Accepted for publication in "Advances in Experimental Medicine and Biology" (May 2007)

PHARMACOLOGICAL AND GENE MODIFICATION-BASED MODELS FOR STUDYING THE IMPACT OF PERINATAL METABOLIC DISTURBANCES IN ADULT LIFE

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Abstract: Genetic modification approaches or pharmacological interventions may be useful for understanding the molecular mechanisms by which nutrient derivatives and metabolites exert their effects in the perinatal period and how they may influence long-term metabolism in adults. Examples for such experimental settings in rodents are targeted disruption of the gene for peroxisome proliferator-activated receptor (PPAR)-α, a lipid sensor and master regulator of lipid catabolism, or maternal treatment with agonists of PPARγ, a master regulator of adipogenesis and target of insulin sensitizing drugs in adults. All these interventions show differential effects in the perinatal period compared to adults and indicate that altered activity of master regulators of metabolism results in profound metabolic alterations in the perinatal period that may influence adult metabolism.

Key words: Fetus, fatty acid, insulin sensitivity, neonate, peroxisome proliferator-activated receptor, thiazolidinedione.

1. Introduction

The primary goal of determining the effects of pre- and post-natal nutrition on offspring development and the potential metabolic disturbances is hampered by the complexities of perinatal nutritional determinants (nutritional components of maternal food, placental transfer of metabolites, changes in milk composition, …). Thus, together with studies looking at the effects of maternal nutrition on the metabolic outcome of neonates and adults, other complementary approaches have been useful for our advancement in the understanding of the mechanisms and molecular processes driving long-term effects of perinatal nutrition changes. Two experimental approaches in rodent models, not involving nutrition primarily, may be useful: gene manipulation via the generation of transgenic or "knockout" (KO) mice, and pharmacologically-driven action on specific metabolic pathways in the perinatal period.

2. A gene manipulation approach to perinatal metabolic alterations and their consequences on adults: the example of PPARα-"knockout" mice.

2.1 The PPAR family of nuclear hormone receptors

Peroxisome proliferator-activated receptor (PPAR)α is a member of the nuclear hormone receptor superfamily and member of the PPAR family of receptors which includes also PPARγ and PPARβ/δ. PPARα acts as a ligand-dependent transcription factor. It regulates the transcription of genes encoding proteins involved in lipid oxidation and, accordingly, it is expressed in tissues with high levels of fat catabolism such as liver, muscle, heart or brown adipose tissue. The natural ligands of PPARα are fatty acids and some types of leukotrienes. Thus, PPARα is considered a master regulator of lipid catabolism and a sensor of the extent of tissue fatty acid available for oxidation. It constitutes a pivotal molecular link between lipid nutrition components and adaptive regulation of gene expression. Other members of the PPAR family are also regulators of gene expression in relation to lipid metabolism. The function of PPARγ is specifically related to the control of adipogenesis and therefore in lipid accumulation, whereas PPARβ/δ is ubiquitously expressed and it has similar effects to PPARα in promoting fatty acid oxidation (Guri et al., 2006).

2.2 Consequences of targeted disruption of PPARα

Multiple metabolic disturbances have been identified in adult mice with targeted disruption of PPARα (PPARα-KO) including impaired adaptation to starvation (hypoglycemica, lowered induction of ketogenesis, …), increased fat in liver and adipose tissue under certain dietary challenges but also protection from diet-induced insulin resistance in the context of obesity (Kersten et al 1999; Leone et al 1999, Fink et al., 2005). Impaired expression of genes for fatty acid oxidation also take place in heart (Murray et al., 2005) but minor alterations occur in skeletal muscle (Muoio et al, 2002). However, it cannot be excluded that disturbances in PPARα-KO adults may result in whole or in part from potential metabolic disturbances due to the lack of PPARα during the fetal or neonatal period and the subsequent effects on metabolism. To clarify this issue, the impact of targeted disruption of PPARα on perinatal metabolism was studied (Yubero et al 2004, Pedraza et al., 2006). Fetuses from PPARα-KO mice did not show major alterations in circulating metabolites or gene expression in PPARα-expressing tissues when compared with littermates. However, the lack of PPARα had a major impact in the neonatal period. PPARα-KO newborns do not show the increase in ketone levels in blood occurring in wild-type pups whereas they show mild hypoglycemia. An overall impairment in gene expression for enzymes involved in lipid catabolism (hydroxymethylglutharyl-CoA synthase, acyl-CoA oxidase) occur in the liver, whereas no major alterations in gluconeogenic genes (phosphoenolpyruvate carboxykinase, glucose-6-phosphatase) take place (Yubero et al., 2004).

