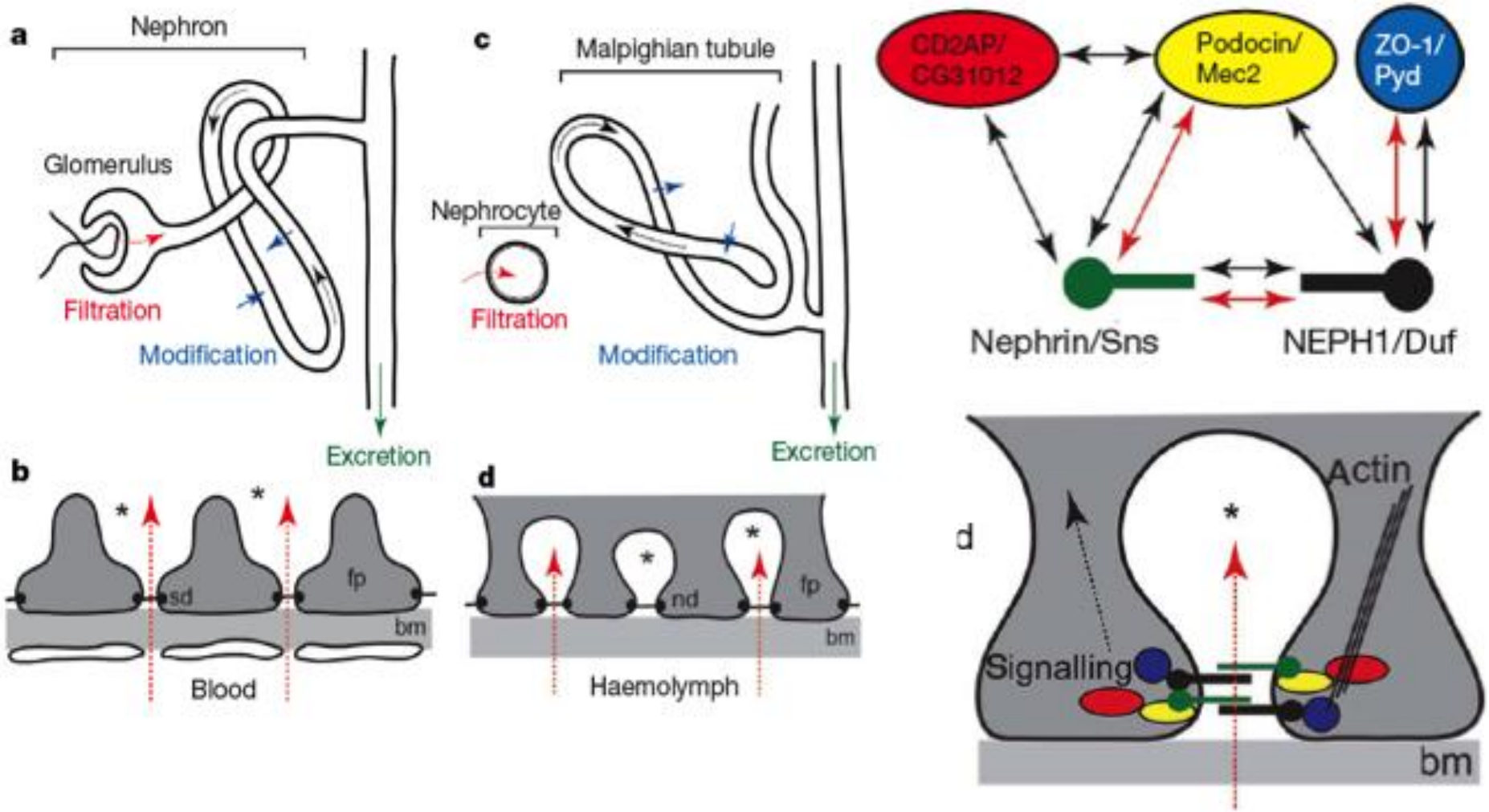
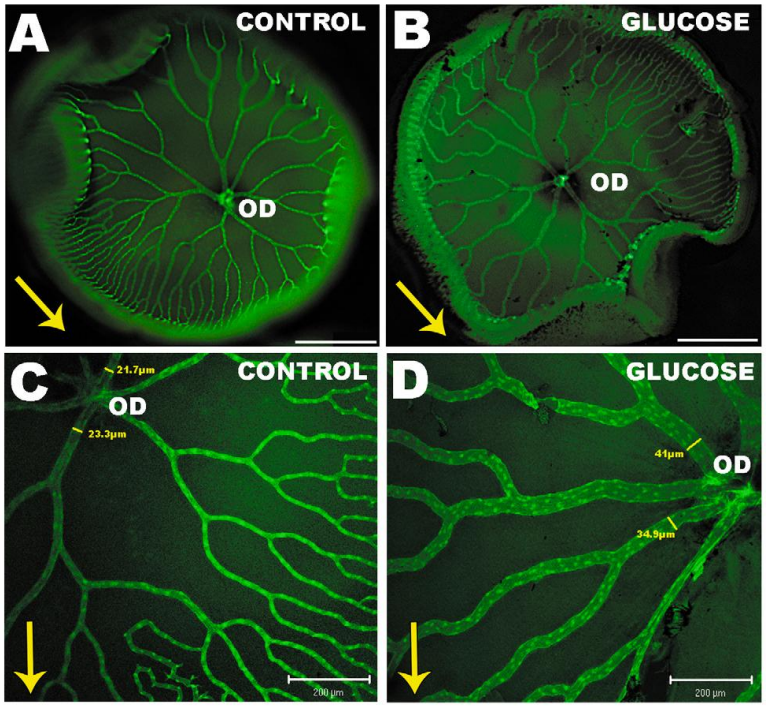
