CC/2012/06

COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET

Background

1. At its meeting on the 20th November 2008, the Committee discussed paper CC/2008/17 "Horizon Scanning 2008." One of the issues raised in this paper was the possible carcinogenic hazard from dietary insulin-like growth factor-1 (IGF-I). The Food Standards Agency (FSA) and the Veterinary Medicines Directorate (VMD) had been contacted regarding the potential effects of dairy produce imported from countries that allowed the use of bovine somatotropin (BST) in cows. This was prompted by issues raised in a book which suggested that consumption of IGF-I in dairy produce could cause increased risks of developing certain cancers, (particularly breast and prostate cancers). The use of BST was said to increase the amount of IGF-I in milk, and there was concern that increased dietary exposure to IGF-I from this source might increase the risk of consumers developing cancer.

2. At its meeting on 23rd July 2009, the COC considered a paper (CC/09/08) which summarised the claims about health effects of IGF-I that were presented in the book "Your Life In Your Hands" (Plant, 2007). Following discussion, the Committee concluded:

a) *"The book presented evidence on the role of IGF-I in cell proliferation and cancer in support of a claim that risks of certain cancers, particularly breast and prostate cancers, are increased by consumption of dairy products and that the increased risk is a result of the presence of IGF-I in milk. The evidence presented was incomplete, prone to bias and of inconsistent quality, so any conclusions drawn from the book must be regarded as provisional and would need to be confirmed following a fuller systematic search of the scientific literature before they could be acted upon.*

b) *"The book identified that IGF-I has a role to play in the normal growth and development of tissues, and that locally high levels of IGF-I or increased sensitivity to IGF-I can also cause cancer cells to multiply.*

c) *"The book did not provide convincing evidence to justify its claim that the IGF-I in milk and dairy products (or in any other food) could cause consumers to have increased risks of developing certain cancers.*

d) *"Information was provided on the amount of IGF-I in milk, but nothing was presented on the amounts of IGF-I in other foods*

e) *"There is a potential for dietary IGF-I to come in contact with the cells lining the gastrointestinal tract. However, no information was presented on the concentrations of IGF-I that these cells could be in contact with.*

f) *"No information was presented on the amount of breakdown of IGF-I that might occur in the gut lumen, although there was evidence from an in vitro study that casein and some other dietary proteins might give some protection from breakdown and there was evidence that partial breakdown to N-terminally truncated forms could increase the potency of IGF-I.*

g) *"No information was presented on the amount of IGF-I that might be absorbed from the gut lumen into the bloodstream.*

h) *"There was evidence that showed that IGF-I could cause increased mitosis and decreased apoptosis to occur in vitro in some cell lines, including several derived from cancer cells. It was also claimed that IGF-I caused differentiation of cells, but the references that were cited presented no evidence from experiments in support of the claim.*

i) *"It was claimed in the book that increased blood concentrations of active IGF-I caused an increased risk of cancer. In support of this claim, the book made reference to several epidemiological studies that showed associations between blood levels of free IGF-I and risks of some cancers."*

3. Additional points made by the Committee were:

- It was considered highly unlikely that dietary IGF-I could elicit an effect in the gastrointestinal tract as it is unlikely that the cells of the intestinal epithelium would respond to luminal growth factors.
- IGF-I is unlikely to be absorbed from the gut to any great extent.

4. The Committee agreed that a systematic review of the risk of cancer from dietary IGF-I would be worthwhile. Members suggested that such a review could include:

   a) Information from the EPIC cohort which showed a positive association between intake of dairy food and serum IGF-I levels (Norat *et al*., 2007; Crowe *et al*., 2009)[1].
   b) Details of a study (Hoppe *et al*., 2004) where boys received protein either in the form of meat or milk, which showed increased IGF-I levels only in those given milk.
   c) Closer scrutiny of the conclusions of an epidemiology study by Ma *et al*., (2001) on colorectal cancer.

[1] Norat *et al*, Crowe *et al*, Hoppe *et al* are discussed in Annex 2 and attached at Annex 4.

d) Relevant information from the preclinical data package for human therapeutic use of IGF-I.
e) Information on whether dietary IGF-I contributes to circulating IGF-I levels.
f) Comparison of the mitogenic potency of IGF-I with a benchmark such as oestradiol.
g) Information on the amount of the high potency truncated form of IGF-I in milk.

5. Resources were not available to perform a systematic review. Instead the secretariat performed a narrative review that incorporated elements of a systematic review: recording details of literature searches and of the selection of articles used. The narrative review covers a wider selection of issues than would have been practical had a systematic review been performed.

6. The Secretariat performed a search of the scientific literature (Annex 1) and obtained relevant papers for use in a narrative review of IGF-I (Annexes 2 and 3). As a result of the large database available, particularly of epidemiology studies, the review has been split into several sections. The current paper presents information on identity, physiological control of IGF-I, human physiological levels of IGF-I, IGF-I in food and tissues, use of IGF-I as a human medicine, toxicology and other safety studies (Annex 2). Annex 3 contains information on the association between blood levels of IGF-I and breast cancer. Conclusions are presented at the end of each section as appropriate and also reproduced at the end of this cover paper. Key papers are included at Annex 4.

7. A future paper will provide information on the association between blood levels of IGF-I and other types of cancer (prostate, lung, colorectal, ovarian, endometrial and malignant melanoma), influence of diet on cancer risk, information relating to possible modes of action and mechanisms of carcinogenicity and mitogenic potency. A draft statement will be produced in due course.

Summary and conclusions

8. To assist members, the summary and conclusions sections from Annexes 2 and 3 are reproduced below in a slightly amended form (to aid clarity).

Annex 2. Conclusions on dietary exposure of humans to IGF-I

9. There are few data available on concentrations of IGF-I in foods derived from animals. No data have been identified on levels in meat, offal or eggs from food-producing animals.

10. A wide range of IGF-1 concentrations (1 to 1850 ng/ml) has been found in cows' milk. The level in milk is affected by genetic (eg. breed of cow) and external factors (eg. diet fed to the cows). The highest measurement was in the first post-partum milking, and this reflects the high level of IGF-I that

is known to occur in colostrum. Levels in the milk from a cow decrease with time after parturition. The colostrum is normally fed to calves and is only rarely eaten by humans. The highest concentration of IGF-I in milk commonly consumed by humans is unlikely to be greater than 100 ng/mL.

11. IGF-I concentrations of 11 to 92 ng/g in muscle, 84 to 89 ng/g in liver and 180 to 816 ng/g in kidney (up to 3469 ng/g in kidneys of diabetic animals) have been reported.

12. Assuming a concentration of 101 ng/g in lean meat, poultry, sausage and milk, consumers mean and high level (97.5%) dietary exposure to IGF-I is 3.97 and 9.56 µg/kg bw (body weight)/day in toddlers and 0.85 and 1.83 µg/kg bw/day in adults. Production of IGF-I has been estimated to be 10,000 µg/day (VPC, 1999) suggesting that dietary IGF-I is less than 1% of endogenous production.

13. A number of epidemiological and intervention studies have indicated that IGF-I levels could be positively associated with milk intake. It has been suggested that this could be due to the protein and/or calcium content of the milk.

14. Following ingestion, the animal-derived foods are mixed with other foods (plant-derived foods are not expected to contain IGF-I), saliva and digestive juices in the lumen of the gastrointestinal tract. Most of the digestive secretions contain lower levels of IGF-I than the animal-derived foods. Table 1 shows levels of 1.3 to 17.2 ng/mL in saliva, 26 ng/mL in gastric juice, 188 ng/mL in jejunal chyme, 28 ng/mL in pancreatic juice and 0.27 to 6.9 ng/mL in bile. Consequently, the digestive secretions, whilst adding to the total load, would dilute the IGF-I in the stomach contents. Plant-derived foods, which do not contain IGF-I, would further dilute the concentration of IGF-I in the gut lumen when consumed at the same time. The concentration of IGF-I in the lumen of the gut is expected to be lower than the concentration in the blood of the consumer (serum/plasma levels of 21.3 to 500 ng/mL have been reported). This suggests that, even if the IGF-I was not digested, any absorption of IGF-I from the gut lumen would need to operate against a concentration gradient.

15. The limited indirect evidence reported above indicates that small amounts of IGF-I are likely to occur in the diet and that such levels would not be absorbed by passive diffusion. Firmer conclusions could be drawn if more information were available on concentrations of IGF-I in foods. Currently only inconsistent data on levels in milk are available. No measurements have been made of levels in other animal-derived foods, such as meat, fat, liver, kidneys, eggs, fish and honey.

Annex 2 Overview and conclusions from safety studies

16. IGF-I is normally rapidly digested in the stomach and small intestines. However some components of the diet (eg. casein) appear to confer some protection from digestion, so some IGF-I might pass through the gut without being broken down. Concentrations of IGF-I in the gut lumen are likely to be lower than the levels in the blood, so passive absorption of IGF-I is not anticipated.

17. In neonatal animals, IGF-I is less readily broken down in the gut. There are limited and inconsistent data to suggest that absorption of IGF-I might occur in young individuals. Neonates are likely to have more systemic exposure to dietary IGF-I, through consumption of maternal milk and to have a greater exposure of the luminal side of the gut to IGF-I than is the case in older individuals. The high concentration of IGF-I in colostrum will provide neonates with a high dietary intake of IGF-I. It is feasible that this high exposure and bioavailability of IGF-I in neonates is related to a normal physiological role of IGF-I in the growth and development of the newborn. Exposures in humans will be variable, since neonatal humans are exposed to IGF-I only in human milk. Formula does not contain IGF-I and as weaning does not occur until 4-6 months of age, when the gut is more mature, some infants would not be exposed to exogenous IGF-I until 4-6 months of age. Current recommendations are that cows' milk is not introduced until 6 months of age.

18. Distribution of IGF-I was to all parts of the body, with well-perfused organs (kidney, liver and lungs) having highest levels. Much of the IGF-I remained in the bloodstream bound to IGF binding proteins (IGFBPs). It is expected that metabolism would be by breakdown to amino acids, which would then be either used to build body proteins or metabolised further by normal processes to produce energy and normal products for excretion (carbon dioxide, urea, water). Excretion of the ultimate products of metabolism was expected to be as exhaled carbon dioxide and in the urine. Excretion/secretion of intact IGF-I in milk, saliva, digestive juices and bile also occurs. Free IGF-I is rapidly removed from plasma (elimination half-life < half-an-hour), but protein-binding can considerably slow down the elimination.

19. Special studies of the trophic effects of IGF-I and related substances on gut tissues showed that oral or parenteral doses could cause growth of the intestines, typically characterised by increases in intestinal weight, intestinal length, mucosal mass, protein synthesis and villus length. A concentration of 750 ng/mL in milk replacer was the lowest oral dose reported to cause intestinal growth, but a level without effect was not detected.

20. Toxicological studies of recombinant human IGF-1 (rhIGF-I) used parenteral dosing; no oral toxicity studies were performed. The lowest dose level reported to cause adverse effects in any of the studies was 0.025 mg/kg bw/day as an intravenous dose (the lowest dose tested in the

study), which caused hypoglycaemia in dogs in a 13-week study. rhIGF-I was not genotoxic in an *in vitro* cytogenetics assay and in an *in vivo* micronucleus test. A carcinogenicity bioassay of subcutaneously administered rhIGF-I showed that rats developed malignant mammary tumours (4 mg/kg bw/day), benign mammary tumours (NOEL = 1 mg/kg bw/day), benign proliferative lesions of the adrenal medulla (at all doses: NOEL<0.25 mg/kg bw/day) and benign skin tumours (NOEL = 1 mg/kg bw/day). A special study of implants of cancer cells into the caeca of mice showed lower numbers of caecal tumours and hepatic metastases in transgenic mice with impaired hepatic production of IGF-I than in normal mice or transgenic mice that had injections of IGF-I. No developmental toxicity was seen when pregnant rats were given up to 16 mg/kg bw/day intravenously, but there was evidence of fetotoxicity in rabbits given 2 mg/kg bw/day intravenously (NOAEL = 0.5 mg/kg bw/day).

21. Several clinical studies of rhIGF-I have been performed in humans as part of its development as a medicinal product. Single subcutaneous or intravenous doses of 0.01 mg/kg bw caused reduced serum glucose and increased serum IGFBP-3. Twice-daily subcutaneous doses of 60 to 120 µg/kg bw given for several years caused decreased serum levels of glucose, ALT and AST, reduced packed cell volume (pcv) and haemoglobin (Hb), but had no effect on electrocardiogram (ECG) measurements. In premature babies, formula supplemented with 100 ng/mL of IGF-I had no effect on serum levels of IGF-I IGFBP-1, IGFBP-3 or growth hormone, but there was decreased gut permeability as compared with controls. There was no evidence from the clinical studies to suggest that treatment with rhIGF-I caused any cancer in treated patients.

22. In conclusion, the results of studies of the safety of rhIGF-I indicate that parenteral doses can be carcinogenic causing malignant mammary tumours in rats, although rhIGF-I does not appear to be genotoxic. It remains unclear whether dietary doses of IGF-I would be carcinogenic. It is also unclear whether absorption of intact IGF-I from the gut lumen can occur, although there is limited evidence that this might occur in neonates. There is also some evidence that some dietary IGF-I might be protected from digestion and might come into contact with the luminal surface of the gastrointestinal tract. However, it is considered likely that normal processes of digestion in the gut lumen and homeostasis of levels in the bloodstream would ensure that tissues are not exposed to elevated levels of diet-derived IGF-I for a sufficiently long time to increase the risk of cancer.

Annex 3. Conclusions on IGF etc levels and risk of breast cancer

23. Studies comparing circulating blood IGF-I levels in women with breast cancer and controls have reported both increased levels of IGF-I in cancer patients and no difference. Since cancers may produce their own growth factors, the results are difficult to interpret.

24. The results of the prospective studies investigating levels of IGF-I and breast cancer risk are also inconsistent. Some studies report an association between IGF-I and others report no association. Where women have been considered in terms of their menopausal status, the associations reported for post and pre-menopausal women have also differed.

25. One explanation for the variation in findings may be the wide range of different study designs and the range of potentially confounding factors that may influence the results. These may include issues such as age, classification of menopausal status, use of exogenous oestrogens by the participants and time from sample collection to diagnosis. Data on ethnicity is often absent which may be important if particular polymorphisms are relevant to IGF-I levels. The choice of assay may also be important since it is unclear to what extent active IGF-I is measured by the different procedures. Rinaldi *et al*., (2005a) used an assay stated to be more specific for active forms and a stronger association was observed than in other studies. However, this has not been repeated by other workers. IGF-I concentrations may be measured and reported as total or free IGF-I or this may not be specified. Some studies adjust the IGF-I results for IGFBP-3 and vice versa, and others present information on the IGF-I/IGFBP-3 molar ratio.

26. Several meta-analyses have been performed. These also produced conflicting results, although were more generally positive. Renehan *et al*., (2004) reported a positive association between IGF-I and risk in pre but not post-menopausal women, Shi *et al*., (2004) in post-menopausal women only, Sugumar *et al*., 2004 a marginally positive association in pre-menopausal women and Key *et al*., (2010) a weak positive association in pre-menopausal women and stronger ones in post-menopausal women as well as an association between IGF-I and oestrogen positivity in the cancer. These references are attached at Annex 4.

27. Results for an association with IGFBP-3 are also inconsistent. It has been suggested that high IGFBP-3 is protective by taking free IGF-I out of circulation, but the results from the studies are inconsistent.

Questions for the Committee

28. Can Members draw any conclusions/comment on:

    i) Whether there are any concerns with regard to the experimental methods used for IGF-I analysis.

    ii)The possible effect of dietary IGF-I intake on blood IGF-I levels, taking into account the likely exposure and absorption in different age groups.

iii) The toxicological and other safety studies supporting the use of IGF-I as a human medicine.

iv) The association between IGF-I levels in blood and the risk of breast cancer (in premenopausal women, postmenopausal women or in all women)

v) Any other issues

Secretariat
March 2012

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POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET

Annex 1. Details of literature search

Secretariat
March 2012

DETAILS OF LITERATURE SEARCH

1. Question to be addressed: “Does the ingestion of IGF-I in the diet cause an increased risk of cancer in consumers?”

2. The starting point for obtaining documents on the dietary effects of IGF-I was the book “Your Life In Your Hands” by Jane Plant. All cited articles that referred to IGF-I were obtained. These articles were often not primary references to original research, so the original reports that were cited in the articles were obtained also.

3. Several searches of the literature were performed on computer by the FSA’s Information Unit. The databases searched included PubMed and the British Library ETOC. Several combinations of keywords were used, including:

- IGF-I (title) AND food,
- IGF-I (title) AND cancer (all fields) filtered by Cancer Cells,
- IGF-I (title) factor AND digestion OR breakdown,
- IGF-I (title) AND absorption, IGF-1 (title) OR insulin-like growth factor AND gut AND lining OR lumen.

4. Less formal searches were also performed using Google.

5. Articles were chosen from the results of the literature search according to the relevance of their titles and/or abstracts to:

- concentrations of IGF-I in foodstuffs,
- endogenous levels of IGF-I
- association of endogenous IGF-I levels with cancers,
- association of eating particular foods with cancer risks,
- toxicological or pharmacokinetic studies of IGF-I,
- possible mechanisms of action.