In heart from neonates, like adults, there are profound changes in gene expression due to the lack of PPARα. They take place shortly after the initiation of suckling but do not occur just at birth. For instance, PPARα-KO neonates show impaired expression of genes which are induced in wild-type pups as a consequence of initiation of milk intake (such as uncoupling proteins (UCP) 2 and 3, pyruvate-dehydrogenase kinase-4, or carnitine palmitoyl-transferase II) (Pedraza et al., 2006). Skeletal muscle appears to be much less sensitive to the lack of PPARα, and only UCP3 and UCP2 gene expression was impaired in PPARα-KO neonates. However, this was different from adults, in which skeletal muscle gene expression appears to be unaltered for both genes. The most likely explanation for these differences in skeletal muscle gene expression between adults and neonates is a redundant compensatory effect of

PPARβ/δ, which may share target genes with PPARα. It is not clear why this compensation occurs only in skeletal muscle from adults and not from neonates or why this does not happen in the heart. Developmental regulation of gene expression for PPARα and PPARβ/δ in these tissues can hardly be an explanation. In skeletal muscle, PPARα gene expression is low in fetuses but it is highly induced just before birth. Neonates show high levels of PPARα gene expression and a progressive decline thereafter. Thus, PPARα gene expression is much lower in neonates than in adults (Brun S et al., 1999). In contrast, PPARβ/δ gene expression in muscle is unchanged when neonates and adults are compared. Thus, in some sense, PPARα expression is more prevalent compared to PPARβ/δ in the neonate than in the adult, and this may explain the particular sensitivity of neonatal skeletal muscle to the lack of PPARα. However, PPARα gene expression in heart is similar in neonates and adults whereas PPARβ/δ is more expressed in adults than in neonates (Pedraza et al, 2006). There is no dramatic difference in the extent of PPARβ/δ expression in skeletal muscle and heart that could explain why compensatory mechanisms may be more efficient in muscle than in heart.

Another tissue showing relevant alterations in gene expression during the neonatal period is brown fat. The naturally-occurring induction of genes involved in fatty acid oxidation after birth is reduced in PPARα-KO mice, and also UCP1 gene, the pivotal gene for induction of thermogenesis, is down-regulated in PPARα-KO neonates (unpublished observations). This is consistent with the identification of UCP1 as a target gene of PPARα-dependent transcriptional control (Barbera et al, 2001).

2.3 The effect of milk intake on neonatal metabolism

The profound disturbances in tissues and systemic metabolism in PPARα-KO neonates can be explained by the sudden requirement of fatty acid catabolism after birth. During pregnancy, glucose coming from placental transfer is the main source of metabolic energy for fetuses. However, after birth, due to the fact that milk in rodents contains large amounts of fat, neonates experience the sudden requirement to activate genes encoding the enzymatic machinery of fatty acid catabolism Thus, the adaptations in fuel usage taking place in adults as a consequence of starvation occur in neonates as a consequence of milk intake. Thus, in neonates with targeted disruption of PPARα, there

is an impaired usage of milk fatty acids by liver, as evidenced by the impaired induction of ketogenesis. Peripheral usage of fatty acids is also impaired, as indicated by the reduction in gene expression for fatty acid catabolism genes in heart, muscle and brown adipose tissue (Fig 1). This observation confirms the importance of PPARα in the control of neonatal thermogenesis. Fatty acid oxidation provides the fuel for thermogenesis to support uncoupled oxidation in brown fat mitochondria and the subsequent heat production. Although the lack of PPARα does not lead to a deep reduction in thermogenesis that challenges neonatal survival, it is expected that PPARα-KO neonates experience, together with an overall impairment of metabolic fuel usage reminiscent of undernutrition, a transient impairment in the control of body temperature.

