

AOP ID and Title:

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AOP 6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss**Short Title: PPARalpha antagonism leading to body-weight loss**

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Status

Author status	OECD status	OECD project	SAAOP status
Open for comment. Do not cite	EAGMST Under Review	2.3	Included in OECD work plan

Abstract

The present AOP describes chemical binding and stabilization of a co-repressor to the peroxisome proliferator-activated receptor α (PPAR α) signaling complex causing a chain of events that includes: antagonism of PPAR α nuclear signaling, decreased transcriptional expression of PPAR α -regulated genes that support energy metabolism, and inhibited metabolic energy production culminating with starvation-like weight loss. The MIE for this AOP involves antagonistic PPAR α binding. The antagonist-binding causing the KE1, stabilization of co-repressor (SMRT or N-CoR) to PPARalpha ligand binding domain suppressing PPAR α nuclear signaling (Nagy et al 1999, Xu et al 2002). PPAR α is a transcriptional regulator for a variety of genes that facilitate systemic energy homeostasis (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). As a result of the MIE and KE1, KE2 occurs where PPARalpha transactivation is inhibited for genes involved in the next 3 key events of the AOP: (KE3) decreased peroxisomal fatty acid beta oxidation (Desvergne and Wahli 1999, Kersten 2014, Dreyer et al 1992, Lazarow 1978), (KE4) decreased mitochondrial fatty acid beta oxidation (Kersten 2014, Brandt et al 1998; Mascaro et al 1998, Aoyama et al 1998, Gulick et al 1994, Sanderson et al 2008), and (KE5) decreased ketogenesis (Cahil 2006, Kersten et al 2014, Sengupta et al 2010, Desvergne and Wahli 1999). KE3 and KE4 result in decreased catabolism of very long chain fatty acids and short through long chain fatty acids, respectively, decreasing key substrate

availability for cellular energy production (Evans et al 2004) coupled with KE5 which decreases the ability to repackage energy substrates to support systemic energy demands (Badman et al 2007, Potthoff 2009). KE6 describes no change or a decrease in circulating ketone bodies under cellular energy deficit conditions, a state where ketogenesis is typically induced thus increasing circulating ketone bodies as metabolic fuel to sustain energy homeostasis (Cahill 2006). Physiological studies of the progression of human starvation have demonstrated the critical importance of ketogenesis, especially production of β -hydroxybutyrate, for meeting systemic energy demands by supplementing glucose to sustain the energy requirements of the brain (Cahill 2006, Owen et al 2005). Sustained negative energy budgets lead to KE7, an increase in muscle protein catabolism, with glutamine and alanine recycled for gluconeogenesis (Felig et al 1970A, Kashiwaya et al 1994). Finally, the AO of body-weight loss occurs, which within the context of dynamic energy budget theory decreases energy allocations to organismal maturation and reproduction (Nisbet et al 2000) and has been demonstrated to negatively affect ecological fitness (Martin et al 1987).

Summary of the AOP

Stressors

Name	Evidence
GW6471	Strong
Nitrotoluenes (hypothesized binding)	Moderate

GW6471

GW6471 - Specifically designed molecules such as the PPAR α antagonists GW6471 can bind to PPAR α selectively recruiting binding of co-repressors to the PPAR α nuclear signaling complex (Xu et al 2002).

Nitrotoluenes (hypothesized binding)

Nitrotoluenes (hypothesized binding) - Recent observations of PPAR α antagonism by nitrotoluenes (including: 2,4-dinitrotoluene and 2-amino-4,6-dinitrotoluene) have demonstrated dose-response relationships for PPAR α nuclear signaling inhibition in *in vitro* investigations which corresponded with dose-responsive decreases in transcriptional expression of genes for which PPAR α acts as a transcriptional regulator, including various lipid metabolism pathways (Wilbanks et al 2014, Gust et al 2015). There is additional evidence that multiple nitrotoluenes including the base structure, 2,4,6-trinitrotoluene and molecules with various substitutions of the nitro groups around that base structure inhibit PPAR α nuclear signaling, and have elevated binding affinity to PPAR α , based on computational docking calculations (Gust, unpublished data).

Molecular Initiating Event

Title	Short name
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Binding of antagonist, PPAR alpha

Binding of antagonist, PPAR alpha

998: Binding of antagonist, PPAR alpha

Short Name: Binding of antagonist, PPAR alpha

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	MolecularInitiatingEvent

Stressors

Name
GW6471

Evidence for Perturbation of this Molecular Initiating Event by Stressor

Antagonist binding of GW6471 causing increased stabilization of the co-repressors SMRT and N-CoR to the PPAR α ligand binding domain has been explicitly demonstrated through x-ray crystallography (Xu et al 2002).

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI
Saccharomyces cerevisiae	Saccharomyces cerevisiae	Strong	NCBI

The fundamental mechanics for nuclear receptor binding as well as demonstration of co-repressor recruitment have been observed to be conserved when comparing humans and yeast (Nagy et al 1999). PPAR α has been cloned from frogs, rats, guinea pigs, and humans where the DNA-binding domain has been shown to be identical across species, however the ligand binding domain has exhibited lower homology, likely adapted to differences in dietary ligands among species (Willson et al 2000). Overall, there is evidence for fairly conserved taxonomic applicability across vertebrates, though care should be given when extrapolating across species.

How this Key Event Works

Binding of molecules to peroxisome proliferator-activated receptor α (PPAR α) can cause either agonistic or antagonistic signaling depending on molecular structure (Xu et al 2001, Xu et al 2002). Certain molecules that can bind to the PPAR α ligand binding domain have been observed to cause conformational changes that induce increased affinity to co-repressors which decrease PPAR α nuclear signaling (Xu et al 2002). Binding of co-repressors such as the silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) to PPAR α is reinforced by the antagonist, which blocks the AF-2 helix from adopting the active conformation, as demonstrated in x-ray crystallography results presented in Xu et al (2002). Thus, molecules that bind to PPAR α that can enhance co-repressor binding act as PPAR α antagonists.

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible? In Xu et al (2002), X-ray crystallography was used to characterize the suppressed PPAR α signaling complex (PPAR α / GW6471 / SMRT) and was compared against the activated PPAR α complex which included binding of PPAR α with the agonist GW409544 and the co-activator, steroid receptor coactivator-1 (SRC-1). For simple PPAR α binding assessment, competitive binding assays are available, however these must be coupled with nuclear signaling activation / inhibition assays to determine if chemicals are agonists or antagonists.

References

- Nagy L, Kao H-Y, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JWR: Mechanism of corepressor binding and release from nuclear hormone receptors. *Genes Dev* 1999, 13(24):3209-3216.
- Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, Oplinger JA, Kliewer SA, Gampe RT, McKee DD et al: Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. *Proceedings of the National Academy of Sciences* 2001, 98(24):13919-13924.
- Willson TM, Brown PJ, Sternbach DD, Henke BR: The PPARs: From Orphan Receptors to Drug Discovery. *J Med Chem* 2000, 43(4):527-550.
- Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT et al: Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPAR[alpha]. *Nature* 2002, 415(6873):813-817.

Key Events

Title	Short name
Decreased, PPARalpha transactivation of gene	Decreased, PPARalpha transactivation of gene

expression	expression
Decreased, Mitochondrial Fatty Acid Beta Oxidation	Decreased, Mitochondrial Fatty Acid Beta Oxidation
Decreased, Ketogenesis (production of ketone bodies)	Decreased, Ketogenesis (production of ketone bodies)
Not Increased, Circulating Ketone Bodies	Not Increased, Circulating Ketone Bodies
Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids	Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids
Increased, Catabolism of Muscle Protein	Increased, Catabolism of Muscle Protein
stabilization, PPAR alpha co-repressor	stabilization, PPAR alpha co-repressor

858: Decreased, PPARalpha transactivation of gene expression

Short Name: Decreased, PPARalpha transactivation of gene expression

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
Mus musculus	Mus musculus	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

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Sex	Evidence
Male	Strong
Female	Strong

Mus musculus (Kersten 2014), *Homo sapiens* in clinical observations (Kersten 2014) and in in vitro assays (reviewed in Kersten 2014).

How this Key Event Works

PPAR α is a nuclear transcription factor that controls the transcription of a variety of genes involved in lipid catabolism and energy production pathways (Desvergne and Wahli 1999, Kersten 2014). Fatty acids serve as the ligands that stimulate PPAR α nuclear signaling where the fatty acids (likely in association with fatty acid binding proteins) bind to the ligand binding domain or PPAR α along with co-activators to the PPAR α regulatory complex initiating the transcription of genes that metabolize the fatty acids (Wolfrum et al. 2001, Desvergne and Wahli 1999, Kersten 2014, Xu et al 2001). PPAR α regulates expression of genes encoding nearly every enzymatic step of fatty acid catabolism including fatty acid uptake into cells, fatty acid activation to acyl-CoAs, and the release of cellular energy from fatty acids through the oxidative breakdown of acyl-CoAs to acetyl-CoA, and in starvation conditions, the repackaging of Acetyl-CoA substrates into ketone bodies via ketogenesis pathways (Kersten 2014, Desvergne and Wahli 1999, Evans et al 2004). A pathway-level schematic for PPAR α transactivation is illustrated in KEGG Pathway map03320 providing the specific gene targets and associated functional responses that are transcriptionally regulated by PPAR α .

Detailed description of important pathways regulated by PPAR α transactivation:

Peroxisomal fatty acid beta oxidation: PPAR α acts as a positive transcriptional regulator for many of the genes involved in peroxisomal fatty acid beta oxidation as well as genes involved in the pre- and post-processing of fatty acids in peroxisomal pathways (Desvergne and Wahli 1999, Kersten 2014). The first gene target identified for PPAR α was Acyl-CoA oxidase (Acox1, Dreyer et al 1992) which represents the first enzyme in peroxisomal long-chain fatty acid oxidation (Kersten 2014) and is also the rate-limiting enzyme in this pathway (Desvergne and Wahli 1999). In addition to Acox1, a variety of additional enzymes involved in peroxisomal fatty acid metabolism are under transcriptional control of PPAR α transactivation including enzymes that facilitate fatty acid uptake into the peroxisome (Abcd1, Abcd2 and Abcd 3), conversion of acyl-CoA/acetyl-CoA to acyl-carnitine/acetyl-carnitine (Crot/Crat), and conversion of acyl-CoAs back to fatty acids via thioesterases (Acots, as reviewed in Kersten 2014). PPAR α also has transcriptional control over enzymes downstream of Acox1 in the peroxisomal beta-oxidation of acyl-CoA pathway including L-bifunctional enzyme (Ehhadh), D-bifunctional enzyme (Hsd17b4), and peroxisomal 3-ketoacyl-CoA thiolase activity (Acaa1a, Acaa1b, as reviewed in Kersten 2014).

Mitochondrial fatty acid beta oxidation: As reviewed in Kersten (2014), the genes (and associated functions) regulated by PPAR α in the mitochondrial processing of fatty acids include the following: (1) Import of acyl-CoAs into the mitochondria is facilitated by PPAR α -induced increases in expression of carnitine palmitoyl-transferases 1a, 1b, and 1 (Cpt1a, Cpt1b, Cpt2) and acyl-carnitine translocase (Slc25a20, Brandt et al 1998; Mascaro et al 1998). (2) The first step of mitochondrial beta-oxidation is catalyzed by length-specific acyl-CoA hydrogenases (Acadvl, Acadl, Acadm, Acads; Aoyama et al 1998, Gulick et al 1994). (3) The three subsequent steps in mitochondrial beta-oxidation that successively release acetyl-CoAs from the hydrocarbon chain are catalyzed by the mitochondrial trifunctional enzyme (Hadha and Hadhb). These enzymes are replaced upon progressive chain shortening by Hadh and Acaa2. (4) the final PPAR α targets include Eci1, Eci2, Decr1, and Hsd17b10 which convert unsaturated and 2-methylated acyl-CoAs into intermediates of beta-oxidation (Sanderson et al 2008, Aoyama et al 1998).

Ketogenesis: Not only does PPAR α induce the upstream production of the raw materials for use in ketogenesis through fatty acid beta-oxidation (see peroxisomal and mitochondrial fatty acid beta oxidation above), but also directly induces key enzymes in the ketogenesis pathway including Hmgcs2, Hmgcl and Acat1 (Kersten et al 2014). PPAR α is recognized as the master transcriptional activator of ketogenic genes (Sengupta et al 2010, Desvergne and Wahli 1999).

How it is Measured or Detected

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A variety of transcript expression assays have been used to demonstrate the effect of PPAR α signaling inhibition on downstream transcript expression (see literature cited above for specific methods within each investigation). A global screen for PPAR α transcriptional targets (especially those involved in fatty acid metabolism) is provided in Rakhshandehroo et al (2007) which utilized microarray based expression screening followed by RT-qPCR and in silico screening of putative PPAR response elements.

References

- Aoyama T, Peters JM, Iritani N, Nakajima T, Furihata K, Hashimoto T, Gonzalez FJ: Altered Constitutive Expression of Fatty Acid-metabolizing Enzymes in Mice Lacking the Peroxisome Proliferator-activated Receptor α (PPAR α). J Biol Chem 1998, 273(10):5678-5684.
- Brandt JM, Djouadi F, Kelly DP: Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. J Biol Chem 1998, 273(37):23786-23792.
- Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocrine Reviews 20(5): 649-688.
- Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W: Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 1992, 68(5):879-887.
- Gulick T, Cresci S, Caira T, Moore DD, Kelly DP: The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. Proc Natl Acad Sci U S A 1994, 91(23):11012-11016.
- Kersten S. 2014. Integrated physiology and systems biology of PPAR α . Molecular Metabolism 2014, 3(4):354-371.
- Mascaró C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D: Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. J Biol Chem 1998, 273(15):8560-8563.
- Rakhshandehroo M, Sanderson LM, Matilainen M, Stienstra R, Carlberg C, de Groot PJ, Muller M, Kersten S: Comprehensive analysis of PPAR α -dependent regulation of hepatic lipid metabolism by expression profiling. PPAR research 2007, 2007:26839.
- Sanderson LM, Boekschoten MV, Desvergne B, Müller M, Kersten S: Transcriptional profiling reveals divergent

roles of PPAR α and PPAR β/δ in regulation of gene expression in mouse liver, vol. 41; 2010.

Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. Nature 2010, 468(7327):1100-1104.

Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, Oplinger JA, Kliewer SA, Gampe RT, McKee DD et al: Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proceedings of the National Academy of Sciences 2001, 98(24):13919-13924.

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT et al: Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPAR[alpha]. Nature 2002, 415(6873):813-817.

860: Decreased, Mitochondrial Fatty Acid Beta Oxidation

Short Name: Decreased, Mitochondrial Fatty Acid Beta Oxidation

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
Mus musculus	Mus musculus	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

Sex	Evidence

Male	Strong
Female	Strong

Human (as reviewed in Brandt et al 1998, Evans et al 2004, Gulick et al 1994, Kersten 2014 and Desvergne and Wahli 1999). Mouse (as measured in Aoyama et al 1998, and as reviewed in Kersten 2014 and Desvergne and Wahli 1999).

How this Key Event Works

Mitochondrial beta-oxidation catabolizes short, medium and long chain fatty acids (<C20) into acetyl-CoA and ATP. The production of acetyl-CoA monomers is important as they serve as fundamental units for metabolic energy production (ATP) via the citric acid cycle followed by electron-transport chain mediated oxidative phosphorylation (Nelson and Cox, 2000A). Acetyl-CoA is also a fundamental units of energy storage via gluconeogenesis (Nelson and Cox, 2000B) and lipogenesis (Nelson and Cox, 2000C).

Mitochondrial processing of fatty acids involves: (1) Import of short, medium and long chain fatty acids (<C20) acyl-CoAs into the mitochondria by carnitine palmitoyl-transferases 1a, 1b, and 1 (Cpt1a, Cpt1b, Cpt2) and acyl-carnitine translocase (Slc25a20, Brandt et al 1998; Mascaro et al 1998, Kersten et al 2014). (2) The first step of beta-oxidation catalyzed by the length-specific acyl-CoA hydrogenases (Acadvl, Acadl, Acadm, Acads; Aoyama et al 1998, Gulick et al 1994, Kersten et al 2014). (3) The three subsequent steps in mitochondrial beta-oxidation that successively release acetyl-CoAs from the hydrocarbon chain are catalyzed by the mitochondrial trifunctional enzyme (Hadha and Hadhb, Kersten et al 2014). These enzymes are replaced upon progressive chain shortening by Hadh and Acaa2 (Kersten et al 2014). (4) The conversion of unsaturated and 2-methylated acetyl-CoAs into intermediates of beta-oxidation are catalyzed by Eci1, Eci2, Decr1, and Hsd17b10 (Sanderson et al 2008, Aoyama et al 1998, Kersten et al 2014).

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

Beta oxidation of fatty acids in mitochondria has been measured using mouse liver homogenates where a radio-labeled fatty acid substrate was reacted for 30 minutes and then centrifuged to separate reaction products for fractional radioactivity measurements (Aoyama et al 1998). Comparative measures of reaction products were also measured where potassium cyanide was added to the reaction mixture to inhibit mitochondrial beta oxidation activity to normalize the contribution of mitochondrial enzymatic reactions to the overall reaction product (Aoyama et al 1998).

Various methods were used for gene expression investigations. Brandt et al (1998) investigated concentration response effects of Oleate, Decanoate and Hexanoate fatty acid chains on mitochondrial carnitine palmitoyl-transferases I (M-CPT I) expression using promoter-reporter plasmid MCPT.Luc.1025 reporter transfected into rat neonate cardiac myocytes. Human M-CPT I was investigate using an analogous method (Brandt et al 1998). Expression of human medium chain acyl-CoA dehydrogenase (MCAD) was investigated using a MCAD.luc.1054 reporter transfected into HepG2 cells in response to fatty acids with various chain lengths (Gulick et al 1994). Investigation of various enzymes involved in hepatic fatty acid metabolism described in Aoyama et al (1998) were

investigated using Western immunoblot quantitation.

References

- Aoyama, T., Peters, J.M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., et al., 1998. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *Journal of Biological Chemistry* 273:5678e5684.
- Brandt, J.M., Djouadi, F., Kelly, D.P., 1998. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. *Journal of Biological Chemistry* 273:23786e23792.
- Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.
- Gulick, T., Cresci, S., Caira, T., Moore, D.D., Kelly, D.P., 1994. The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proceedings of the National Academy of Sciences of the United States of America* 91:11012e11016.
- Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.
- Mascaro, C., Acosta, E., Ortiz, J.A., Marrero, P.F., Hegardt, F.G., Haro, D., 1998. Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *Journal of Biological Chemistry* 273:8560e8563.
- Nelson DL, Cox MM 2000A. The Citric Acid Cycle. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p567-592.
- Nelson DL, Cox MM 2000B. Carbohydrate Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p722-764.
- Nelson DL, Cox MM 2000C. Lipid Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p770-814.
- Sanderson, L.M., de Groot, P.J., Hooiveld, G.J., Koppen, A., Kalkhoven, E., Muller, M., et al., 2008. Effect of synthetic dietary triglycerides: a novel research paradigm for nutrigenomics. *PLoS One* 3:e1681.

861: Decreased, Ketogenesis (production of ketone bodies)

Short Name: Decreased, Ketogenesis (production of ketone bodies)

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization

Molecular

Evidence Supporting Applicability of this Event**Taxonomic Applicability**

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	Strong	NCBI
Homo sapiens	Homo sapiens	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified
Adults	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Evidence provided for human in Cahill (2006), Owen et al (2005) and Williamson et al (1962). Evidence for mouse provided in Kersten et al (1999).

How this Key Event Works

The liver plays a key role in processing the fundamental energy substrate, acetyl-CoA, into metabolic currencies that contribute to the systemic cellular energy needs of the whole organism. The liver represents a key organ involved in systemic energy distribution given its ability to synthesize glucose (an ability shared only with the kidney, Gerich et al 2001) as well as its exclusive role in the generation of ketone bodies (Cahill 2006, Sengupta et al 2010, Kersten 2014). This is especially important for the metabolic energy needs of the brain which can only use glucose and the ketone body, β -hydroxybutyrate for cellular energy production (Cahill 2006, Owen 2005, Kersten 2014). Therefore, ketogenesis is critical to supporting general systemic energy homeostasis in fasting events (Cahill 2006, Evans et al 2004, Sengupta et al 2010). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009, Sengupta et al 2010). The Badman et al (2007) study indicated that metabolism of fatty acid substrates (measured as liver triglycerides) that would otherwise contribute to β -hydroxybutyrate production was additionally inhibited under PPAR α knockout.

In a fasting state, humans transition from the use of exogenous glucose to glucose derived from glycogen within 4

hours with a steadily increasing proportion of glucose usage that is derived from gluconeogenesis up to 2 days (Cahill 2006). Beyond 2 days of fasting, ketone body production (β -hydroxybutyrate) increasingly supports the energy demands of the brain (Cahill 2006).

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

The quantification of β -hydroxybutyrate described in Cahill 2006 was measured in a cell-free system catalyzed by D(-)-p-hydroxybutyric dehydrogenase where all components of the reaction [D(-)-p-hydroxybutyrate + DPN⁺ = acetoacetate + DPNH + H⁺] were able to be quantitatively determined (Williamson et al 1962).

Serum β -hydroxybutyrate was measured using Stanbio Laboratory small-scale enzymatic assays in Badman et al (2007) and by Wako Chemicals D-3-hydroxybutyric acid kit in Potthoff et al (2009).

SMART micro-FPLC (Amersham Biosciences) consisting of a Superose 6 PC 3.2/30 column (Amersham Biosciences) equilibrated in 13 PBS buffer was conducted where triglyceride and cholesterol fractions were investigated by enzymatic assay (Wako Diagnostics) as described in Badman et al (2007).

Clinical observations of ketone bodies have been simplified by the development of urine test strips that can provide quantitative values for the ketone bodies aceto-acetate, acetone and 3-hydroxybutyrate using reflectometry (Penders et al 2005).

References

- Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPAR α and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.
- Cahill Jr., G.F., 2006. Fuel metabolism in starvation. *Annual Review of Nutrition* 26:1e22.
- Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.
- Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001, 24(2):382-391. Kersten S. 2014. Integrated physiology and systems biology of PPAR α . *Molecular Metabolism* 2014, 3(4):354-371.
- Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W: Peroxisome proliferator-activated receptor α mediates the adaptive response to fasting. *J Clin Invest* 1999, 103(11):1489-1498.
- Owen OE: Ketone bodies as a fuel for the brain during starvation. *Biochem Mol Biol Educ* 2005, 33(4):246-251.
- Penders J, Fiers T, Giri M, Wuyts B, Ysewyn L, Delanghe JR: Quantitative measurement of ketone bodies in urine using reflectometry. *Clin Chem Lab Med* 2005, 43(7):724-729.
- Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et

al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. Proceedings of the National Academy of Sciences 2009, 106(26):10853-10858.

Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. Nature 2010, 468(7327):1100-1104.

Williamson DH, Mellanby J, Krebs HA: Enzymic determination of d(-)- β -hydroxybutyric acid and acetoacetic acid in blood. Biochem J 1962, 82(1):90-96.

862: Not Increased, Circulating Ketone Bodies

Short Name: Not Increased, Circulating Ketone Bodies

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization
Tissue

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
Mus musculus	Mus musculus	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified
Adults	Strong

Sex Applicability

Sex	Evidence
Male	Strong

Evidence for mouse provided in (Badman et al 2007, Potthoff 2009). Evidence for human provided in (Cahill 2006, Owen et al 2005, Gerich et al 2001).

How this Key Event Works

A fundamental process in all biological systems is the production of metabolic fuel for use in meeting the energy demands of cells and the systemic energy needs of multi-cellular organisms. Physiological studies of the progression of human starvation have identified that the preferred metabolic fuel is glucose in the fed state and progressing through two days of fasting, afterward ketone bodies become increasingly important for meeting energy demands (Cahill 2006, Owen et al 2005). Substrates derived from carbohydrates, fats and protein can contribute to gluconeogenesis (Cahill 2006, Gerich et al 2001) whereas substrates derived from fatty acids are the primary contributors to ketogenesis (Desvergne and Wahli 1999). Mobilization of fatty acids as a metabolic fuel source increase dramatically during fasting to support both gluconeogenesis and ketogenesis (Evans et al 2004). Cahill (2006) and colleagues have demonstrated the importance of ketone body production, especially β -hydroxybutyrate, for maintaining energy homeostasis during starvation. β -hydroxybutyrate serves as an alternative substrate to glucose for providing energy to the brain in the starvation state, providing ATP at higher efficiency relative to the glucose substrate (Cahill 2006). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009, Sengupta et al 2010). The Badman et al (2007) study indicated that metabolism of fatty acid substrates (measured as liver triglycerides) that would otherwise contribute to β -hydroxybutyrate production was inhibited under PPAR α knockout. Increased concentrations of circulating ketone bodies is indicative of potential metabolic fuel deficits in fasting animals (Cahill 2006), and a lack of increase in circulating ketone bodies during fasting, especially in conjunction with elevated blood triglycerides, indicates impaired ketogenesis and potentially impaired bioenergetic potential. Although the potential therapeutic implications of increased ketone body metabolism via ketogenic diets for various disease states has been discussed (Veech 2004), no studies were found demonstrating effects on whole organism responses to impaired ketogenesis over long-term starvation events.

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

The quantification of β -hydroxybutyrate described in Cahill 2006 was measured in a cell-free system catalyzed by D(-)-p-hydroxybutyric dehydrogenase where all components of the reaction [D(-)-fl-hydroxybutyrate + DPN+ = acetoacetate + DPNH + H+] were able to be quantitatively determined (Williamson et al 1962).

Serum β -hydroxybutyrate was measured using Stanbio Laboratory small-scale enzymatic assays in Badman et al (2007) and by Wako Chemicals D-3-hydroxybutyric acid kit in Potthoff et al (2009).

SMART micro-FPLC (Amersham Biosciences) consisting of a Superose 6 PC 3.2/30 column (Amersham Biosciences) equilibrated in 13 PBS buffer was conducted where triglyceride and cholesterol fractions were investigated by enzymatic assay (Wako Diagnostics) as described in Badman et al (2007).

Clinical observations of ketone bodies have been simplified by the development of urine test strips that can provide

quantitative values for the ketone bodies aceto-acetate, acetone and 3-hydroxybutyrate using reflectometry (Penders et al 2005).

References

- Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.
- Cahill GF, Jr. Fuel metabolism in starvation. *Annu Rev Nutr* 2006, 26:1-22.
- Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.
- Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001, 24(2):382-391. Owen OE: Ketone bodies as a fuel for the brain during starvation. *Biochem Mol Biol Educ* 2005, 33(4):246-251.
- Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.
- Veech RL: The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids* 2004, 70(3):309-319.
- Williamson DH, Mellanby J, Krebs HA: Enzymic determination of d(-)- β -hydroxybutyric acid and acetoacetic acid in blood. *Biochem J* 1962, 82(1):90-96.

859: Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids

Short Name: Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
Mus musculus	Mus musculus	Strong	NCBI
Rattus rattus	Rattus rattus	Strong	NCBI

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Human (as reviewed in Kersten 2014 and Desvergne and Wahli 1999). Rat (as measured by Lazarow 1978). Mouse (as reviewed in Kersten 2014 and Desvergne and Wahli 1999).

How this Key Event Works

Peroxisomes participate in a variety of lipid metabolic pathways including the beta-oxidation of very long-straight chain (<20 C in length) or branched –chain acyl-CoAs (Lazarow 1978, Kersten 2014). The peroxisomal beta-oxidation pathway is not directly coupled to the electron transport chain and oxidative phosphorylation, therefore the first oxidation reaction loses energy to heat (H₂O₂ production) while in the second step, energy is captured in the metabolically accessible form of high-energy electrons in NADH (Mannaerts and Van Veldhoven 1993, Desvergne and Wahli 1999). The peroxisomal beta-oxidation pathway provides fatty acid chain shortening where two carbons are removed in each round of oxidation in the form of acetyl-CoA (Desvergne and Wahli 1999). The acetyl-CoA monomers serve as fundamental units for metabolic energy production (ATP) via the citric acid cycle followed by electron-transport chain mediated oxidative phosphorylation (Nelson and Cox, 2000A) as well as serve as the fundamental units for energy storage via gluconeogenesis (Nelson and Cox, 2000B) and lipogenesis (Nelson and Cox, 2000C). The shortened chain fatty acids (<20C) can then be transported to the mitochondria to undergo mitochondrial beta-oxidation for complete metabolism of the carbon substrate for cellular energy production (Desvergne and Wahli 1999).