6. The selected articles were obtained. Further relevant articles were cited in the articles that had been obtained and copies of these too were ordered.

7. It was not possible to obtain original copies of the full reports of toxicological studies submitted in support of the authorisation of use of IGF-I as a medicine for human patients. However, published summaries of the studies were available.

8. Not all of the obtained articles were cited in the reviews conducted by the Secretariat (Annex 2, 3 and to follow). Some did not meet the selection criteria (the same as the above list of relevances), despite their titles. Some repeated information given elsewhere. Wherever possible, the primary source of information was used.

A total of 413 articles have been obtained to date.
Of these, 106 articles were cited in the review in Annex 2.
Of these, 47 articles were cited in the review in Annex 3.

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ANNEX 2 REVIEW OF THE SAFETY OF DIETARY IGF-I

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Identity

1. Insulin-like growth factor-I (IGF-I) is a peptide that consists of 70 amino acids in a single chain with three intramolecular disulfide bridges. It has a molecular weight of 7649 daltons. The amino acid sequence of IGF-I is highly conserved in mammalian species and is identical in humans, cattle and pigs. IGF-I from other species is biologically active in humans (European Commission, 1999). Thus IGF-I in animal-derived foods will be active in humans.

2. The IGF-I in blood is produced mainly by the liver but IGF-I is also produced by other tissues (Chan *et al*., 1998a). It has a variety of autocrine, paracrine and endocrine functions.

Physiological control of levels of free IGF-I

3. It has been postulated that high levels of IGF-I activity in the blood increases the risk of developing certain cancers. Support for this hypothesis is given by the observation that people with acromegaly have high endogenous levels of production of growth hormone and IGF-I, and also have a high prevalence of colorectal cancer (VPC, 1999). It has also been noted that the use of tamoxifen against breast cancer reduced serum concentrations of IGF-I (Pollak *et al*., 1992). These authors proposed that tamoxifen blocked oestrogen receptors in the hypothalamic-pituitary axis, causing inhibition of release of growth hormone, which led to reduced hepatic production of IGF-I.

4. The rate of secretion of IGF-I and the degree to which it is protein bound in the bloodstream are affected by a complex interaction of physical factors (energy intake, body mass index and physical activity) and levels of hormones, including insulin, growth hormone (somatotropin), oestrogen, testosterone, T3 and T4 (Holly *et al*., 1998; Yu and Rohan, 2002). Sex steroid hormones can also affect the activity of IGF-I by directly affecting pituitary secretion of growth hormone or by altering levels of IGF-binding proteins (Holly, 1998). Oestrogens can increase the sensitivity of breast cancer cells to IGF-I (Westley and May, 1994). Binding of hormones to the androgen receptor switches off production of the IGF-I binding protein, IGFBP-3, resulting in an increased amount of free IGF-I in the bloodstream (Marcelli *et al*., 1995). IGF-I can cause increases in the levels of some hormones, for instance, when IGF-I was used to treat Laron dwarfism, patients experienced an increase in male hormone levels (Fox., 2002).

5. Sandhu *et al*., (2002) reviewed the available information on the physiological interactions of insulin, IGF-I and IGF-binding proteins. The amount of free IGF-I circulating in the bloodstream is regulated by IGF-binding proteins. Six IGF-binding proteins have been identified (IGFBP-1 to IGFBP-6), which have different affinities for different insulin-like growth factors. In the bloodstream, IGF-I is most commonly (>90%) bound to IGFBP-3.

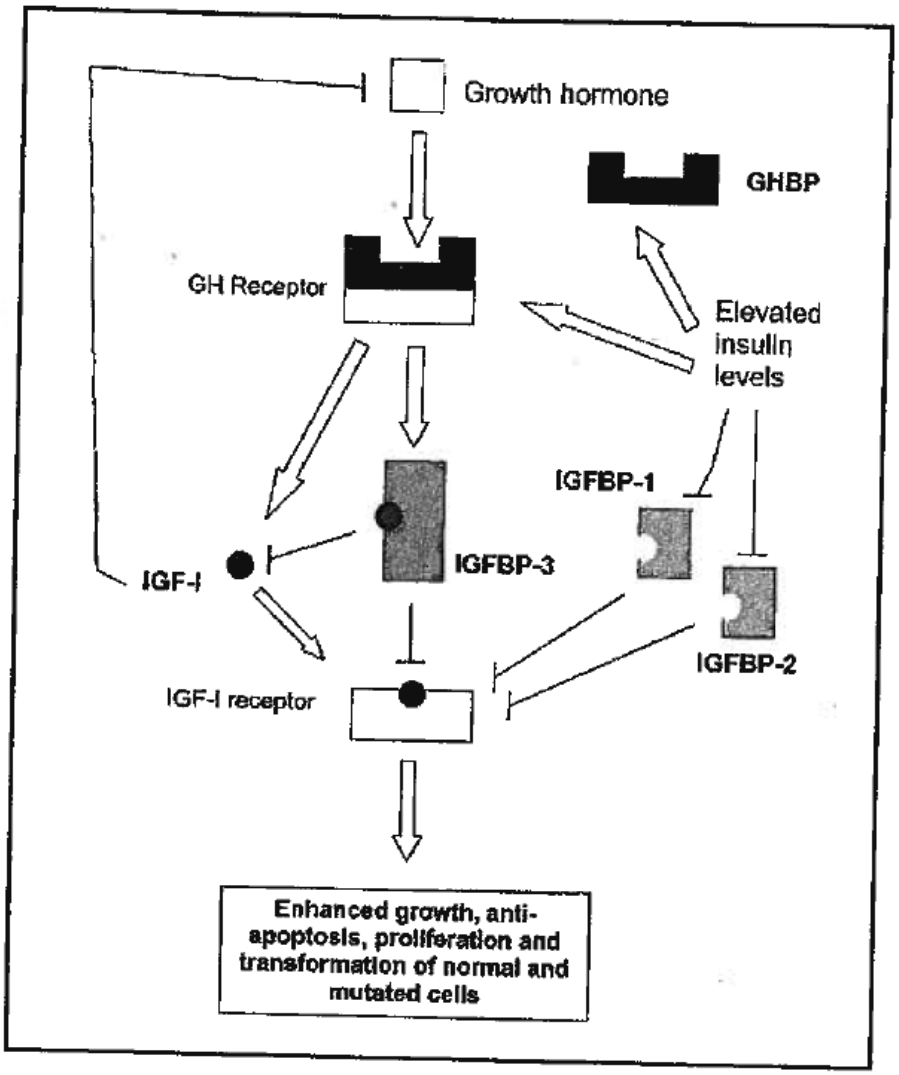

Fig. 1. Biologic interactions at the pituitary and hepatic levels among insulin, growth hormone (GH), insulin-like growth factor-I (IGF-I), and insulin-like growth factor binding proteins (IGFBPs). **Open arrows** denote stimulation, and **thin black lines** denote inhibition. Elevated insulin levels (**at right**) may indirectly increase the bioavailability of IGF-I (**solid circles**) by suppressing the production of IGFBP-1 and, to a lesser extent, IGFBP-2 (**shaded symbols**). In turn, increased IGF-I bioavailability may increase negative feedback effect on GH (**open square**), leading to reduced GH secretion and lower hepatic production of IGF-I and IGFBP-3. However, elevated insulin levels may also increase hepatic GH receptor (**open bar**) number and activity, reflected by increases in levels of circulating growth hormone binding protein (GHBP). This effect may lead to a rise in GH-regulated hepatic IGF-I and IGFBP-3 production, with a greater increase in levels of circulating IGF-I relative to IGFBP-3. Thus, along with genetic, hormonal, and other environmental factors, the relative magnitude of these two opposing effects of insulin on IGF-I production could determine levels of circulating IGF-I. Over time, excessive IGF-I bioavailability might increase the risk of colorectal cancer by promoting the survival of transformed and mutated cells that would normally undergo apoptosis.

6. The levels of free IGF-I in the bloodstream are controlled by a feedback mechanism, involving IGF-binding proteins, insulin and growth hormone. Production of IGF-I and IGFBP-3 (with relatively more IGF-I being produced than IGFBP-3) by the liver is stimulated by growth hormone (somatotropin) binding to its receptor. Insulin inhibits the production of IGFBP-1 and, to a lesser extent, IGFBP-2 by the liver. Lower amounts of IGFBPs available for binding cause an elevation of circulating levels of free IGF-I. In turn, free IGF-I inhibits the secretion of growth hormone by the pituitary. Elevated insulin levels can also increase hepatic growth hormone receptor number and activity (reflected by an increased level of growth hormone-binding protein), causing increased production of IGF-I and, to a lesser extent, IGFBP-3, resulting in an increase in the level of free IGF-I. The relative magnitude of the two opposing effects of insulin on levels of free IGF-I form the basis of its homeostasis. This is set out in Fig 1 taken from Sandhu *et al*., (2002).

7. Most of the IGF-I in blood exists as a ternary complex with an IGF-binding protein plus a glycoprotein called the acid-labile subunit (ALS), which does not cross the vascular barrier (Rajaram *et al*., 1997; Guidi *et al*., 2007). Whereas free IGF-I is prone to degradation in the blood stream, the ternary complex is more stable and protects the IGF-I from degradation (Wu *et al.,* 2008). IGF-I is made available to leave the bloodstream to affect surrounding tissues by the action of IGFBP-3 protease which is present in serum and is produced by vascular endothelial cells (Rajaram *et al*., 1997). The free IGF-I may subsequently bind to small molecular weight IGFBPs (such as IGFBP-4, which is normally present in excess in the serum) which can cross the vascular barrier and protect the IGF-I from degradation during its journey to the target tissue. In the tissue space, IGFBP-4 protease released by the target tissue can make the IGF-I available to local receptors. Tissue-specific regulation of IGFBP proteolysis may provide a mechanism for controlling bioavailablity of IGF-I to receptors. Local growth factors, such as TGFβ, can regulate IGFBP-4 proteolysis. Rajaram *et al*. (1997) speculated that some effectors may increase the local production of IGFBP proteases in specific tissues and in turn may degrade the inhibitory IGFBPs in extracellular fluid and thus increase the amount of free IGF-I available for receptor interaction. See Fig 2 taken from Zhou *et al*., (2003)

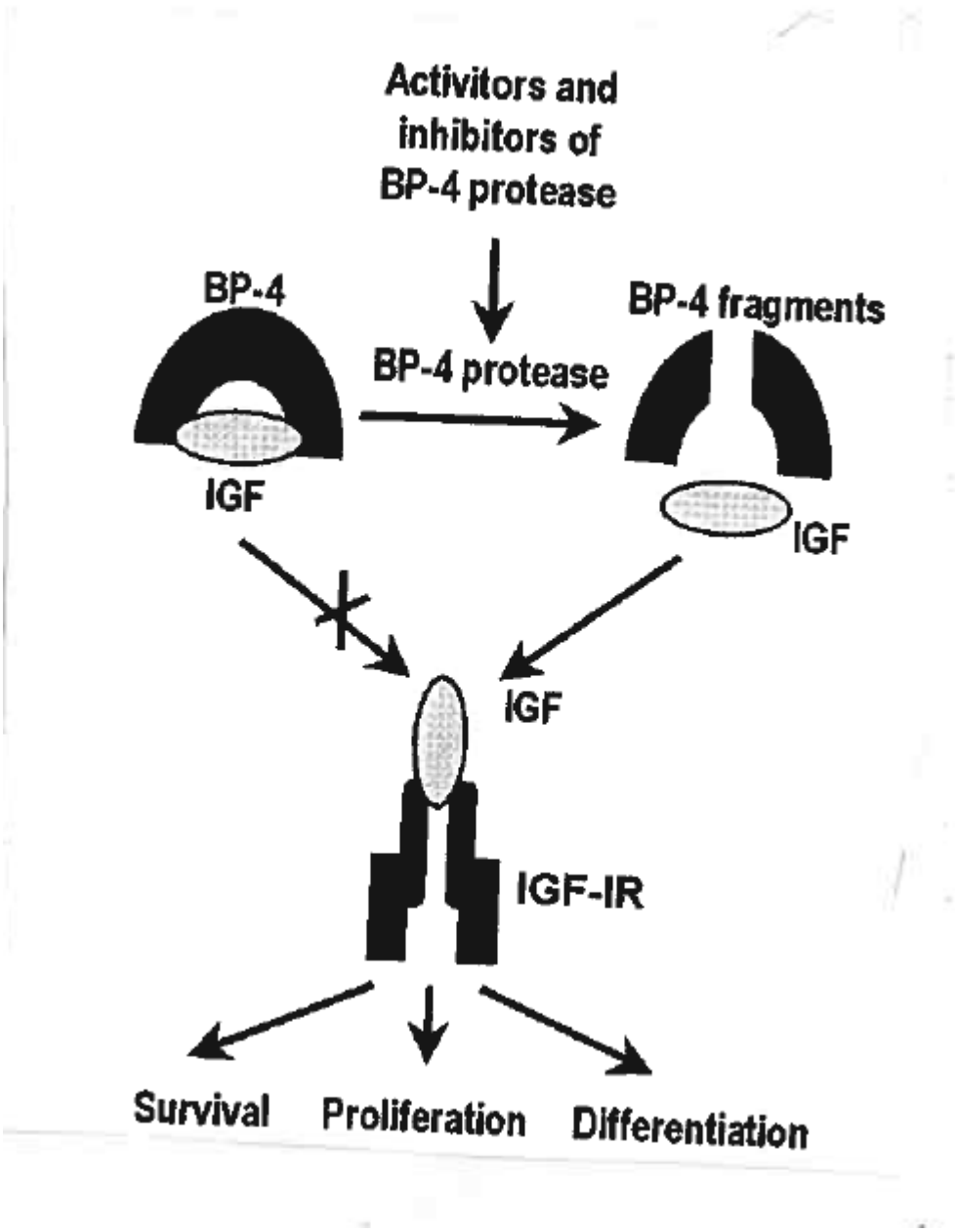

Fig 2. IGF-dependent actions of IGFBP-4 (BP-4). The mitogenic activity of IGFs mediated by IGF-IR (IGF-I receptor) is inhibited by the binding of IGFBP-4. Proteolysis of IGFBP-4 results in the release of IGF from the IGF/IGFPB-4 complex and a potentiation of IGF effects.

Human physiological levels of IGF-1 and its binding proteins

Factors affecting blood levels:

8. The circulating levels of IGF-I and its binding proteins are variable, depending on factors such as age, gender, race, diet, exercise, smoking status and levels of certain hormones such as insulin, somatotropins and oestrogens (Kaklamani *et al*., 1999; Sandhu *et al*., 2002; Holmes *et al*.,

2002a; Chang *et al*., 2002). Some examples of serum and plasma levels of IGF-I that have been measured are given in Table 1, and more data are given in the results of various human studies that are reported later in this document under the headings “Toxicological and other safety studies – Human clinical studies”, “Association between blood level of IGF- and cancer risk” and “Influence of diet on IGF-I levels and cancer risk”.

9. Circulating levels of IGF-I can change in different physiological states. They increase in the second half of pregnancy, fall slightly during sleep, and fall during acute fasting and chronic protein-calorie deprivation (Underwood *et al.,* 1980).

10. On a molar basis, human serum levels of IGFBP-3 (about 105 nM on average) were found to be about three to four times greater than those of IGF-I (about 30 nM or 230 ng/mL) (Rajaram *et al*., 1997). Mean serum levels of the other IGFBPs were lower (1-15 nM).

Table 1: Concentrations of IGF-I in human body fluids and tissues (NB. only a selection of values are given for the levels in human blood serum/plasma, the results of many further measurements are given in the text)

Fluid	**Source**	**Average concentration of IGF-I ± SD** (ng/mL or ng/g wet weight)	**Reference**
Serum	Pre-pubertal children	80-200	Juul *et al*., 1994b
Serum	Pubertal children	500	Juul *et al*., 1994b
Plasma	Pubertal children	200	Perdue, 1984
Serum	20-year-old humans	290	Juul *et al*., 1994a
Serum	70-year-old humans	160	Juul *et al*., 1994a
Serum	Women aged 70-79 years	124±52	Cappola *et al*., 2001
Serum	80-year-old humans	100	Juul *et al.,* 1994b
Serum	Human: elderly osteoporosis patients	21.3	Schürch *et al.,* 1998
Serum	Men	274	Kaklamani *et al*., 1999
Serum	Women	221	Kaklamani *et al*., 1999
Serum	Caucasian men	224	Platz *et al*., 1999
Serum	African-American men	205	Platz *et al*., 1999

Fluid	**Source**	**Average concentration of IGF-I ± SD** (ng/mL or ng/g wet weight)	**Reference**
Serum	Asian men	208	Platz *et al*., 1999
Plasma	Human	315 ± 27	Costigan *et al*., 1988
Saliva	Human (normal)	2.3 ± 0.3	Costigan *et al*., 1988
Saliva	Human (with growth hormone deficiency)	1.3 ± 0.2	Costigan *et al*., 1988
Saliva	Human (acromegaly patients)	17.2 ± 6.3	Costigan *et al*., 1988
Saliva	Human	6.9 ± 0.8 (0.9 ± 0.1 nM)	Chaurasia *et al*., 1994
Gastric juice	Human	26 ± 12 (3.5 ± 1.6 nM)	Chaurasia *et al*., 1994
Jejunal chyme	Human	188 ± 73 (24.6 ± 9.6 nM)	Chaurasia *et al*., 1994
Pancreatic juice	Human	28 ± 10 (3.6 ± 1.3 nM)	Chaurasia *et al*., 1994
Milk	Women	6 to 17.6	Outwater et al., 1997
Bile	Human	6.9 ± 0.8 (0.9 ± 0.1 nM)	Chaurasia *et al*., 1994
Bone	Human – women in their 30s	0.42	Seck *et al*., 1998
Bone	Human – women in their 80s	0.27	Seck *et al*., 1998

Age

11. Endogenous levels of circulating IGF-I increase throughout childhood, reaching a peak plasma concentration of approximately 200 ng/mL at about 12 years-of-age in girls and 14 years in boys (Perdue 1984; Yu and Rohan, 2002). After puberty these levels fall to about a half or a third of the peak value and then gradually decrease with age. Whilst some authors have reported that levels in women are generally higher than in men of the same age (Underwood *et al*., 1980). Other authors (Yu and Rohan, 2002) report that there is no significant difference. Table 2 (taken from Rajaram *et al*., 1997) shows how circulating levels of the IGFs and IGFBPs change in puberty and with age.