2.4 Implications for future studies

These profound alterations in PPARα-KO neonates also have important overall implications for experimental studies using targeted disruption of genes to analyze their function in metabolism. It is essential that adult mice with targeted disruption of a given gene are always studied in comparison with wild-type controls that do not experience any gene disruption-mediated alteration in nutrition during pregnancy or lactation. For instance, using the PPARα-KO mice as example, the comparison of adult PPARα-KO mice with wild-type controls, each one kept on separate mice strains and therefore experiencing differential exposure to maternal metabolic alterations during pregnancy or lactation, is not correct. Despite the almost complete absence of direct studies, it may be expected that the lack of PPARα, a master gene of lipid metabolism, may affect maternal lipid metabolism, milk composition and even mammary gland development (Yang et al 2006.) Changes in the metabolic status of PPARα-KO adults may therefore be the result not of the intrinsic effects of the lack of PPARα on adult metabolic regulation but of the long-term effects of altered perinatal metabolism.

Thus, it is essential that maternal behaviour and nutritional delivery to fetuses and pups are the same if strict analysis of the effects of disruption of a given gene in adults is to be undertaken. A potential approach would be to compare wild-type and PPARα-KO littermates raised by mating male and female PPARα-KO heterozygotes. In such case, although maternal pregnancy and lactation will be performed by an heterozygote female, it would be at least common to wild-type and PPARα-KO

littermates. A further control of these aspects, although of a higher experimental complexity, will be the use of wild-type foster mothers to lactate litters composed of wild-type and PPARα-KO pups to ensure a common normal lactation. However, ultimate differential nutrition during lactation caused by a potential effect of the lack of PPARα on suckling behaviour would not be controlled by these experimental settings.

3. A pharmacological approach to the effects of perinatal metabolic alterations and their consequences in adults: the effects of a PPARγ agonist during pregnancy on neonatal and adult rats.

Another potential way of investigating the effects of metabolic disturbances during the perinatal period on adults using non-nutritional approaches is to modulate targets of nutritional regulation by means of specific drugs. An example of such approach would be the analysis of the action of thiazolidinediones (TZDs) during pregnancy on neonatal and adult life. TZDs are used as anti-diabetic drugs in adults because they ameliorate insulin resistance. TZDs act as activators of PPARγ, the member of the PPAR family of transcription factors expressed preferentially in adipose tissues and involved in promoting adipocyte differentiation.

Pregnant rats were treated for four days with an oral dose of 50 mg of the TZD englitazone/kg of body mass daily from day 16 of gestation. Neonates from englitazone-treated pregnant rats showed insulin-resistance, as evidenced by reduced glucose/insulin ratio, high free fatty acid levels, enhanced ketonaemia and low plasma IGF-I levels. In liver, lipoprotein lipase activity and Akt[A1] phosphorylation were increased (Sevillano et al. 2005). A profound alteration occurred in brown adipose tissue from neonates: there was a massive accumulation of triacylglycerols in the tissue together with an overall repression of specific marker genes of brown versus white adipose tissue phenotype such as UCP1, PPARγ-coactivator-1α or cytochrome oxidase subunit IV (unpublished observations). Thus, the response of neonates to maternal antidiabetic drug treatment is the opposite of what would be expected and this may have consequences on the adult. In fact, preliminary data studying neonates who experienced the maternal treatment with the TZD during pregnancy, indicate permanent systemic alterations as well as disturbances in adipose tissue development in the adult life. There are several hypothesis that could be considered to explain the profound effects of TZD

treatment during pregnancy on neonatal metabolism. First, it should be considered that some extent of insulin resistance is part of the maternal metabolic adaptations to pregnancy, and TZDs would impair the physiological insulin resistance associated with gestation. It is considered that such insulin resistance in pregnancy favours the channelling of glucose to the fetuses and the alternative usage of other fuels by maternal tissues. However, there are no clear signs of lack of glucose availability in fetuses from treated mothers and, in fact, lipid accumulation in brown adipose tissue is likely to result from enhanced lipogenesis from glucose in the late fetal period. Transient reductions in glucose availability after each TZD administration may increase fetal insulin and therefore activate the lipogenic pathway. Another possibility is a direct effect of englitazone on fetal development. Although not extensively studied, TZDs are considered to be capable of crossing the placenta (O'Moore-Sullivan et al, 2002). and therefore they may act directly on target genes through PPARγ activation in fetal tissues. Fetuses do not possess white fat and brown fat is probably the main site of PPARγ gene expression in fetuses. It may be suggested that the lack of the main site of action of PPARγ activation in the adult, white fat, may be responsible for the lack of anti-diabetic effect of TZDs in the perinatal period. However, it is clear that brown fat responds to the treatment in a way reminiscent of trans-differentiation into a "white fat-like" phenotype. How this may influence overall insulin sensitivity and cause insulin resistance in neonates deserves further study. In any case, the response of brown adipose tissue in developing fetuses to englitazone cannot be explained solely as a direct effect because *in vitro* treatment of brown pre-adipocytes with englitazone does not impair brown fat-specific gene expression but rather the opposite, and enhances it (unpublished observations).