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

Spectroscopic analysis of the characteristic absorption bands for fatty acid substrates and fatty acid beta oxidation products were examined for peroxisomal fractions purified from rat livers by differential and of equilibrium density centrifugation (Lazarow 1978). Additionally, NAD reduction assays were conducted for acyl-CoA substrates with varying chain lengths where increased oxidation was observed for substrates with long chain length relative to short

chain acyl-CoAs (Lazarow 1978).

References

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.

Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.

Lazarow PB: Rat liver peroxisomes catalyze the beta oxidation of fatty acids. *J Biol Chem* 1978, 253(5):1522-1528.

Mannaerts GP, Van Veldhoven PP 1993 Metabolic role of mammalian peroxisomes. In: Gibson G, Lake B (eds) *Peroxisomes: Biology and Importance in Toxicology and Medicine*. Taylor & Francis, London, pp 19–62.

Nelson DL, Cox MM 2000A. The Citric Acid Cycle. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p567-592.

Nelson DL, Cox MM 2000B. Carbohydrate Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p722-764.

Nelson DL, Cox MM 2000C. Lipid Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p770-814.

863: Increased, Catabolism of Muscle Protein

Short Name: Increased, Catabolism of Muscle Protein

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization
Organ

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
Mus musculus	Mus musculus	Strong	NCBI

Rattus rattus	Rattus rattus	Moderate	NCBI
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Life Stage Applicability

Life Stage	Evidence
Adults	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Evidence for mouse provided in (Cahill 2006, Marliss et al 1971, Felig et al 1970A, 1970B). Evidence for rat provided in Kashiwaya et al 1994, Goodman et al 1966). Evidence for human provided in (Cahill 2006).

How this Key Event Works

After two to three days of fasting in humans, dietary glucose has been long-since expended and contribution to blood glucose from glycogen metabolism is reduced to zero (Cahill 2006). At this point, about two fifths of fatty acid metabolism in the whole body is dedicated to hepatic ketogenesis, largely in support of the energy demands of the brain, however the brain is still significantly supported by glucose derived from gluconeogenesis (Cahill 2006). As fatty acid stores are depleted, gluconeogenesis from other substrates becomes increasingly important including muscle protein catabolism in situ for supporting muscle function as well as releasing glutamine (Marliss et al 1971) and alanine (Felig et al 1970A) which can be recycled to glucose by gluconeogenesis in the kidney (Goodman et al 1966, Kashiwaya et al 1994, Cahill 2006). Renal gluconeogenesis from glutamine and alanine supports two fifths of new glucose production while the remaining three fifths is produced in liver from, (a) alanine derived from muscle and nonhepatic splanchnic bed, (b) recycled lactate and pyruvate from red blood cells and renal medulla, (c) glycerol from adipose lipolysis and (d) small amounts of β -hydroxybutyrate are recycled to glucose (Cahill 2006). Blood concentrations of alanine exert control over hepatic glucose production and thus also represent a diagnostic of alanine contribution from muscle to support gluconeogenesis (Cahill 2006, Felig et al 1970B). In prolonged starvation events, the catabolism of muscle protein for gluconeogenesis in order to support systemic energy needs results in loss of muscle mass which contributes to loss of overall body weight.

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

Glutamate and glutamine were measured in fresh plasma taken from human subjects that were fasted and those in a postabsorptive state using enzymatic assays (Marliss et al 1971).

In Kashiwaya et al (1994), perfused rat hearts were prepared for metabolic flux experiments. Measurement of enzyme kinetics involved in glycolysis and gluconeogenesis were measured using fluorometric procedures measuring the oxidation or reduction of pyridine nucleotides. Radio-labeled substrates were used to track metabolite flux during glycolysis / gluconeogenesis.

Goodman et al provided in vitro assessment of gluconeogenic capacity of renal cortex in rats. Glutamic acid and other ketogenic substrates were added and measure in the system and measured as net glucose content.

All amino acids were measured in Felig et al (1970A), however the analytical methods that were references were not found using Google Scholar search.

References

Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr 2006, 26:1-22.

Felig P, Pozefsky T, Marliss E, Cahill GF, Jr.: Alanine: key role in gluconeogenesis. Science 1970A, 167(3920):1003-1004.

Felig P, Marliss E, Pozefsky T, Cahill GF, Jr.: Amino acid metabolism in the regulation of gluconeogenesis in man. Am J Clin Nutr 1970B, 23(7):986-992.

Goodman AD, Fuisz RE, Cahill GF: Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. J Clin Invest 1966, 45(4):612-619.

Kashiwaya Y, Sato K, Tsuchiya N, Thomas S, Fell DA, Veech RL, Passonneau JV: Control of glucose utilization in working perfused rat heart. J Biol Chem 1994, 269(41):25502-25514.

Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF: Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. J Clin Invest 1971, 50(4):814-817.

1000: stabilization, PPAR alpha co-repressor

Short Name: stabilization, PPAR alpha co-repressor

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	KeyEvent

Biological Organization

Level of Biological Organization
Molecular

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
yeast	Saccharomyces cerevisiae	Moderate	NCBI

The fundamental mechanics for co-repressor antagonism of nuclear signaling by SMRT and N-CoR have been observed to be equivalent when comparing humans and yeast (Nagy et al 1999). Therefore, the taxonomic applicability should be broad across eukaryotes. However, knowledge of the specific amino acid sequence for PPAR α in the species of interest relative to human (for which the present evidence is based) is valuable for understanding the confidence in extrapolating relationships across species.

How this Key Event Works

The transcription co-repressors, silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) have been observed to compete with transcriptional co-activators for binding to nuclear receptors (including PPAR α) thus suppressing basal transcriptional activity (Nagy et al 1999, Xu et al 2002). Binding motifs for the co-repressors and co-activators to PPAR α have been observed to be conserved, however the co-repressor blocks the AF-2 helix from adopting the active conformation, as demonstrated in x-ray crystallography results presented in Xu et al (2002). PPAR α agonists and antagonists have been developed to selectively enhance co-activator or co-repressor binding, respectively (Xu et al 2001, Xu et al 2002). Regarding the present key event, the prior binding of a PPAR α antagonists such as GW6471 can stabilize the binding of the co-repressors to the PPAR α signaling complex suppressing nuclear signaling and thus downstream transcription of PPAR α -regulated genes.

Wilbanks et al. (2014) and Gust et al (2015) demonstrated inhibition of human PPAR α nuclear signaling in in vitro nuclear signaling bioassays in response to 2,4-dinitrotoluene(2,4-DNT) and 2-amino-4,6-dinitrotoluene (2A-DNT), respectively. However, it is unknown if this response was manifested through the co-repressor binding stabilization that was identified in (Xu et al 2002).

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

In Xu et al (2002), X-ray crystallography was used to characterize the suppressed PPAR α signaling complex (PPAR α / GW6471 / SMRT) and was compared against the activated PPAR α complex which included binding of PPAR α with the agonist GW409544 and the co-activator, steroid receptor coactivator-1 (SRC-1).

The effects of 2,4-DNT and 2A-DNT on PPAR signaling was investigated using nuclear receptor reporter assays were conducted for (PPARalpha, PPARgamma, PPARsigma, and RXRalpha human cell-based assays, (Indigo Biosciences).

References

Gust KA, Nanduri B, Rawat A, Wilbanks MS, Ang CY, Johnson DR, Pendarvis K, Chen X, Quinn Jr. MJ, Johnson MS, Burgess SC, Perkins EJ. 2015. Systems Toxicology Identifies Mechanistic Impacts of 2-amino-4,6-dinitrotoluene (2A-DNT) Exposure in Northern Bobwhite. BMC Genomics. 16:587 doi:10.1186/s12864-015-1798-4

Nagy L, Kao H-Y, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JWR: Mechanism of corepressor binding and release from nuclear hormone receptors. Genes Dev 1999, 13(24):3209-3216.

Wilbanks, M., Gust, K.A., Atwa, S., Sunesara, I., Johnson, D., Ang, C.Y., Meyer., S.A., and Perkins, E.J. 2014. Validation of a genomics-based hypothetical adverse outcome pathway: 2,4-dinitrotoluene perturbs PPAR signaling thus impairing energy metabolism and exercise endurance. Toxicological Sciences. 141(1):44-58.

Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, Oplinger JA, Kliewer SA, Gampe RT, McKee DD et al: Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proceedings of the National Academy of Sciences 2001, 98(24):13919-13924.

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT et al: Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPAR[alpha]. Nature 2002, 415(6873):813-817.

Adverse Outcomes

Title	Short name
Decreased, Body Weight	Decreased, Body Weight

864: [Decreased, Body Weight](#)

Short Name: Decreased, Body Weight

AOPs Including This Key Event

AOP ID and Name	Event Type
6: Antagonist binding to PPARalpha leading to starvation-like body-weight loss	AdverseOutcome

Biological Organization

Level of Biological Organization
Individual

Evidence Supporting Applicability of this Event

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI
Mus musculus	Mus musculus	Strong	NCBI
Colinus virginianus	Colinus virginianus	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	Moderate

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

Evidence for human (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). Evidence for mice (Badman et al 2007, Sanderson et al 2010, Wilbanks et al 2014, Xu et al 2012, Kersten et al 1999). Evidence for birds (Martin et al 1987).

How this Key Event Works

If caloric intake is less than caloric use over time, an individual will lose body weight. This is a basic principle in human dieting as well as an important principle related to individual health and ecological fitness of animal populations. Dynamic energy budget theory has provided useful insights on how organisms take up, assimilate and then allocate energy to various fundamental biological processes including maintenance, growth, development and reproduction (Nisbet et al 2000). Regarding energy allocation, somatic maintenance must first be met before then growth may occur, followed by maturation and then finally, surplus energy is dedicated to reproduction (Nisbet et al 2000). As an example of the importance of energy allocation to ecological fitness, a review by Martin et al (1987) demonstrated that energy availability (availability of food) was the predominant limiting factor in reproductive success and survival for both young and parents in a broad life history review for bird species. This is a likely scenario for many organisms.

Various physiological processes act to maintain and prioritize energy allocations in individuals. The influence of PPARalpha on systemic energy metabolism and energy homeostasis has been broadly established (see reviews by Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). Inhibition of PPARalpha predominantly impairs lipid metabolism with respect to overall energy metabolism whereby energy release from fatty acid substrates is decreased. PPARalpha has been demonstrated to play a critical role in stimulating fatty acid oxidation and ketogenesis during fasting resulting in increased ketone body levels in plasma (Badman et al 2007, Kersten 2014) a response that is eliminated in PPARalpha knockout mice (Badman et al 2007, Sanderson et al 2010). A reviews by Cahill (2006) and Wang et al (2010) summarize the critical adaptive response of ketogenesis during fasting for maintaining systemic energy homeostasis by providing ketone bodies to energetically fuel a diverse range of tissues, especially the brain. Not only does PPARalpha induce the upstream production of the raw materials for use

in ketogenesis through fatty acid beta-oxidation, but also directly induces key enzymes in the ketogenesis pathway including Hmgcs2, Hmgcl and Acat1 (Kersten 2014).

Kersten et al (1999) and Badman et al (2007) demonstrated that PPARalpha-null mice were unable to actively mobilize fatty acid oxidation, and further, Kersten et al (1999) demonstrated that these mice were unable to meet energy demands during fasting and leading to hypoglycemia, hyperlipidemia, hypoketonemia and fatty liver. Observations from toxicological and toxicogenomic research have implicated nitrotoluenes as potential PPAR antagonists in birds (Rawat et al 2010), rats (Deng et al 2011) and mice (Wilbanks et al 2014), an effect that additionally corresponded with weight loss in rats (Wilbanks et al 2014) and weight loss, loss of muscle mass and emaciation in birds (Quinn et al 2007). These combined results indicate that inhibition of PPARalpha signaling and the resultant decrease in fatty acid oxidation and ketogenesis can detrimentally impair systemic energy budgets leading to starvation-like effects and resultant weight loss. As reviewed in the introductory paragraph of this adverse outcome description, impaired energy availability leading to inability to meet somatic maintenance needs and causing negative growth are likely to have detrimental effects on survivorship, reproduction and ecological fitness.

How it is Measured or Detected

Methods that have been previously reviewed and approved by a recognized authority should be included in the Overview section above. All other methods, including those well established in the published literature, should be described here. Consider the following criteria when describing each method: 1. Is the assay fit for purpose? 2. Is the assay directly or indirectly (i.e. a surrogate) related to a key event relevant to the final adverse effect in question? 3. Is the assay repeatable? 4. Is the assay reproducible?

Dynamic energy budget model development and validation demonstrated against various parameter values and population studies (Nisbet et al 2000).

Food availability, animal weights, brood sizes, adult survival, and juvenile survival measured in Martin et al (1987).

Whole body animal weights were measured for mice in Wilbanks et al (2014). Whole body weights, organ weights and breast muscle weights measured in Quinn et al (2007).

In vitro human PPARalpha nuclear-receptor activation/inhibition assays have been used to determine if chemicals interfere with PPARalpha nuclear signaling (Wilbanks et al 2014, Gust et al 2015).

Transcript Expression of PPARalpha as well as transcript expression for genes in which PPARalpha acts as a transcriptional regulator (Wilbanks et al 2014, Deng et al 2011, Rawat et al 2010, and studies reviewed in Kersten 2014).

Regulatory Examples Using This Adverse Outcome

Weight loss in wild populations has direct implications on fitness as demonstrated dynamic energy budget modeling (Nisbet et al 2000). Thus weight loss can be used as a metric for populations sustainability. For individuals, rapid weight loss of greater than 20% total body weight is considered indicative of a moribund condition in laboratory animals for many Institutional Animal Care and Use Committees as established by American Association for Laboratory Animal Science ([1]).

References

Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is

regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.

Cahill GF, Jr.: Fuel metabolism in starvation. *Annu Rev Nutr* 2006, 26:1-22.

Deng Y, Meyer SA, Guan X, Escalon BL, Ai J, Wilbanks MS, Welti R, Garcia-Reyero N, Perkins EJ (2011) Analysis of common and specific mechanisms of liver function affected by nitrotoluene compounds. *PLoS One* 6(2): e14662.

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.

Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.

Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.

Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W: Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* 1999, 103(11):1489-1498.