Table 2: Changes in serum levels of IGFs and IGFBPs in puberty and with age. (Rajaram *et al*., 1997).

Substance	**Puberty**	**Aging**
IGF-I	Increase	Decrease
IGF-II	Increase	Decrease
IGFBP-1	Decrease	Increase
IGFBP-2	No change	Increase
IGFBP-3	Increase	Decrease
IGFBP-4	Not known	Increase
IGFBP-5	Increase	Decrease
IGFBP-6	Not known	Not known

12. Juul *et al*., (1994a and 1994b) investigated the serum levels of IGF-I and IGFBP-3 in 1030 healthy Caucasian children, adolescents and adults in Denmark, looking at the influence of age, gender, stage of puberty, testicular size and body mass index. Mean serum IGF-I concentration increased slowly in prepubertal children from 80-200 ng/mL, peaking with the onset of puberty at approximately 500 ng/mL. In healthy adults, serum levels of IGF-I and IGFBP-3 remained fairly constant, but concentrations of both substances and the IGF-I/IGFBP-3 ratio decreased with age. In 20-year-olds, mean levels were 290 ng/mL for IGF-I, 4100 ng/mL for IGFBP-3 and 0.3 for IGF-I/IGFBP-3 molar ratio; whereas in 70-year-olds the values were respectively 160 ng/mL, 3100 ng/mL and 0.18. In 80 year-olds, the mean level of IGF-I was 100 ng/mL. No gender differences were noted other than the average age of onset of puberty: 14.5 years in girls and 15.5 years in boys. Testicular volume was positively associated with serum IGF-I concentration. There was no significant correlation of IGF-I with body mass index (BMI) in prepubertal children, but serum IGF-I concentrations predicted the rate of increase in height in the following year

Sex, ethnicity, smoking, oestrogen use

13. In a healthy Greek population (Kaklamani *et al*., 1999), serum levels of IGF-I were found to be higher in men (274±131 ng/mL) than in women (221±126 ng/mL) when compared by student t-test (p = 0.04), whereas levels of IGFBP-3 were lower (2172 ± 479 ng/mL in men; 2414± 621 ng/mL in women, p = 0.02). Serum levels of IGF-I were negatively associated with age. Lower serum levels of IGF-I and of IGFBP-3 were associated with smoking (mean IGFBP-3 = 2137 in smokers and 2388 ng/mL in non-smokers). Mean serum levels of IGF-I in smokers and non-smokers were not presented but serum IGF-I levels were noted to be positively associated with pack-year history of smoking (p =0.038) when analysed by multi-variate linear regression.

14. Platz *et al*. (1999) reported racial differences in the amount of circulating IGF-I and IGFBP-3 in middle-aged men. Caucasians had the highest median IGF-I level (224 ng/mL), followed by Asians (208 ng/mL) and

African-Americans (205 ng/mL). Median molar ratio of IGF-I to IGFBP-3 was greatest in Caucasians and lowest in Asians.

15. Jernström *et al*., (2001) measured plasma IGF-I levels in 503 black and white women aged 17-35 and examined the effect that oral contraceptive use had on these levels. Black women had significantly higher levels of IGF-I than white women (330 ng/ml vs 284 ng/ml; $p = 0.001$). IGF-I levels were significantly suppressed by oral contraceptive use in white women (301 ng/ml vs 267 ng/ml; $p = 0.0003$) but not in black women.

16. Holmes *et al*. (2002) examined the cross-sectional association of plasma IGF-I and IGFBP-3 with age, smoking, physical activity and reproductive/menopausal factors in 1037 women from across the USA from the Nurses' Health Study (NHS) cohort. Age was inversely associated with IGF-I levels. Higher BMI was associated with higher IGFBP-3 levels. Current use of hormone replacement therapy (HRT) was associated with lower IGF-I, with oral oestrogen use being associated with the lowest levels of IGF-I. Levels of IGF-I were negatively associated with the number of pregnancies a woman had had.

17. Chang *et al*. (2002) performed a cross-sectional study of 210 mean and 171 post-menopausal women who were serving as controls in an ongoing lung cancer case-control study at the University of Texas M. D. Anderson Cancer Center. They found that women on HRT had lower levels of circulating IGF-I, with the association being particularly strong in smokers. BMI and height were positively associated with IGF-I levels. Caloric intake was negatively associated with IGF-I in men and level of physical exercise was positively associated with IGFBP-3 levels in men. In women, miscarriage number and menopausal age were positively associated with IGFBP-3 levels.

Diet

18. In a cross sectional study by Diorio *et al*., (2008) Plasma intact and total IGBP-3 and IGF-I levels were measured by ELISA in 737 pre-menopausal women attending mammography screening. Means ± standard deviation of intact and total IGBP-3 levels were 1,044 ± 234 and 4,806 ± 910 ng/ml respectively. Intact and total IGBP-3 levels were positively correlated with age and smoking (this contrasts with the findings of Kaklamani *et al*., (1999) in respect of smoking. Levels of intact IGBP-3 were negatively correlated with waist to hip ratio (WHR), parity and alcohol intake and positively correlated with energy intake. In contrast, total IGFBP-3 was correlated with WHR, parity, BMI, physical activity and IGF-I levels and negatively correlated with percent and absolute breast density.

19. Blood levels of IGF-I can be affected by diet. The serum levels of IGF-I (initial mean = 125 ng/mL) rose by 10% in subjects given three additional 8-ounce servings per day of low-fat milk for 12 weeks (Heaney *et al*., 1999) and serum IGFBP-4 decreased by 4% over the same period whilst IGFBP-4 in controls rose by 7.9%. In a study of 159 adolescents in the

Dietary Intervention Study in Children (DISC), IGF-1 was significantly associated with total protein, lactose and calcium and sodium; IGFBP3 with total fat (inverse) lactose, fibre and calcium; IGF-1/IGFBP3 with lactose and calcium; and IGFBP3 with vegetable protein (Kerver *et al*, 2010); these analyses were adjusted for energy, age and time since menarche. Multivariate analysis showed that significant predictors of IGF-1 were energy and calcium, the significant predictors of IGFBP3 and IGFBP1 were calcium and vegetable protein. Dietary intake was assessed by three 24 hour dietary recalls.

20. Norat *et al*., 2007 reported a study of 2109 women from the control arm of a study on breast cancer nested in the European Investigation into Cancer (EPIC) cohort (see Kaaks *et al*., 2005 for details of this study). Diet was assessed by validated questionnaire and serum hormone concentrations by Enzyme Linked Immunosorbent Assay (ELISA). The relationship between serum IGF-I, IGFBP-3 and intake of nutrients and foods was explored by linear regression in models adjusted for energy intake, BMI, smoking, physical activity, centre and laboratory batch. It was reported that serum IGF-I levels were positively related to protein intake ($p_{trend} = < 0.001$) but not to energy, fat or carbohydrate intake. Positive relationships were observed with the intake of milk ($p_{trend} = < 0.007$), cheese ($p_{trend} = < 0.001$), calcium ($p_{trend} = < 0.03$), magnesium ($p_{trend} = < 0.003$), phosphorus ($p_{trend} = < 0.001$), potassium ($p_{trend} = < 0.002$), vitamin B6 ($p_{trend} = < 0.06$), vitamin B2, ($p_{trend} = < 0.001$).The association between yoghurt intake and IGF-I was not significant ($p_{trend} = < 0.07$). Inverse relationships were observed with vegetable ($p_{trend} = < 0.02$) and beta-carotene intake ($p_{trend} = < 0.02$). IGFBP-3 was not related with most of the nutrients or food in the study. It was concluded that in the population assessed, circulating IGF-I levels were modestly associated with intakes of protein and minerals and with milk and cheese. The difference in mean IGF-I concentrations when comparing women in the 5th quintile of milk intake (>400g/day) and women in the first quintile (<25.1g/day) was 8% (233 and 216 ng/ml respectively). For cheese intake the difference was 3%. (230 and 224 ng/ml respectively). When the relationship of milk intake and IGF-I was adjusted for protein intake it weakened (($p_{trend} = < 0.04$ and disappeared after adjustment for dietary calcium ($p_{trend} = < 0.10$).

21. In a further study among the EPIC cohort, the association between dietary intake and serum concentrations of IGF-I, IGFBP-1, IGFBP-2 and IGFBP-3 was assessed in a cross-sectional analysis of 4, 371 men and women (Crowe *et al*., 2007). The mean age of the participants was 59.9 ± 6.5 and 55.7 ± 8.4 for men and women respectively. Diet was assessed by country-specific validated questionnaire and serum hormone concentrations were measured by ELISA. The relationships between serum IGFs and IGFBPs and diet were assessed by multiple linear regression adjusted for sex, age, BMI, smoking, alcohol and energy intake, Each 1 SD increment in total and dairy protein and calcium intake was associated with an increase of 2.5, 2.4 and 3.3% in IGF-I concentration respectively ($p_{trend} = < 0.001$ for all). There were no significant associations between the intake of protein and calcium from

non-dairy sources and IGF-I. The authors concluded that the intake of either dairy protein or calcium was an important determinant of IGF-I and IGFBP-2 concentrations, but that it was more likely that the effect was due to the dairy protein. Because of the strong correlation between protein and calcium, it was not possible to adjust the association for these variables. It was considered that the most likely explanation was that some component in dairy products was responsible for increasing serum IGF-I concentrations as there was no evidence for an association between the intake of protein and calcium from non-dairy sources and IGF-I. In addition there was little evidence from the literature that calcium supplementation alone increased IGF-I levels, suggesting that the association could be due to dairy protein. It was noted that the inverse association between IGFBP-2 and total and dairy protein and calcium was consistent with the results of other authors and could be due to the inhibition of the growth promoting effect of IGF-I by IGFBP-2 which had been demonstrated *in vitro*.

22. In a study by Hoppe *et al*., 24 8-year old boys were asked to consume 1.5L skimmed milk or the same amount of protein as lean meat (250g) daily for 7 days. The rest of the diet was free choice. This was assessed by a 3-day weighed diet record. Protein intake increased by 61% in the milk group (4 g/kg bw/day) and 54% (3.8 g/kg bw/day) in the meat group. The high milk intake increased serum IGF-I by 19% ($p < 0.001$) from 209.3 ± 54.9 to 249.0 ± 66.8 ng/ml and the ratio IGF-I/IGFBP-3 by 13% ($p < 0.0001$). There were no increases in these parameters in the meat group. There were no differences in anthropometrical variables between the two groups at base line, but the milk group gained an average of 550 g weight at the end of the study; there were no changes in the meat group. IGFBP-3 increased from 3612 ± 409 to 3806 ± 560 ng/ml in the milk group ($p = <0.05$) and from 3120 ± 560 to 3213 ± 597 which was not significant. However, the baseline levels of IGFBP-3 were significantly different in the two groups. There were differences in the habitual diets of the two groups with the meat group having a higher proportion of their energy intake from carbohydrate and a lower proportion from fat than the milk group. It was suggested that the increase in both parameters could be due to a direct effect on the liver where both were manufactured, possibly via growth hormone secretion.

23. In a randomised double-blind trial, 82 elderly patients (mean age 80.7) with a recent osteoporotic hip fracture received a 20 g/day protein supplement (90% milk proteins) or an isocaloric placebo for 6 months in addition to a single vitamin D dose at baseline and calcium supplementation followed by a 6 month follow up (Schürch *et al.,* 1998). Compared with the controls, the patients who received a placebo had a significant increase in IGF-I levels (85.6 ± 14.8% and 34.1 ± 7.2 % respectively; difference 51.5 percentage points [95%CI 18.6-84.4 percentage points] $p = <0.003$). Attenuation of proximal bone loss and shorter rehabilitation time was also reported. Baseline IGF-I levels were 73.4 and 82.6 ng/ml in the control and treatment groups respectively

which increased by 24.5 and 45.1 ng/ml at 6 months. At the 12 month follow up, IGF-I levels were 23.1 and 34.5 ng/ml higher than at baseline.

24. Serum IGF-I levels are low in starvation (Pollak, 2000). Low serum levels of IGF-I and IGFBP-3 have been found in humans with acute (fasting) or chronic (marasus & kwashiorkor) dietary restrictions. These findings were not due to decreased growth hormone secretion as concentrations and pulse frequency of growth hormones were increased (Thissen *et al*., 1994; Sandhu *et al*., 2002). It was suggested that the decline of circulating levels was due to a decrease in hepatic production of IGF-I and IGFBP-3 by decreased hepatic growth hormone receptor number and function, both of which are partially regulated by insulin levels. Protein restriction also had the effect of lowering serum IGF-I and in addition the balance of essential amino acid affected levels. In prolonged dietary restriction (especially protein restriction) or in diabetes there were also elevated levels of IGFBP-2 that were related to low insulin levels (Sandhu *et al*., 2002). Obese people seem to be resistant to the effects of dietary restriction on IGF-I levels: energy restriction of 18-20 kcal/kg bw/day for a week reduced serum IGF-1 by 38% in normal subjects but had no effect on IGF-I levels in obese subjects (Thissen *et al*., 1994). Low levels of IGFBP-2 are found in chronically obese people, possibly as a result of hyperinsulinaemia (Sandhu *et al*., 2002). The results of studies investigating associations between diet, circulating levels of IGF-I and risk of cancer will be summarised and discussed in a forthcoming document which will include a section covering “Influence of diet on IGF-I levels and cancer risk”.

Physical activity

25. Rosendal *et al*. (2002) investigated the effects of 11 weeks of intense physical training on circulating levels of IGF-I and its binding proteins in untrained and highly physically trained people. Proteolysis of IGFBP-3 was increased in previously untrained people but not in those who were already highly trained. There were decreased serum levels of total IGF-I, free IGF-I, and IGFBP-4 in both untrained and highly trained individuals. Serum levels of IGFBP-2 were decreased and IGFBP-3 increased in untrained people but there was no effect on these parameters in well-trained individuals. Levels of IGFBP-1 were unaffected in both groups.

Acromegaly

26. Serum levels of IGF-I and IGFBP-3 were elevated in patients with active acromegaly due to excessive secretion of growth hormone (somatotropin), with the ratio being proportional to the serum growth hormone level (Juul *et al*., 1994a). Serum levels of IGFBP-1 and IGFBP-2 levels were low and IGF-II levels remain similar to normal. The ranges of values for 56 acromegaly patients were: 46 to 1860 ng/mL for IGF-I; 311 to 1132 ng/mL for IGF-II; 0.29 to 55.1 ng/mL for IGFBP-1; 61 to 1825 ng/mL for IGFBP-2; 1392 to 10209 ng/mL for IGFBP-3; and 0.133 to 1.081 for IGF-I/IGFBP-3 molar ratio. Perdue (1984) reported IGF-I serum levels of 193± 58, 24±14

and 712± 245 ng/mL in normal adults, hypopituitary patients and acromegalics, respectively.

Other issues

27. Renehan *et al*. (2003) noted that higher results were obtained for measurement in EDTA plasma (173 ng/mL), as compared with those in serum (108 ng/mL) or heparin plasma (109 ng/mL). Details are not always available on the methods used to obtain plasma, making the results of different studies using plasma difficult to compare. The majority of studies measure IGF and IGFBPs in serum rather than plasma.

Levels in tissues and fluids other than blood:

28. There are few data on levels of IGF-I in human tissues and fluids other than blood. Chaurasia *et al*. (1994) measured 0.9± 0.1 nM (6.9± 0.8 ng/mL) of IGF-I in saliva, 3.5± 1.6 nM (26±12 ng/mL) in gastric juice, 24.6±9.6 nM (188±73 ng/mL) in jejunal chyme, 3.6±1.3 nM (28±10 ng/mL) in pancreatic juice and 0.9±0.1 nM (6.9± 0.8 ng/mL) in bile.

29. Costigan *et al*. (1988) measured IGF-I in saliva from healthy humans, patients with growth hormone deficiency and acromegaly patients, finding levels of 2.3± 0.3, 1.3± 0.2 and 17.2± 6.3 ng/mL, respectively.