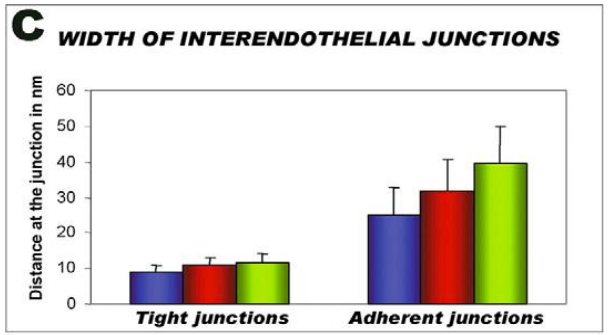
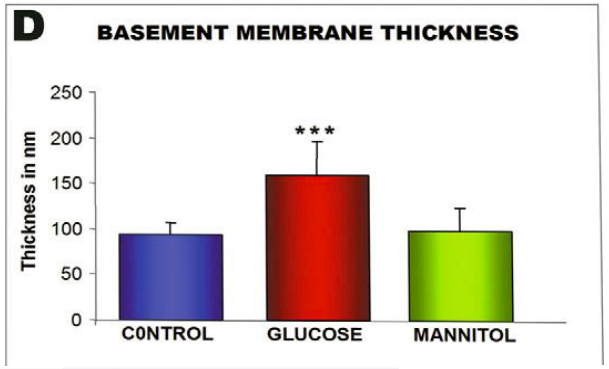