4. Conclusions

In summary, it is possible, either by genetic modification or pharmacological intervention, to act during the perinatal period on master regulators of metabolism that, like PPARs, behave as intracellular sensors of nutritional signals. Such experimental approaches may help to delineate the molecular events that may be disturbed as a consequence of altered perinatal nutrition that would cause long-standing consequences in adult metabolism.

Acknowledgments
Supported by grants from Ministerio de Educación y Ciencia (SAF2005-01722), Fondo de Investigaciones Sanitarias, Ministerio de Sanidad y Consumo (PI052336 and PI060352) and Universidad San Pablo-CEU (PC10/06). Spain.

Abbreviations

PPAR, peroxisome proliferator-activated receptor; KO, knock out; TZD, thiazolidinedione; UCP, uncoupling protein

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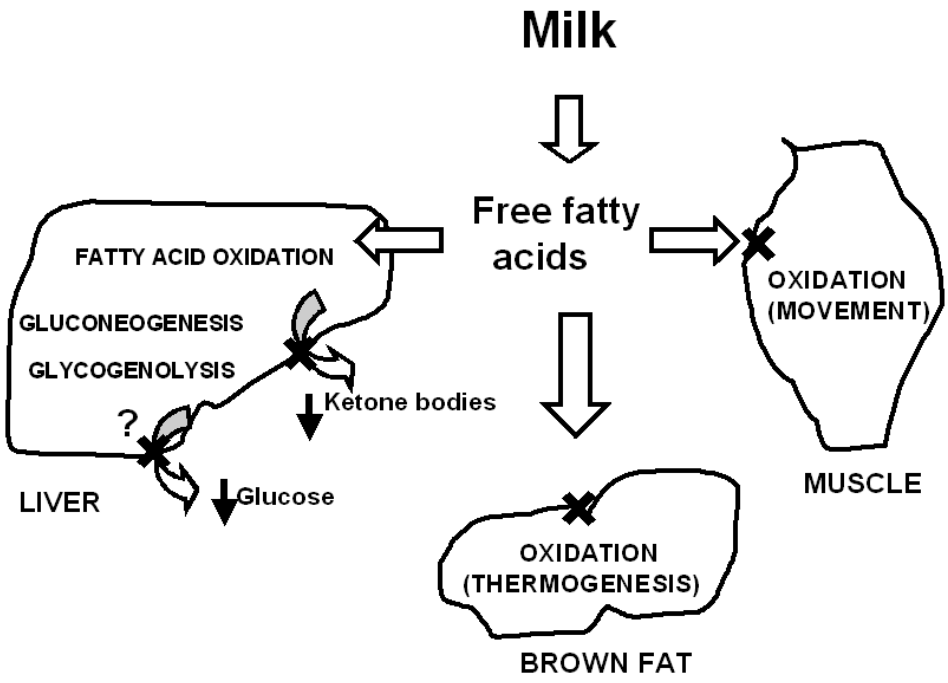
Legend to Fig 1

Metabolic fate of milk derived fatty acids during suckling and disruption caused by the lack of PPARα. The X marks indicate metabolic consequences of impaired fatty acid utilization due to the lack of PPARα in suckling neonates.


Milk
Free fatty acids
FATTY ACID OXIDATION
GLUCONEOGENESIS
GLYCOGENOLYSIS
?
Ketone bodies
Glucose
LIVER
OXIDATION (MOVEMENT)
MUSCLE
OXIDATION (THERMOGENESIS)
BROWN FAT