30. Human milk contained 6 to 17.6 ng/mL of IGF-I (Outwater *et al*., 1997).

31. Seck et al. (1998) measured IGF-I in the iliac crest bone matrix using biopsies from 533 women. High cancellous (spongy) bone volume was positively associated with the level of IGF-I in the bone matrix. The level of IGF-I in bone was unaffected by menopause. There was a wide range of values for IGF-I in bone (0.05 – 0.88 ng/mL). There was an inverse relationship between bone matrix IGF-I levels and age, with levels in women in their 80s (mean = 0.27 ng/mL) being 35% lower than those in women in their 30s (mean = 0.42 ng/mL). Serum IGF-I levels were only weakly associated with bone levels of IGF-I, and only when adjusted for serum IGFBP-3 levels.

32. Different combinations of IGFBPs have been detected in various body fluids including blood, milk, urine, cerebrospinal fluid, follicular fluid, amniotic fluid, lymph and seminal fluid (Rajaram *et al*., 1997). The levels of IGFs and IGFBPs (measured semi-quantitatively in arbitrary units) were lower in lymph than in serum. It was noted that the IGF-I/IGFBP-3 complex did not elute from the bloodstream to the lymph.

IGF-I in foods and tissues

33. Most animal-derived foods contain IGF-I. There is information available on concentrations of IGF-I in milk, but few data are available on levels of IGF-I in other animal-derived foods, such as meat, edible offal or eggs. There are some data available on levels in blood and in tissues of laboratory animals.

34. There has been wide variability in the results for IGF-I levels found in milk and in other matrices. Some of the variability would have been due to differences in levels between different groups of animals, but the use of different analytical methods by different groups of workers was also a probable source of uncertainty in the results. An additional problem is that the reporting of the results of analyses has not always made clear whether the results refer to free IGF-I or total IGF-I (including the protein-bound fraction). Information was usually not available on levels of the highly active N-terminally truncated forms of IGF-I.

Milk

35. IGF-I is the major growth factor present in colostrum and milk. Other growth factors in bovine milk include IGF-2 and transforming growth factors TGF-β1 and TGF-β2 (Ginjala and Pakkanen, 1998). Four of the six known IGF-binding proteins (IGFBP-1, -2, -3 and -4) have also been detected in milk (Burrin, 1997).

36. It has been noted (European Commission, 1999) that about 3% of the IGF-I in milk is in N-terminally truncated forms (missing a few amino acids). These have a reduced affinity for IGF-binding proteins and have been approximately 10 times more potent as mitogens than is normal IGF-I in *in vitro* assays (Burrin, 1997; European Commission, 1999).

37. Daxenberger *et al*. (1998) measured naturally-occurring IGF-I concentrations in 5777 random milk samples from the dairy cow population of Bavaria (various breeds, including Simmental, Brown Swiss and Holstein-Friesian). A wide range of concentrations was detected: 1 to >83 ng/mL. The distribution was skewed to the right with a median of 4.4 ng/mL and 90th- and 95th-percentiles of 9.5 and 12.5 ng/mL, respectively. IGF-I concentrations did not correlate with region, season, the cow's milk performance or milk fat content, but there were positive correlations between IGF-I levels and the protein content and the somatic cell count of the milk.

38. Daxenberger *et al*. (1998) also reported that IGF-I levels in milk were strongly dependent on the lactational state of the cow, with relatively high concentrations occurring at the beginning (colostrum phase) and end of lactation. Ginjala and Pakkanen (1998) reported that IGF-I levels in milk from Ayrshire cows, showed initial high levels which decreased with subsequent milkings. At the first milking after calving, the concentration

ranged from 248 to 1850 ng/mL. A steady decline in the concentration of IGF-I correlated with a reduction in the total protein content, with IGF-I levels reaching 27 to 101 ng/mL by the fifth milking. The data limited provided suggest that levels were comparable at milkings 4 and 5 and (presumably) thereafter.

Table 3: Amounts of IGF-I found in cows' milk

Sample	**Concentration of IGF-I (ng/mL)**	**Reference**
Cows' milk (raw or pasteurised)	About 7	Miller *et al*. 1989
Bulked cows' milk in USA	1 to 8	Mepham *et al*., 1994
Milk from BST-treated cows	Up to 30	Outwater *et al*., 1997
Milk from cows in Bavaria	1 to >83 (median = 44)	Daxenberger *et al*., 1998
1st post-partum milking from Ayrshire cows	248 to 1850	Ginjala and Pakkanen, 1998
5th post-partum milking from Ayrshire cows	27 to 101	Ginjala and Pakkanen, 1998

39. It was reported (Mepham *et al*., 1994, Outwater *et al*., 1997; Daxenberger *et al*., 1998; Guidi *et al*., 2007) that treatment of cows with bovine somatotropin (BST) increased the concentration of IGF-I in milk from individual animals by two- to five-fold, but the inter-animal variation in natural levels of IGF-I is so large that it would be difficult to use milk IGF-I as a reliable marker of use of BST. Milk from BST-treated cows contained up to 30 ng/mL of IGF-I, whereas bulked milk in the USA contained 1 to 8 ng/mL (Mepham *et al*., 1995; Outwater *et al*., 1997). Administration of BST to lactating cows caused increased serum levels of IGF-I and the IGF-binding protein, IGFBP3; whereas levels of IGFBP-2 were decreased in serum but increased in lymph (Cohick *et al*., 1992).

40. High levels of IGF-I are also found in milks from high-yield breeds of cows (European Commission, 1999), and in colostrum.

41. Miller *et al.* (1989) reported that pasteurisation (heating to 175 C for 45 seconds) did not reduce the concentration of IGF-I in cows' milk from its initial value of approximately 7 ng/mL, whereas more extreme heat treatment (250 C for 5 minutes) reduced the IGF-I concentration to less than 1 ng/mL. IGF-I was not detected in infant formulae and it has been suggested that this is because it is either destroyed or removed during processing (Burrin, 1997).

Tissues and other body fluids – food animals

42. Plasma IGF-I concentrations of 4.5 to 24.9 ng/ml were measured in different groups of broiler chickens (Decuyper, *et al*., 1993). In male pigs aged 8 to 32 weeks, plasma IGF-I concentrations varied between 150 and 240 ng/mL but the levels were not related to age (Zamaratskaia *et al*., 2004). In young male goats, a mean IGF-I level of 232 ng/mL was found in the plasma from a group given a high energy diet compared with 81 ng/mL in those given a low energy diet (Shen *et al*., 2004). Simpson, *et al.,* (1997) measured mean plasma levels of IGF-I of 164.1 and 98.4 ng/mL in Brahman and Angus cows, respectively, and levels of approximately 220 ng/mL (with an 11% increase in binding of IGF-I to plasma proteins) in both breeds when implanted with oestradiol-17β to promote growth. Silage-fed Holstein-Friesian steers had plasma IGF-I levels of approximately 280 ng/mL (Juniper *et al.,* 2006 & 2008). Lee *et al.,* (2005) measured serum levels of 133 and 157 ng/mL IGF-I in Holstein steers fed low protein (9.5%) or high protein (21.9%) diets, respectively. Four Holstein-Friesian calves had a mean serum IGF-I level of 18.9 nM (145 ng/mL) shortly after birth, which fell to 16.5 nM (126 ng/mL) at 1½ hours of age and 13.6 nM (104 ng/mL) at 32 hours of age (Kirovski *et al*., 2008). Funston *et al*. (1995) found levels of approximately 20 and 70 ng/mL in serum and anterior pituitary of mature beef cows.

43. Rahimi *et al*. (2010) measured IGF-I in plasma of fasted and fed rainbow trout (*Oncorhynchus mykiss*). Levels in the fasted fish were between 7 and 10 ng/mL. In fish given 180 to 440 g/tank/day (23 fish per tank) for 5 weeks of feed, the levels were between 14 and 19 ng/mL with no clear dose-relationship. Small and Peterson (2005) found 45.7 ng/mL of IGF-I in pooled plasma from rainbow trout and 53.9 and 19.7 ng/mL in pooled plasma from hybrid striped bass (*Morone chrysops* x *M. saxatilis*) and channel catfish (*Ictalurus punctatus*), respectively.

Tissues and other body fluids – laboratory animals:

44. Sjögren *et al*. (1999) found levels of 350±17 ng/mL in serum from untreated Mx-Cre 31 strain mice. They also demonstrated that 75% of the IGF-I in mouse serum was derived from the liver.

45. Untreated guinea pigs had mean plasma levels of 950 ng/mL of total IGF-I, 190ng/mL of free IGF-I and 750 ng/mL of IGF-II (Conlon *et al.,* 1995).

46. Gomes *et al*., (2003) measured IGF-I in tissues from adult male Wistar rats (about 250 g bodyweight) fed either protein-free diet or a protein-sufficient diet (12% casein). Mean concentrations of 1923 ng/mL, 90 ng/mg and 89 ng/mg were detected in plasma, muscle and liver, respectively, from rats fed protein-sufficient diet. Lower levels of IGF-I were found in plasma (768 ng/mL) and muscle (83 ng/mg) from the rats given the protein deficient diet, but liver IGF-I was unaffected (84 ng/mg). On re-feeding of the protein-sufficient diet, levels of IGF-I in the rats that had been given the low protein diet returned to normal: plasma =

1954±150 ng/mL, muscle = 92±7 ng/mg and liver = 89±5 ng/mg. The results of an earlier study (Tirapegui, 1999) also showed that IGF-I levels in serum and muscle were lower in newly-weaned Wistar rats (about 50 g bodyweight; sex not specified) fed a low protein diet (5% casein) as compared with those fed a high protein diet (12% casein), with levels of 573 ng/mL, 0.011 ng/mg and 0.039 ng/mg in serum, muscle and cartilage, respectively, from rats fed 12% casein. It should be noted that the two articles report very different concentrations of IGF-I in rat l muscle. The levels reported by Gomes *et al*. (2010) are about 1000 times (150 to 8200 times) higher than the levels reported for normal rat muscle (discounting results for diabetic rats and rats with unilateral nephrotomy) by other authors, including Tirapegui (1999) who were said by Gomes *et al*. (2010) to have obtained similar results to them (11 ng/g in muscle of rats fed a 12% casein diet) suggesting that the results should have been reported as ng/g rather than ng/mg.

47. IGF-I has been found in fluids secreted into the gut lumen, including saliva, gastric juice, jejunal chime, pancreatic juice and bile (see Table 4). It was shown that IGF-binding proteins were present in human saliva at or below the limit of detection (< 0.06 µgeq/ml) in an amniotic fluid binding protein radioimmunoassay (Costigan *et al*., 1988). Binding proteins were not detected in rat bile analysed by chromatography or Western ligand blotting (limit of detection not stated) (Kong *et al*,. 1995).

48. Levels of IGF-I in rat kidneys were found to be 816 ± 21 ng/g in adult male Wistar rats (Flyvbjerg *et al*.,1988) and 543 ± 21 ng/g in young male Sprague-Dawley rats (Bach and Jerums, 1990) but these levels were greatly increased in diabetes or after unilateral nephrectomy. In normal male Wistar rats, the IGF-I was mainly located in the cortical collecting ducts, but in diabetic rats it was also in the cells of the thick ascending limbs of the loops of Henle, with the extractable IGF-I rising from 180 ng/g to 720 ng/g 4 days after the induction of diabetes (Sayed-Ahmed *et al*., 1993).

49. Levels of 55 ± 5ng/g of IGF-I were measured in untreated skeletal muscle from female Sprague-Dawley rats (Adams and McCue, 1998). Lang *et al*. (2009) compared IGF-I levels in young and mature male F344 rats, finding 36 & 34 ng/mL in muscle from young and mature rat, respectively, and 90 & 80 ng/mL in plasma. In mice, background concentrations of approximately 35 ng/mL were found in hamstring muscle (Takahashi *et al*., 2003).

Table 4: Concentrations of IGF-I in body fluids and tissues of animals

Fluid	**Animal**	**Average concentration of IGF-I ± SD** (ng/mL or ng/g wet weight)	**Reference**
Serum	Mice – Mx-Cre 31 strain	350 ± 17	Sjögren, *et al*. 1999
Serum	Male mice – TGS-p53 strain (normal diet)	367 ± 31	Dunn *et al*., 1997
Serum	Male mice – TGS-p53 strain (20% restricted diet)	271 ± 34	Dunn *et al*., 1997
Serum	Young Wistar rat (12% casein diet)	573	Tirapegui 1999
Plasma	Young Fischer F344 rat	90	Lang *et al.,* 2009
Plasma	Mature Fischer F344 rat	80	Lang *et al*., 2009
Plasma	Adult male Wistar rat (protein-sufficient diet)	1923 ± 369	Gomes *et al*., 2010
Plasma	Adult male Wistar rat (protein-deficient diet)	768 ± 218	Gomes *et al*., 2010
Plasma	Adult male Wistar rat (protein-deficient followed by protein-sufficient diet)	1952 ±150	Gomes *et al*., 2010
Plasma	Guinea-pig	950 (total): 190 (free)	Conlon *et al.,* 1995
Plasma	Broiler chicken	4.5 to 24.9	Decuypere *et al.,* 1993
Plasma	Chicken (Cobb broiler sire line)	14	Kita *et al*., 1996
Plasma	Large White pigs (fasted)	10.0	Morovat *et al*., 1994
Plasma	Large White pigs (fed)	13.5	Morovat *et al*., 1994
Plasma	Piglets (fasted)	5.9 ±1.9	Morovat *et al*., 1994
Plasma	Piglets (fed)	11.2 ±1.5	Morovat *et al*., 1994
Plasma	Male pig (8-32 weeks-old)	150 to 240	Zamaratskaia *et al*., 2004
Plasma	Young male goat (low energy diet)	81	Shen *et al*., 2004
Plasma	Young male goat (high energy diet)	232	Shen *et al*., 2004
Serum	Beef cows	20	Funston *et al.,* 1995
Plasma	Holstein-Friesian calves	104 to 145	Kirovski *et al*., 2008

Fluid	**Animal**	**Average concentration of IGF-I ± SD** (ng/mL or ng/g wet weight)	**Reference**
		(18.9 - 13.6 nM)	
Plasma	Holstein-Friesian steers (silage fed)	~280	Juniper *et al*., 2006 & 2008
Plasma	Holstein-Friesian steers (9.5% protein diet)	133	Lee *et al*., 2005
Plasma	Holstein-Friesian steers (21.9% protein diet)	157	Lee *et al*., 2005
Plasma	Brahman cows	98.4	Simpson *et al.,* 1997
Plasma	Brahman cows with oestradiol-17β implants	220	Simpson *et al.,* 1997
Plasma	Angus cows	164.1	Simpson *et al.,* 1997
Plasma	Angus cows with oestradiol-17β implants	220	Simpson *et al.,* 1997
Plasma	Rainbow trout (fasted)	7 to 10	Rahimi *et al*., 2010
Plasma	Rainbow trout (well-fed)	14 to 19	Rahimi *et al*., 2010
Plasma	Rainbow trout	45.7	Small & Peterson, 2005
Plasma	Channel catfish	19.5	Small & Peterson, 2005
Plasma	Hybrid bass	53.9	Small & Peterson, 2005
Milk	Mouse	10 to 15	Burrin, 1997
Milk	Cow	See Table 3	-
Bile	12-day-old Sprague-Dawley rat	133 ± 15.9	Kong *et al.*, 1995
Bile	33-day-old Sprague-Dawley rat	79.4 ± 10.5	Kong *et al.*, 1995
Bile	Adult Sprague-Dawley rat	45.3 ± 12.7	Kong *et al.*, 1995
Anterior pituitary	Beef cows	70	Funston *et al.,* 1995
Liver	Adult male Wistar rat (protein-sufficient diet)	89000 ± 5000	Gomes *et al*., 2010
Liver	Adult male Wistar rat (protein-deficient diet)	84000± 4000	Gomes *et al*., 2010
Liver	Adult male Wistar rat (protein-deficient followed by protein-sufficient diet)	89000 ± 5000	Gomes *et al*., 2010
Kidney	Post-pubertal young male Sprague-Dawley rat (non-diabetic)	543 ± 21	Bach and Jerums, 1990

Fluid	**Animal**	**Average concentration of IGF-I ± SD** (ng/mL or ng/g wet weight)	**Reference**
Kidney	Diabetic post-pubertal young male Sprague-Dawley rat	1082 ±156	Bach and Jerums, 1990
Kidney	Adult male Wistar rat (normal)	180	Sayed-Ahmed *et al*., 1993
Kidney	Adult male Wistar rat – diabetic	720	Sayed-Ahmed *et al*., 1993
Kidney	Male Wistar rat (non-diabetic)	816 ± 21	Flyvbjerg *et al*., 1988
Kidney	Diabetic male Wistar rat	3469 ± 312	Flyvbjerg *et al*., 1988
Kidney	Male Wistar rat after unilateral nephrectomy	2122 ± 214	Flyvbjerg *et al*., 1988
Skeletal muscle	Mice	35	Takahashi *et al*., 2003
Skeletal muscle	Sprague-Dawley rat	55 ± 5	Adams and McCue, 1998
Skeletal muscle	Young Fischer F344 rat	36 (free IGF-I)	Lang *et al*., 2009
Skeletal muscle	Mature Fischer F344 rat	34 (free IGF-I)	Lang *et al*., 2009
Skeletal muscle	Young Wistar rat (12% casein diet)	11	Tirapegui 1999
Skeletal muscle	Adult male Wistar rat (12% casein diet)	90000 ± 5000	Gomes *et al*., 2010
Skeletal muscle	Adult male Wistar rat (protein-deficient diet)	83000 ± 5000	Gomes *et al*., 2010
Skeletal muscle	Adult male Wistar rat (protein-deficient followed by protein-sufficient diet)	92000 ± 7000	Gomes *et al*., 2010
Cartilage	Young Wistar rat (12% casein diet)	39	Tirapegui, 1999

50. The measured levels of IGFs and IGFBPs are very dependent upon the methodology used. (Methods that have been used include: Western ligand binding, Western immunoblotting, RIA, and immunoradiometric assay.) Furthermore, the higher levels of IGF-I found in human EDTA plasma as compared with heparin plasma and serum, that were found in human blood samples by Renehan *et al*. (2003), suggest that the results for circulating levels of IGF-I can be affected by the type of plasma/serum analysed. Thus comparisons of results from different studies should be regarded with caution.