Oscillating Periods of Hyperglycemia in Zebrafish Induces Microvascular Changes and Retinopathy

Alvarez et al Disease Models and Mech 2010



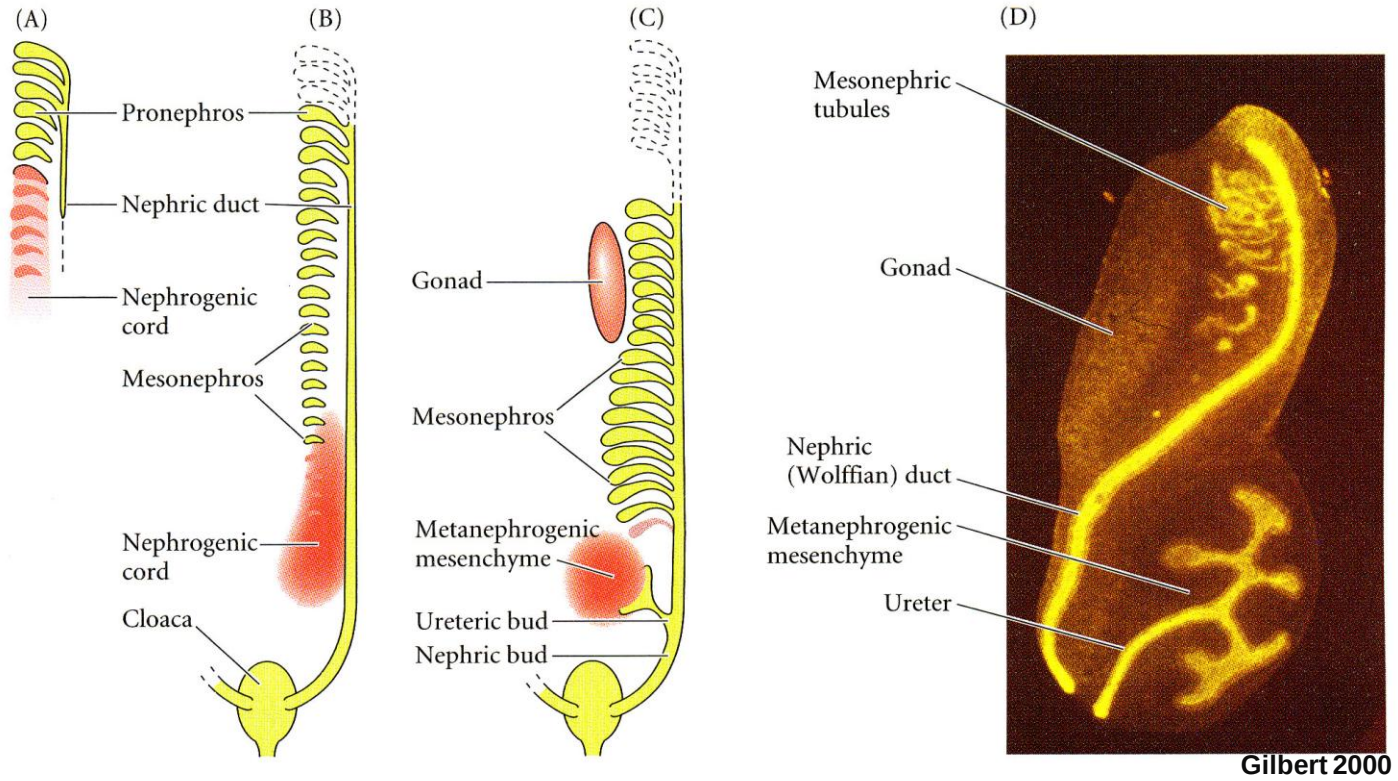




DR hallmarks		Human diabetes	Glucose-treated zebrafish
Vascular	Opening of retinal blood barrier junctions	Confirmed*	Confirmed
	Increased VEGF expression	Confirmed	Confirmed
	Dilated retinal vessels	Confirmed	Confirmed
	Basement membrane thickening in retinal capillaries	Confirmed*	Confirmed
	Loss of retinal pericytes	Confirmed*	X
	Retinal neovascularisation	Confirmed	X
	Glial activation	Confirmed*	X
Neuronal	Impaired photoreceptor physiology	Confirmed	Confirmed
	Cone morphology degeneration	?*	Confirmed
	Apoptosis in GCL and INL	?*	X

