Proceedings of the Nutrition Society (2001), **60**, 63–72
DOI: 10.1079/PNS200071

Nutrition–hormone receptor–gene interactions: implications for development and disease

M. J. Dauncey*, P. White, K. A. Burton and M. Katsumata
Developmental Genetics Programme, The Babraham Institute, Cambridge CB2 4AT, UK

Nutrition profoundly alters the phenotypic expression of a given genotype, particularly during fetal and postnatal development. Many hormones act as nutritional signals and their receptors play a key role in mediating the effects of nutrition on numerous genes involved in differentiation, growth and metabolism. Polypeptide hormones act on membrane-bound receptors to trigger gene transcription via complex intracellular signalling pathways. By contrast, nuclear receptors for lipid-soluble molecules such as glucocorticoids (GC) and thyroid hormones (TH) directly regulate transcription via DNA binding and chromatin remodelling. Nuclear hormone receptors are members of a large superfamily of transcriptional regulators with the ability to activate or repress many genes involved in development and disease. Nutrition influences not only hormone synthesis and metabolism but also hormone receptors, and regulation is mediated either by specific nutrients or by energy status. Recent studies on the role of early environment on development have implicated GC and their receptors in the programming of adult disease. Intrauterine growth restriction and postnatal undernutrition also induce striking differences in TH-receptor isoforms in functionally-distinct muscles, with critical implications for gene transcription of myosin isoforms, glucose transporters, uncoupling proteins and cation pumps. Such findings highlight a mechanism by which nutritional status can influence normal development, and modify nutrient utilization, thermogenesis, peripheral sensitivity to insulin and optimal cardiac function. Diet and stage of development will also influence the transcriptional activity of drugs acting as ligands for nuclear receptors. Potential interactions between nuclear receptors, including those for retinoic acid and vitamin D, should not be overlooked in intervention programmes using I or vitamin A supplementation of young and adult human populations.

Development: Hormones: Intrauterine growth restriction: Nuclear receptors: Nutrition

Nutritional status can profoundly alter the phenotypic expression of a given genotype, and hormone receptors play a key role in mediating the effects of nutrition on numerous genes involved in development. Whereas polypeptide hormones act on membrane-bound receptors, receptors for lipophilic hormones act directly within the nucleus to regulate transcription. Nutrition influences not only hormone synthesis and metabolism but also hormone receptors, and regulation is mediated either by specific nutrients or by energy status.

The present review briefly mentions some of the many hormones involved in development, and then illustrates the complexity of nutrition–hormone receptor–gene interactions by focusing on nuclear hormone receptors. As paradigms for nuclear hormone action, the receptors for glucocorticoids (GC; GR) and especially those for thyroid hormones (TH; TR) are considered. These hormones have long been recognized to play crucial roles in regulating differentiation, growth and metabolism. Advances in molecular cloning and structural analysis, combined with sophisticated gene-targeting studies, have revealed new insights into the developmental functions of these hormone systems. Recent findings on tissue-specific and developmental-stage-specific regulation of nuclear TR isoforms, and their modulation by intrauterine growth restriction and postnatal undernutrition, are particularly relevant to the understanding of development and disease. They highlight a mechanism by which nutritional status can influence normal development and modify nutrient utilization, thermogenesis, insulin resistance and optimal cardiac function. Interactions

Abbreviations: GC, glucocorticoid; GH, growth hormone; GR, glucocorticoid receptor; IGF, insulin-like growth factor; RXR, retinoid X receptor; TH, thyroid hormone; TR, thyroid hormone receptor.

***Corresponding author:** Dr M. J. Dauncey, fax +44 1223 496023, email joy.dauncey@bbsrc.ac.uk

between diet and stage of development in influencing the transcriptional activity of drugs acting as ligands for nuclear receptors is mentioned. Finally, the potential significance of interactions between members of the superfamily of nuclear receptors in nutrient-intervention programmes is discussed.

Hormone receptors and gene transcription

All stages of development involve complex and coordinated patterns of signalling at the intracellular, cell–cell and cell–environment levels. Numerous hormones are involved in this communication network and, in relation to the external environment, they act as especially powerful nutritional signals and are themselves subject to modification by nutritional status. Specific receptors and receptor isoforms enable a given circulating level of hormone to have both tissue-specific and developmental-stage-specific effects. The polypeptide hormones such as growth hormone (GH), insulin-like growth factors (IGF) and insulin act on membrane-bound receptors to trigger gene transcription via complex intracellular signalling pathways (Argetsinger & Carter-Su, 1996; Czech & Corvera, 1999). By contrast, nuclear receptors for lipid-soluble molecules such as GC and TH act predominantly by directly regulating transcription via DNA binding and chromatin remodelling (Collingwood *et al*. 1999; McNally *et al*. 2000).

Superfamily of nuclear receptors

The nuclear hormone receptors are members of a large superfamily of transcriptional regulators with the ability to activate or repress numerous genes. The superfamily of nuclear receptors includes not only GR and TR, but also those for retinoids, vitamin D and the sex steroids (Lazar, 1993; Kumar & Thompson, 1999). Knowledge of nuclear receptors has increased considerably during the last decade, with identification of a group termed orphan receptors, i.e. receptors originally identified without knowledge of their specific ligand, such as peroxisome proliferator-activated receptors and farnesoid X receptors, the functions of which are currently being elucidated (Desvergne & Wahli, 1999; Gustafsson, 1999). Nuclear receptors are of particular interest in the present context, because the cognate ligand for each receptor is usually dependent on the supply of a specific dietary component for its synthesis, e.g. I and tyrosine for TH, cholesterol for vitamin D and GC, vitamin A as the ligand for all-*trans*-retinoic acid receptors, and fatty acids for peroxisome proliferator-activated receptors.

Mechanism of action of nuclear hormone receptors

Nuclear receptors usually act by remodelling of chromatin, and this process is dependent on their common structure which has several specific functional domains (Fig. 1). The highly-conserved DNA-binding domain binds to specific response elements in the promoter region of the target gene. The carboxy-terminal region contains a hormone- or ligand-binding region and sites necessary for dimerization and for recruitment of coactivators and corepressors (Collingwood *et al*. 1999). Transcription of target genes involves receptor binding to DNA together with hormone binding to the receptor. This process results in recruitment of coactivator proteins, an open chromatin structure is formed and gene expression is initiated. The precise modes of action of the various nuclear hormone receptors differ. To illustrate the complexity of nuclear receptor action and indicate the many functional aspects that may be subject to nutritional modulation, the specific mechanisms by which GC and TH act at the molecular level will be outlined.