Martin TE: Food as a limit on breeding birds: a life-history perspective. *Annu Rev Ecol Syst* 1987:453-487.

Nisbet R, Muller E, Lika K, Kooijman S: From molecules to ecosystems through dynamic energy budget models. *J Anim Ecol* 2000, 69(6):913-926.

Quinn MJ Jr, Bazar MA, McFarland CA, Perkins EJ, Gust KA, Gogal Jr RM, Johnson MS (2007) Effects of subchronic exposure to 2,6-dinitrotoluene in the northern bobwhite (*Colinus virginianus*). *Environmental Toxicology and Chemistry* 26(10):2202-2207.

Rawat A, Gust KA, Deng Y, Garcia-Reyero N, Quinn MJ, Jr., Johnson MS, Indest KJ, Elasri MO, Perkins EJ (2010) From raw materials to validated system: the construction of a genomic library and microarray to interpret systemic perturbations in Northern bobwhite. *Physiological Genomics* 42(2):219-235.

Sanderson, L.M., Boekschoten, M.V., Desvergne, B., Muller, M., Kersten, S., 2010. Transcriptional profiling reveals divergent roles of PPARalpha and PPARbeta/delta in regulation of gene expression in mouse liver. *Physiological Genomics* 41:42e52.

Wang YX: PPARs: diverse regulators in energy metabolism and metabolic diseases. *Cell Res* 2010, 20(2):124-137.

Wilbanks, M., Gust, K.A., Atwa, S., Sunesara, I., Johnson, D., Ang, C.Y., Meyer, S.A., and Perkins, E.J. 2014. Validation of a genomics-based hypothetical adverse outcome pathway: 2,4-dinitrotoluene perturbs PPAR signaling thus impairing energy metabolism and exercise endurance. *Toxicological Sciences*. 141(1):44-58.

Scientific evidence supporting the linkages in the AOP

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Decreased, PPARalpha transactivation of gene expression	directly leads to	Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids	Strong	Strong
Decreased, PPARalpha transactivation of gene	directly leads to	Decreased, Mitochondrial Fatty Acid Beta Oxidation	Strong	Strong

expression				
Decreased, PPARalpha transactivation of gene expression	directly leads to	Decreased, Ketogenesis (production of ketone bodies)	Moderate	Moderate
Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids	indirectly leads to	Decreased, Mitochondrial Fatty Acid Beta Oxidation	Moderate	Moderate
Decreased, Mitochondrial Fatty Acid Beta Oxidation	directly leads to	Decreased, Ketogenesis (production of ketone bodies)	Strong	Moderate
Decreased, Ketogenesis (production of ketone bodies)	directly leads to	Not Increased, Circulating Ketone Bodies	Strong	Moderate
Not Increased, Circulating Ketone Bodies	indirectly leads to	Increased, Catabolism of Muscle Protein	Moderate	Weak
Increased, Catabolism of Muscle Protein	directly leads to	Decreased, Body Weight	Strong	Strong
Binding of antagonist, PPAR alpha	indirectly leads to	stabilization, PPAR alpha co-repressor	Strong	Moderate
stabilization, PPAR alpha co-repressor	directly leads to	Decreased, PPARalpha transactivation of gene expression	Strong	Moderate

Decreased, PPARalpha transactivation of gene expression leads to Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
humans	Homo sapiens	Strong	NCBI
rat	Rattus norvegicus	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The relationships described herein have been primarily established in human and rodent models.

How Does This Key Event Relationship Work

PPAR α is a transcriptional regulator for a variety of genes that facilitate systemic energy homeostasis (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). Inhibition of PPAR α transactivation (KE1) results in decreased transcriptional expression for genes that catalyze the peroxisomal fatty acid beta oxidation pathway (Desvergne and Wahli 1999, Kersten 2014, Dreyer et al. 1992, Lazarow 1978). The processes of the KE, peroxisomal fatty acid beta-oxidation, are fairly well described in the literature including good coverage of the gene products that catalyze the metabolic reactions (Kersten 2014) with reasonable characterization of metabolic flux (Mannaerts and Van Veldhoven 1993, Desvergne and Wahli 1999), thus the WOE scores for KER were in the medium to medium-high range.

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

PPAR α knock out nullifies downstream expression of transcripts for genes involved in peroxisomal beta-oxidation of fatty acids (Kersten et al 2014).

Uncertainties or Inconsistencies

The KER relationship between the KE, “decreased PPAR α transactivation of gene expression” and the KE, “decreased peroxisomal fatty acid beta oxidation” is well supported by the literature (see references above). Few uncertainties remain, and few inconsistencies have been reported.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

A large body of research demonstrated that PPAR α nuclear signaling directly controls transcriptional expression for genes catalyzing peroxisomal beta-oxidation of very long chain fatty acids (>20C), mitochondrial beta-oxidation of short, medium and long chain fatty acids (<20C), and ketogenesis (as reviewed in Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999, Sanderson et al 2010). The majority of the research described in these reviews was established using gene knock outs, so there is not much dose-response information available describing the KE, “decreased PPAR α transactivation of gene expression” -> the KE, “decreased peroxisomal fatty acid beta oxidation”.

References

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.

Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* 68(5):879-887.

Evans RM, Barish GD, Wang YX. 2004. Ppars and the complex journey to obesity. *Nat Med* 10:355-361.

Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.

Lazarow PB: Rat liver peroxisomes catalyze the beta oxidation of fatty acids. *J Biol Chem* 1978, 253(5):1522-1528.

Mannaerts GP, Van Veldhoven PP (1993) Metabolic role of mammalian peroxisomes. In: Gibson G, Lake B (eds.) *Peroxisomes: Biology and Importance in Toxicology and Medicine*. Taylor & Francis, London, pp 19–62.

Decreased, PPARalpha transactivation of gene expression leads to Decreased, Mitochondrial Fatty Acid Beta Oxidation

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The relationships described herein have been primarily established in human and rodent models.

How Does This Key Event Relationship Work

PPAR α is a transcriptional regulator for a variety of genes that facilitate systemic energy homeostasis (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). The KE “Inhibition of PPAR α transactivation” results in the KE, “decreased transcriptional expression for genes that catalyze mitochondrial fatty acid beta oxidation” (Kersten 2014, Brandt et al. 1998; Mascaro et al. 1998, Aoyama et al. 1998, Gulick et al. 1994, Sanderson et al. 2008). A robust literature-base is available for mitochondrial fatty acid beta-oxidation including broad investigation of key enzymes (Brandt et al. 1998; Mascaro et al. 1998, Kersten 2014, Sanderson et al. 2008, Aoyama et al. 1998) and detailed examination of metabolic flux (Aoyama et al. 1998, Badmann et al. 2007, Potthoff et al. 2009), thus the KER received relatively high scores (see weight of evidence section on main page <https://aopkb.org/aopwiki/index.php/Aop:6>).

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

Blocking PPAR α signaling has been shown to inhibit expression of transcripts / enzymes involved in both peroxisomal and mitochondrial beta-oxidation causing impaired fatty acid catabolism, fatty acid accumulation in the liver and impaired cellular energy state during fasting events (Badman et al 2007, Kersten et al 1999).

Uncertainties or Inconsistencies

The KER between the KE, “decreased PPAR α transactivation of gene expression” -> the KE “decreased mitochondrial fatty acid beta-oxidation” is well supported by the literature (see references above). Few uncertainties remain, and few inconsistencies have been reported.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

A large body of research demonstrated that PPAR α nuclear signaling directly controls transcriptional expression for genes catalyzing mitochondrial beta-oxidation of short, medium and long chain fatty acids (<20C) (as reviewed in Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999, Sanderson et al 2010). The majority of the research described in these reviews was established using gene knock outs, so there is not much dose-response information available describing the KER between the KE, “decreased PPAR α transactivation of gene expression” -> the KE “decreased mitochondrial fatty acid beta-oxidation”.

References

Aoyama, T., Peters, J.M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., et al., 1998. Altered constitutive expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *Journal of Biological Chemistry* 273:5678e5684.

Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.

Brandt JM, Djouadi F, Kelly DP: Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. *J Biol Chem* 1998, 273(37):23786-23792.

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.

Evans RM, Barish GD, Wang YX. 2004. Ppars and the complex journey to obesity. *Nat Med* 10:355-361.

Gulick T, Cresci S, Caira T, Moore DD, Kelly DP (1994) The peroxisome proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proceedings of the National Academy of Sciences USA* , 91(23):11012-11016.

Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.

Mascaró C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D: Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J Biol Chem* 1998, 273(15):8560-8563.

Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.

Sanderson LM, Boekschoten MV, Desvergne B, Muller M, Kersten S (2010) Transcriptional profiling reveals divergent roles of PPARalpha and PPARbeta/delta in regulation of gene expression in mouse liver. *Physiological Genomics* 41:42e52.

Decreased, PPARalpha transactivation of gene expression leads to Decreased, Ketogenesis (production of ketone bodies)

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	Strong	NCBI
Homo sapiens	Homo sapiens	Strong	NCBI
Rattus rattus	Rattus rattus	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	Moderate

Sex Applicability

Sex	Evidence
Male	Moderate
Female	Moderate

The relationships described herein have been primarily established in human and rodent models.

How Does This Key Event Relationship Work

PPAR α is a transcriptional regulator for a variety of genes that facilitate systemic energy homeostasis (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). The KE, “decreased PPAR α transactivation of gene expression” results in the KE, “decreased transcriptional expression for genes that catalyze ketogenesis” (Cahil

2006, Kersten et al. 2014, Sengupta et al. 2010, Desvergne and Wahli 1999). Enzyme description (Kersten 2014, Sengupta et al. 2010) and metabolic flux examinations (Sengupta et al. 2010) additionally providing fairly robust characterization in support of the KER.

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

Given that inhibition of PPAR α transactivation results in downstream inhibition of transcriptional expression for the genes that catalyze ketogenesis, as well as ketone body production (Badman et al 2007, Potthoff 2009, Sengupta 2010), that KE occurs prior to the KE of decreased ketogenesis.

Uncertainties or Inconsistencies

A critical data gap regarding this AOP is an absence of studies that have investigated the effects null mutants for ketogenesis on the physiology and individual performance during long term starvation relative to wild type individuals.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

Enzyme description (Kersten 2014, Sengupta et al. 2010) and metabolic flux examinations (Sengupta et al. 2010) additionally providing fairly robust characterization in support for the KE of decreased ketogenesis. Little dose-response information is available regarding decreased transcriptional expression of genes involved in ketogenesis and ketone body production.

References

Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPAR α and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.

Cahill Jr., G.F., 2006. Fuel metabolism in starvation. *Annual Review of Nutrition* 26:1e22.

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.

Evans RM, Barish GD, Wang YX. 2004. Ppars and the complex journey to obesity. *Nat Med* 10:355-361.

Kersten S. 2014. Integrated physiology and systems biology of PPAR α . *Molecular Metabolism* 2014, 3(4):354-371.

Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.

Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010, 468(7327):1100-1104.

Decreased, Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids leads to Decreased, Mitochondrial Fatty Acid Beta Oxidation

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

Sex	Evidence
Male	Moderate
Female	Moderate

The evidence provided is primarily derived from human and rodent models.

How Does This Key Event Relationship Work

Peroxisomes participate in a variety of lipid metabolic pathways including the beta-oxidation of very long-straight chain (<20 C in length) or branched –chain acyl-CoAs (Lazarow 1978, Kersten 2014). The peroxisomal beta-oxidation pathway is not directly coupled to the electron transport chain and oxidative phosphorylation, therefore the first oxidation reaction loses energy to heat (H₂O₂ production) while in the second step, energy is captured in the metabolically accessible form of high-energy electrons in NADH (Mannaerts and Van Veldhoven 1993, Desvergne and Wahli 1999). The peroxisomal beta-oxidation pathway provides fatty acid chain shortening where two carbons are removed in each round of oxidation in the form of acetyl-CoA (Desvergne and Wahli 1999). The shortened chain fatty acids (<20C) can then be transported to the mitochondria to undergo mitochondrial beta-oxidation for complete metabolism of the carbon substrate for cellular energy production (Desvergne and Wahli 1999). Mitochondrial beta-oxidation catabolizes short, medium and long chain fatty acids (<C20) into acetyl-CoA and ATP. The production of acetyl-CoA monomers is important as they serve as fundamental units for metabolic energy production (ATP) via the citric acid cycle followed by electron-transport chain mediated oxidative phosphorylation (Nelson and Cox, 2000A). Acetyl-CoA is also a fundamental units of energy storage via gluconeogenesis (Nelson and Cox, 2000B) and lipogenesis (Nelson and Cox, 2000C).

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

As described in the previous sections, there is a fundamental linkage between KEs given that the KE, “peroxisomal fatty acid beta oxidation of fatty acids” produces raw materials that can be used in the KE, “mitochondrial fatty acid beta oxidation”. It is less clear how essential the latter is to a sustainable throughput of the former.

Uncertainties or Inconsistencies

The degree to which the KE, “peroxisomal fatty acid beta oxidation of fatty acids” contributes to the KE, “mitochondrial fatty acid beta oxidation” under a broad range of nutrient levels and types is not well characterized.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

As discussed in the previous sections, the degree to which the KE, “peroxisomal fatty acid beta oxidation of fatty acids” contributes to the KE, “mitochondrial fatty acid beta oxidation” is not well described, neither are modulators of the response-response relationship. We are not currently aware of any models available to extrapolate results among KEs.

References

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.

Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.

Lazarow PB: Rat liver peroxisomes catalyze the beta oxidation of fatty acids. *J Biol Chem* 1978, 253(5):1522-1528.

Mannaerts GP, Van Veldhoven PP 1993 Metabolic role of mammalian peroxisomes. In: Gibson G, Lake B (eds) *Peroxisomes: Biology and Importance in Toxicology and Medicine*. Taylor & Francis, London, pp 19–62.

Nelson DL, Cox MM 2000A. The Citric Acid Cycle. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p567-592.

Nelson DL, Cox MM 2000B. Carbohydrate Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p722-764.

Nelson DL, Cox MM 2000C. Lipid Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p770-814.

Decreased, Mitochondrial Fatty Acid Beta Oxidation leads to Decreased, Ketogenesis (production of ketone bodies)

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links

human	Homo sapiens	Strong	NCBI
rat	Rattus norvegicus	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	Moderate

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The relationships described herein have been primarily established in human and rodent models.