51. Most of the analyses for IGF-I measured total IGF-I which might not necessarily reflect the availability of IGF-I to receptors. In some assays IGF is acid extracted to release it from binding proteins but details are not always provided on whether this was done.

52. When discussing the differences in the results for IGFBP-3 between studies, Stattin *et al*. (2004) noted that the commercial ELISAs (eg obtained from DSL and used in a number of epidemiological studies – see Annex 3) mostly measured specific intact forms of IGFBP-3 whereas the DSL radioimmunometric assay might detect more or different forms combined.

Conclusions on dietary exposure of humans to IGF-I

53. There are few data available on concentrations of IGF-I in foods derived from animals. No reported data have been identified on levels in meat, offal or eggs from food-producing animals.

54. A wide range of IGF-1 concentrations has been found in cows' milk: from 1 to 1850 ng/mL. The level in milk is affected by genetic (eg. breed of cow) and external factors (eg. diet fed to the cows). The highest measurement was in the first post-partum milking, and this reflects the high level of IGF-I that is known to occur in colostrum. Levels in the milk from a cow decrease with time after parturition. The colostrum is normally fed to calves and is only rarely eaten by humans. The highest concentration of IGF-I in milk commonly consumed by humans is unlikely to be greater than 100 ng/mL. Little information is available on the concentration of the truncated form of IGF-I in foods.

55. Gomes *et al*. (2010) reported very high levels of IGF-I in the liver (84 - 89 mg/mg) and muscle (83 - 92 ng/mg) of rats. As noted previously, the units for these measurements might have been reported incorrectly and should have been ng/g rather than ng/mg. If so, the laboratory animal data shown in Table 4 indicate concentrations of 11 to 92 ng/g in muscle, 84 to 89 ng/g in liver and 180 to 816 ng/g in kidney (up to 3469 ng/g in kidneys of diabetic animals).

56. Assuming a concentration of 101 ng/g in lean meat, poultry, sausage and milk and using consumption data from the National Diet and Nutrition Survey (NDNS), consumers' mean and high level (97.5%) dietary exposure to IGF-I is 3.97 and 9.56 µg/kg bw (body weight)/day in toddlers and 0.85 and 1.83 µg/kg bw/day in adults. Production of IGF-I has been estimated to be 10,000 µg/day (VPC, 1999) suggesting that dietary IGF-I would represent less than 1% compared to endogenous production.

57. A number of epidemiological and intervention studies have indicated that IGF-I levels could be associated with milk intake. It has been suggested that this could be due to the protein and/or content of the milk.

58. Following ingestion, the animal-derived foods are mixed with other foods (plant-derived foods are not expected to contain IGF-I), saliva and digestive juices in the lumen of the gastrointestinal tract. Most of the digestive secretions contain lower levels of IGF-I than the animal-derived foods. Table 1 shows levels of 1.3 to 17.2 ng/mL in saliva, 26 ng/mL in gastric juice, 188 ng/mL in jejunal chyme, 28 ng/mL in pancreatic juice and 0.27 to 6.9 ng/mL in bile. Consequently, the digestive secretions would dilute the concentration of IGF-I in animal-derived foods once consumed. Concurrent consumption of plant-derived foods, which do not contain IGF-I, would further dilute the concentration of IGF-I in the gut lumen. The concentration of IGF-I in the lumen of the gut is expected to be lower than the concentration in the blood of the consumer (serum/plasma levels were 21.3 to 500 ng/mL). This suggests that, even if the IGF-I was not digested, any absorption of IGF-I from the gut lumen would need to operate against a concentration gradient.

59. The limited indirect evidence reported above indicates that small amounts of IGF-I are likely to occur in the diet and that such levels would not be absorbed by passive diffusion. Firmer conclusions could be drawn if more information were available on concentrations of IGF-I in foods. Currently only inconsistent data on levels in milk are available. No measurements have been made of levels in other animal-derived foods, such as meat, fat, liver, kidneys, eggs, fish and honey.

Use of IGF-1 as a human medicine

60. IGF-I is not used in any human or veterinary medicines intended for oral administration. However, recombinant human IGF-I (rhIGF-I) is present in two medicines that are administered by injection to human patients.

61. The medicinal product “Increlex” contains rhIGF-I (under the International Non-proprietary Name [INN] of “mecasermin”) as its active ingredient. Use of Increlex as an orphan medicinal product has been permitted in the UK since August 2005 (COMP, 2007). In the EU, Increlex has been designated as an orphan medicinal product[2] since May 2006 for the treatment of primary growth hormone insensitivity syndrome (COMP, 2007) and of primary IGF-I deficiency due to molecular or genetic defects (COMP, 2009a). In June 2007, Increlex was granted marketing

[2] EU designated orphan medicinal products are medicines that are still under investigation. Such products may not have been fully tested for quality, safety and efficacy. Demonstration of quality, safety and efficacy is necessary before a product can be granted a marketing authorisation.
The criteria for granting orphan medicinal product designations are:

- The seriousness of the condition,
- The existence of alternative methods of diagnosis, prevention or treatment, and
- Either the rarity of the condition (affecting not more than five in 10,000 people in the Community) or the insufficient returns on investment.

authorisation for long-term treatment of growth failure in children with severe primary IGF-I deficiency or with growth hormone gene deletion who have developed neutralising antibodies to growth hormone (EMEA, 2007a). This marketing authorisation was granted under exceptional circumstances as the indication did not fall within the scope of the orphan indication and as the indicated disease has a low prevalence in the EU population (not more than 1 in 10,000). In the USA, use of Increlex as an orphan medicinal product has been permitted since December 1995 and it has had marketing approval since August 2005 (EMEA, 2007a).

62. The other medicine is “Iplex”, which contains a complex of rhIGF-I and its binding protein, IGFBP-3 (under the INN name of “mecasermin rinfabate”) as its active ingredient. Iplex was introduced in the UK in June 2006 with designation as an orphan medicinal product (COMP, 2009b), but it was removed from the Community Register of Designated Orphan Medicinal Products in November 2006 at the request of the sponsor. A sponsor applied for marketing authorisation for use of Iplex for the treatment of patients with primary growth hormone insensitivity and those with growth hormone gene deletion, but the sponsor withdrew its application in March 2007. In April 2009, Iplex was given a positive opinion for use throughout the EU as a designated orphan medicinal product to treat primary IGF-I deficiency due to molecular or genetic defects (COMP, 2009b) and to prevent retinopathy of prematurity (COMP, 2009c).

Toxicological and other safety studies

63. Several studies were performed in laboratory animals to demonstrate the safety of parenteral use of mecasermin (rhIGF-I) as a human medicinal product named Increlex. The full reports of the studies were not available, but summaries of the results of the studies were published by EMEA as a European Public Assessment Report (EMEA, 2007b). None of the studies used the oral route for administering rhIGF-I.

64. In addition to the studies supplied with the Increlex submission (EMEA, 2007b), there are a few additional studies reported in the open literature. These are included in the relevant sections below.

<u>Digestion and exposure of the gut lining to undigested IGF-I</u>

65. IGF-I is a peptide. As such it is expected that ingested IGF-I would be subjected to pH changes that might denature it, and to digestive enzymes that would break it down to amino acids. Cells of the body could potentially be exposed to dietary IGF-I either directly or systemically following absorption from the gut. Cells lining the gut could be exposed to any IGF-I that survives the digestion process. In neonates, some dietary IGF-I reaches the gut lining in biologically active form, and it is feasible that the IGF-I in colostrum might play an important role in the growth and differentiation of the neonate gut (European Commission, 1999).

66. The luminal surface of the gut is exposed to IGF-I in the contents of the lumen as a result of its presence in saliva, gastric juice, jejunal chyme, pancreatic juice and bile (see Table 4) as well as its presence in various ingested foods. The tissues of the gut are also exposed to IGF-I in the blood. The concentrations of IGF-I in rat bile were highest in neonates – see Table 4 for results (Kong *et al*., 1995).

67. It has been suggested (Juskevich *et al*., 1990; Daxenberger *et al*., 1998) that IGF-I does not retain bioactivity if delivered orally because of rapid proteolysis in the gastrointestinal lumen. Xian *et al*., 1995, measured degradation half-lives of 2 to 8, 2, 2 and 16 to 38 minutes *in vivo* in ligated gut segments of adult rat stomach, duodenum, ileum and colon, respectively. This indicates that, in adult rats, there was rapid proteolysis of IGF-I by digestive juices in the stomach and small intestine. However, there is some evidence that the digestion of IGF-I might be incomplete in some individuals, leaving some active IGF-I in the gut lumen to directly expose the cells that line the enteric surface of the gut. IGF-I was more readily digested by gastrointestinal juices in adults than in neonates: Rao *et al.* (1998) found degradation in luminal flushings from the duodenum, jejunum, mid-jejunum and ileum of adult rats was several times greater (3.3 to 35 times, depending on the region) than in 12-day-old rats. No breakdown of IGF-I had occurred in flushings from the stomachs of adult rats (Rao *et al.,* 1998). Koldovský *et al*. (1992) found that in adult rats IGF-I was rapidly degraded in flushings from the jejunum and ileum, but slower in flushings from stomach or duodenum; but IGF-I was not broken down by flushings from the guts of newborn rats.

68. There is some evidence to suggest that some dietary components may protect IGF-I from digestion and that the luminal surface of the intestines might be exposed to active IGF-I. *In vitro* studies with fluids from the stomach and jejunum showed that acidic proteins, such as casein and (to a lesser degree) bovine serum albumin, protected IGF-I from proteolysis (Xian *et al*., 1995). Binding of IGF-I to IGFBP-3 or heparin did not protect the IGF-I from proteolysis by gut luminal flushings. An *in vivo* study of ^{125}I-labelled IGF-I in rats indicated that casein and IGF-antibodies protected IGF-I from degradation in the adult rat gut lumen (Xian *et al*., 1995). In addition, Rao *et al*. (1993) found a heat-labile soluble fraction in rat milk that inhibited the *in vitro* digestion of IGF-I by luminal flushings from the mid-jejunum. Koldovský *et al*. (1993) found three separate substances in the soluble fraction of rat milk that inhibited peptidases and it was postulated that these might protect IGF-I in milk from digestion in the gut lumen.

69. Indirect evidence that IGF-I can remain active in the gut lumen is given by observations that oral doses of IGF-I have effects on the gut. (An alternative explanation is that sufficient IGF-I might be absorbed to raise blood levels to such an extent that the gut tissues are affected.) Oral doses of IGF-I remained receptor-active in gastrointestinal tissues of suckling rats for at least 30 minutes post-ingestion (Philipps *et al*., 1995).

Feeding infant rats with IGF-I increased the migration of enterocytes from crypts in the small intestine (Philipps *et al*., 1997). Oral doses of 1 µg IGF-I/animal/day for 6 days stimulated brush border enzymes in rat pups (Burton *et al*., 1994). When the Veterinary Products Committee (VPC) assessed the safety of use of BST in cows, it could not exclude the possibility that IGF-I in ingested milk might reach the cells lining the gut and cause increased risk of colorectal cancer in consumers (VPC, 1999).

70. Dietary IGF-I can only affect other tissues of the body if it is absorbed from the gut. There is evidence that some absorption of active IGF-I might occur in neonate rats. A study of suckling rats (Philipps *et al*., 2000) showed that milk-borne IGF-I can be absorbed intact and may exert effects on the liver and other tissues. Oral doses of IGF-I given to suckling rats caused increased bodyweight gain and increased the weights of some organs (Philipps *et al*., 1995). The guts of neonates are capable of absorbing intact macromolecules that would not be absorbed by adults and their guts also have relatively low levels of acidity and proteolytic activity that would favour the survival of IGF-I in the gut lumen. There is less evidence that IGF-I is absorbed from the guts of adult animals. The VPC (1999) noted that no study using oral dosing had reliably demonstrated an increase in the IGF-I level in blood and estimated that consumption of milk would add only 0.1% to circulating IGF-I levels even if completely absorbed.

<u>Pharmacokinetics in animals</u>

Absorption:

71. There is some evidence that some dietary components might protect IGF-I from breakdown in the gut lumen and that some IGF-I might be available for absorption (see the section entitled “Digestion and exposure of the gut lining to undigested IGF-I”).

72. Philipps *et al*., (2000) showed that milk-borne IGF-I could be absorbed by suckling Sprague-Dawley rats. A dose level of 7 ng/rat of ^{125}I radio-labelled rhIGF-I was administered by oral gavage in rat milk to a total of 22 ten-to-twelve-day-old rats. Blood was sampled at 5, 10, 20 or 30 mins post-dosing and the blood was analysed for IGF-I by gel chromatography, reversed-phase HPLC and binding to placental membranes bearing IGF receptors. Intact receptor-binding ^{125}I-rhIGF-I was present in the portal blood collected at each time-point. After 5 mins the mean proportions of the administered radiolabel were 23% in the portal blood and 31% in cardiac blood. This result indicated that in young rats there was rapid and extensive absorption of IGF-I from the gastrointestinal lumen. The same group (Philipps *et al.,* 1997) had earlier shown that oral doses of IGF-I (a concentration of 500 ng/mL in milk substitute) given to suckling rats, increased serum IGF-I (90 ng/mL in controls *vs*. 220 ng/mL in those given dietary IGF-I: $p<0.05$), serum IGF-binding proteins, bodyweight gain, brain and liver weights and caused increased migration of enterocytes along the

villi of the duodenum and proximal jejunum, giving indirect evidence of the absorption of IGF-I by suckling rats.

73. Burrin *et al*. (1996a) reported that oral doses of des(1-3) human IGF-I were not absorbed in neonatal mice, although intestinal changes were seen. Litters of 7 pups were suckled from birth until 8 days-old, on mothers who were either wild-type (n=7) or transgenic mice (n=6) of a strain (WAP-IGF) that had about 50 µg/mL of des(1-3) human IGF-I in its milk (not detected in the milk from the wild-type dams). At the age of 8 days, protein synthesis was measured in 3 pups from each litter, using a bolus dose of ^{3}H-phenylalanine. Both total (5.9 ± 0.8 *vs*. 3.8 ± 0.9 mg/day) and fractional protein synthesis (72 ± 9 *vs*. 58 ±10 %/day) were increased in the pups suckled on WAP-IGF dams. Villus height was also increased in the intestines of these pups (350 ± 17 *vs*. 314 ± 16 µm) as were intestinal weight (45 ± 4 *vs*. 39±3 g) and protein content (8.3 ± 1.1 *vs*. 6.5 ±0.7 mg/g bw). There were no treatment-related effects on body weight, weights of liver, spleen, and kidneys and liver protein synthesis. IGF-I was not detected in the plasma of any of the pups. These results are consistent with the hypothesis that active IGF-I (or its truncated form in this instance) can reach the lumen of the intestines and that effects on intestinal tissue are the result of exposure from the luminal side rather than due to exposure from the blood following absorption.

74. In newborn piglets, different investigators obtained essentially opposite results for absorption of IGF-I. Xu and Wang (1996) found that 3 µg/kg bw doses of radiolabelled rhIGF-I given to newborn and 3-day-old piglets by stomach tube caused radiolabel to appear in blood sampled one hour later reaching a peak concentration at 2-3 hrs post-dosing. More radiolabel was found in blood from the newborn piglets than in the 3-day-olds. Radiolabel was found in all organs tested, including the gut, pancreas, spleen, liver, kidney, diaphragm, brain and lung, with highest levels in the gut and kidneys. The absorption appeared to be independent of gut closure, as oral fluorescent isothiocyanate dextran (FITC-D70s – a marker for assessment of gut closure), was passed into the urine of newborn piglets but not into urine from 3-day-old piglets. In contrast, Donovan, *et al*. (1997) found that gastric tube doses of approximately 1 µg/piglet (equivalent to 1 µg/kg bw) radiolabelled rhIGF-I were not passed into the bloodstream of newborn piglets. In another study of piglets (Houle *et al.,* 1996), up to 1000 µg/L of IGF-I fed in milk-replacement formula for 14 days caused no significant change in circulating levels of IGF-I, IGF-II or IGF binding proteins.