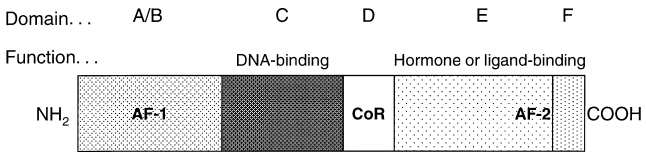

Fig. 1. Primary structure of the nuclear hormone receptor superfamily illustrating major functional domains common to all members. This group of transcription factors includes receptors for steroids, thyroid hormones and retinoids. The most-highly-conserved region is the DNA-binding domain, followed by the carboxy-terminal (COOH) hormone- or ligand-binding domain. The amino-terminal (NH_2) domain is particularly variable in both size and primary sequence among the superfamily. AF-1 and AF-2, ligand-independent and ligand-dependent transactivation functions respectively; CoR, co-repressor-binding site.

In the absence of ligand, GR is located in the cytoplasm where it exists in the inactive state bound to heat-shock proteins. Binding of GC releases these proteins and the receptor is then translocated to the nucleus where it regulates transcription by binding as a GR –GR homodimer to specific DNA response elements. In addition to direct DNA recognition, GR can also act as a monomer to modulate transcriptional activation via protein–protein interactions (Tronche *et al*. 1998).

The major actions of TH are exerted at the genomic level via a group of receptors which regulate the expression of numerous target genes, usually by remodelling of chromatin structure (Wu & Koenig, 2000). Unliganded TR are located in the nucleus and can interact directly with chromatin to modulate gene transcription either in the presence or absence of TH (Fig 2). Binding to specific TH response elements is usually by heterodimerization with retinoid X receptors (RXR). In the presence of TH (Fig. 2(b)), the TR–RXR heterodimer recruits a coactivator protein complex with histone acetyl transferase activity. Acetylation of histones results in a relaxed open chromatin structure, enabling access of basal transcription factors and RNA polymerase to DNA, hence activating gene transcription. In the absence of TH (Fig. 2(c)), the TR–RXR heterodimer binds to a group of corepressor proteins, leading to recruitment of histone deacetylase, a closed form of chromatin and silencing of transcription.

TR add a further level of complexity to transcriptional regulation because they occur as a series of isoforms encoded by two distinct proto-oncogenes, c-*erb*A-α, and c-*erb*A-β, each of which regulates a specific set of target genes. Alternative splicing of the TRα mRNA transcript produces two carboxy-terminal variants (TRα1 and TRα2) and the TRβ gene produces two amino-terminal variants, TRβ1 and TRβ2. Whereas TRα1, TRβ1 and TRβ2 can bind TH and transactivate TH response elements, the TRα2

https://doi.org/10.1079/PNS200071 Published online by Cambridge University Press

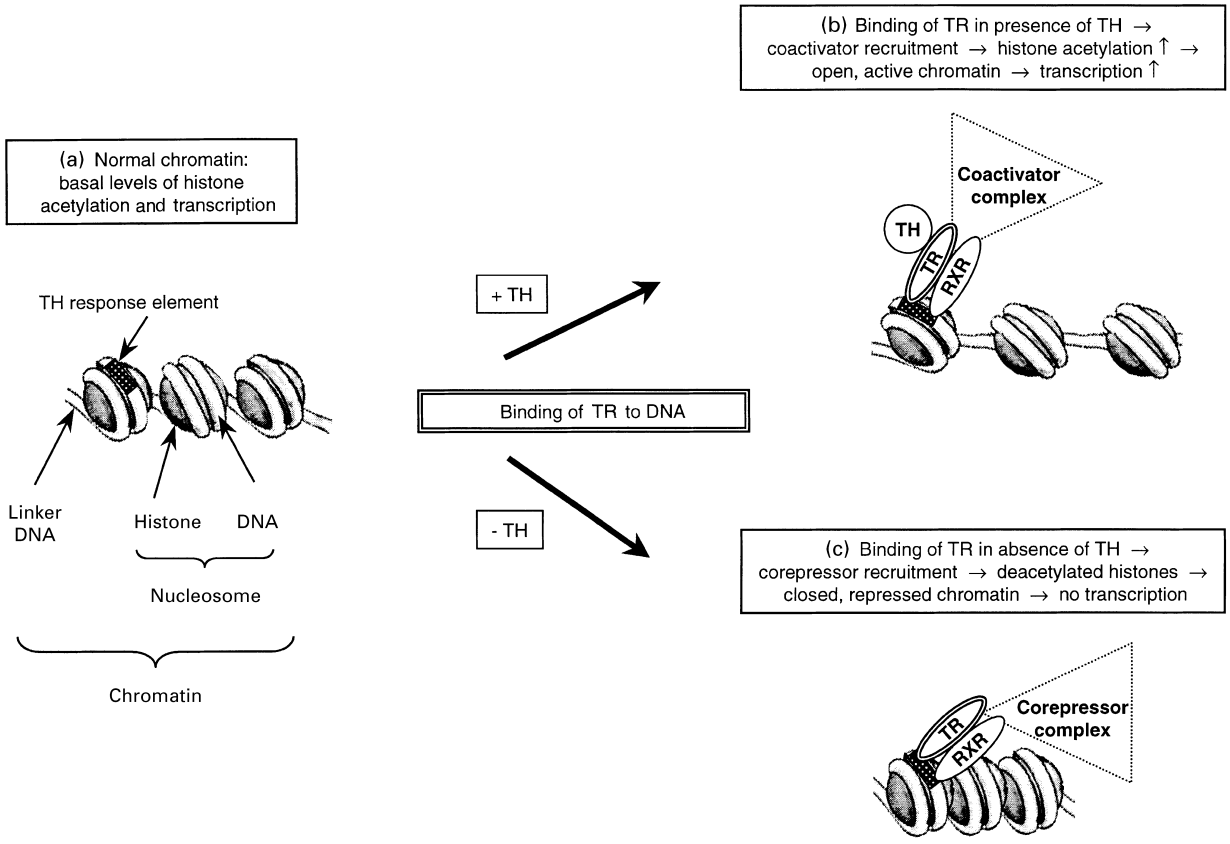

Fig. 2. Model of the proposed action of nuclear thyroid hormone (TH) receptors (TR). Binding of TR to TH response elements of target genes can occur in the presence (+ TH) or absence (- TH) of hormone. TR preferentially binds to DNA as a heterodimer with the retinoid X receptor (RXR). Binding to DNA results in recruitment of coactivator or corepressor protein complexes, modification of chromatin structure and the subsequent activation or repression of transcription. Note that this mode of action operates for the TRα1 and TRβ isoforms. Structural changes in the carboxy-terminal domain of the TRα2 isoform prevent TH binding, resulting in inhibition of transcription even in the presence of TH. ↑, process increased.

variant cannot bind TH and hence is not strictly a receptor for TH. Structural changes in the TRα2 hormone-binding domain and lack of AF-2 transactivation function prevent TH binding and transactivation (Lazar, 1993; Tagami *et al.* 1998). The precise functions of TRα2 are unknown, but it is thought to compete with the TH-binding TR isoforms for specific DNA response elements and thus inhibit transcription. Recent findings outlined in the section on developmental actions of TH and TR (pp. 66–67) lend support to this hypothesis.