How Does This Key Event Relationship Work

The KE, “mitochondrial fatty acid beta oxidation” catabolizes short, medium and long chain fatty acids (<C20) into acetyl-CoA and ATP. The production of acetyl-CoA monomers is important as they serve as fundamental units for metabolic energy production (ATP) via the citric acid cycle followed by electron-transport chain mediated oxidative phosphorylation (Nelson and Cox, 2000A). Acetyl-CoA is also a fundamental units of energy storage via gluconeogenesis (Nelson and Cox, 2000B) and lipogenesis (Nelson and Cox, 2000C). The liver plays a key role in processing the fundamental energy substrate, acetyl-CoA, into metabolic currencies that contribute to the systemic cellular energy needs of the whole organism. The liver represents a key organ involved in systemic energy distribution given its ability to synthesize glucose (an ability shared only with the kidney, Gerich et al 2001) as well as its exclusive role in the generation of ketone bodies (Cahill 2006, Sengupta et al 2010, Kersten 2014). This is especially important for the metabolic energy needs of the brain which can only use glucose and the ketone body, β -hydroxybutyrate for cellular energy production (Cahill 2006, Owen 2005, Kersten 2014). Therefore, the KE, “ketogenesis (production of ketone bodies)” is critical to supporting general systemic energy homeostasis in fasting events (Cahill 2006, Evans et al 2004, Sengupta et al 2010).

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

As described in the previous sections, there is a fundamental linkage between KEs given that the KE, “mitochondrial fatty acid beta oxidation” produces raw materials that are used in the KE, “ketogenesis (production of ketone bodies)”. It is less clear how essential the former is to a sustainable throughput of the latter especially given that the latter can utilize substrates that can be produced by various other cellular energy processing pathways in addition to mitochondrial fatty acid beta oxidation.

Uncertainties or Inconsistencies

Additional investigations tracing substrate processing, specifically from sources resulting from the KE, “mitochondrial fatty acid beta oxidation” under control as well as starvation conditions would supplement current understanding of the connections between the KE, “mitochondrial fatty acid beta oxidation” and the KE, “ketogenesis (production of ketone bodies)”.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

As discussed in the previous sections, the degree to which the, KE “mitochondrial fatty acid beta oxidation” affects the KE, “ketogenesis (production of ketone bodies)” is not well described, neither are modulators of the response-response relationships. Certainly, the pathways are interrelated and connected by PPARalpha as the master regulator of each process, so additional modulators related to resource availability and cellular signaling require exploration. We are not currently aware of any models available to extrapolate results among KEs.

References

- Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.
- Cahill GF, Jr. Fuel metabolism in starvation. *Annu Rev Nutr* 2006, 26:1-22.
- Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.
- Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001, 24(2):382-391.
- Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.
- Nelson DL, Cox MM 2000A. The Citric Acid Cycle. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p567-592.
- Nelson DL, Cox MM 2000B. Carbohydrate Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p722-764.
- Nelson DL, Cox MM 2000C. Lipid Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p770-814.
- Owen OE: Ketone bodies as a fuel for the brain during starvation. *Biochem Mol Biol Educ* 2005, 33(4):246-251.
- Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.
- Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010, 468(7327):1100-1104.

Decreased, Ketogenesis (production of ketone bodies) leads to Not Increased, Circulating Ketone Bodies

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	Moderate

Sex Applicability

Sex	Evidence
Male	Moderate
Female	Moderate

The relationships described herein have been primarily established in human and rodent models.

How Does This Key Event Relationship Work

The KE, “ketogenesis (production of ketone bodies)” is critical to supporting general systemic energy homeostasis in fasting events (Cahill 2006, Evans et al 2004, Sengupta et al 2010). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009, Sengupta et al 2010). The Badman et al (2007) study indicated that metabolism of fatty acid substrates (measured as liver triglycerides) that would otherwise contribute to β -hydroxybutyrate production was additionally inhibited under PPAR α knockout.

In a fasting state, humans transition from the use of exogenous glucose to glucose derived from glycogen within 4 hours with a steadily increasing proportion of glucose usage that is derived from gluconeogenesis up to 2 days (Cahill 2006). Beyond 2 days of fasting, ketone body production (β -hydroxybutyrate) increasingly supports the energy demands of the brain (Cahill 2006).

Physiological studies of the progression of human starvation have identified that the preferred metabolic fuel is glucose in the fed state and progressing through two days of fasting, afterward ketone bodies become increasingly important for meeting energy demands (Cahill 2006, Owen et al 2005). Substrates derived from carbohydrates, fats and protein can contribute to gluconeogenesis (Cahill 2006, Gerich et al 2001) whereas substrates derived from fatty acids are the primary contributors to ketogenesis (Desvergne and Wahli 1999). Mobilization of fatty acids as a metabolic fuel source increase dramatically during fasting to support both gluconeogenesis and ketogenesis (Evans et al 2004). Cahill (2006) and colleagues have demonstrated the importance of ketone body production, especially β -hydroxybutyrate, for maintaining energy homeostasis during starvation. β -hydroxybutyrate serves as an alternative substrate to glucose for providing energy to the brain in the starvation state, providing ATP at higher efficiency relative to the glucose substrate (Cahill 2006). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009, Sengupta et al 2010). The Badman et al (2007) study indicated that metabolism of fatty acid substrates (measured as liver triglycerides) that would otherwise contribute to β -

hydroxybutyrate production was inhibited under PPAR α knockout. Increased concentrations of circulating ketone bodies is indicative of potential metabolic fuel deficits in fasting animals (Cahill 2006), and a lack of increase in circulating ketone bodies during fasting, especially in conjunction with elevated blood triglycerides, indicates impaired ketogenesis and potentially impaired bioenergetic potential.

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

As described in the bullets above, there is fairly robust empirical support for this KER, including temporal concordance associated with starvation events.

Uncertainties or Inconsistencies

The data is fairly robust. Additional specific systems level investigations with PPAR α signaling knockouts would be useful for understanding non-starvation related ketogenic processes.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

The results presented in the references cited above provide many of the quantitative relationships among KEs. Multiple signaling pathways are involved in the starvation response, so additional response-response relationships are likely to play a role in the systemic response as well as interaction with the KE, “decreased ketogenesis (production of ketone bodies)” and the KE, “no increase of circulating ketone bodies”. We are not currently aware of any models available to extrapolate results among KEs.

References

- Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPAR α and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.
- Cahill Jr., G.F., 2006. Fuel metabolism in starvation. *Annual Review of Nutrition* 26:1e22.
- Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688. Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.
- Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001, 24(2):382-391.
- Owen OE: Ketone bodies as a fuel for the brain during starvation. *Biochem Mol Biol Educ* 2005, 33(4):246-251.
- Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.
- Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010, 468(7327):1100-1104.

Not Increased, Circulating Ketone Bodies leads to Increased, Catabolism of Muscle Protein

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	Moderate

Sex Applicability

Sex	Evidence
Male	Moderate
Female	Moderate

The relationships described herein have been primarily established in human and rodent models.

How Does This Key Event Relationship Work

A fundamental process in all biological systems is the production of metabolic fuel for use in meeting the energy demands of cells and the systemic energy needs of multi-cellular organisms. Physiological studies of the progression of human starvation have identified that the preferred metabolic fuel is glucose in the fed state and progressing through two days of fasting, afterward ketone bodies become increasingly important for meeting energy demands (Cahill 2006, Owen et al 2005). Substrates derived from carbohydrates, fats and protein can contribute to gluconeogenesis (Cahill 2006, Gerich et al 2001) whereas substrates derived from fatty acids are the primary contributors to ketogenesis (KE5, Desvergne and Wahli 1999). Mobilization of fatty acids as a metabolic fuel source increase dramatically during fasting to support both gluconeogenesis and ketogenesis (Evans et al 2004). Cahill (2006) and colleagues have demonstrated the importance of ketone body production, especially β -hydroxybutyrate, for maintaining energy homeostasis during starvation. β -hydroxybutyrate serves as an alternative substrate to glucose for providing energy to the brain in the starvation state, providing ATP at higher efficiency relative to the glucose substrate (Cahill 2006). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009, Sengupta et al 2010). The Badman et al (2007) study indicated that metabolism of fatty acid substrates (measured as liver triglycerides) that would otherwise contribute to β -hydroxybutyrate production was inhibited under PPAR α knockout. Increased concentrations of circulating ketone bodies is indicative of potential metabolic fuel deficits in fasting animals (Cahill 2006), and a lack of increase in circulating ketone bodies during fasting, especially in conjunction with elevated blood triglycerides, indicates impaired ketogenesis and potentially impaired bioenergetic potential.

After two to three days of fasting in humans, dietary glucose has been long-since expended and contribution to blood glucose from glycogen metabolism is reduced to zero (Cahill 2006). At this point, about two fifths of fatty acid metabolism in the whole body is dedicated to hepatic ketogenesis, largely in support of the energy demands of the brain, however the brain is still significantly supported by glucose derived from gluconeogenesis (Cahill 2006). As fatty acid stores are depleted, gluconeogenesis from other substrates becomes increasingly important including muscle protein catabolism in situ for supporting muscle function as well as releasing glutamine (Marliss et al 1971) and alanine (Felig et al 1970A) which can be recycled to glucose by gluconeogenesis in the kidney (Goodman et al 1966, Kashiwaya et al 1994, Cahill 2006). Renal gluconeogenesis from glutamine and alanine supports two fifths of new glucose production while the remaining three fifths is produced in liver from, (a) alanine derived from muscle and nonhepatic splanchnic bed, (b) recycled lactate and pyruvate from red blood cells and renal medulla, (c) glycerol from adipose lipolysis and (d) small amounts of β -hydroxybutyrate are recycled to glucose (Cahill 2006). Blood concentrations of alanine exert control over hepatic glucose production and thus also represent a diagnostic of alanine contribution from muscle to support gluconeogenesis (Cahill 2006, Felig et al 1970B). In prolonged starvation events, the catabolism of muscle protein (KE6) for gluconeogenesis in order to support systemic energy needs results in loss of muscle mass which contributes to loss of overall body weight.

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

As described in the bullets above, there is fairly robust empirical support for this KER, although dedicated assays providing mechanistic / quantitative relationships between amino acid recycling for energy production relative to ketogenesis-based energy production are still needed.

Uncertainties or Inconsistencies

See previous section.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

Although the KER, for the KE, “no increase in circulating ketone bodies” -> the KE, “increased, catabolism of muscle protein” lacks a mechanistic connection, a strong correlative relationship exists between the KEs regarding energy homeostasis. Discovering response-response relationships regarding this complex signaling network represents a key basic research question related to systemic energy metabolism. We are not currently aware of any models available to extrapolate results among KEs.

References

Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.

Cahill GF, Jr. Fuel metabolism in starvation. *Annu Rev Nutr* 2006, 26:1-22.

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.

Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.

Felig P, Pozefsky T, Marliss E, Cahill GF, Jr.: Alanine: key role in gluconeogenesis. *Science* 1970A, 167(3920):1003-1004.

Felig P, Marliss E, Pozefsky T, Cahill GF, Jr.: Amino acid metabolism in the regulation of gluconeogenesis in man. *Am J Clin Nutr* 1970B, 23(7):986-992.

Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001, 24(2):382-391.

Goodman AD, Fuisz RE, Cahill GF: Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. *J Clin Invest* 1966, 45(4):612-619.

Kashiwaya Y, Sato K, Tsuchiya N, Thomas S, Fell DA, Veech RL, Passonneau JV: Control of glucose utilization in working perfused rat heart. *J Biol Chem* 1994, 269(41):25502-25514.

Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF: Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. *J Clin Invest* 1971, 50(4):814-817.

Owen OE: Ketone bodies as a fuel for the brain during starvation. *Biochem Mol Biol Educ* 2005, 33(4):246-251.

Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.

Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010, 468(7327):1100-1104.

Veech RL: The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids* 2004, 70(3):309-319.

Williamson DH, Mellanby J, Krebs HA: Enzymic determination of d(-)- β -hydroxybutyric acid and acetoacetic acid in blood. *Biochem J* 1962, 82(1):90-96.

Increased, Catabolism of Muscle Protein leads to Decreased, Body Weight

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Adults	Strong
Juvenile	Strong

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

This KER is generally applicable to animal systems.

How Does This Key Event Relationship Work

After two to three days of fasting in humans, dietary glucose has been long-since expended and contribution to blood glucose from glycogen metabolism is reduced to zero (Cahill 2006). At this point, about two fifths of fatty acid metabolism in the whole body is dedicated to hepatic ketogenesis, largely in support of the energy demands of the brain, however the brain is still significantly supported by glucose derived from gluconeogenesis (Cahill 2006). As fatty acid stores are depleted, gluconeogenesis from other substrates becomes increasingly important including muscle protein catabolism in situ for supporting muscle function as well as releasing glutamine (Marliss et al 1971) and alanine (Felig et al 1970A) which can be recycled to glucose by gluconeogenesis in the kidney (Goodman et al 1966, Kashiwaya et al 1994, Cahill 2006). Renal gluconeogenesis from glutamine and alanine supports two fifths of new glucose production while the remaining three fifths is produced in liver from, (a) alanine derived from muscle and nonhepatic splanchnic bed, (b) recycled lactate and pyruvate from red blood cells and renal medulla, (c) glycerol from adipose lipolysis and (d) small amounts of β -hydroxybutyrate are recycled to glucose (Cahill 2006). Blood concentrations of alanine exert control over hepatic glucose production and thus also represent a diagnostic of alanine contribution from muscle to support gluconeogenesis (Cahill 2006, Felig et al 1970B). In prolonged starvation events, the catabolism of muscle protein (KE6) for gluconeogenesis in order to support systemic energy needs results in loss of muscle mass which contributes to loss of overall body weight.

Dynamic energy budget theory has provided useful insights on how organisms take up, assimilate and then allocate energy to various fundamental biological processes including maintenance, growth, development and reproduction (Nisbet et al 2000). Regarding energy allocation, somatic maintenance (maintaining homeostasis) must first be met before then growth may occur, followed by maturation and then finally, surplus energy is dedicated to reproduction (Nisbet et al 2000). If somatic maintenance cannot be sustained, energy substrates must be generated using standing biomass from non-essential organs, such as skeletal muscle, to maintain homeostasis ultimately leading to the ultimate AO of weight loss (Cahill 2006). As an example of the importance of energy allocation to ecological fitness, a review by Martin et al (1987) demonstrated that energy availability (availability of food) was the predominant limiting factor in reproductive success and survival for both young and parents in a broad life history review for bird species. This is a likely scenario for many organisms.