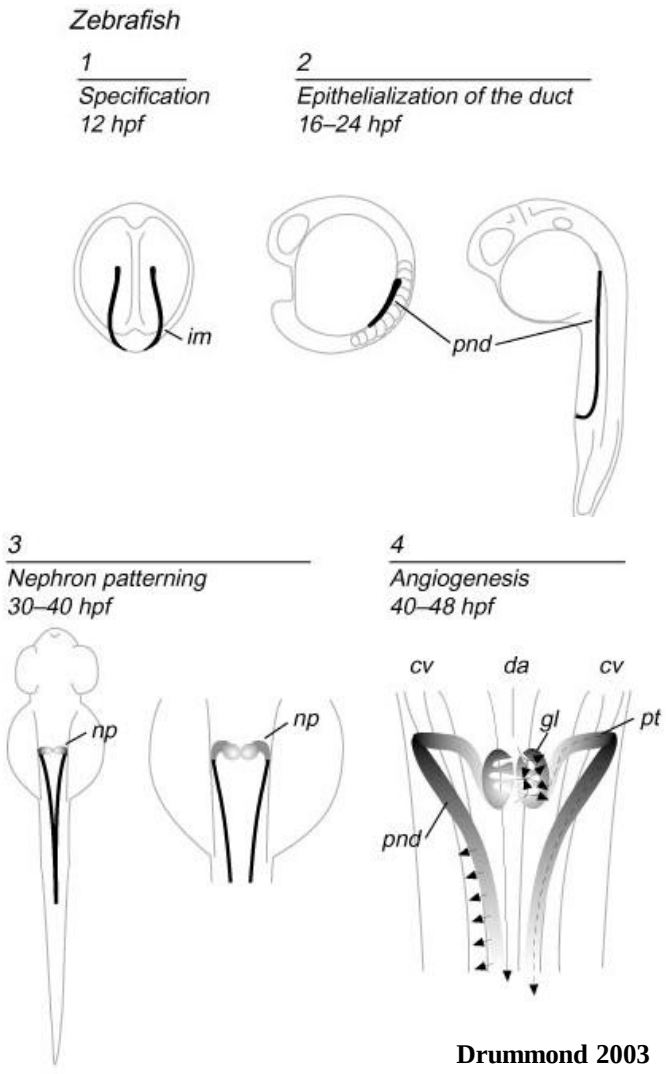
Mammalian kidney progresses through three stages…