Hormones, nuclear receptors and development

Hormones and their receptors exert marked tissue-specific and age-specific effects on development (Dauncey, 1995). During fetal development, the coordinated actions of IGF, insulin, TH and GC play central roles in the control of differentiation, growth and maturation (Fowden, 1995). By contrast with its essential role postnatally, pituitary GH does not appear to have a major role in controlling fetal growth; hepatic GH receptor expression is either absent or extremely low and the GH–IGF axis does not mature until around birth. However, GH receptor expression is high in fetal skeletal muscle, suggesting direct actions of prenatal GH in muscle differentiation and metabolism (Duchamp *et al.* 1996). Interactions between hormones also occur. For example, TH exert a striking tissue-specific influence on prenatal GH receptor gene expression: hypothyroidism induces down regulation in liver but a marked up regulation in muscle.

The following sections discuss the widespread developmental actions of GC, TH and their receptors, and focus especially on regulation of gene expression in brain and functionally-distinct muscles. This discussion emphasises the potentially significant role of nutrition in modulating neurodevelopment, cardiac function, and the numerous actions of skeletal muscles. Not only is muscle essential for locomotion, postural maintenance, breathing and thermogenesis, but it plays a key role in determining nutrient oxidation rates and is the main peripheral site of insulin action.

Developmental actions of glucocorticoids and glucocorticoid receptors

The main effects of GC (predominantly cortisol or corticosterone, depending on the species) before birth are on tissue differentiation and maturation (Fowden *et al.* 1998). They act directly to alter gene transcription or post-translational processing of gene products, and may also initiate the

https://doi.org/10.1079/PNS200071 Published online by Cambridge University Press

transition from fetal to adult mode of growth regulation by inducing the switch from hepatic IGF-II to IGF-I. The prepartum surge in GC appears to have an important maturational role in initiating the perinatal switch from the fetal to adult mode of somatotrophic regulation; during late gestation GC regulate hepatic GH receptors and IGF-I gene expression, and preferentially increase the class 2 transcript of the IGF-I gene (Li *et al*. 1996, 1998*b*). During infancy and later life GC have a wide range of functions in many tissues and play a key role in homeostasis. They are important regulators of glucose, fat and protein metabolism and, by contrast with their prenatal role, act as growth inhibitors. GC also have marked influences on cognitive function (McEwen & Sapolsky, 1995) and affect neuronal activity at several levels including membrane polarity, neurotransmitter release and neuronal survival.

Gene-targeting studies have revealed important insights into the developmental role of GR (Tronche *et al*. 1998). The majority of GR^{null} mutants, i.e. mice with complete inactivation of the GR gene, die a few minutes after birth because of lung atelectasis (incomplete expansion of the lungs at birth), and there are defects in the function of many other tissues including liver and adrenal gland. Conditional mutations of the GR gene using the Cre/*loxP* system circumvents the problem of early death by generating tissue-specific mutations. Absence of GR in the nervous system is not lethal, demonstrating that the lung atelectasis in GR^{null} mutants is not neuronal in origin. By contrast, absence of GR feedback regulation in the hypothalamus of mice with the nervous-system-specific mutation profoundly alters the equilibrium of the hypothalamic–pituitary–adrenal axis; secretion of corticotrophin-releasing hormone increases, leading in turn to elevated circulating GC levels. Mutants not only exhibit growth retardation and redistribution of adipose tissue but they are less anxious, suggesting direct involvement of GR in emotional behaviour. These findings highlight the importance of GR to development of the nervous system, and are directly relevant to an understanding of mechanisms by which nutrition modulates neurodevelopment and cognitive function (Dauncey & Bicknell, 1999).

Developmental actions of thyroid hormones and thyroid hormone receptors

The widespread actions of hormones during development are exemplified by the striking effects of TH (thyroxine and 3,5,3′-triiodothyronine) on differentiation, growth and metabolism of many tissues and cell types (Fisher *et al*. 1977; Dauncey, 1990; Fowden, 1995). Particularly well recognised are the effects of TH on myelination and development of the central nervous system, and TH deficiency during the perinatal period results in severe mental and physical retardation (Oppenheimer & Schwartz, 1997; Chan & Kilby, 2000). Since I is essential for TH synthesis, I deficiency during fetal and early postnatal life can also result in brain damage and mental retardation (Delange, 2000). Moreover, Se has essential roles in TH metabolism, and has the potential to play a major part in the outcome of I deficiency (Arthur, 1999).

The differential temporal and spatial distributions of TRα and TRβ, together with coexpression at comparable levels in some brain regions, suggest different roles for TR isoforms during brain development and in the mature animal (Mellstrom *et al*. 1991). However, the target genes of TH that play crucial roles in brain development are as yet unclear, and the rat cerebellum is currently proving useful as a model system for studying TH action in brain development (Koibuchi & Chin, 2000).

TH have direct actions on protein turnover, affecting both protein synthesis and protein breakdown, and they are essential for muscle development (Muscat *et al*. 1995; Dauncey & Gilmour, 1996). Recent studies also suggest that the relative distribution of TRα1 and TRα2 isoforms plays a pivotal role in regulating the phenotypic and functional differences between the wide diversity of muscle types (White & Dauncey, 1999). Prenatally, TH act via muscle-specific regulatory factors to induce myoblasts to exit from the cell cycle and differentiate to form myotubes and mature myofibres. Perinatal sequential transitions from embryonic to fetal to adult myosin heavy-chain isoforms are followed by switching between mature myosins. In skeletal muscle TH induce type I slow fibres to become type II fast fibres, and similarly in cardiac muscle they induce switching from β-myosin to α-myosin. Other genes specifically regulated by TH include glucose transporters, cation pumps and uncoupling proteins (Castello *et al*. 1994; Dauncey & Harrison, 1996; Gong *et al*. 1997). These diverse actions enable TH to exert a powerful influence on many aspects of muscle function including contractility, nutrient utilization and metabolic activity.

Gene-inactivation studies have shown that the four TR isoforms probably regulate individual tissue-specific functions and common functions *in vivo* (Forrest & Vennstrom, 2000). Table 1 describes some of the major phenotypes of targeted TR inactivation, and highlights the widespread influences of these receptors during development. Disruption of either TRα1 or TRα2 alone does not produce a lethal phenotype, but reveals that TRα1 plays a pivotal role in determining basal heart rate, irrespective of TH status (Wikstrom *et al*. 1998). However, simultaneous disruption of both TRα isoforms results in a lethal phenotype postnatally (Fraichard *et al*. 1997). Deletion of both TRβ isoforms results in relatively mild effects on phenotype, similar to those associated with TH resistance in humans (Chatterjee, 1997). Overall, the effects of TR deletion are not always as profound as those occurring in response to hypothyroidism and a reduction in circulating TH levels. This situation is probably because TR deletion simply results in basal levels of transcription, whereas TR in the absence of TH represses basal transcription (see Fig. 2). Thus, although the transgenic approach is of considerable value, it does not necessarily lead to a complete understanding of hormone receptor action.