Weight of Evidence

Biological Plausibility

Biological plausibility of this KER is strong given the supporting relationships cited in the literature described in the previous bullets above.

Empirical Support for Linkage

Include consideration of temporal concordance here

During starvation, the loss of muscle mass to sustain systemic energy requirements has been broadly documented as a source of body weight loss (Cahill 2006). Body weight loss can occur via component weight loss from nearly all organ systems. In starvation conditions, loss of muscle mass is essentially always connected to overall body-weight loss. Regarding temporal concordance, the process is reversed after feeding resumes.

Uncertainties or Inconsistencies

No uncertainties presented.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

Specific thresholds for translating the KE, “increased, catabolism of muscle protein” -> the AO, “decreased body weight” are species specific. Models for investigating these relationships are available including dynamic energy budget models (Nisbet et al 2000) as well as variety of detailed caloric models for humans.

References

Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr 2006, 26:1-22.

Felig P, Pozefsky T, Marliss E, Cahill GF, Jr.: Alanine: key role in gluconeogenesis. Science 1970A, 167(3920):1003-1004.

Felig P, Marliss E, Pozefsky T, Cahill GF, Jr.: Amino acid metabolism in the regulation of gluconeogenesis in man. Am J Clin Nutr 1970B, 23(7):986-992.

Kashiwaya Y, Sato K, Tsuchiya N, Thomas S, Fell DA, Veech RL, Passonneau JV: Control of glucose utilization in working perfused rat heart. J Biol Chem 1994, 269(41):25502-25514.

Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF: Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. J Clin Invest 1971, 50(4):814-817.

Martin TE: Food as a limit on breeding birds: a life-history perspective. Annu Rev Ecol Syst 1987:453-487.

Nisbet R, Muller E, Lika K, Kooijman S: From molecules to ecosystems through dynamic energy budget models. J Anim Ecol 2000, 69(6):913-926.

Binding of antagonist, PPAR alpha leads to stabilization, PPAR alpha co-repressor

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	Strong	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

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Sex	Evidence
Male	Strong
Female	Strong

The majority of the studies cited herein provide evidence for human and rat, however much of the signaling architecture is also present in yeast (Krogdams et al 2002).

How Does This Key Event Relationship Work

Binding of molecules to peroxisome proliferator-activated receptor α (PPAR α) can cause either agonistic or antagonistic signaling depending on molecular structure (Xu et al 2001, Xu et al 2002). Certain molecules that can bind to the PPAR α ligand binding domain have been observed to cause conformational changes that induce increased affinity to co-repressors which decrease PPAR α nuclear signaling (Xu et al 2002). The transcription co-repressors, silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) have been observed to compete with transcriptional co-activators for binding to nuclear receptors (including PPAR α) thus suppressing nuclear signaling activity (Nagy et al 1999, Xu et al 2002). Regarding the present MIE, PPAR α antagonists such as GW6471 which leads to the KE where increased binding and stabilization of the co-repressors to the PPAR α signaling complex suppressing nuclear signaling.

Weight of Evidence

Biological Plausibility

The biological plausibility is high given the crystal structure resolved for the bound group of GW6471, the co-repressor SMRT, and PPAR α where the ligand binding domain of PPAR α was set in the inactive conformation (Xu et al 2002).

Empirical Support for Linkage

Include consideration of temporal concordance here

The inclusion of GW6471 was observed to recruit binding of the co-repressors SMRT and NCOR to PPAR α in a positive dose-responsive manner (Xu et al 2002). Additionally, the application of the antagonist GW6471 was observed to displace the PPAR α agonist GW409544 thus reducing PPAR α signaling (Xu et al 2002). The MIE occurs in advance of co-repressor recruitment and changes in PPAR α signaling (Xu et al 2002).

Uncertainties or Inconsistencies

Regarding the present MIE, GW6471 has highly specific binding to the SMRT and N-CoR binding domains (Nagy et al 1999, Xu et al 2002). The degree to which other chemicals cause PPAR α antagonism by this specific MIE needs to be explored. For example, Wilbanks et al. (2014) and Gust et al (2015) demonstrated inhibition of human PPAR α nuclear signaling in in vitro nuclear signaling bioassays in response to 2,4-dinitrotoluene (2,4-DNT) and 2-amino-4,6-dinitrotoluene (2A-DNT), respectively. However, it is unknown if this response was manifested through the co-repressor binding stabilization that was identified in (Xu et al 2002).

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

A concentration-response curve has been developed for GW6471 recruiting binding of the SMRT and N-CoR co-repressors to the PPAR α complex (Xu et al 2002).

References

Gust KA, Nanduri B, Rawat A, Wilbanks MS, Ang CY, Johnson DR, Pendarvis K, Chen X, Quinn Jr. MJ, Johnson MS, Burgess SC, Perkins EJ (2015) Systems Toxicology Identifies Mechanistic Impacts of 2-amino-4,6-dinitrotoluene (2A-DNT) Exposure in Northern Bobwhite. BMC Genomics. In Press.

Krogsdam AM, Nielsen CA, Neve S, Holst D, Helledie T, Thomsen B, et al. 2002. Nuclear receptor corepressor-dependent repression of peroxisome-proliferator-activated receptor delta-mediated transactivation. Biochem J 363:157-165.

Nagy L, Kao H-Y, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JWR: Mechanism of corepressor binding and release from nuclear hormone receptors. Genes Dev 1999, 13(24):3209-3216.

Wilbanks, M., Gust, K.A., Atwa, S., Sunesara, I., Johnson, D., Ang, C.Y., Meyer., S.A., and Perkins, E.J. 2014. Validation of a genomics-based hypothetical adverse outcome pathway: 2,4-dinitrotoluene perturbs PPAR signaling thus impairing energy metabolism and exercise endurance. Toxicological Sciences. 141(1):44-58.

Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, et al. 2001. Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. Proceedings of the National Academy of Sciences 98:13919-13924.

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT et al: Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPAR[alpha]. Nature 2002, 415(6873):813-817.

stabilization, PPAR alpha co-repressor leads to Decreased, PPARalpha transactivation of gene expression

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Strong	NCBI
yeast	Saccharomyces cerevisiae	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
Not Otherwise Specified	Not Specified

Sex Applicability

Sex	Evidence
Male	Strong
Female	Strong

The majority of the studies cited herein provide evidence for human and rat, however much of the signaling architecture is also present in yeast (Krogsdam et al 2002).

How Does This Key Event Relationship Work

The transcription co-repressors, silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) have been observed to compete with transcriptional co-activators for binding to nuclear receptors (including PPAR α) thus suppressing basal transcriptional activity (Nagy et al 1999, Xu et al 2002). Regarding the present MIE, PPAR α antagonists such as GW6471 stabilize the binding of co-repressors to the PPAR α signaling complex suppressing nuclear signaling and thus downstream transactivation-transcription of PPAR α -regulated genes. Given that PPAR α trans-activation induces catabolism of fatty acids, this signaling pathway has been broadly demonstrated to play a key role in energy homeostasis (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999).

Weight of Evidence

Biological Plausibility

The biological plausibility is high given the crystal structure resolved for the bound group of GW6471, the co-repressor SMRT, and PPAR α where the ligand binding domain of PPAR α was set in the inactive conformation (Xu et al 2002). Additionally, Krogsdam et al (2002) have established dose-response relationships for increasing N-CoR activity with decreased fold induction of PPAR α transactivation potential.

Empirical Support for Linkage

Include consideration of temporal concordance here

See supporting evidence in previous bullets. The binding of the co-repressor to the PPAR α complex occurs in advance of suppression of PPAR α transactivation (Xu et al 2002).

Uncertainties or Inconsistencies

Given the observations that co-repressors can inhibit PPAR α nuclear signaling (Xu et al 2002) and downstream transactivation potential (Krogsdam et al 2002), each in a dose-responsive manner, this provides strong evidence for the present KER. It should be noted however that there are a variety of structural elements included in the PPAR α nuclear signaling complex, including the action of co-activators (Xu et al 2001), so there is potential for modifiers in the signaling cascade.

Quantitative Understanding of the Linkage

Is it known how much change in the first event is needed to impact the second? Are there known modulators of the response-response relationships? Are there models or extrapolation approaches that help describe those relationships?

Krogsdam et al (2002) have established dose-response relationships for increasing N-CoR activity with decreased fold induction of PPAR α transactivation potential.

References

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.

Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-3.

Kersten S. 2014. Integrated physiology and systems biology of PPAR α . *Molecular Metabolism* 2014, 3(4):354-371.

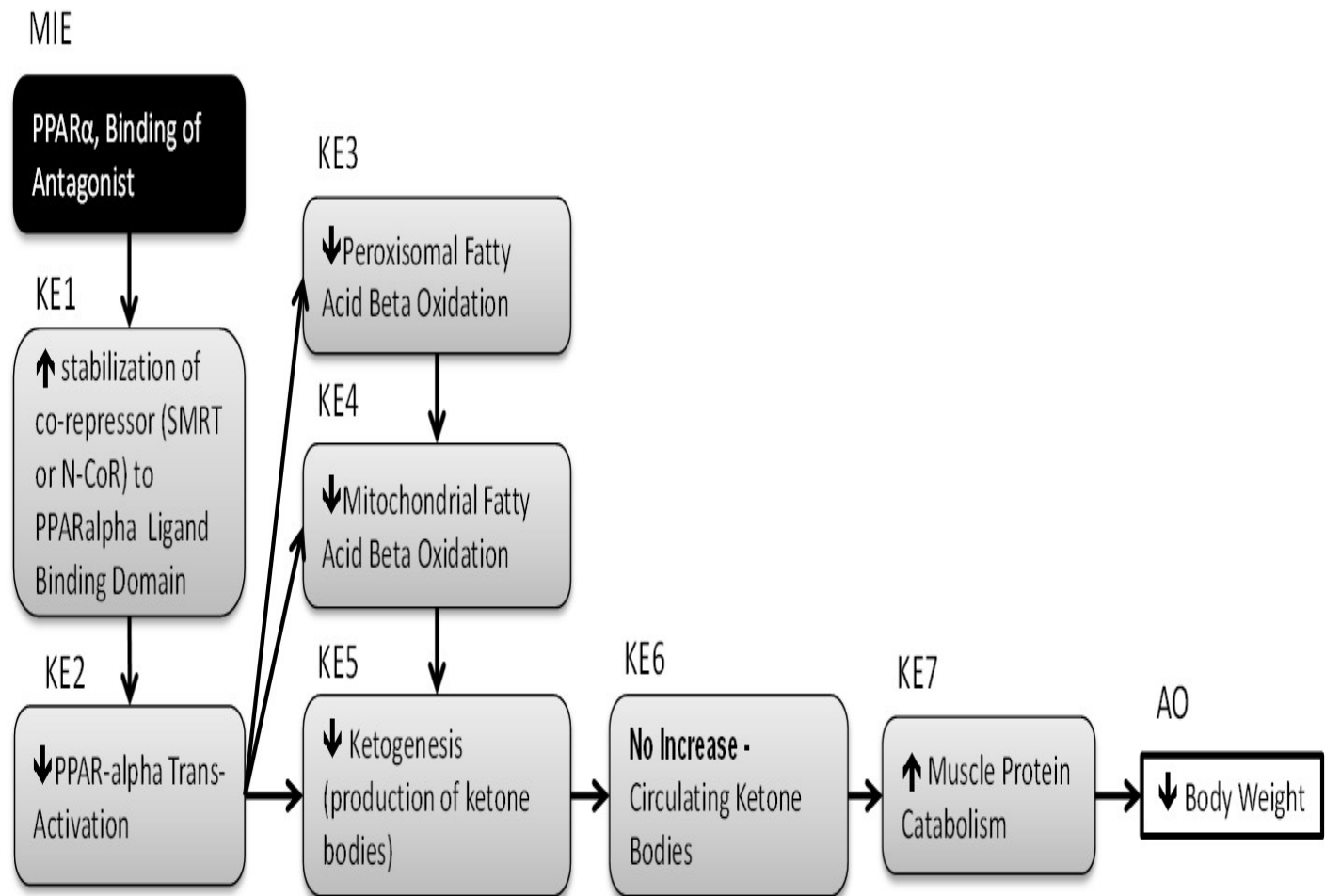
Krogsdam AM, Nielsen CA, Neve S, Holst D, Helledie T, Thomsen B, et al. 2002. Nuclear receptor corepressor-dependent repression of peroxisome-proliferator-activated receptor delta-mediated transactivation. *Biochem J* 363:157-165.

Nagy L, Kao H-Y, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JWR: Mechanism of corepressor binding and release from nuclear hormone receptors. *Genes Dev* 1999, 13(24):3209-3216.

Xu HE, Lambert MH, Montana VG, Plunket KD, Moore LB, Collins JL, et al. 2001. Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors. *Proceedings of the National Academy of Sciences* 98:13919-13924.

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT et al: Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPAR[alpha]. *Nature* 2002, 415(6873):813-817.

Graphical Representation



Overall Assessment of the AOP

The domain of applicability, the essentiality of the key events and weight of evidence for the overall hypothesized AOP are provided in the following:

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
Adult	Strong

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Mus musculus	Mus musculus	Strong	NCBI
Colinus virginianus	Colinus virginianus	Moderate	NCBI
Pimephales promelas	Pimephales promelas	Moderate	NCBI
Rattus norvegicus	Rattus norvegicus	Moderate	NCBI
Homo sapiens	Homo sapiens	Strong	NCBI

Sex Applicability

Sex	Evidence
Female	Strong
Male	Moderate

1) rats and mice-females,. 2) Northern bobwhite quail-juvenile males. 3) fathead minnow-adult, sex not determined. 4) carp-juvenile, sex not determined. 5) human, male & female

Essentiality of the Key Events

Rationale for essentiality calls:

• **MIE: PPAR alpha, Binding of antagonist:** Regarding the present MIE, molecules can bind to the PPAR α regulatory complex affecting the binding of co-activators and co-repressors. Specifically designed molecules such as the PPAR α antagonists GW6471 can bind to PPAR α selectively recruiting binding of co-repressors to the PPAR α nuclear signaling complex (Xu et al 2002).