…zebrafish kidney through two stages



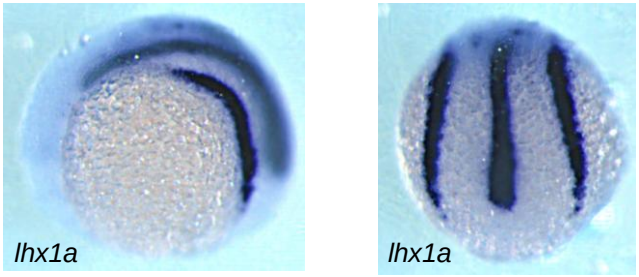
Gilbert 2000

The zebrafish pronephric kidney is a simple structure

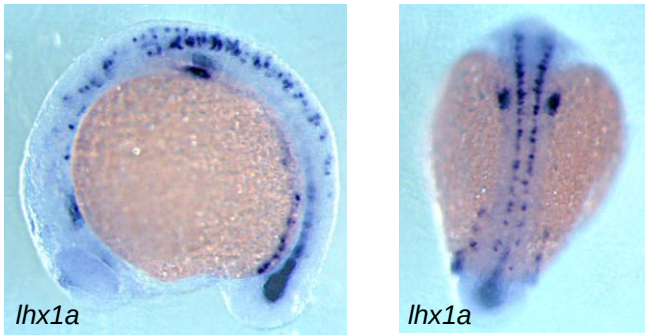


Drummond 2003

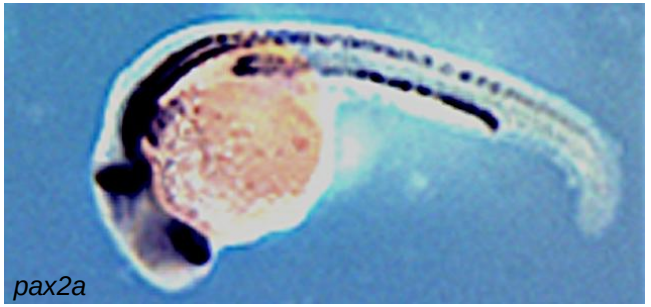
5 Somites



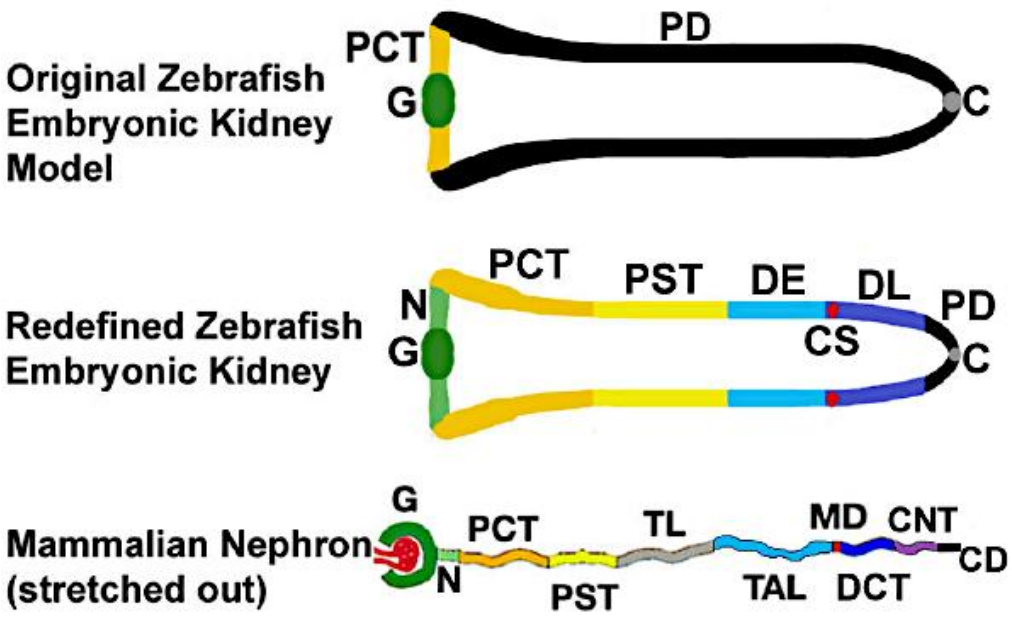
15 Somites



24 hours



The redefined zebrafish larval kidney



G = glomerulus; N = neck; PCT = proximal convoluted tubule; PST = proximal straight tubule; DE=distal early; CS=corpuscle of stannius; DL=distal late; PD=pronephric duct; C=cloaca

H. sapiens and *M. musculus*	*C. elegans*	*D. melanogaster*	*D. rerio*
Endocrine pancreas production of insulin and glucagon regulate sugar and lipid metabolism.	There is no orthologous structure. Mutations in multiple components of insulin signaling affect gut granule lipid mass, life span, and reproduction.	Insulin-like proteins, and the counter-regulatory adipokinetic hormone perform similar functions to human proteins, but are produced in physically separate parts of the brain. Glucose is not the principle carbohydrate in hemolymph.	Well-characterized endocrine pancreas present and functional by the late larval stage [114].
INSR, *Insr*. Multiple syndromes in humans marked by severe insulin resistance, and growth retardation. *Insr*$^{-/-}$ mice are small, severely insulin resistant, and die within 72 hours of diabetic ketoacidosis [96].	DAF-2. Insulin/IGF receptor. Mutation leads to dauer arrest and longevity [23].	*InR*. Mutations have variable effect on cell size and longevity. Insulin receptor substrate-1 ortholog *chico* is small and long-lived [97].	*insra* and *insrb* genes present. Role in metabolic regulation not known.