Major differences occur with respect to the relative abundance of the TR isoforms in functionally distinct muscles (White & Dauncey, 1999). In fast muscles TRα1 predominates and TRα1:TRα2 is high, whereas in slow muscles, the non-TH-binding TRα2 predominates (Fig. 3). These differences have important functional consequences;

https://doi.org/10.1079/PNS200071 Published online by Cambridge University Press

Table 1. Major phenotypic effects of disrupting genes for the thyroid hormone (TH) receptor (TR) isoforms

Gene disrupted*	Viability	Phenotype	Reference
TRα1 + TRα2	Death at 4–5 weeks	Growth arrest at 2 weeks Progressive hypothyroidism Small intestine: delayed maturation Bone: delayed development Post-weaning lethality (rescued by 1 week with T_3 treatment)	Fraichard *et al.* (1997)
TRα1	Viable	Growth rate normal Mild hypothyroidism Heart rate ↓ (no response to T_3 treatment) Body temperature ↓ (restored by T_3 treatment) Skeletal muscle: slow myosin ↑, fast myosin ↓, Ca^{2+}-ATPase ↓, longer contraction and relaxation times	Wikstrom *et al* (1998), Johansson *et al.* (2000) Yu *et al.* (2000)
TRβ1 + TRβ2	Viable	Growth rate normal Plasma TH levels elevated; goitre Heart rate ↑ (no response to T_3 treatment) Auditory function defective Skeletal muscle: slow and fast myosins not affected, less fatigue-resistant	Forrest *et al.* (1996), Weiss *et al.* (1998), Johansson *et al.* (1999, 2000), Yu *et al.* (2000)
TRβ2	Viable	Plasma TH levels elevated Auditory function normal Basal GH gene expression slightly decreased Response to T_3 blunted	Abel *et al.* (1999)
TRα1 + TRβ	Viable	Growth rate reduced; lower body weight all ages Plasma TH levels grossly elevated; very large goitre Bone: delayed development Heart rate ↓ (no response to T_3 treatment) Body temperature ↓ Skeletal muscle: slow myosin ↑, fast myosin ↓ GH gene expression profoundly down regulated	Gothe *et al.* (1999), Johansson *et al.* (1999), Yu *et al.* (2000)
TRα + TRβ	Death at 4–5 weeks	Growth arrest at 2 weeks Plasma TH levels elevated (more than in $TR\beta^{null}$); goitre Ileum: pronounced malformation Bone: delayed development Post-weaning lethality	Gauthier *et al.* (1999)

T_3, 3,5,3′-triiodothyronine; ↓, decrease; ↑, increase; GH, growth hormone.
*Disruption of TRα2 results in viability; its other effects and those of TRβ1 disruption have not yet been described. Severity of phenotype in response to disruption of TRα gene alone or TRα plus TRβ genes may result from an artefact; it has been suggested that expression of partial TRα products may be causing the lethality (Forrest & Vennstrom, 2000).

preponderance of TRα1 in *longissimus* will result in a high proportion of type II fast myosin for rapid movement, while high levels of TRα2 in cardiac muscle will enhance slow sustained contractility. Recent findings on the ontogeny of porcine TR expression also indicate that the relative levels of TRα1 and TRα2 are important in regulating TH action (P White, K A Burton, A L Fowden and M J Dauncey, unpublished results). During cardiac development TRα2 expression is two to four times greater than that of TRα1, and as development progresses the TRα isoforms decrease gradually to reach low levels at 7 weeks postnatally. This developmental expression pattern is strikingly different from that in the fast-twitch skeletal muscle *longissimus*. Taken together, these novel findings highlight a key role for TR isoforms in regulating tissue-specific TH sensitivity and muscle phenotype during development. Moreover, nutritional modulation of specific TR isoforms will have a marked effect on optimal physiological function of cardiac and skeletal muscles.

Nutritional regulation of hormones and hormone receptors

Numerous investigations have shown that nutrition markedly influences the synthesis and metabolism of many hormones involved in development, growth and metabolism (Dauncey, 1995; Brameld, 1997; Holness, 1999). Effects are exerted both by specific nutrients and by changes in overall food intake, as occurs during undernutrition or intrauterine growth restriction. A further mechanism by which nutrition modulates hormone action is by regulation of hormone receptors. Particularly important is the finding that the response to nutrition can be tissue-specific, because this factor enables highly specific and diverse functional responses to a given circulating hormone level. Postnatal undernutrition down regulates GH receptor gene expression in liver, whereas it is up regulated in muscle (Dauncey *et al*. 1994; Weller *et al*. 1994). This situation will affect the GH–IGF-I axis and limit growth by reducing hepatic IGF-I synthesis, while simultaneously enhancing the anti-