Distribution:

75. EMEA (2007b) briefly reported a distribution study of male rats given intravenous doses of ^{125}I-labelled rhIGF-I in which the radioactivity was mainly restricted to highly perfused organs and organs of elimination (kidney and liver). Another distribution/excretion study (Kong *et al.,* 1997) was performed in 10- to 12-day-old Sprague-Dawley rats given an intravenous dose of 5-10 ng/animal. Rats were killed at 5, 30 and 120

minutes after injection, and bile was collected for 2 hours, for measurements of tissue IGF-I and radioactivity. In the 2 hours, 2% of the radioactivity had been passed into the urine. Much of the radioactivity remained in the blood stream, largely as intact receptor-reactive IGF-I. The highest levels of radioactivity and IGF-I were found in the kidney, blood, liver and lungs (in that order) with lesser amounts in other organs.

Metabolism:

76. Any dietary IGF-I that might be absorbed would be metabolised in the same way as endogenously produced IGF-I, with the peptide structure being broken down to amino acids that might be incorporated into body proteins or broken down further to produce energy, carbon dioxide, urea and water. EMEA (2007b) noted that metabolism of IGF-I occurs in the liver and kidneys. Homeostatic mechanisms exist to control the levels of IGF-I (see the section entitled “Physiological control of levels of IGF-I”).

Clearance:

77. Excretion of the ultimate products of metabolism would be expected to be by exhaled air (carbon dioxide) and urine (urea). It was noted (EMEA, 2007b) that, although 95-99% of the IGF-I is normally bound to IGF-binding proteins (IGFBP-1 to -6) in blood, with high doses of IGF-I larger proportions of free IGF-I were present. A high degree of protein binding slows down the rate of clearance of IGF-I from the blood, as protein-bound IGF-I is cleared more slowly (0.5 to 48 mL/min/kg) than free IGF-I (168 to 204 mL/min/kg).

<u>Pharmacokinetic studies in humans</u>

78. Nine studies were performed in volunteers (EMEA, 2007b): three using subcutaneous dosing and six using intravenous dosing. It was noted that oral dosing was not used as IGF-I is a peptide that would be expected to be broken down in the gastrointestinal tract. There were no differences between male and female subjects in the pharmacokinetics of IGF-I (EMEA, 2007b).

79. Bioavailability following subcutaneous dosing was approximately 100%, with no significant metabolism occurring at the injection site (EMEA, 2007b).

80. EMEA (2007b) reported that rhIGF-I was extensively (over 80%) bound to proteins in the blood, with much of it occurring as a ternary complex with IGFBP-3 and acid-labile subunit (ALS). Some binding to IGFBP-1, IGFBP-2 and IGFBP-4 also occurs, and may be important for regulation of the activity of IGF-I (see the section entitled “Physiological control of levels of IGF-I”). The amounts of these binding proteins were greatly reduced in patients with primary IGF-I-deficiency (IGFD), resulting in increased clearance of IGF-I in these patients relative to healthy subjects. The

apparent volume of distribution (V_D) of total IGF-I in IGFD patients was 0.26 L/kg. (It was not possible to measure V_D in healthy people as the endogenous level of IGF-I in blood was so high).

81. In healthy subjects, the apparent terminal elimination rate of total IGF-I was 20 to 30 hours and the plasma elimination half-life of free IGF-I was reported as being less than half an hour (EMEA, 2007b). Elsewhere (Thissen *et al*., 1994), a serum elimination half-life of 12-15 hours was reported. The difference in results may have been due to the elimination of bound IGF-I being much slower than that of free IGF-I. The rate of clearance of total IGF-I was inversely proportional to the amount of IGFBP-3 in the blood and systemic clearance (CL/F) was 0.04 L/hour/kg at an IGFBP-3 level of 3 µg/mL (EMEA, 2007b).

82. IGF-I has been detected in human milk, saliva, digestive juices and bile (see Table 1), which represent minor routes for elimination of IGF-I.

Single dose toxicity

83. Single dose toxicity studies of rhIGF-I were performed in CD-1 mice, Sprague-Dawley rats, New Zealand White rabbits, Beagle dogs and Rhesus monkeys using subcutaneous and intravenous routes (EMEA, 2007b). The primary findings from these studies were the anticipated reductions in serum glucose and clinical manifestations associated with hypoglycaemia.

Repeat dose toxicity studies

84. Repeat dose studies of duration of up to 26 weeks were performed in Sprague-Dawley rats and Beagle dogs (EMEA, 2007b).

Rats

85. For subcutaneous administration, a NOAEL of 1.0 mg/kg bw/day was identified in a 26-week study. The rats dosed with 4.0 mg/kg bw/day of rhIGF-I showed histopathological changes to the thymus (increased cortical area in males), decreases in serum glucose and beta-globulin (males only), and urinalysis changes.

86. With intravenous dosing, no histopathological effects were seen at doses of up to 4.0 mg/kg bw/day for up to 13 weeks, although various changes were seen in the serum biochemistry and urinalysis findings at this dose and to a lesser degree at 1.0 and 0.25 mg/kg bw/day.

Dogs

87. For subcutaneous administration, a NOAEL of 0.15 mg/kg bw/day was identified in a 26-week study, with increased organ weights (adrenal, thymus, heart, salivary glands) and adrenal medullary hyperplasia and fibrosis being caused at doses of 0.3 mg/kg bw/day or greater. Serum glucose was reduced at all dose levels (the lowest dose being 0.1 mg/kg bw/day) and 1.0 mg/kg bw/day caused hypoglycaemic convulsions. Full recovery was evident from doses of 0.3 mg/kg bw/day or less.

88. Intravenous doses of 0.025 to 0.5 mg/kg bw/day were given for up to 13 weeks. Increased mortality due to hypoglycaemia was seen at 0.5 mg/kg bw/day, but not at 0.125 mg/kg bw/day or less. Reduced concentrations of glucose and changes to levels of electrolytes were seen in serum and increased adrenal weights were seen at all dose levels.

Genotoxicity

89. No evidence of genotoxicity of rhIGF-I was seen in an *in vitro* cytogenetics assay in Chinese lung fibroblasts and in an *in vivo* mouse micronucleus test utilising intravenous dosing (EMEA, 2007b).

Carcinogenicity

90. In a 2-year carcinogenicity study of rhIGF-I (EMEA, 2007b), Sprague-Dawley rats were given daily subcutaneous doses of 0, 0.25, 1.0, 4.0 or 10.0 mg/kg bw. Mortality was increased at 1.0 mg/kg bw/day or greater, but it was decreased in females given 0.25 mg/kg bw/day. Benign proliferative lesions of the adrenal medulla (hyperplasia and/or benign phaeochromocytoma) were each increased in males and females given 1.0 mg/kg bw/day or greater, and combined lesions of the adrenal medulla were elevated in females only at 0.25 mg/kg bw/day. Benign epithelial neoplasms of the skin (primary keratoacanthoma and squamous cell papilloma) were increased in males given 4.0 mg/kg bw/day or more, and a marginal increase in benign skin neoplasms was seen in females given 10.0 mg/kg bw/day. The incidences of palpable masses (primarily in the mammary gland region) were increased in males and females at doses of 4.0 mg/kg bw/day or greater in weeks 84-105. The overall incidence, multiplicity and type of mammary gland neoplasms (fibroadenoma, carcinoma and galactocoeles) were similar across all female dose groups, but when adjusted for survival statistically significant increases in the incidences of mammary fibroadenomas (4.0 and 10.0 mg/kg bw/day) and carcinomas (10.0 mg/kg bw/day) were observed. In males, there was a marginal increase in mammary gland carcinomas at 10.0 mg/kg bw/day. It was noted (EMEA, 2007b) that the increased incidences of tumours that commonly occur spontaneously in Sprague-Dawley rats could be caused by direct mitogenic or anti-apoptotic effects of IGF-I and/or indirect contributing factors including effects on calcium metabolism, blood

glucose, body weight, and food consumption. There was uncertainty about the clinical relevance of the benign adrenal medullary phaeochromocytomas given the marked difference between background tumour patterns in rats and humans, and it was suggested that altered calcium metabolism, repeated hypoglycaemic episodes and increased food intake and body weight could act to increase the incidence of these lesions. An NOEL of 1.0 mg/kg bw/day was identified for the increased incidences of benign skin neoplasms and benign mammary neoplasms. Malignant tumours (mammary carcinomas) were seen only at the highest dose level (NOEL = 4 mg/kg bw/day).

Reproduction toxicity

91. In a “multi-week” rat repeat dose rat fertility/developmental study (EMEA, 2007b), male and female rats were given intravenous doses of 0, 0.25, 1.0 or 4.0 mg/kg bw/day of rhIGF-I. There were no adverse effects on reproductive performance or on the fetal development.

92. In a developmental study (EMEA, 2007b), rhIGF-I was administered intravenously at daily doses of 0, 1.0, 4.0 or 16.0 mg/kg bw to pregnant rats on days 7 to 17 of gestation. No test article related effects on fetuses or pups were observed.

93. In a rabbit developmental study (EMEA, 2007b), rhIGF-I was administered as daily intravenous doses of 0, 0.125, 0.5 or 2.0 mg/kg bw on days 6 to 18 of gestation. At 2 mg/kg bw, there was a statistically significant increase in the number of dead or resorbed fetuses per implantation. No other significant adverse effects were seen. The NOAEL for this study was 0.5 mg/kg bw/day.

Special studies of trophic effects on the gut

Parenteral studies

94. Parenteral doses of IGF-I given to rodents have been shown to stimulate intestinal growth, increase crypt cell proliferation, increase intestinal protein synthesis (Lo *et al*., 1996) and are associated with increased mucosal thickness and intestinal length (Burrin, 1997). Steeb *et al*. (1994) dosed female Hooded Wistar rats (~90g bodyweight) with infusions of 0, 44, 111 or 278 µg/rat/day of IGF-I or long R^3 IGF-I (an analogue of IGF-I with arginine replacing glutamate at position 3 and with a NH_2-terminal extension) delivered by an implanted osmotic mini-pump. After 14 days of treatment, both peptides had caused a dose-dependent increase in bodyweight and gastrointestinal tissue weight and increases in crypt depth and villus height, with the effects being most pronounced with long R^3 IGF-I. Infusions of 2.5 mg/kg bw/day of long R^3 IGF-I given to young female Hooded Wistar rats for 3 days increased bodyweight and wet tissue weight of small and large intestines (+20%), but IGF-I had no

significant effect on these parameters although it caused an increased tritiated thymidine labelling index in the jejunum and ileum (Steeb *et al*., 1995). In a further experiment (Steeb *et al.,* 1997), infusions of up to 12.5 mg/kg bw/day of IGF-I caused increased gut weight and length and increased its mucosal thickness in weanling rats. Lo *et al*. (1996) showed that 800 µg/rat/day of IGF-I increased protein synthesis in the jejunal mucosa and muscularis in Sprague Dawley rats (~230 g bodyweight) on total parenteral nutrition (with the IGF-I being administered through the TPN catheter). Infusion of a 10 nM (7.649 mg/mL) solution of rhIGF-I into the ileal lumen of weaned rats caused increased mucosal growth as characterised by a doubling of mass and a 300% increase in mucosal activity of the growth-related enzyme ornithine decarboxylase (Olanrewaju *et al*., 1992).

Oral studies

95. Burrin *et al*. (1996a) demonstrated the intestinal trophic effects of orally administered IGF-I in suckling mice using a transgenic mouse model, which exhibited targeted over-expression of des(1-3) human IGF-I in the mammary gland. Large doses of des(1-3) human IGF-I were administered to nursing females, resulting in concentrations of about 50 µg/mL in their milk (about 1000 times the normal levels of IGF-I). At 8 days of age, increases in the weight of the small intestine, protein synthesis and villus height were found in pups suckling from the treated mice. The weights of liver, spleen and kidney and liver protein synthesis were unaffected by treatment. The authors concluded that milk-borne IGF-I increases intestinal protein accretion in neonatal mice via stimulation of protein synthesis. They further concluded that milk-borne des(1-3) IGF-I is neither absorbed from the intestines of neonatal mice nor does it stimulate peripheral organ growth in neonatal mice that had been given rat whey acid protein promoter to stimulate intestinal growth.

96. Burrin *et al*. (1996b) found that an oral dose of 3.5 mg/kg bw/day of rhIGF-I given to neonatal piglets caused a small increase in intestinal mucosal growth and was associated with increased mucosal thickness secondary to a lengthening of the villi rather than an increase in crypt depth. Xu *et al.* (1994) reported crypt cell proliferation, without any effect on mucosal mass or intestinal length, in newborn piglets fed for 24 h with milk containing 2 µg/mL rhIGF-I (a level intended to be similar to that in porcine colostrum) as compared with controls fed un-supplemented milk. When 750 ng/mL of rhIGF-I was fed to calves in milk replacer (a food given to calves in place of maternal milk), it caused intestinal growth and increased the IGF-I binding capacity of the intestine (Baumrucker *et al*., 1994). Houle *et al*. (1996) found that oral administration of 0.1 to 1 mg/L of IGF-I to neonatal piglets for 14 days increased activities of disaccharidases in the jejunum and decreased peptidase activity.

Special studies in transgenic mice

97. Transgenic hepatic IGF-I gene deletion mice have been created that show a 75% reduction in circulating IGF-I levels. “Colon 38” cells (from an adenocarcinomas created in C57BL/6 mice) were orthotopically transplanted into the caecum of groups of 82 transgenic and 74 non-transgenic mice. Approximately half of each group were given a twice-daily intraperitoneal injection of 2 mg/kg bw of rhIGF-I for six weeks while the remainder received injections of saline. The rhIGF-I injections increased serum levels of IGF-I and IGFBP-3 in the transgenic and the non-transgenic animals. Treatment of the transgenic and non-transgenic mice with rhIGF-I led to significantly increased rates of tumour development and metastatis to the liver. There were significantly lower numbers of caecal tumours and of hepatic metastases in the transgenic mice as compared with the non-transgenic mice. The intensity of histochemical staining for vascular endothelial growth factor[3] in the caecal tumours and the degree of vascularisation of these tumours reflected the level of serum IGF-I (Wu *et al.,* 2002).

Human clinical studies

98. High circulating levels of IGF-I are associated with skeletal growth. Elevated serum levels were associated with bone growth in adolescents (Perdue, 1984). High serum levels of IGF-I are found in acromegaly patients (Perdue, 1984; Juul *et al*., 1994a).

99. EMEA (2007b) reported that an investigation of effects of rhIGF-I on carbohydrate metabolism and cardiovascular function had been incorporated into one of the pharmacokinetic studies that had been performed on healthy volunteers. The results of this pharmacokinetics study showed that single subcutaneous doses of 0.1 to 0.12 mg/kg bw did not increase appetite and did not produce systemic cardiovascular effects. The rhIGF-I decreased blood glucose levels with a greater effect when 0.01mg/kg bw or more was given as a bolus intravenous dose than when identical dose levels were given subcutaneously. It was noted that serum IGFBP-3 concentrations increased following rhIGF-I administration. In IGF-I deficiency (IGFD) patients, a single dose of rhIGF-I caused an initial reduction in serum growth hormone followed by an increase, but in healthy subjects there was no effect on growth hormone levels.

100. EMEA (2007b) reported on clinical studies that were conducted to evaluate the long-term efficacy and safety of mecasermin (rhIGF-I) in children with severe primary IGF-I deficiency (IGFD). A total of 121 patients were given twice-daily subcutaneous rhIGF-I doses of 60 to 120 µg/kg bw for several years. It was shown that a minimum twice-daily dose of at least 80 µg/kg bw was needed to effectively treat primary IGFD. In

[3] The article refers to vascular epithelial growth factor (VEGF), but it is presumed that this was a mistake and that they really meant vascular **endothelial** growth factor (VEGF)

the most extensive of the clinical studies (open-label, multi-centre study design), 76 patients had been given 80 to 120 µg/kg bw for a mean duration of 3.9 ± 3.2 years (a median of 3 years), representing 321 patient-years of exposure. 49% of subjects had one or more episodes of hypoglycaemia during their treatment. Snoring was reported in 22% of subjects and tonsillar hypertrophy with upper airway congestion in 17%, mainly in the first 2 years of treatment. Lipohypertrophy was seen at the injection site in 32% of patients. Other adverse effects reported in this study included headache (28%) and middle ear infusions (13%). Electrocardiograms showed no effect on heart size, but one patient had tricuspid insufficiency that the investigator considered to be possibly related to the treatment. Ultrasound showed normal spleen sizes but increased renal length (more than two times the normal average) was seen in two subjects (2.6%). Facial appearance, cephalometric measurements and mandibular cephalometric x-rays showed no evidence of acromegaloid growth of facial bones or other abnormalities of facial appearance. Routine haematology evaluations were available for 23 subjects, showing that about a third of the subjects had anaemia (low haematocrit and haemoglobin) at the start of the study, but the anaemia was resolved in most subjects during the course of treatment. Blood biochemistry data were available for 37 subjects, showing elevated ALT and AST (3 to 5 times normal) in two subjects (5.4%) on two consecutive visits, subsequently returning to normal later. Among the 76 patients in the study, there was one adverse event report coded to “neoplasia” in the MedDRA system, which represented a report of a new wart.