• **Key Event 1: PPAR alpha co-repressor, Increased** - The binding of co-repressors to the PPAR α signaling complex suppresses nuclear signaling and thus downstream transcription of PPAR α -regulated genes. GW6471 binding to the co-repressor is reversible thus allowing the co-repressor to leave the ligand binding domain of PPAR α , restoring normal function (Xu et al 2002).

• **Key Event 2: PPARalpha transactivation of gene expression, Decreased** - As described in a variety of reviews, PPARalpha represents a master regulator of energy metabolism which specifically promotes fatty oxidation for energy production & distribution (Evans et al 2004, Kersten 2014, Lefebvre et al 2006, Desvergne and Wahli 1999).

Both PPAR α knock outs and PPAR α antagonism decreased transcriptional expression of gene targets involved in peroxisomal fatty acid beta oxidation (Kersten et al 1999, Desvergne and Wahli 1999), mitochondrial fatty acid beta oxidation (Brandt et al 1998; Mascaro et al 1998, Kersten 2014), and ketogenesis (Sengupta et al 2010, Desvergne and Wahli 1999, Kersten 2014).

•Key Event 3: Peroxisomal Fatty Acid Beta Oxidation of Fatty Acids, Decreased – The essentiality of peroxisomal fatty acid beta oxidation to maintaining the systems-level energy demands of the organism is not well known. This key event serves to metabolize very long chain fatty acids that are accumulated from the diet and to some degree acts as a supporting pathway to the mitochondrial fatty acid beta oxidation pathway (Kersten 2014). Given that both pathways are inhibited during PPAR α antagonism, it is difficult to separate the contribution to systemic energy sustainment supported by peroxisomal fatty acid beta oxidation alone.

•Key Event 4: Mitochondrial Fatty Acid Beta Oxidation, Decreased – This key event is essential for deriving metabolic energy from fatty acid substrates thus supporting a large component of overall organismal energy demands (Evans et al 2004, Kersten 2014, Desvergne and Wahli 1999). Short, medium and long chain fatty acids (<C20) are catabolized by mitochondrial beta-oxidation. PPAR α regulates nearly every enzymatic step in the uptake as well as the oxidative breakdown of acyl-CoAs to acetyl-CoA (Kersten 2014). The acetyl-CoA monomers serve as fundamental units for metabolic energy production (ATP) via the citric acid cycle followed by electron-transport chain mediated oxidative phosphorylation (Nelson and Cox, 2000A) as well as serve as the fundamental units for energy storage via gluconeogenesis (Nelson and Cox, 2000B) and lipogenesis (Nelson and Cox, 2000C). PPAR α knockout studies have demonstrated impaired mitochondrial fatty acid oxidation leading to fatty acid accumulation in the liver (Badmann et al 2007) as well as an inability to meet systemic energy demands (Kersten et al, 1999).

•Key Event 5: Ketogenesis (production of ketone bodies), decreased - The liver represents a key organ involved in systemic energy distribution given its ability to synthesize glucose (an ability shared only with the kidney, Gerich et al 2001) as well as its exclusive role in the generation of ketone bodies (Cahill 2006, Sengupta et al 2010, Kersten 2014). This is especially important for the metabolic energy needs of the brain which can only use glucose and the ketone body, β -hydroxybutyrate for cellular energy production (Cahill 2006, Owen 2005, Kersten 2014). Therefore, ketogenesis is critical to supporting general systemic energy homeostasis in fasting events (Cahill 2006, Evans et al 2004, Sengupta et al 2010). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009, Sengupta et al 2010). The Badman et al (2007) study indicated that metabolism of fatty acid substrates (measured as liver triglycerides) that would otherwise contribute to β -hydroxybutyrate production was additionally inhibited under PPAR α knockout.

•Key Event 6: Circulating Ketone Bodies, Not Increased - Physiological studies of the progression of human starvation have identified that the preferred metabolic fuel is glucose in the fed state and progressing through two days of fasting, afterward ketone bodies become increasingly important for meeting energy demands (Cahill 2006, Owen et al 2005). Substrates derived from carbohydrates, fats and protein can contribute to gluconeogenesis (Cahill 2006, Gerich et al 2001) whereas substrates derived from fatty acids are the primary contributors to ketogenesis (Desvergne and Wahli 1999). Cahill (2006) and colleagues have demonstrated the importance of ketone body production, especially β -hydroxybutyrate, for maintaining energy homeostasis during starvation by serving as an alternative substrate to glucose for providing energy to the brain in the starvation state (Cahill 2006). Interference with ketogenesis, for example by PPAR α inhibition, has been demonstrated to inhibit β -hydroxybutyrate production (measured in serum) during fasting events in mice (Badman et al 2007, Potthoff 2009). Under normal conditions, activated ketogenesis occurring during fasting events is rapidly deactivated when blood glucose concentrations increase to normal levels and resultant elevated circulating ketone bodies are reduced correspondingly (Cahill 2006).

•Key Event 7: Catabolism of Muscle Protein, Increased - After two to three days of fasting in humans, dietary

glucose has been long-since expended and contribution to blood glucose from glycogen metabolism is reduced to zero (Cahill 2006). At this point, about two fifths of fatty acid metabolism in the whole body is dedicated to hepatic ketogenesis, largely in support of the energy demands of the brain, however the brain is still significantly supported by glucose derived from gluconeogenesis (Cahill 2006). As fatty acid stores are depleted, gluconeogenesis from other substrates becomes increasingly important including muscle protein catabolism in situ for supporting muscle function as well as releasing glutamine (Marliss et al 1971) and alanine (Felig et al 1970A) which can be recycled to glucose by gluconeogenesis in the kidney (Goodman et al 1966, Kashiwaya et al 1994, Cahill 2006). In prolonged starvation events, the catabolism of muscle protein for gluconeogenesis in order to support systemic energy needs results in loss of muscle mass which contributes to loss of overall body weight. This loss is rapidly reversible upon input of alternative metabolic fuel for example by nutrient assimilation from feeding.

•**Adverse Outcome: Loss of body weight** - If caloric intake is less than caloric use over time, an individual will lose body weight. Dynamic energy budget theory has provided useful insights on how organisms take up, assimilate and then allocate energy to various fundamental biological processes including maintenance, growth, development and reproduction (Nisbet et al 2000). Regarding energy allocation, somatic maintenance must first be met before then growth may occur, followed by maturation and then finally, surplus energy is dedicated to reproduction (Nisbet et al 2000). The influence of PPARalpha on systemic energy metabolism and energy homeostasis has been broadly established (see reviews by Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). PPARalpha has been demonstrated to play a critical role in stimulating fatty acid oxidation and ketogenesis during fasting resulting in increased ketone body levels in plasma (Badman et al 2007, Kersten 2014) a response that is eliminated in PPARalpha knockout mice (Badman et al 2007, Sanderson et al 2010). Kersten et al (1999) and Badman et al (2007) demonstrated that PPARalpha-null mice were unable to actively mobilize fatty acid oxidation, and further, Kersten et al (1999) demonstrated that these mice were unable to meet energy demands during fasting and leading to hypoglycemia, hyperlipidemia, hypoketonemia and fatty liver. Observations from toxicological and toxicogenomic research have implicated nitrotoluenes as potential PPAR antagonists in birds (Rawat et al 2010), rats (Deng et al 2011) and mice (Wilbanks et al 2014), an effect that additionally corresponded with weight loss in rats (Wilbanks et al 2014) and body weight loss, loss of muscle mass and emaciation in birds (Quinn et al 2007). These combined results indicate that inhibition of PPARalpha signaling and the resultant decrease in fatty acid oxidation and ketogenesis can detrimentally impair systemic energy budgets leading to starvation-like effects and resultant weight loss. In the absence of PPARalpha knockout, and upon removal of PPARalpha antagonist dosing, normal bioenergetic physiology can potentially be attained.

Weight of Evidence Summary

"Biological Plausibility"

Binding of molecules to peroxisome proliferator-activated receptor α (PPAR α) can cause either agonistic or antagonistic signaling depending on molecular structure (Xu et al 2001, Xu et al 2002). Certain molecules that can bind to the PPAR α ligand binding domain have been observed to cause conformational changes that induce increased affinity to co-repressors which decrease PPAR α nuclear signaling (Xu et al 2002) representing the MIE for this AOP.

The transcription co-repressors, silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) have been observed to compete with transcriptional co-activators for binding to nuclear receptors (including PPAR α) thus suppressing basal transcriptional activity (Nagy et al 1999, Xu et al 2002). Regarding the KE1, the binding of co-repressors such as the silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) to PPAR α is reinforced by the MIE, which blocks the AF-2 helix from adopting the active conformation, as demonstrated in x-ray crystallography results presented in Xu et al (2002). Thus, molecules that bind to PPAR α that can enhance co-repressor binding act as PPAR α antagonists.

Given that PPAR α trans-activation induces catabolism of fatty acids, this signaling pathway has been broadly

demonstrated to play a key role in energy homeostasis (Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999). In fact, PPAR α regulates expression of genes encoding nearly every enzymatic step of fatty acid catabolism including fatty acid uptake into cells, fatty acid activation to acyl-CoAs, the release of cellular energy from fatty acids through the oxidative breakdown of acyl-CoAs to acetyl-CoA, and in starvation conditions, the repackaging of Acetyl-CoA substrates into ketone bodies (Kersten 2014, Desvergne and Wahli 1999, Evans et al 2004, Sengupta et al 2010).

A large body of research demonstrated that PPAR α nuclear signaling directly controls transcriptional expression for genes catalyzing peroxisomal beta-oxidation of very long chain fatty acids (>20C), mitochondrial beta-oxidation of short, medium and long chain fatty acids (<20C), and ketogenesis (as reviewed in Kersten 2014, Evans et al 2004, Desvergne and Wahli 1999, Sanderson et al 2010). Peroxisomal beta-oxidation reactions shorten very long chain fatty acids from dietary sources releasing acetyl-CoA subunits (a primary metabolic fuel source) and shortened-chain fatty acids that can subsequently be catabolized by mitochondrial fatty acid beta oxidation reaction (as reviewed in Kersten et al 2014 and Desvergne and Wahli 1999).

Fatty acids shortened via peroxisomal beta-oxidation as well as fatty acids released from adipose tissue stores can be catabolized in mitochondrial beta-oxidation reactions to acetyl-CoA, NADH and ATP (Aoyama et al 1998). Within the mitochondria, the acetyl-CoA substrates can be used to maximize ATP production through full substrate oxidation via the citric acid cycle followed by oxidative phosphorylation by the electron transport chain (Nelson and Cox 2000A, Desvergne and Wahli 1999). This demonstrates importance of PPAR α signaling for inducing cellular energy release from fatty acids.

Blocking PPAR α signaling has been shown to inhibit expression of transcripts / enzymes involved in both peroxisomal and mitochondrial beta-oxidation causing impaired fatty acid catabolism, fatty acid accumulation in the liver and impaired cellular energy state during fasting events (Badman et al 2007, Kersten et al 1999). During periods of fasting, acetyl-CoA generated during either peroxisomal or mitochondrial beta-oxidation of fatty acids in the liver can each contribute to ketogenesis (Kersten 2014, Sengupta 2010). The liver represents a key organ involved in systemic energy distribution given its ability to synthesize glucose and catalyze the formation of ketone bodies, especially β -hydroxybutyrate, via ketogenesis (Cahil 2006, Kersten 2014). β -hydroxybutyrate is especially important for the metabolic energy needs of the brain which is unable to utilize fatty acids for cellular energy production (Owen 2005, Kersten 2014) as well as supporting general systemic energy homeostasis in fasting events (Cahil 2006, Evans et al 2004).

Not only does PPAR α signaling stimulate the release of cellular energy from fatty acids, it also regulates the transcription of enzymes that catalyze the repackaging of that cellular energy to ketone bodies via ketogenesis (Sengupta et al 2010, Desvergne and Wahli 1999). Inhibition of PPAR α signaling has been demonstrated to inhibit transcriptional expression of genes that catalyze ketogenesis as well as ketone body production (Badman et al 2007, Potthoff 2009, Sengupta 2010) affecting circulating levels of ketone bodies for systemic use. Kersten et al (1999) demonstrated that PPAR α is induced in fasted mice mobilizing the oxidation of fatty acids for energy production. In that study, PPAR α -null mice did not actively induce fatty acid oxidation or ketogenesis leaving the mice unable to meet energy demands during fasting and leading to hypoglycemia, hyperlipidemia, hypoketonemia and fatty liver. In such energy deficits, muscle protein catabolism is induced to where the amino acids glutamine and alanine serve as substrates for gluconeogenesis in the kidney to supplement cellular energy production / distribution (Cahill 2006, Marliss et al 1971, Felig et al 1970A, Goodman et al 1966, Kashiwaya et al 1994). Specifically, organism level responses associated with exposure to PPAR α antagonists include effects characteristic of a starvation response including decreased exercise endurance (Wilbanks et al 2014) body weight loss (Wilbanks et al 2014, Quinn et al 2007), loss of muscle mass and emaciation (Quinn et al 2007).

In general, if caloric intake is less than caloric use over time, an individual will lose body weight. This is a basic principle in human dieting as well as an important principle related to individual health and ecological fitness of animal populations. Dynamic energy budget theory has provided useful insights on how organisms take up,

assimilate and then allocate energy to various fundamental biological processes including maintenance, growth, development and reproduction (Nisbet et al 2000). Regarding energy allocation, somatic maintenance must first be met before then growth may occur, followed by maturation and then finally, surplus energy is dedicated to reproduction (Nisbet et al 2000).

As an example of the importance of energy allocation to ecological fitness, a review by Martin et al (1987) demonstrated that energy availability (availability of food) was the predominant limiting factor in reproductive success and survival for both young and parents in a broad life history review for bird species. This is a likely scenario for many organisms.

"Concordance of dose-response relationships:"

Dose-response relationships have been developed for GW6471 and the relative binding of PPAR α co-repressors and co-activators to the PPAR α nuclear signaling complex where the proportion of co-repressors increases dramatically with increasing GW6471 concentration (Xu et al 2002). Correspondingly, the relative activity of PPAR α decreased to zero with increasing GW6471 concentrations (Xu et al 2002). Additionally, recent observations of PPAR α antagonism by nitrotoluenes have demonstrated dose-response relationships for PPAR α nuclear signaling inhibition in in vitro investigations which corresponded with dose-responsive decreases in transcriptional expression of genes involved in lipid metabolism pathways (Wilbanks et al 2014, Gust et al 2015). These results corresponded with an dose-responsive relationship where increasing nitrotoluene dose caused decreased muscle mass, decreased body weight and increased emaciation in chronic dosing studies (Quinn et al 2007).