https://doi.org/10.1079/PNS200071 Published online by Cambridge University Press

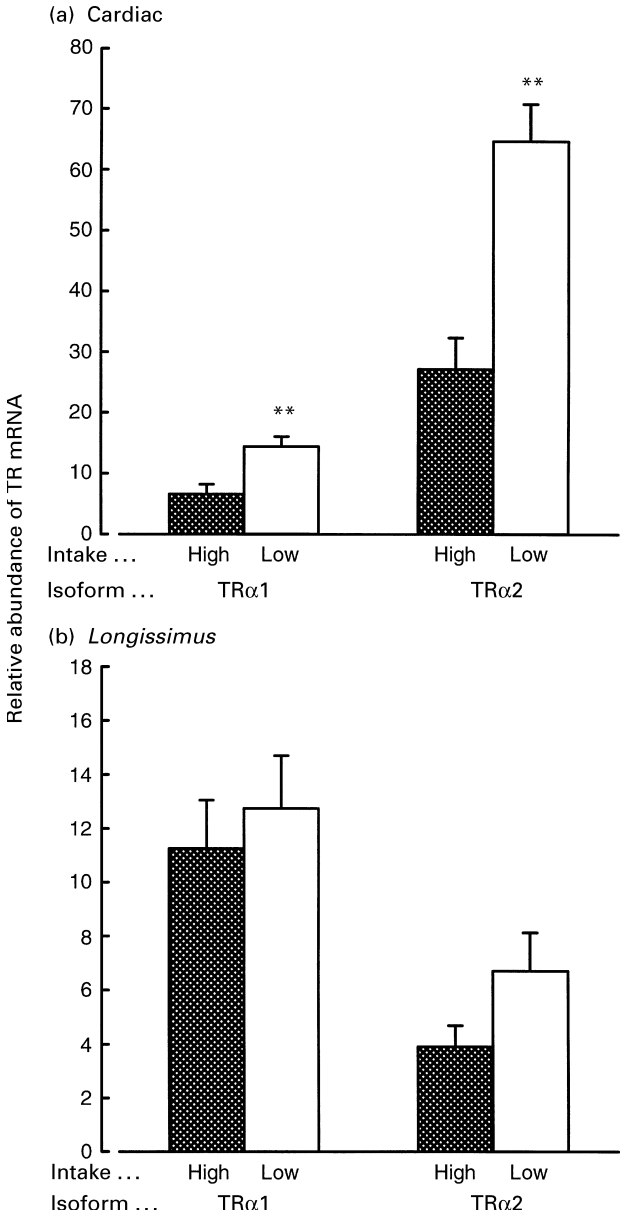

Fig. 3. Differential expression of thyroid hormone receptor (TR) α isoforms in functionally-distinct muscles and its regulation by postnatal undernutrition. Results are for cardiac (a) and *longissimus* (b), slow and fast muscles respectively, from 6-week-old littermate pigs which had been kept at thermal neutrality for the previous 3 weeks on a high (▩) or low (▭) food intake (60 or 20 g food/kg per d respectively). All animals grew during this period, but the rate was much greater on the high intake. Note the very different scales on the *y*-axis for the two graphs. Values are means with their standard errors represented by vertical bars. Mean values for isoform expression were significantly different from those for the high-intake group: $^{**}P < 0{\cdot}01$. (From White & Dauncey, 1998, 1999; MJ Dauncey, P White and KA Burton, unpublished results).

lipogenic, lipolytic and diabetogenic actions of GH in muscle. There are also striking muscle-specific differences both in postnatal GH receptor gene expression, and in its regulation by nutritional status, that are related to the metabolic, contractile and functional properties of different muscles (Katsumata *et al*. 2000). Recent findings on nutritional regulation of nuclear receptors are described later (pp. 68–69). They highlight mechanisms by which early nutrition may exert permanent or long-term effects on optimal development. The probability is that the diverse actions of hormone receptors on cellular development persist long after subsequent optimization of nutrition has restored hormone receptor expression to its appropriate level.

Glucocorticoid receptors and nutrition

Energy restriction may exert many of its effects on modulating disease and longevity via the hypothalamic–pituitary–adrenal axis (Duffy *et al*. 1997). A reduction in food intake alters the patterns of pulsatile, circadian and ultradian GC release and increases plasma GC levels (Vance & Thorner, 1989), whereas overfeeding reduces GC levels (Lewis *et al*. 1992). There is a fine balance between the beneficial and harmful effects of GC, which is related in part to the stage of development, and the magnitude and duration of any changes (Fowden *et al*. 1998). Intrauterine programming of the hypothalamic–pituitary–adrenal axis has been implicated in the link between low birth weight and later disease (Nyirenda *et al*. 1998; Phillips *et al*. 1998), and elevated basal GC levels may cause hippocampal damage and hippocampus-dependent learning and memory deficits in later life (Lupien *et al*. 1998).

Investigations on the nutritional regulation of GR are limited. Nevertheless, altered regulation of developmental GR expression may affect the later incidence of many diseases, including hypertension and the insulin resistance syndrome. Maternal low-protein diet in the rat results in persistent up regulation of GR expression in central and peripheral tissues (Bertram *et al*. 1999). Moreover, GR expression is differentially regulated in skeletal myocytes from men with contrasting levels of insulin resistance and obesity (Donovan & Whorwood, 1999). GR are present in several brain areas, and are most abundant in the hippocampus where they play an essential role in GC feedback inhibition (Dauncey & Bicknell, 1999). Their precise role in neurodevelopment and long-term health or disease has yet to be established. An important area for future investigation is to determine the extent to which diet can influence tissue-specific GR expression during prenatal, postnatal and adult life.

Thyroid hormone receptors and nutrition

Nutrition has a major influence both on the hypothalamic–pituitary–thyroid axis and on nuclear TH binding in peripheral tissues. A low energy intake reduces thyroid gland activity, plasma TH levels and total TR numbers, and the decrease in nuclear TH binding is even greater when metabolic demand is increased by lowering the environmental temperature (Dauncey, 1990; Swoap *et al*. 1994; Morovat & Dauncey, 1995). Intrauterine growth restriction also down regulates TR (Dauncey, 1995). Thus, when energy is restricted, because of either a low intake or a high expenditure, the reduction in TR will limit responsiveness to TH and result in reduced growth and metabolism.

https://doi.org/10.1079/PNS200071 Published online by Cambridge University Press

Ligand-binding studies provide no information on the expression of specific receptor isoforms, and this factor is especially important in the case of TR, in which the TRα2 variant does not bind TH. Investigations on the role of energy status in regulating porcine TR isoform expression have revealed new insights into mechanisms by which nutrition can influence gene expression (White & Dauncey, 1998; P White, KA Burton and MJ Dauncey, unpublished results). Studies on intrauterine growth restriction and postnatal undernutrition indicate that responses are markedly dependent on stage of development and muscle type. Particularly significant are results for cardiac muscle; intrauterine growth restriction down regulated TRα1 by 50 %, whereas postnatal undernutrition induced a striking 140 % increase in TRα2 (Fig. 3). These nutritionally-induced changes in cardiac TRα isoforms may profoundly affect myocardial function; either a low level of the TH-binding TRα1 or a high level of the non-TH-binding TRα2 isoform, combined with a reduction in plasma TH levels, would reduce cardiac α-myosin transcription, leading in turn to a lower intrinsic contractile ability and operational heart rate. Undernutrition also induces marked changes in TR in skeletal muscle. Expression of TRα2 in *longissimus* of small-for-gestational-age piglets is more than two-fold greater than that in controls, and TRα2:TRα1 increases. This preferential up regulation of TRα2 expression would reduce TH binding and decrease transcription of target genes, resulting in reduced growth and metabolism, energy conservation and impairment of muscle phenotype and function (White *et al*. 2000).

Potential significance of nutrition–nuclear receptor interactions

Not only are interactions between nutrition and hormone receptors critical for optimal development, but there is currently considerable interest in specific targeting of nuclear receptors by novel pharmaceutical agents. The therapeutic action of thiazolidinediones in decreasing insulin resistance and blood glucose levels is probably mediated by their action as ligands for peroxisome proliferator-activated receptor-γ (Barroso *et al*. 1999). Interactions between different hormones and their receptors illustrate the complexity of mechanisms involved in nutritional regulation of gene expression. For example, GC increase RXRα expression and enhance TH action in primary-cultured rat hepatocytes (Yamaguchi *et al*. 1999). Moreover, up regulation of the insulin-dependent glucose transporter in selected muscles by postnatal undernutrition is probably induced by the concomitant increase in GC levels and not to changes in TH status (Li *et al*. 1998*a*; Katsumata *et al*. 1999).

The importance of nutrition–hormone–drug interactions should not be underestimated. Interactions between dietary I, TR and amiodarone, for example, have major consequences for TH metabolism and optimal cardiac function. Amiodarone is a powerful anti-arrhythmic drug that probably exerts its major effect by antagonism with TH at the receptor level (Drvota *et al*. 1995). It acts on cardiomyocytes to block ion channels and adrenergic receptors, and hence prolongs action potential and refractory period and reduces heart rate. This drug contains 370 mg I/g and a major side effect is induction of thyroid dysfunction; hypothyroidism is frequently encountered in I-sufficient geographical regions, whereas thyrotoxicosis is more common in I-deficient areas (Loh, 2000). This finding may be explained by an excess of I preventing thyroidal I uptake, by blocking the I pump. Moreover, the presenting clinical features are age-dependent, and probably reflect changes in peripheral responsiveness to TH. The additional finding that some types of amiodarone-induced thyrotoxicosis benefit from treatment with GC further highlights the complexity of the interactions between nutrition and nuclear receptors.