101. The US Food and Drugs Administration (FDA, 2010) reported on five studies: Study 1200, Study 1202, Mayo Clinic Study, Cephalon Treatment IND[4] and the Kyowa Hakko Study. The studies were presented to FDA in support of an application for authorisation of use of rhIGF-I to treat amyltrophic lateral sclerosis (ALS or Lou Gehrig’s disease). The FDA did not authorise this use of IGF-I.

- Study 1200 (Lai *et al*., 1997) was a multi-centre double-blind clinical trial performed in the United States and Canada in which 266 patients with ALS were randomly assigned to groups that received rhIGF-I at dosages of 0 (placebo), 0.05 or 0.1 mg/kg bw/day by twice-daily subcutaneous injections for up to 9 months. The health scores (Appel ALS scale) for the patients given the highest dose were improved over the placebo group with a 26% slower progression of functional impairment, but there was no significant effect at the low dose level. The mortalities in the placebo, low dose and high dose groups were, respectively, 8%, 12% and 9%.

- Study 1202 (Borasio *et al*., 1998) was a multi-centre clinical trial performed in Europe in which ALS patients were given either 0.1 mg/kg bw/day of rhIGF-I (124 patients) or a placebo (59 patients) as twice-

[4] A treatment IND is a treatment protocol that is added to an existing investigational new drug application (IND), which allows physicians to treat qualifying patients according to the protocol, and which provides additional data on the drug's safety and effectiveness.

daily subcutaneous injections for up to 9 months. The trial was double-blinded. No significant difference was seen between the health scores (Appel ALS scale) of the two groups. The mortalities in the placebo and rhIGF-I groups were 8% and 15%, respectively.

- The Mayo Clinic Study (Sorenson *et al*., 2008) was a multi-centre clinical trial (randomised double-blind) performed at 20 clinics in the United States. A total of 330 ALS patients were randomly allocated to groups given either 0.1 mg/kg bw/day of rhIGF-I or a placebo as twice-daily subcutaneous injections (0.5 mg/kg bw per injection) for 2 years. The primary outcome measurement was the rate of change in the manual muscle testing score (MMT). There were no significant differences in MMT scores, ALS functional rating or mortality between the two groups.

- The Cephalon Treatment IND (unpublished report submitted to FDA, 14th August 1998) consisted of a trial in ALS patients aged 18 years or over. As there was insufficient rhIGF-I available to treat all eligible patients, the use of the drug in patients was decided by a lottery. Effects of treatment with twice-daily subcutaneous injections of 0.1 mg/kg bw/day of rhIGF-I (183 patients) were compared with observations of those who did not receive rhIGF-I (1533 patients). The survival rates of patients receiving rhIGF-I (mortality = 41/183 = 22.4%) were lower than in those who were not treated (mortality = 262/1533 = 17.1%), but the difference was not statistically significant (p = 0.14).

- The Kyowa Hakko Study (unpublished report submitted to FDA) was a randomised placebo-controlled, parallel group, clinical trial in patients with ALS (Appel ALS scores of 40-80 at screening) that was performed in Japan. Patients received twice daily subcutaneous injections of either rhIGF-I (to give a dose of 0.1 mg/kg bw/day) or a placebo. The duration of the study was intended to be 9 months, but the trial was stopped when interim mortality data showed an increased mortality in rhIGF-I-treated patients. The mortality in rhIGF-I-treated patients was 47/78 (60.3%), as compared with 36/71 (50.7%) in the placebo group. The difference in mortality was not statistically significant for the untreated data (p = 0.14), but was significant when the data were adjusted for baseline covariates (p = 0.03).

102. Corpeleijn *et al.* (2008) performed a prospective, double-blind, randomised controlled study of sixty premature babies of birth weight 750 to 1250 g. Babies with congenital abnormalities, congenital metabolic disorders or abnormal liver and/or kidney function were excluded from the study. During the first 28 days following admission to a neonatal intensive care unit, the babies were fed on either standard infant formula or the same formula supplemented with 100 ng/ml of IGF-I. There were no statistically significant differences in serum concentrations of IGF-I, of the IGF-binding proteins IGFBP-1 and IGFBP-3, or of growth hormone (somatotropin) between the groups at any point during the study. The only treatment-related significant effect seen in the study was a decrease in gut

permeability in the IGF-I treated babies, as indicated by a mean lactulose/mannitol excretion ratio of 0.06 in the IGF-I group as compared to 0.12 in the placebo group (p = 0.022). The measured parameters that were unaffected by treatment included growth rate (measured by knee-heel length, calf circumference, skin-fold thickness and bodyweight), risk of mortality (indicated by Clinical Risk Index for Babies), feeding tolerance, days to regain birth weight, days to full enteral feeding, proportion of enteral intake of total intake and gut maturation (as measured by lactulose/lactose ratio).

103. EMEA (2007b) noted that there were no reports of any neoplasms (other than the wart mentioned above) developing in any of the subjects in any of the human studies of rhIGF-I, which involved a total of 1516 subjects enrolled in sponsored Increlex clinical trials for indications including primary IGFD, type 1 and type 2 diabetes mellitus, HIV cachexia or as healthy individuals in pharmacokinetic studies. In addition, clinical studies conducted by manufacturers of rhIGF-I products other than Increlex reported no neoplasms developing in 57 European, Ecuadorian and Middle Eastern children treated with rhIGF-I for severe primary IGFD.

Overview and conclusions from safety studies

104. IGF-I is normally rapidly digested in the stomach and small intestines. However some components of the diet (eg. casein) appear to confer some protection from digestion, so some IGF-I might pass through the gut without being broken down. Concentrations of IGF-I in the gut lumen are likely to be lower than the levels in the blood, so passive absorption of IGF-I is not anticipated. In neonatal animals, IGF-I is less readily broken down in the gut. There is limited and inconsistent data to suggest that absorption of IGF-I might occur in young individuals. Neonates are likely to have more systemic exposure to dietary IGF-I, through consumption of maternal milk and to have a greater exposure of the luminal side of the gut to IGF-I than is the case in older individuals. The high concentration of IGF-I in colostrum will provide neonates with a high dietary intake of IGF-I. It is feasible that this high exposure and bioavailability of IGF-I in neonates is related to a normal physiological role of IGF-I in the growth and development of the newborn. Exposures in humans will be variable, since neonatal humans are exposed to IGF-I only in human milk. Formula does not contain IGF-I and as weaning does not occur until 4-6 months of age, when the gut is more mature; some infants would not be exposed to exogenous IGF-I until 4-6 months of age. Current recommendations are that cows' milk is not introduced until 6 months of age.

105. Distribution was to all parts of the body, with well-perfused organs (kidney, liver and lungs) having highest levels. Much of the IGF-I remained in the bloodstream bound to IGFBPs. It is expected that metabolism would be by breakdown to amino acids, which would then be either used to build body proteins or metabolised further by normal processes to produce energy and normal products for excretion (carbon dioxide, urea, water).

Excretion of the ultimate products of metabolism was expected to be as exhaled carbon dioxide and in the urine. Excretion/secretion of intact IGF-I in milk, saliva, digestive juices and bile also occurs. Free IGF-I is rapidly removed from plasma (elimination half-life < half-an-hour), but protein-binding can considerably slow down the elimination.

106. Special studies of the trophic effects of IGF-I and related substances on gut tissues showed that oral or parenteral doses could cause growth of the intestines, typically characterised by increases in intestinal weight, intestinal length, mucosal mass, protein synthesis and villus length. A concentration of 750 ng/mL in milk replacer (a food given to calves in place of milk) was the lowest oral dose reported to cause intestinal growth, but a level without effect was not detected.

107. Toxicological studies of rhIGF-I used parenteral dosing; no oral toxicity studies were performed. The lowest dose level reported to cause adverse effects in any of the studies was 0.025 mg/kg bw/day as an intravenous dose (the lowest dose tested in the study), which caused hypoglycaemia in dogs in a 13-week study. rhIGF-I was not genotoxic in an *in vitro* cytogenetics assay and in an *in vivo* micronucleus test. A carcinogenicity bioassay of subcutaneously administered rhIGF-I showed that rats developed malignant mammary tumours (4 mg/kg bw/day), benign mammary tumours (NOEL = 1 mg/kg bw/day), benign proliferative lesions of the adrenal medulla (at all doses: NOEL<0.25 mg/kg bw/day) and benign skin tumours (NOEL = 1 mg/kg bw/day). A special study of implants of cancer cells into the caeca of mice showed lower numbers of caecal tumours and hepatic metastases in transgenic mice with impaired hepatic production of IGF-I than in normal mice or transgenic mice that had injections of IGF-I. No developmental toxicity was seen when pregnant rats were given up to 16 mg/kg bw/day intravenously, but there was evidence of fetotoxicity in rabbits given 2 mg/kg bw/day intravenously (NOAEL = 0.5 mg/kg bw/day).

108. Several clinical studies of rhIGF-I have been performed in humans as part of its development as a medicinal product. Single subcutaneous or intravenous doses of 0.01 mg/kg bw caused reduced serum glucose and increased serum IGFBP-3. Twice-daily subcutaneous doses of 60 to 120 µg/kg bw given for several years caused decreased serum levels of glucose, ALT and AST, reduced packed cell volume (pcv) and haemoglobin (Hb), but had no effect on electrocardiogram (ECG) measurements. In premature babies, formula supplemented with 100 ng/mL of IGF-I had no effect on serum levels of IGF-I IGFBP-1, IGFBP-3 or growth hormone, but there was decreased gut permeability as compared with controls. There was no evidence from the clinical studies to suggest that treatment with rhIGF-I caused any cancer in treated patients.

109. In conclusion, the results of studies of the safety of rhIGF-I indicate that parenteral doses can be carcinogenic causing malignant mammary

tumours in rats, although rhIGF-I does not appear to be genotoxic. It remains unclear whether dietary doses of IGF-I would be carcinogenic. It is also unclear whether absorption of intact IGF-I from the gut lumen can occur, although there is limited evidence that this might occur in neonates. There is also some evidence that some dietary IGF-I might be protected from digestion and might come into contact with the luminal surface of the gastrointestinal tract. However, it is considered likely that normal processes of digestion in the gut lumen and homeostasis of levels in the bloodstream would ensure that tissues are not exposed to elevated levels of diet-derived IGF-I for a sufficiently long time to increase the risk of cancer.

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COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET

Annex 3

Association between blood level of IGF-I and breast cancer risk

Secretariat
March 2012

Association between blood level of IGF-I and breast cancer risk

Clinical observations

Receptor status

1. Breast cancer specimens have been reported to show a positive correlation between ER status and IGF receptor status and a negative correlation between ER status and IGFBP-3 expression (reviewed Stoll., 1997). Ectopic growth hormone expression has been demonstrated in a majority of specimens of normal and malignant tissue, which could contribute to breast cancer risk by increasing the local level of bioavailable IGF-I.

Genetic polymorphisms

2. In a systematic review, Fletcher *et al*., (2005) reported that a single sequence length polymorphism 1kb 5' to IGF-I was examined in relation to circulating levels of IGF-I (12 studies) or breast cancer risk (4 studies); but there was no evidence of any effect. For an A/C polymorphism 5' to IGFBP-3, the three available studies were consistent for a modest effect on circulating IGF-I levels but there was no evidence for a direct effect on breast cancer risk in the single study available.

3. Patel *et al*., (2008) examined the association between common genetic variations in the IGF-I, IGFBP-I and IGFBP-3 genes in relation to circulating IGF-I and IGFBP-3 levels and breast cancer risk within the NCI Breast and Prostate Cancer Consortium (BPC3). This included 6912 breast cancer cases and 8891 matched controls comprised primarily of Caucasian women drawn from 6 large cohorts (the American Cancer Society Cancer Prevention Study-II, EPIC, Nurses' Health Study Harvard Women's Health Study, the Hawaii-Los Angeles Multiethnic Cohort Study and the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial Cohort). Thirty haplotype tagging single nucleotide polymorphisms (SNPs) were selected to provide high coverage of common haplotypes in the regions surrounding IGF-I and its binding proteins IGFBP-I and IGFBP-3. Specific IGF-I SNPs individually accounted for up to a 5% change in circulating IGF-I levels and individual IGFBP-3 SNPs were associated with up to 12% change in circulating IGFBP-3 levels but no associations were observed between these polymorphisms and breast cancer.

4. Known polymorphic variants in *IGF-I*, *AIB1* and *CYP3A4* genes were analysed by Jernström *et al*., (2001) (see paragraph 16 for study details) . Among oral contraceptive users, the IGF-I level was positively associated with the absence of the *IGF-I* 19-repeat allele, the presence of the *CYP3A4* variant allele and with the presence of the *AIB1* 26-repeat allele. After adjusting for the genotypes, ethnic group was no longer a significant predictor of the IGF-I level. The authors suggested that the high incidence of pre-menopausal breast cancer among black women could be mediated through genetic modifiers of circulating levels of IGF-I.

5. In a further case control study from the NHS II cohort, Su *et al.*, (2010) examined the association between genetic variants and circulating levels of IGF-I and IGFBP-3 with proliferative benign breast disease (BBD) a marker of increased cancer risk. The participants were 359 pathology-confirmed BBD cases and 359 matched controls. Thirty single nucleotide polymorphism (SNPs) in IGF-I, IGFBP-I and IGFBP-3 were selected using a haplotype tagging approach and genotyped in cases and controls. Circulating IGF-1 levels were not associated with proliferative BBD risk. Higher circulating IGFBP-3 levels were significantly associated with proliferative BBD risk (highest vs lowest quartile OR1.70, 95%CI 1.06-2.72) p-trend= 0.03. Certain IGFBP-3 SNPs were associated with lower proliferative BBD risk and others with higher circulating IGFBP-3 levels; suggestive evidence indicated that these could be associated with higher risk of proliferative BBD.

Observational studies

6. The results of the available single studies and meta- analyses are summarised in Table 1 below. The studies are described in the following paragraphs. It should be noted that these follow chronologically, but where additional studies have been done on the same cohort they are grouped together.

Table 1: Summary of results of epidemiology studies of breast cancer risk associated with IGF-I and related substances

Subjects	**Number of subjects**	**Was blood taken prospectively?**	**How was IGF-I measured and was it free?**	**Association between IGF-I levels and breast cancer**	**Main results**	**Reference**
Breast cancer patients treated with tamoxifen	69 patients-	No	Not stated – probably RIA	-	It was noted that tamoxifen treatment caused a reduction in serum IGF-I	Pollak *et al*., 1992
French breast cancer patients, aged 20-80	47 cases; 134 controls	No	RIA on plasma or acid ethanol extract (removes 90% of binding proteins).	Positive	Higher median levels of IGF-I in cases (152 ng/mL) than in controls (115 ng/mL)	Peyrat, *et al*., 1993
Dutch breast cancer patients aged 38-75 y	150 cases; 441 controls	No	RIA	Positive	Elevated IGF-I in pre-menopausal patients but not in post-menopausal patients. RR=7.34 for IGF-I/IGFB-3 ratio, comparing upper and lower quintiles.	Bruning *et al*., 1995
Female nurses (USA) aged 30-55 y	397 cases; 620 controls	Yes	ELISA	Positive	RR=7.28 in pre-menopausal women ≤50y with IGF-I>207ng/mL, compared to those with IGF-I<158ng/mL	Hankinson *et al*., 1998
Female nurses (USA) aged 30-55 y	800 cases; 1129 controls	Yes	ELISA after acid extraction	Positive	RR=2.5 in pre-menopausal women ≤50y with IGF-I>187ng/mL, compared to those with IGF-I<176ng/mL. No association in post-menopausal women.	Schernhammer *et al*., 2005 (update /expansion of Hankinson *et al*., 1998)
Female Nurses aged 25-42y	317 cases; 634 controls	Yes	Commercial ELISA after acid extraction	None	No association between IGF-1, IGFBP-I or IGFBP-3 and breast cancer risk in largely pre-menopausal women.	Schernhammer *et al.,* 2006
Chinese breast cancer patients (age	63 cases; 27 controls	No	RIA after acid extraction	None	No significant difference between IGF-I in cases (149 ng/mL) and controls (174 ng/mL).	Ng *et al*., 1998

