Nocturnin: A Night Acting Protein is Modulated by a High-Fat Diet in Rats

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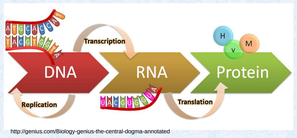
Background and Significance

We hypothesized that iron deficiency caused by a HFD reduces the ability to make heme and therefore hinders the synthesis of the Nocturnin protein ultimately decreasing overall fat metabolism.



http://genius.com/Biology-genius-the-central-dogma-annotated

Figure 1: The Central Dogma of Biology

Iron is a metal electrolyte needed for biological molecules. Heme, made of ferrous iron and porphyrin, is a subgroup of Hemoglobin, a major component of blood. Nocturnin is an important protein in Circadian Rhythm. Which is the day/night cycles in animals and is important for all biological functions. These cycles are easily disturbed by diet, habits, and health.
Prostaglandins are important physiological lipids that can have both detrimental and beneficial effects.

Methods

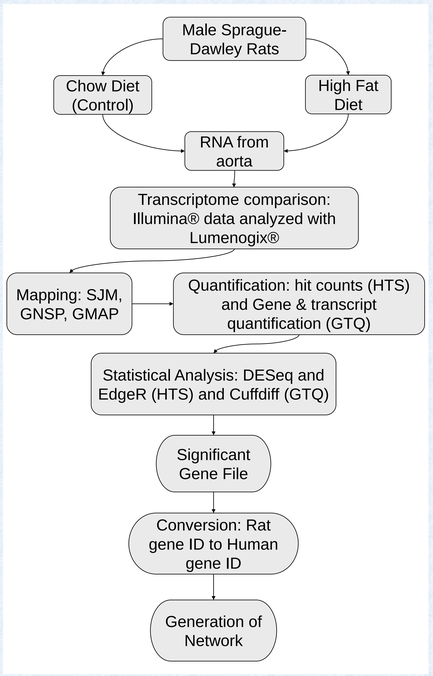


Figure 2. Overall workflow to generate gene expression data. Experiments with Sprague-Dawley rats were performed at ASU. Transcriptome comparison, matching to the reference genome, database management, and extraction of relevant data was done at the National Center for Genome Resources in Santa Fe. Network Generation was done at New Mexico Tech.

Results

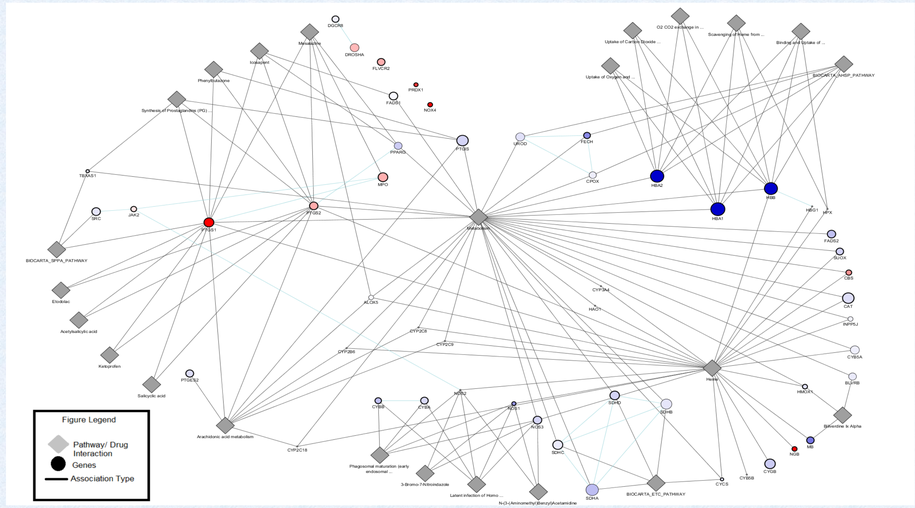


Figure 3, Cytoscape network produced using GeneMANIA. The network was merged with Log2 fold-change data from a transcriptome analysis experiment comparing Sprague-Dawley rats fed a high fat diet to those fed a regular Chow diet.