Nutrient–nuclear hormone receptor interactions

Precise interactions between members of the nuclear receptor superfamily and their diverse range of ligands remain to be established. The heterodimerization properties of some of the receptors ensure a complexity of transcriptional responses to nutritional and hormonal stimuli (Glass, 1996). RXR are receptors for the vitamin A metabolite 9-*cis*-retinoic acid and are also cofactors which heterodimerize with many members of the nuclear receptor superfamily, including those for all-*trans*-retinoic acid, TH, vitamin D and peroxisome proliferator-activated receptors (Rowe, 1997). The vitamin D receptor is thought to heterodimerize with RXR but not with TR, and distinct repressive actions of TR on vitamin D receptor-mediated signalling have been demonstrated; a TH-independent action, presumably via direct competition with TR–RXR for DNA binding, and a TH-dependent repression, probably by diversion of RXR from vitamin D receptor–RXR heterodimers to TR–RXR heterodimers (Thompson *et al*. 1999). These interactions may provide a partial explanation for the observed association between hyperthyroidism and bone demineralization or osteoporosis. Moreover, the finding that vitamin D interferes with transactivation of the GH gene by TH and retinoic acid further highlights the potential significance of interactions between nutrients and hormones during development (Garcia-Villalba *et al*. 1996).

During early development both TR and retinoic acid receptors are expressed. Although this stage of development is predominantly sensitive to retinoic acid rather than to TH, unliganded TRα1 in mouse embryonic stem cells inhibits retinoic acid-responsiveness and retinoic acid-stimulated neural differentiation (Lee *et al*. 1994). Moreover, vitamin A may suppress the thyroid-stimulating hormone β-subunit transcription directly, via a retinoic acid receptor–RXR-mediated mechanism (Breen *et al*. 1995). Important interactions between TR, GR and retinoid acid receptors are also suggested by studies on TH, I and vitamin A status (Garcin *et al*. 1984; Filteau *et al*. 1994; Coustaut *et al*. 1996). Potential interactions between members of the superfamily of nuclear receptors, and their cognate ligands, should not be ignored in intervention programmes using I or vitamin A supplementation of young and adult human populations.

Concluding remarks

Considerable advances in understanding of the molecular structure and function of hormone receptors have occurred

https://doi.org/10.1079/PNS200071 Published online by Cambridge University Press

during the last decade. Advances have been dependent on new techniques of molecular and structural analysis combined with sophisticated gene-targeting studies. Less clear-cut is our understanding of the mechanisms by which nutritional status regulates phenotypic expression via genetic and epigenetic events involved in hormone receptor action. The next decade should see significant advances in knowledge of the precise mechanisms by which nutrition modulates hormone receptor function throughout fetal, infant and adult life. Insight should also be gained into the relative contributions of nutrition and genotype to optimal development. Studies should focus not only on specific nutrients but also on energy status and overall food intake. It is well recognized that intrauterine growth restriction and postnatal undernutrition can impair both immediate and long-term development, with profound consequences for optimal health and disease (Lucas, 1994; Barker, 1995; Dauncey, 1997; Desai & Hales, 1997). Detailed understanding of nutrition–hormone receptor–gene interactions will lead to improvements in preventative and therapeutic strategies, from the nutritional to the molecular level.

Acknowledgements

The Babraham Institute is supported by the Biotechnology and Biological Sciences Research Council, P.W. was supported by a Medical Research Council Postgraduate Studentship, and M.K. was supported by a Japanese Science and Technology Agency Postdoctoral Fellowship.

References

Abel ED, Boers ME, Pazos-Moura C, Moura E, Kaulbach H, Zakaria M, Lowell B, Radovick S, Liberman MC & Wondisford F (1999) Divergent roles for thyroid hormone receptor beta isoforms in the endocrine axis and auditory system. *Journal of Clinical Investigation* **104**, 291–300.

Argetsinger LS & Carter-Su C (1996) Mechanism of signaling by growth hormone receptor. *Physiological Reviews* **76**, 1089–1107.

Arthur JR (1999) Functional indicators of iodine and selenium status. *Proceedings of the Nutrition Society* **58**, 507–512.

Barker DJ (1995) The Wellcome Foundation Lecture, 1994. The fetal origins of adult disease. *Proceedings of the Royal Society of London* **262**B, 37–43.

Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, Maslen GL, Williams TD, Lewis H, Schafer AJ, Chatterjee VK & O'Rahilly S (1999) Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension. *Nature* **402**, 880–883.

Bertram C, Trowern AR, Dunn R & Whorwood CB (1999) Maternal low protein diet, but not carbenoxolone treatment, results in persistent up-regulation of expression of glucocorticoid receptor in both central and peripheral tissues. *Journal of Endocrinology* **163**, P92.

Brameld JM (1997) Molecular mechanisms involved in the nutritional and hormonal regulation of growth in pigs. *Proceedings of the Nutrition Society* **56**, 607–619.

Breen JJ, Matsuura T, Ross AC & Gurr JA (1995) Regulation of thyroid-stimulating hormone beta-subunit and growth hormone messenger ribonucleic acid levels in the rat: effect of vitamin A status. *Endocrinology* **136**, 543–549.

Castello A, Rodriguez-Manzaneque JC, Camps M, Perez-Castillo A, Testar X, Palacin M, Santos A & Zorzano A (1994) Perinatal hypothyroidism impairs the normal transition of GLUT4 and GLUT1 glucose transporters from fetal to neonatal levels in heart and brown adipose tissue. Evidence for tissue-specific regulation of GLUT4 expression by thyroid hormone. *Journal of Biological Chemistry* **269**, 5905–5912.

Chan S & Kilby MD (2000) Thyroid hormone and central nervous system development. *Journal of Endocrinology* **165**, 1–8.

Chatterjee VK (1997) Resistance to thyroid hormone. *Hormone Research* **48**, 43–46.

Collingwood TN, Urnov FD & Wolffe AP (1999) Nuclear receptors: coactivators, corepressors and chromatin remodeling in the control of transcription. *Journal of Molecular Endocrinology* **23**, 255–275.

Coustaut M, Pallet V, Garcin H & Higueret P (1996) The influence of dietary vitamin A on triiodothyronine, retinoic acid, and glucocorticoid receptors in liver of hypothyroid rats. *British Journal of Nutrition* **76**, 295–306.

Czech MP & Corvera S (1999) Signaling mechanisms that regulate glucose transport. *Journal of Biological Chemistry* **274**, 1865–1868.

Dauncey MJ (1990) Thyroid hormones and thermogenesis. *Proceedings of the Nutrition Society* **49**, 203–215.