"Temporal concordance among the key events and adverse effect:"

Co-repressor binding was observed prior to inhibition of PPAR α signaling (Xu et al 2002). PPAR α knock out nullifies downstream expression of transcripts for genes involved in peroxisomal beta-oxidation of fatty acids, mitochondrial beta-oxidation of fatty acids, and ketogenesis pathways relative to wild types (Kersten et al 2014). Peroxisomal beta-oxidation of very long chain fatty acids into long chain fatty acids occurs prior to import into mitochondria and progression of mitochondrial beta-oxidation (Lazarow 1978, Kersten 2014). Mitochondrial beta-oxidation of long chain fatty acids occurs prior to generation of ketone bodies via ketogenesis (Sengupta et al 2010, Badman et al 2007). Ketogenesis occurs prior to increases in circulating ketone bodies (Sengupta et al 2010, Badman et al 2007, Cahill 2006). Increases in circulating ketone bodies can be observed prior to loss of muscle mass to muscle-protein catabolism given that this linkage is not directly connected. Muscle protein catabolism derives amino acids that are recycled to glucose via renal gluconeogenesis (Goodman et al 1966, Kashiwaya et al 1994, Cahill 2006). Catabolism of muscle protein occurs prior to body weight loss (Quinn et al 2007).

"Consistency:"

The transcription co-repressors, silencing mediator for retinoid and thyroid hormone receptors (SMRT) and nuclear receptor co-repressor (N-CoR) competition with transcriptional co-activators for binding to nuclear receptors (including PPAR α) has been observed in humans as well as yeast (Nagy et al 1999) suggest broad taxonomic applicability for this MIE. The evidence of PPAR α as a regulator of fatty acid metabolism is well described in the literature (for example, Kersten 2014, Evans 2004, Desvergne and Wahili 1999), and is consistent across many species including human, mouse, rat, Northern bobwhite, fathead minnow and carp (Kersten et al 1999, Kersten 2014, Wintz et al 2006, Gust et al 2015, Deng et al 2011, Wilbanks et al 2014, Xu and Jing, 2012). Inhibition of PPAR α via gene knockout or treatment with PPAR α antagonist consistently results in decreased fatty acid metabolism with indicators of increased serum triglycerides, fatty livers and steatosis (Kersten 2014, Evans 2004, Desvergne and Wahili 1999, Kersten et al 1999, Wintz et al 2006, Deng et al 2011). Given PPAR α 's central role in systemic energy metabolism, studies of PPAR α antagonism have shown decreased potential for sustaining

energy needs of the organism (Kersten et al 1999) leading to decreased burst exercise performance (Wilbanks et al 2014) and weight loss (Wilbanks et al 2014, Quinn et al 2007). Research thus far suggest that PPARalpha transcriptional regulation pathway as well as the metabolic pathways for which PPARalpha acts as a regulator indicates that the progression of key events through to the adverse outcome will tend to be evolutionarily conserved for within mammals and likely across animal phyla.

"Uncertainties, inconsistencies, and data gaps:"

A critical data gap regarding this AOP is an absence of studies that have investigated the effects null mutants for ketogenesis on the physiology and individual performance during long term starvation relative to wild type individuals. Additionally, knowledge about feedback mechanisms between ketogenesis vs gluconeogenesis would be beneficial for interpreting systemic energy metabolism. Regarding the antagonistic action of nitrotoluenes on PPARalpha nuclear signaling (Wilbanks et al 2014, Gust et al 2015), receptor-binding assays would be beneficial to determine if this class of chemicals is binding the SMRT and N-CoR co-repressors, similar to the antagonistic action of GW6471 (Xu et al 2002).

The weight of evidence scoring for this AOP is fully described in Collier et al (2015)

Quantitative Consideration

Given the complex nature of PPARalpha's functioning within a multi-subunit transcription factor regulating the transcriptional expression of a multitude of genes that facilitate lipid metabolism, to our knowledge, the relationship between PPARalpha signaling and individual gene expression has not yet been quantitatively modeled. However, the gene regulatory networks structure is well established (KEGG Pathway, map03320) and numerous empirical observations of the positive relationship between PPARalpha signaling with transcript expression and downstream metabolic pathways (Kersten 2014, Desvergne and Wahli 1999), there is opportunity to develop a quantitative gene signaling model for this system. For peroxisomal and mitochondrial fatty acid beta-oxidation pathways and ketogenesis, a variety of enzyme kinetics information is available for modeling (see reviews by Kersten 2014, Desvergne and Wahli 1999) as well as basic knowledge of the reaction stoichiometry of each metabolic reactions that can contribute to metabolic energy substrates for systemic use. Resultant models should be integrated with the work of Kashiwaya et al (1994) who have developed a detailed quantitative model for the metabolic flux of glucose including the influence of ketone bodies and insulin action on the dynamics of glycolysis versus gluconeogenesis. Dynamic energy budget (DEB) models (Nisbet et al 2000) have strong utility for integrating the dynamics of energy input and allocation to organismal processes of importance for characterizing/predicting the condition of the individual (ie. growth and maturation) as well as population-level responses (ie. allocation of energy to reproduction). DEB modeling has great potential for integrating suborganismal processes into individual and population level outcomes (Ananthasubramaniam et al 2015) and could serve to integrate data from dose-responsive relationships among PPARalpha antagonistic nitrotoluenes and fatty acid metabolism, muscle loss and body weight loss (Rawat et al 2010, Deng et al 2011, Wilbanks et al 2014, Quinn et al 2007, Xu and Jin 2012) thus supporting development of a semi-quantitative or quantitative AOP.

References

Ananthasubramaniam B, McCauley E, Gust KA, Kennedy AJ, Muller EB, Perkins EJ, Nisbet RM: Relating suborganismal processes to ecotoxicological and population level endpoints using a bioenergetic model. *Ecol Appl* 2015, 25(6):1691-1710.

Aoyama, T., Peters, J.M., Iritani, N., Nakajima, T., Furihata, K., Hashimoto, T., et al., 1998. Altered constitutive

expression of fatty acid-metabolizing enzymes in mice lacking the peroxisome proliferator-activated receptor alpha (PPARalpha). *Journal of Biological Chemistry* 273:5678e5684.

Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E: Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell metabolism* 2007, 5(6):426-437.

Brandt JM, Djouadi F, Kelly DP: Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor alpha. *J Biol Chem* 1998, 273(37):23786-23792.

Cahill Jr., G.F., 2006. Fuel metabolism in starvation. *Annual Review of Nutrition* 26:1e22.

Collier ZA, Gust KA, Gonzalez-Morales B, Linkov I, Perkins EJ (Eligible for publication, pending revisions) A Weight of Evidence Assessment Approach for Adverse Outcome Pathways. *Regul Toxicol Pharm*.

Deng Y, Johnson DR, Guan X, Ang CY, Ai J, Perkins EJ (2010) In vitro gene regulatory networks predict in vivo function of liver. *BMC Systems Biology* 4:153.

Deng, Y., Meyer, S. A., Guan, X., Escalon, B. L., Ai, J., Wilbanks, M.S., Welti, R., Garcia-Reyero, N. and Perkins, E. J. (2011) Analysis of common and specific mechanisms of liver function affected by nitrotoluene compounds. *PLoS One* 6, e14662.

Desvergne B, Wahli W (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocrine Reviews* 20(5): 649-688.

Evans RM, Barish GD, Wang YX: PPARs and the complex journey to obesity. *Nat Med* 2004, 10(4):355-361.

Felig P, Pozefsky T, Marliss E, Cahill GF, Jr.: Alanine: key role in gluconeogenesis. *Science* 1970A, 167(3920):1003-1004.

Felig P, Marliss E, Pozefsky T, Cahill GF, Jr.: Amino acid metabolism in the regulation of gluconeogenesis in man. *Am J Clin Nutr* 1970B, 23(7):986-992.

Gerich JE, Meyer C, Woerle HJ, Stumvoll M: Renal gluconeogenesis: its importance in human glucose homeostasis. *Diabetes Care* 2001, 24(2):382-391. Goodman AD, Fuisz RE, Cahill GF: Renal gluconeogenesis in acidosis, alkalosis, and potassium deficiency: its possible role in regulation of renal ammonia production. *J Clin Invest* 1966, 45(4):612-619.

Gust KA, Nanduri B, Rawat A, Wilbanks MS, Ang CY, Johnson DR, Pendarvis K, Chen X, Quinn Jr. MJ, Johnson MS, Burgess SC, Perkins EJ (2015) Systems Toxicology Identifies Mechanistic Impacts of 2-amino-4,6-dinitrotoluene (2A-DNT) Exposure in Northern Bobwhite. *BMC Genomics*. In Press.

Ide T, Shimano H, Yoshikawa T, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Yatoh S, Iizuka Y, Tomita S, et al (2003) Cross-talk between peroxisome proliferator-activated receptor (PPAR) alpha and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. II. LXRs suppress lipid degradation gene promoters through inhibition of PPAR signaling. *Mol Endocrinol* 17(7):1255-1267.

Kashiwaya Y, Sato K, Tsuchiya N, Thomas S, Fell DA, Veech RL, Passonneau JV: Control of glucose utilization in working perfused rat heart. *J Biol Chem* 1994, 269(41):25502-25514.

Kersten S. 2014. Integrated physiology and systems biology of PPARalpha. *Molecular Metabolism* 2014, 3(4):354-371.

Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W: Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* 1999, 103(11):1489-1498.

- Lazarow PB: Rat liver peroxisomes catalyze the beta oxidation of fatty acids. *J Biol Chem* 1978, 253(5):1522-1528.
- Lefebvre P, Chinetti G, Fruchart J-C, Staels B (2006) Sorting out the roles of PPAR α in energy metabolism and vascular homeostasis. *Journal of Clinical Investigation*, 116(3), 571–580.
- Marliss EB, Aoki TT, Pozefsky T, Most AS, Cahill GF: Muscle and splanchnic glutamine and glutamate metabolism in postabsorptive and starved man. *J Clin Invest* 1971, 50(4):814-817.
- Martin TE: Food as a limit on breeding birds: a life-history perspective. *Annu Rev Ecol Syst* 1987:453-487.
- Mascaró C, Acosta E, Ortiz JA, Marrero PF, Hegardt FG, Haro D: Control of human muscle-type carnitine palmitoyltransferase I gene transcription by peroxisome proliferator-activated receptor. *J Biol Chem* 1998, 273(15):8560-8563.
- Nagy L, Kao H-Y, Love JD, Li C, Banayo E, Gooch JT, Krishna V, Chatterjee K, Evans RM, Schwabe JWR: Mechanism of corepressor binding and release from nuclear hormone receptors. *Genes Dev* 1999, 13(24):3209-3216.
- Nelson DL, Cox MM 2000A. The Citric Acid Cycle. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p567-592.
- Nelson DL, Cox MM 2000B. Carbohydrate Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p722-764.
- Nelson DL, Cox MM 2000C. Lipid Biosynthesis. *Lehninger Principles of Biochemistry*. 3rd Edition. Worth Publishers. New York, NY. p770-814.
- Nisbet R, Muller E, Lika K, Kooijman S: From molecules to ecosystems through dynamic energy budget models. *J Anim Ecol* 2000, 69(6):913-926. Owen OE: Ketone bodies as a fuel for the brain during starvation. *Biochem Mol Biol Educ* 2005, 33(4):246-251.
- Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA et al: FGF21 induces PGC-1 α and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proceedings of the National Academy of Sciences* 2009, 106(26):10853-10858.
- Quinn MJ Jr, Bazar MA, McFarland CA, Perkins EJ, Gust KA, Gogal Jr RM, Johnson MS (2007) Effects of subchronic exposure to 2,6-dinitrotoluene in the northern bobwhite (*Colinus virginianus*). *Environmental Toxicology and Chemistry* 26(10):2202-2207.
- Rawat A, Gust KA, Deng Y, Garcia-Reyero N, Quinn MJ, Jr., Johnson MS, Indest KJ, Elasri MO, Perkins EJ (2010) From raw materials to validated system: the construction of a genomic library and microarray to interpret systemic perturbations in Northern bobwhite. *Physiological Genomics* 42(2):219-235.
- Sanderson, L.M., Boekschoten, M.V., Desvergne, B., Muller, M., Kersten, S., 2010. Transcriptional profiling reveals divergent roles of PPAR α and PPAR β/δ in regulation of gene expression in mouse liver. *Physiological Genomics* 41:42e52.
- Sengupta S, Peterson TR, Laplante M, Oh S, Sabatini DM: mTORC1 controls fasting-induced ketogenesis and its modulation by ageing. *Nature* 2010, 468(7327):1100-1104.
- Wang YX: PPARs: diverse regulators in energy metabolism and metabolic diseases. *Cell Res* 2010, 20(2):124-137.
- Wilbanks, M., Gust, K.A., Atwa, S., Sunesara, I., Johnson, D., Ang, C.Y., Meyer, S.A., and Perkins, E.J. 2014. Validation of a genomics-based hypothetical adverse outcome pathway: 2,4-dinitrotoluene perturbs PPAR signaling thus impairing energy metabolism and exercise endurance. *Toxicological Sciences*. 141(1):44-58.
- Wintz, H., Yoo, L. J., Loguinov, A., Wu, Y., Steevens, J. A., Holland, R. D., Beger, R. D., Perkins, E. J., Hughes, O.

and Vulpe, C. D. (2006) Gene expression profiles in fathead minnow exposed to 2,4-DNT correlation with toxicity in mammals. *Toxicol. Sci.* 94, 71–82.

Wolfrum C, Borrmann CM, Borchers T, Spener F (2001) Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha - and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. *Proc Natl Acad Sci USA* 98(5):2323-2328.

Xu HE, Stanley TB, Montana VG, Lambert MH, Shearer BG, Cobb JE, McKee DD, Galardi CM, Plunket KD, Nolte RT et al: Structural basis for antagonist-mediated recruitment of nuclear co-repressors by PPAR[alpha]. *Nature* 2002, 415(6873):813-817.

Xu, J. and Jing, N. (2012) Effects of 2,4-dinitrotoluene exposure on enzyme activity, energy reserves and condition factors in common carp (*Cyprinus carpio*). *J. Hazard. Mater.* 203–204, 299–307.