Network Gene	Gene Name	Function
Downregulated Genes		
HBA1	Hemoglobin alpha 1	protein coding
HBA2	Hemoglobin alpha 2	protein coding
HBB	Hemoglobin beta	protein coding
FECH	Ferrochelatase	proteing coding for catalyzes the insertion of the ferrous form of iron into protoporphyrin IX in the heme synthesis pathway
MB	Myoglobin	protein coding for intracellular oxygen storage and transcellular facilitated diffusion of oxygen
NOS1	Nitric Oxide Synthase 1	protein coding for synthesize nitric oxide from L-arginine
PTGIS	Prostaglandin I2 Synthase	catalyzes the conversion of prostglandin H2 to prostacyclin (prostaglandin I2), a potent vasodilator and inhibitor of platelet aggregation
UROD	Uroporphyrinogen Decarboxylase	enzyme catalyzes part of heme synthetic pathway
CAT	Catalase	a heme enzyme that converts the reactive oxygen species hydrogen peroxide to water and oxygen
CYB5A	Cytochrome B5 Type A	protein encoded by this gene is a membrane-bound cytochrome that reduces ferric hemoglobin
SUOX	Sulfite Oxidase	heme enzyme catalyzes the oxidation of sulfite to sulfate
HMOX1	Heme Oxygenase 1	essential enzyme in heme catabolism
BLVRB	Biliverdin Reductase B	final step in heme metabolism in mammals is catalyzed by the cytosolic biliverdin reductase enzymes A and B
FADS2	Fatty Acid Desaturase 2	enzymes regulate unsaturation of fatty acids through the introduction of double bonds
PPARG	Peroxisome Proliferator-Activated Receptor Gamma	nuclear receptor regulator of adipocyte differentiation
SRC	SRC Proto-Oncogene	proto-oncogene Mutations in this gene could be involved in the malignant progression of colon cancer
PTGES2	Prostaglandin E Synthase 2	protein encoded by this gene is a membrane-associated prostaglandin E synthase, which catalyzes the conversion of prostaglandin H2 to prostaglandin E2
SDHA	Succinate Dehydrogenase Complex, Subunit A	major catalytic subunit of succinate-ubiquinone oxidoreductase, a complex of the mitochondrial respiratory chain
SDHB	Succinate Dehydrogenase Complex, Subunit B	nuclear-encoded subunits that comprise succinate dehydrogenase, also known as mitochondrial complex I, a key enzyme complex of the tricarboxylic acid cycle and aerobic respiratory chains of mitochondria
SDHC	Succinate Dehydrogenase Complex, Subunit C	nuclear-encoded subunits that comprise succinate dehydrogenase, also known as mitochondrial complex II, a key enzyme complex of the tricarboxylic acid cycle and aerobic respiratory chains of mitochondria
SDHD	Succinate Dehydrogenase Complex, Subunit D	member of complex II of the respiratory chain, which is responsible for the oxidation of succinate
Upregulated Genes		
PTGS1	Prostaglandin-Endoperoxide Synthase 1	one of two genes that encode enzymes catalyzing the conversion of arachinodate to protaglandin; regulates angiogenesis in endothelial cells; inhibited by NSAIDs
PTSG2	Prostaglandin-Endoperoxide Synthase 2	one of two genes that encode enzymes catalyzing the conversion of arachinodate to protaglandin; acts as a dioxygenase and as a peroxidase
MPO	Myeloperoxidase	heme protein synthesized during myeloid differentiation
PRDX1	Peroxiredoxin 1	encodes a member of the peroxiredoxin family of antioxidant enzymes, which reduce hydrogen peroxide and alkyl hydroperoxides
NOX4	NADPH Oxidase	encodes a member of the NOX-family of enzymes that functions as the catalytic subunit in the NADPH oxidase complex
DROSHA	Ribonuclease Type III	plays a role in RNA maturation and decay pathways in eukaryotic and prokaryotic cells
NGB	Neuroglobin	encodes an oxygen-binding protein; may be involved in increasing oxygen availability and providing protection under hypoxic/ischemic conditions
CBS	Cystathionine-Beta-Synthase	only pyridoxal phosphate-dependent enzyme that contains heme; regulates hydrogen sulfide;acts as a neuromodulator in the brain to protect neurons against hypoxic injury
FLYCR2	Feline Leukemia Virus Subgroup C Cellular Receptor	protein coding; acts as an importer of heme; important for growth and calcium metabolism

Table 1, Table for important network genes from Fig.3.. Network observed for highly up-regulated, down-regulated, and relevant genes. Table generated using information from genecards.

Conclusions & Future Work

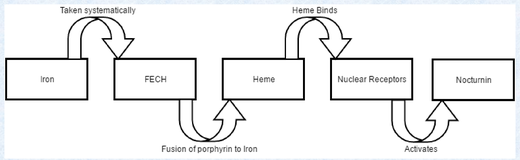


Figure 4. Hypothesized connection between Iron, Heme, and Nocturnin. Iron is absorbed in the small intestine and fused by ferrochelators with porphyrin to make Heme with binds to nuclear receptors and activates Nocturnin.

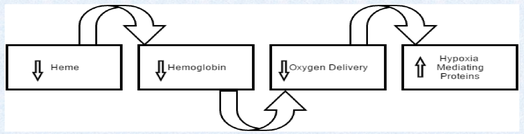


Figure 5. Hypothesized connection between Heme and hypoxia. Genes involved in Heme production are down-regulated which can lead to hypoxia.

An additional conclusion was made regarding the upregulation of two enzymes involved in the biosynthesis of prostaglandins. The up-regulation is logical in rats fed a HFD because certain fats are high in arachidonic acid, a precursor molecule of prostaglandins. Future studies should be done with iron supplements in the HFD. We would expect to see normal synthesis of Nocturnin, normal fat metabolism, and possibly less fluctuations in the Circadian Rhythm.

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References

1. *S. Kojima, et al. Changes in poly(A) tail length dynamics from the loss of the circadian deadenylase Nocturnin. Sci Reports.* ***5*** *(2015)*
2. *S. Raghuram, et al. Identification of heme as the ligand for the orphan nuclear receptors REV-ERBalpha and REV-ERBbeta. Nat Struct Mol Biol.* ***14*** *(2007)*
3. *L. Yin, et al. Nuclear receptor REV-ERBalpha: a heme receptor that coordinates circadian rhythm and metabolism. Nucl Recept Signal.* ***8*** *(2010)*
4. *T. Sonnweber, et al. High-fat diet causes iron deficiency via hepcidin-independent reduction of duodenal iron absorption. J Nutr Biochem.* ***23*** *(2012)*