Dauncey MJ (1995) From whole body to molecule: an integrated approach to the regulation of metabolism and growth. *Thermochimica Acta* **250**, 305–318.

Dauncey MJ (1997) From early nutrition and later development … to underlying mechanisms and optimal health. *British Journal of Nutrition* **78**, Suppl. 2, S113–S123.

Dauncey MJ & Bicknell RJ (1999) Nutrition and neurodevelopment: mechanisms of developmental dysfunction and disease in later life. *Nutrition Research Reviews* **12**, 231–253.

Dauncey MJ, Burton KA, White P, Harrison AP, Gilmour RS, Duchamp C & Cattaneo D (1994) Nutritional regulation of growth hormone receptor gene expression. *FASEB Journal* **8**, 81–88.

Dauncey MJ & Gilmour RS (1996) Regulatory factors in the control of muscle development. *Proceedings of the Nutrition Society* **55**, 543–559.

Dauncey MJ & Harrison AP (1996) Developmental regulation of cation pumps in skeletal and cardiac muscle. *Acta Physiologica Scandinavica* **156**, 313–323.

Delange F (2000) The role of iodine in brain development. *Proceedings of the Nutrition Society* **59**, 75–79.

Desai M & Hales CN (1997) Role of fetal and infant growth in programming metabolism in later life. *Biological Reviews of the Cambridge Philosophical Society* **72**, 329–348.

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

Donovan SJ & Whorwood CB (1999) Regulation of glucocorticoid receptor (GR) α and β by cytokines and glucocorticoids in skeletal myocytes from men with contrasting levels of insulin resistance and obesity. *Journal of Endocrinology* **163**, P93.

Drvota V, Bronnegard M, Hagglad J, Barkhem T & Sylven C (1995) Downregulation of thyroid hormone receptor subtype mRNA levels by amiodarone during catecholamine stress in vitro. *Biochemical and Biophysical Research Communications* **211**, 991–996.

Duchamp C, Burton KA, Herpin P & Dauncey MJ (1996) Perinatal ontogeny of porcine growth hormone receptor gene expression is modulated by thyroid status. *European Journal of Endocrinology* **134**, 524–531.

Duffy PH, Leakey JE, Pipkin JL, Turturro A & Hart RW (1997) The physiologic, neurologic, and behavioral effects of caloric

restriction related to aging, disease, and environmental factors. *Environmental Research* **73**, 242–248.
Filteau SM, Sullivan KR, Anwar US, Anwar ZR & Tomkins AM (1994) Iodine deficiency alone cannot account for goitre prevalence among pregnant women in Modhupur, Bangladesh. *European Journal of Clinical Nutrition* **48**, 293–302.
Fisher DA, Dussault JH, Sack J & Chopra IJ (1977) Ontogenesis of hypothalamic-pituitary-thyroid function and metabolism in man, sheep, and rat. *Recent Progress in Hormone Research* **33**, 59–116.
Forrest D, Hanebuth E, Smeyne RJ, Everds N, Stewart CL, Wehner JM & Curran T (1996) Recessive resistance to thyroid hormone in mice lacking thyroid hormone receptor β: evidence for tissue-specific modulation of receptor function. *EMBO Journal* **15**, 3006–3015.
Forrest D & Vennstrom B (2000) Functions of thyroid hormone receptors in mice. *Thyroid* **10**, 41–52.
Fowden AL (1995) Endocrine regulation of fetal growth. *Reproduction, Fertility and Development* **7**, 351–363.
Fowden AL, Li J & Forhead AJ (1998) Glucocorticoids and the preparation for life after birth: are there long-term consequences of the life insurance? *Proceedings of the Nutrition Society* **57**, 113–122.
Fraichard A, Chassande O, Plateroti M, Roux JP, Trouillas J, Dehay C, Legrand C, Gauthier K, Kedinger M, Malaval L, Rousset B & Samarut J (1997) The T3Rα gene encoding a thyroid hormone receptor is essential for post-natal development and thyroid hormone production. *EMBO Journal* **16**, 4412–4420.
Garcia-Villalba P, Jimenez-Lara AM & Aranda A (1996) Vitamin D interferes with transactivation of the growth hormone gene by thyroid hormone and retinoic acid. *Molecular and Cellular Biology* **16**, 318–327.
Garcin H, Higueret P & Amoikon K (1984) Effects of a large dose of retinol or retinoic acid on the thyroid hormones in the rat. *Annals of Nutrition and Metabolism* **28**, 92–100.
Gauthier K, Chassande O, Plateroti M, Roux JP, Legrand C, Pain B, Rousset B, Weiss R, Trouillas J & Samarut J (1999) Different functions for the thyroid hormone receptors TRα and TRβ in the control of thyroid hormone production and post-natal development. *EMBO Journal* **18**, 623–631.
Glass CK (1996) Some new twists in the regulation of gene expression by thyroid hormone and retinoic acid receptors. *Journal of Endocrinology* **150**, 349–357.
Gong DW, He Y, Karas M & Reitman M (1997) Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *Journal of Biological Chemistry* **272**, 24129–24132.
Gothe S, Wang Z, Ng L, Kindblom JM, Barros AC, Ohlsson C, Vennstrom B & Forrest D (1999) Mice devoid of all known thyroid hormone receptors are viable but exhibit disorders of the pituitary-thyroid axis, growth, and bone maturation. *Genes and Development* **13**, 1329–1341.
Gustafsson JA (1999) Seeking ligands for lonely orphan receptors. *Science* **284**, 1285–1286.
Holness MJ (1999) The impact of dietary protein restriction on insulin secretion and action. *Proceedings of the Nutrition Society* **58**, 647–653.
Johansson C, Gothe S, Forrest D, Vennstrom B & Thoren P (1999) Cardiovascular phenotype and temperature control in mice lacking thyroid hormone receptor-β or both α1 and β. *American Journal of Physiology* **276**, H2006–H2012.
Johansson C, Lannergren J, Lunde PK, Vennstrom B, Thoren P & Westerblad H (2000) Isometric force and endurance in soleus muscle of thyroid hormone receptor-α1- or -β-deficient mice. *American Journal of Physiology* **278**, R598–R603.
Katsumata M, Burton KA, Li J & Dauncey MJ (1999) Suboptimal energy balance selectively up-regulates muscle GLUT gene expression but reduces insulin-dependent glucose uptake during postnatal development. *FASEB Journal* **13**, 1405–1413.
Katsumata M, Cattaneo D, White P, Burton KA & Dauncey MJ (2000) Growth hormone receptor gene expression in porcine skeletal and cardiac muscles is selectively regulated by postnatal undernutrition. *Journal of Nutrition* **130**, 2482–2488.
Koibuchi N & Chin WW (2000) Thyroid hormone action and brain development. *Trends in Endocrinology and Metabolism* **11**, 123–128.
Kumar R & Thompson EB (1999) The structure of the nuclear hormone receptors. *Steroids* **64**, 310–319.
Lazar MA (1993) Thyroid hormone receptors: multiple forms, multiple possibilities. *Endocrine Reviews* **14**, 184–193.
Lee LR, Mortensen RM, Larson CA & Brent GA (1994) Thyroid hormone receptor α inhibits retinoic acid-responsive gene expression and modulates retinoic acid-stimulated neural differentiation in mouse embryonic stem cells. *Molecular Endocrinology* **8**, 746–756.
Lewis DS, Jackson EM & Mott GE (1992) Effect of energy intake on postprandial plasma hormones and triglyceride concentrations in infant female baboons (Papio species). *Journal of Clinical Endocrinology and Metabolism* **74**, 920–926.
Li J, Forhead AJ, Fowden AL & Dauncey MJ (1998*a*) Regulation of glucose transporter (GLUT) 4 gene expression in ovine skeletal muscle during fetal development. *Journal of Physiology* **507**, 53P–54P.
Li J, Owens JA, Owens PC, Saunders JC, Fowden AL & Gilmour RS (1996) The ontogeny of hepatic growth hormone receptor and insulin-like growth factor I gene expression in the sheep fetus during late gestation: developmental regulation by cortisol. *Endocrinology* **137**, 1650–1657.
Li J, Saunders JC, Fowden AL, Dauncey MJ & Gilmour RS (1998*b*) Transcriptional regulation of insulin-like growth factor-II gene expression by cortisol in fetal sheep during late gestation. *Journal of Biological Chemistry* **273**, 10586–10593.
Loh KC (2000) Amiodarone-induced thyroid disorders: a clinical review. *Postgraduate Medical Journal* **76**, 133–140.
Lucas A (1994) Role of nutritional programming in determining adult morbidity. *Archives of Disease in Childhood* **71**, 288–290.
Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, Thakur M, McEwen BS, Hauger RL & Meaney MJ (1998) Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nature Neuroscience* **1**, 69–73.
McEwen BS & Sapolsky RM (1995) Stress and cognitive function. *Current Opinion in Neurobiology* **5**, 205–216.
McNally JG, Muller WG, Walker D, Wolford R & Hager GL (2000) The glucocorticoid receptor: rapid exchange with regulatory sites in living cells. *Science* **287**, 1262–1265.
Mellstrom B, Naranjo JR, Santos A, Gonzalez AM & Bernal J (1991) Independent expression of the alpha and beta c-erbA genes in developing rat brain. *Molecular Endocrinology* **5**, 1339–1350.
Morovat A & Dauncey MJ (1995) Regulation of porcine skeletal muscle nuclear 3,5,3′-triiodothyronine receptor binding capacity by thyroid hormones: modification by energy balance. *Journal of Endocrinology* **144**, 233–242.
Muscat GE, Downes M & Dowhan DH (1995) Regulation of vertebrate muscle differentiation by thyroid hormone: the role of the myoD gene family. *BioEssays* **17**, 211–218.
Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A & Seckl JR (1998) Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose

intolerance in adult offspring. *Journal of Clinical Investigation* **101**, 2174–2181.

Oppenheimer JH & Schwartz HL (1997) Molecular basis of thyroid hormone-dependent brain development. *Endocrine Reviews* **18**, 462–475.

Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ & Walker BR (1998) Elevated plasma cortisol concentrations: a link between low birth weight and the insulin resistance syndrome? *Journal of Clinical Endocrinology and Metabolism* **83**, 757–760.

Rowe A (1997) Retinoid X receptors. *International Journal of Biochemistry and Cell Biology* **29**, 275–278.

Swoap SJ, Haddad F, Bodell P & Baldwin KM (1994) Effect of chronic energy deprivation on cardiac thyroid hormone receptor and myosin isoform expression. *American Journal of Physiology* **266**, E254–E260.

Tagami T, Kopp P, Johnson W, Arseven OK & Jameson JL (1998) The thyroid hormone receptor variant α2 is a weak antagonist because it is deficient in interactions with nuclear receptor corepressors. *Endocrinology* **139**, 2535–2544.

Thompson PD, Hsieh JC, Whitfield GK, Haussler CA, Jurutka PW, Galligan MA, Tillman JB, Spindler SR & Haussler MR (1999) Vitamin D receptor displays DNA binding and transactivation as a heterodimer with the retinoid X receptor, but not with the thyroid hormone receptor. *Journal of Cellular Biochemistry* **75**, 462–480.

Tronche F, Kellendonk C, Reichardt HM & Schutz G (1998) Genetic dissection of glucocorticoid receptor function in mice. *Current Opinion in Genetics and Development* **8**, 532–538.

Vance ML & Thorner MO (1989) Fasting alters pulsatile and rhythmic cortisol release in normal man. *Journal of Clinical Endocrinology and Metabolism* **68**, 1013–1018.

Weiss RE, Murata Y, Cua K, Hayashi Y, Seo H & Refetoff S (1998) Thyroid hormone action on liver, heart, and energy expenditure in thyroid hormone receptor beta-deficient mice. *Endocrinology* **139**, 4945–4952.

Weller PA, Dauncey MJ, Bates PC, Brameld JM, Buttery PJ & Gilmour RS (1994) Regulation of porcine insulin-like growth factor I and growth hormone receptor mRNA expression by energy status. *American Journal of Physiology* **266**, E776–E785.

White P, Cattaneo D & Dauncey MJ (2000) Postnatal regulation of myosin heavy chain isoform expression and metabolic enzyme activity by nutrition. *British Journal of Nutrition* **84**, 185–194.

White P & Dauncey MJ (1998) Postnatal undernutrition markedly upregulates cardiac α1 and α2 thyroid hormone receptor gene expression. *Proceedings of the Nutrition Society* **57**, 79A.

White P & Dauncey MJ (1999) Differential expression of thyroid hormone receptor isoforms is strikingly related to cardiac and skeletal muscle phenotype during postnatal development. *Journal of Molecular Endocrinology* **23**, 241–254.

Wikstrom L, Johansson C, Salto C, Barlow C, Campos Barros A, Baas F, Forrest D, Thoren P & Vennstrom B (1998) Abnormal heart rate and body temperature in mice lacking thyroid hormone receptor α1. *EMBO Journal* **17**, 455–461.

Wu Y & Koenig RJ (2000) Gene regulation by thyroid hormone. *Trends in Endocrinology and Metabolism* **11**, 207–211.

Yamaguchi S, Murata Y, Nagaya T, Hayashi Y, Ohmori S, Nimura Y & Seo H (1999) Glucocorticoids increase retinoid-X receptor alpha (RXRalpha) expression and enhance thyroid hormone action in primary cultured rat hepatocytes. *Journal of Molecular Endocrinology* **22**, 81–90.

Yu F, Gothe S, Wikstrom L, Forrest D, Vennstrom B & Larsson L (2000) Effects of thyroid hormone receptor gene disruption on myosin isoform expression in mouse skeletal muscles. *American Journal of Physiology* **278**, R1545–R1554.