Table 2. Some Parallels and Differences among Model Organisms and Human Metabolism, as Compared by Organ System

H. sapiens and *M. musculus*	*C. elegans*	*D. melanogaster*	*D. rerio*
Central and peripheral nervous system regulation of metabolism; autonomic and hormonal modulation of acute energy needs.	Multiple studies identifying specific neuronal populations' involvement in regulating feeding behavior and gut granule lipid mass. A hypothalamus-like structure is not present.	Several hormone-producing areas within the brain have been implicated in regulating energy metabolism.	The vertebrate brain, cranial nerves, and peripheral nervous systems are robust in this animal. Development and function of the hypothalamus and pituitary gland are active areas of investigation. Pituitary hormone target-organs including thyroid gland, adrenal (interrenal) gland, and gonads are present.
Endocrine pancreas production of insulin and glucagon regulate sugar and lipid metabolism.	There is no orthologous structure. Mutations in multiple components of insulin signaling affect gut granule lipid mass, life span, and reproduction.	Insulin-like proteins, and the counter-regulatory adipokinetic hormone perform similar functions to human proteins, but are produced in physically separate parts of the brain. Glucose is not the principle carbohydrate in hemolymph.	Well-characterized endocrine pancreas present and functional by the late larval stage [114].
Liver and intestinal integration of glucose and lipid metabolism; lipoprotein production and transport.	Intestinal cells and skeletal muscle perform nutrient storage functions. No liver-like structure is present.	Oenocytes function as hepatocytes in converting fat body derived FAs into ketone bodies during fasting. Lipoprotein particles produced using highly conserved microsomal triglyceride transfer protein and vitellogenins are present in the gonad. Fat body-derived DAG is transported in high-density lipophorin particles [87,100].	Conventional vertebrate body plan is present. The liver and intestine use microsomal triglyceride transfer protein to produce beta lipoprotein particles. Vitellogenins and lipovitellins are present in the maternally supplied yolk, and in female adults producing eggs after spawning. Dietary and liver-derived lipids are probably supplied to other tissues using apolipoprotein B.
Adipose tissue storage of neutral lipids. Production of hormones.	Neutral lipids are stored in intestinal epithelium gut granules, lysosome-like structures. Gut granule lipid mass is regulated by many genes known to be involved in adipocyte biology. Life span and fertility affected by gut granule lipid mass and insulin signaling.	Larval and adult fat body cells store neutral lipids, respond to and produce hormones based on food supply. Fat mass sensed by brain and integrated into regulation of life span and reproduction. Human and mouse adipogenesis program employs similar gene networks as fat body.	In late larval and early juvenile stages, mesoderm-derived adipose tissue is seen [115]. Regulation of adipose mass and hormone production have not been characterized extensively.

#2 - Potential Impact CLINICAL RELEVANCE

- Benefits of model organisms are: genetically tractable, and quick, given relatively short life spans.
- How do you define relevance?
- Move in both directions. Targets in humans understand mechanisms in lower organisms
- Model Organisms will allow for analysis of specific pathways. These must be selected judiciously to identify or to address specific mechanisms that might be challenging to study in more complex organisms
- There has to be plan to move the field forward from discovery to translation

Model Organisms

- Could potentially be a platform for high throughput screening for complications or potential therapeutic targets
- Ultimately these would need to validated.
- Non Human primates take as long as people therefore after initial toxicology, the case is made to take these straight to humans
- Should large mammal work be centralized into shared resources, such as pigs, which might be good models for vascular complications

Making Models More Relevant

- Humanizing key pathways in relevant models.

#3- Barriers?
All Models have Caveats

- Mice are not necessarily great models of humans either
- Should we be considering larger mammals again, pigs, non-human primates etc.
- This depends on the complications and the relevance of the model to a given complication e.g. vascular complications.
- Large animals not appropriate for early stage development, but could be relevant for example could be a source of stem cells for example.

4 - Resources
nPOD-c

- Using existing human study cohorts to procure tissue and other samples, e.g. EDIC cohort.
- Use preclinical tissue data to compare with these data sets

Biomarkers

- Validating pathways found in model organisms to determine if relevant biomarkers might exist.
- Developing diagnostics such as pathway specific markers such as antibodies, liposomes etc.

How two move things forward

- Foster interactions between pathophysiological experts with model organism individuals might be the most productive approach.
- Foster workshops to encourage interaction and cross fertilization