Subjects	Number of subjects	Was blood taken prospectively?	How was IGF-I measured and was it free?	Association between IGF-I levels and breast cancer	Main results	Reference
not given)						
US pre-menopausal breast cancer patients (mean age 42.6 y)	99 cases; 99 controls	No	RIA after acid extraction	None	No significant association between IGF-I and cancer, but OR of 2.05 for comparison of highest quintile of IGFBP-3 levels versus the lowest quintile.	Del Giudice *et al*., 1998
US breast cancer patients, aged <40 to 49	76 cases	No	Commercial immunoradio-metric assay	Positive	Increased breast cancer risk in upper two tertiles of IGF-I levels as compared with the lower tertile (OR=2.4 & 1.8).	Bohlke *et al*., 1998
US women mean age 74 (54.6 at recruitment)	45 cases 393 controls	No	RIA stated not to cross react	None	IGF-I 120.22 ng/ml in cases; 126.96 ng/ml in controls. Not significant.	Jernström & Barrett-Connor., 1999
US women mean age 52 (largely African-American or Hispanic)	130 cases 42 controls	No	RIA after acid extraction	Positive	IGF-I 150.9 ng/ml in benign disease cases; 142 ng/ml in matched controls, 128 ng/ml in cancer patients and 126 ng/ml in matched controls. Not significant	Vadgama *et al*., 1999
NZ women undergoing surgery for breast lesions	12 benign 31 malignant + matched controls	No	RIA after acid extraction	None	IGF-I 111.9 ng/ml in cases; 92.1 ng/ml in controls. Significant (p = 0.019)	Holdaway *et al*., 1999
US breast cancer patients, aged <40 to 49	83 cases 69 controls	No	Commercial immunoradio-metric assay	None	IGF-I 161.52 ng/ml in cases and 157.95 ng/ml in controls. Not significant	Mantzoros *et al*., 1999
Breast cancer patients aged	75 cases; 75 controls	No	Commercial immunoradio-	None	No association between IGF-I and breast cancer in pre- or post-	Petridou *et al*., 2000

Subjects	Number of subjects	Was blood taken prospectively?	How was IGF-I measured and was it free?	Association between IGF-I levels and breast cancer	Main results	Reference
<45 to >75 y			metric assay of serum samples		menopausal women	
American women aged 35-65 y	115 cases; 486 controls	Yes	RIA after acid extraction	Positive	Adjusted RR=2.3 in women ≤50y with IGF-I>265ng/mL, compared to those with IGF-I<168ng/mL	Toniolo *et al*., 2000
American women "pre-menopausal"	138 cases; 259 controls	Yes	In house RIA or 2 commercial ELISAs after acid extraction	Positive	Variable ORs depending on assay and adjustments used. Increased risk in women with elevated IGF-I and IGFBP-3 levels.	Extension of above study. Rinaldi *et al*., 2005a
US, Swedish and Italian women pre-menopausal aged 35-47	220 cases; 434 controls	Yes	Commercial ELISA on plasma or serum	Not significant but authors consider Positive	Mean levels 01.5 and 93.6 ng/ml in cases and controls. OR 1.41 for highest vs lowest quintile, lower if adjusted.	Rinaldi *et al*., 2005b - re-analysis of Toniolo *et al*., 2000, Kaaks *et al*., 2002, Muti *et al* 2002.
Black & white American women aged 31-67 y	40 cases	No		Positive	Adjusted OR=2.00 for women with greater than the median level of IGF-I (OR=6.31 for free IGF-I)	LI *et al*., 2001
Italian women aged 39-69 y	133 cases; 532 controls	Yes	Commercial immunoradio-metric assay of free and total IGF-I	Positive	Adjusted RR=3.12, comparing upper & lower quartiles of free IGF-I	Muti *et al*., 2002
Chinese breast cancer patients, aged 48.5±8.3 y	300 cases; 300 controls	No	Commercial ELISA	Positive	Plasma IGF-I was higher in cases (143 ng/mL) than in controls (127 ng/mL)	Yu *et al*., 2002
American breast cancer patients, aged	126 cases; 126 controls	Yes	Commercial immunoradio-metric assay	Positive	Elevated IGF-I and IGFBP-3 were associated with raised risk of breast cancer in pre-menopausal women.	Krajcik *et al*., 2002

Subjects	Number of subjects	Was blood taken prospectively?	How was IGF-I measured and was it free?	Association between IGF-I levels and breast cancer	Main results	Reference
19-73 y			after acid extraction		Elevated IGFBP-2 was associated with reduced breast cancer risk in post-menopausal women.	
Dutch women aged 29-73	513 cases; 987 controls	Yes	Commercial immunoradio-metric assay after acid extraction	None	Small association between IGF-1 and breast cancer risk in post-menopausal women (OR s 1.73 to 1.9) in 1 of 3 cohorts only; reduced when adjusted for hormone use. No association in pre-menopausal women.	Kaaks *et al.,* 2002
Swedish women aged 20-69 at enrolment. Mean age 57 in study.	149 cases; 333 controls	Yes	Commercial immunoradio-metric assay after acid extraction	None	No association between IGF-1, IGFBP-1,-2,-3 or IGF-I/IGFBP-3 ratio and breast cancer risk in post-menopausal women.	Keinan-Boker *et al.,* 2003
Danish women aged 50-64	412 cases; 397 controls	Yes	Non-competitive time-resolved immunofluoro-metric assay (DELFIA) after acid extraction.	None	No association between IGF-I and risk but there was an association between IGF-II and IGFBP-3 and breast cancer risk in post-menopausal women with ER positive tumours.	Grønbæk *et al.,* 2004
Meta analysis	-	-	-	*Positive*	*High levels of IGF-I & IGFBP-3 were associated with increased risk of pre-menopausal breast cancer eg (OR 1.96), but not of post-menopausal breast cancer (OR 0.97) other analyses performed.*	*Renehan et al., 2004*
Meta analysis	-	-	-	*Positive*	*IGF-I levels higher for risk in post-menopausal women only (OR 1.39).*	*Shi et al., 2004*
Meta analysis	-	-	-	*Marginally positive*	*Higher levels of IGF-I but not IGFBP-3 group were associated with increased risk of pre-menopausal breast cancer*	*Sugumar et al., 2004*

Subjects	Number of subjects	Was blood taken prospectively?	How was IGF-I measured and was it free?	Association between IGF-I levels and breast cancer	Main results	Reference
					OR 1.74.	
Guernsey women, ≥35 at recruitment, mean age 57 at diagnosis	117cases; 350 controls	Yes	Commercial ELISA	None	Non-significant association for IGF-I adjusted for IGFBP-3 in pre-menopausal women. Association between IGFBP-3 adjusted for IGF-1 and breast cancer risk in premenopausal women. No associations in post-menopausal women	Allen *et al.,* 2005
Swedish women 19-43y	212 cases; 369 controls	Yes	Commercial immunoradio-metric assay	Positive	Association between IGF-I and increased risk (OR 1.7) but not with IGF-II.	Lukanova *et al*., 2006
Swedish women 19-43y	244 cases; 453 controls	Yes	Commercial chemiluminescence based immunoassay	Positive	Association between IGF-I and increased risk (OR 1.73; 95% CI 1.14-2.63).	Chen *et al*., 2010. Same cohort as above.
Swedish women 22-37y	719 cases; 1434 controls	Yes	Commercial chemiluminescence based immunoassay	None	No association between IGF-I and increased risk (OR 1.08; 95% CI 0.80-1.47).	Toriola *et al*., 2011. Same cohort as above.
European women aged 50 or more at diagnosis	243 cases; 243 controls	Yes	ELISA	Positive	No overall association, but an association between IGF-1 adjusted for IGFBP-3 and post-menopausal breast cancer risk in the youngest premenopausal women and oldest post-menopausal women.	Rollison *et al.,* 2006
European women aged 35-69	1081 cases; 2098 controls	Yes	Commercial ELISA after acid extraction	Positive	Association between IGF-1 and IGFBP-3 and breast cancer risk in post-menopausal women (OR 1.38), but no association in pre-menopausal women.	Rinaldi *et al.,* 2006
Australian women aged 27-75y at baseline	423 cases; 1901 controls	Yes	Commercial ELISA	Positive	No overall association but IGF-1 and IGFBP-3 associated with increased breast cancer risk in older women (HR 1.61 for women aged >60).	Baglietto *et al.,* 2007

Subjects	Number of subjects	Was blood taken prospectively?	How was IGF-I measured and was it free?	Association between IGF-I levels and breast cancer	Main results	Reference
Norwegian women aged 40-42	325 cases; 647 controls	Yes	RIA after acid extraction	Positive	No overall association but modest increase in risk associated in women aged <50	Vatten *et al*., 2008
US women aged 54-74	835 cases; 816 controls	Yes	Commercial ELISA for total and free IGF-I	Positive	Free IGF-1 associated with a modest (but not linear) increase risk in postmenopausal women not using HR. No associations for total IGF-I or IGFBP-3.	Gunter *et al*, 2009.
Taiwanese women aged 24-72	297 cases; 593 controls	No	Commercial immunoradio-metric asay	Positive	High IGF-1 associated with increased risk of pre but not postmenopausal breast cancer (OR 1.86). No association with IGFBP-3.	Wu *et al*, 2009.
US women aged 55-74	389 cases; 470 controls	Yes	Commercial ELISA for total IGF-I	None? (Authors considered positive but not statistically significant)	IGF-1 and IGF-I/IGFBP-3 associated with increased risk of postmenopausal breast cancer (OR 1.28 for IGFI) No association with IGFBP-3.	Schairer *et al*, 2010.
US women aged 25-79	184 cases; 522 controls	No	Commercial IGF-I (IGFBP-3 blocked) RIA	Positive	IGFBP-3 associated with increased risk of breast cancer. No association for IGF-I in Hispanic women, but association in NHW women	Rollison *et al*, 2010.
Meta analysis	*-*	*Yes*	*-*	*Positive*	*IGF-1 weakly positively associated with increased risk in pre-menopausal women and strongly positively associated with increased risk in post-menopausal women. IGF-1 positively associated with increased risk of (oestrogen positive) breast cancer, but not of (oestrogen-negative) breast cancer*	*Key et al., 2010*

7. In a randomised, blinded placebo-controlled clinical trial involving 69 patients Pollak, *et al*. (1992) observed that use of the drug tamoxifen in human breast cancer patients reduced serum concentrations of IGF-I. Tamoxifen treatment (dose not stated) produced a statistically significant reduction in serum IGF-I, with mean levels decreasing from 1.4 u/mL to 0.9 u/mL (*sic*). The authors suggested that the action of tamoxifen against breast cancer was mediated by it blocking oestrogen receptors in the hypothalamic-pituitary axis to interfere with release of growth hormone and subsequently to reduce production of IGF-I by the liver, with the resultant lowering of serum IGF-I levels reducing the mitotic effect of IGF-I on the cancer cells. The results of this study are consistent with the idea that serum IGF-I levels affect the growth of human breast cancers.

8. In a small clinical study (Peyrat *et al*., 1993), statistically significantly ($p<0.001$) higher levels of plasma IGF-I were found in blood from a group of 47 women with breast cancer than in 134 control samples of plasma taken from the local blood transfusion service, employees and patients at an old people's home. The subjects (aged 20-80 years with an average age of 47.8 years) were an unselected sequence of female breast cancer patients undergoing surgery for locoregional disease at a health centre in Lille, France. The control plasma samples were taken from women having a similar range of ages to the cancer patients. The plasma IGF-I was measured by two different ways by methods using radioimmunoassay. Direct measurement of plasma IGF-I gave median concentrations of 152 ng/mL in the cases and 115 ng/mL in controls; measurement in ethanol extracts from plasma showed median concentrations of 26 ng/mL in subjects and 20 ng/mL in controls. The design of the study was such that it did not look into the temporal relationship between plasma IGF-I levels and breast cancer risk.

9. An association between high plasma levels of IGF-I and risk of breast cancer was seen in a case-control study (Bruning *et al*., 1995), but the design of the study was such that it was not possible to look into whether there was a temporal relationship. The cases were 150 women, aged 38 to 75 years, with early-stage breast cancer (Stage I or II), who were consecutively admitted to the Netherlands Cancer Institute for either surgery or post-operative irradiation. Controls were 441 women without breast cancer who were of the same age group. Blood samples were taken on admission and were analysed for IGF-I and IGFBP-3. For pre-menopausal women, serum IGF-I levels were significantly higher in cases than in controls ($p=0.025$), but there was no significant difference in post-menopausal women. There was no association with IGFBP-3 levels in either pre- or post-menopausal women; mean IGF-I and IBFBP-3 levels were not included in the paper. The mean IGF-I/IGFBP-3 ratio was significantly ($p=0.002$) higher in pre-menopausal cases than in pre-menopausal controls (4.06 compared to 3.88), but there was no significant difference ($p>0.05$) in post-menopausal women (3.77 and 3.80 in controls and cases respectively). The authors suggested that the risk of breast cancer was related to the level of free-IGF-I rather than by menopausal

status. The relative risk (RR) values increased with increasing ratio of IGF-I/IGFBP-3, as summarised in Table 2 for data adjusted for age, menopausal status, family history, pre-menopausal BMI, height, waist to hip ratio (WHR), albumin, C-peptide, testosterone and c-reactive protein (CRP).

Table 2: RR per quintile of IGF-I/IGFBP-3 (Bruning *et al*., 1995)

IGF-I/IGFBP-3	**RR**	**95% CI**
<0.3458	1	Referent
0.3458-0.4130	2.02	0.47-8.68
0.4131-0.4808	3.22	0.71-14.57
0.4808-0.5682	5.38	1.25-23.22
>0.5682	7.34	1.67-32.16

10. Hankinson *et al*. (1998) reported a case control study nested within the prospective Nurse's Health Study cohort. The subjects were female American registered nurses aged 30 to 55 years-old. Plasma concentrations of IGF-I and IGFBP-3 were measured by ELISA in blood samples collected in 1989-1990; it was noted that the ELISA was highly correlated with the RIA acid extraction method used by Pollak *et al*., (1990). The invasive breast cancers investigated in the study were diagnosed after the blood collection but before 1st June 1994. The plasma IGF-I results for 397 women<with breast cancer were compared with those for 620 age-matched controls. There was no association between IGF-I concentration and breast cancer risk in the whole study group. In pre-menopausal women aged 50 years or less, the median plasma IGF-I concentrations were significantly (p<0.01) higher in the cases (206 ng/mL) than in the controls (175 ng/mL), but there was no significant difference (p>0.05) in post-menopausal or total pre-menopausal women. In comparisons of tertiles of levels of plasma IGF-I, there was a statistically significant trend (p<0.05) for an increased risk of breast cancer in pre-menopausal women (adjusted for IGFBP-3 concentrations) and premenopausal women of 50 years of age or less (adjusted and unadjusted). There was an increased risk of breast cancer (relative risk (RR; 95% CI) = 2.33; 1.06-5.16 (unadjusted) p_{trend}=0.08 or 2.88; 1.21-6.85 p_{trend} =0.02 (adjusted)) in pre-menopausal women with a high plasma level of IGF-I (>207 ng/mL), but not in post-menopausal women (RR = 0.85; 0.53-1.38 p_{trend} =0.63 (unadjusted); 0.89; 0.51-1.55 p_{trend} =0.99 (adjusted)). The risk was larger in pre-menopausal women aged 50 years or less. The relative risk (RR) in such women with a plasma IGF-I of more than 207 ng/mL was 4.58; 1.75-12.0 p_{trend} =0.02 (unadjusted) or 7.28; 2.40-22.0 p =0.01(adjusted), as compared with the risk in premenopausal women of 50 years or less having IGF-I levels of less than 158 ng/mL.

11. This study was updated by Schernhammer *et al*., (2005). The number of cases was doubled and the associations of IGFBP-3, IGFBP-1 and free IGF-I levels (assayed by ELISA after acid extraction; the IGFBP-3 was

also assayed by an in house RIA which produced highly correlated results) with breast cancer risk reported. In this expanded study, a total of 800 women with invasive or *in situ* breast cancer were identified following blood collection; of these, 27% were pre-menopausal. The cases were age-matched with 1129 controls. Among postmenopausal women, neither IGF-I, IGFBP-3, IGFBP-1 nor free IGF was associated with breast cancer risk. Among pre-menopausal women, IGFBP-3, IGFBP-1 and free IGF were also not associated with breast cancer risk. However, high plasma IGF-1 was associated with a modestly elevated breast cancer risk (lowest vs highest tertile RR,1.6; 95%CI,1.0-2.5 p_{trend} =0.07) among the pre-menopausal women, with a stronger association among pre-menopausal women aged 50 or less (RR,2.5; 95%CI,1.4-4.5 p_{trend} =0.01); further adjustment for IGFBP-3 did not greatly change these estimates.

12. Schernhammer *et al*., (2006) reported a further case-control study nested within the Nurses' Health Study II (NHSII) in which plasma concentrations of IGF-I, IGFBP-3, IGFBP-I and growth hormone were measured in samples collected between 1996 and 1999. A total of 317 women were identified who were diagnosed with invasive or *in situ* breast cancer between sample collection and June 1, 2003. 75% of the women were pre-menopausal at the time of sample collection. Each woman was age matched to 2 controls. IGF-I was measured by ELISA after acid extraction to release the binding proteins. Overall plasma IGF-I, IGFBP-I, IGFBP-3 and GH levels were not associated with breast cancer risk. The ORs (95%CI) (top vs bottom quartile) were IGF-I, 0.98, (0.69-1.39); IGFBP-I, 0.95 (0.63-1.41); IGFBP-3, 1.10 (0.78-1.54); GH, 1.09, (0.82-1.46). The risks were similar for pre-menopausal women aged 45 years or less. The risk associated with IGF-I was not affected by adjustment for IGFBP-I or IGFBP-3 or vice versa. Adjustment for additional breast cancer risk factors also did not change the risk estimates.

13. Ng *et al*. (1998) collected serum from 63 patients with breast cancer and 27 with benign breast disease (controls) randomly selected from a national breast screening trial. The serum was analysed by radioimmunoassay for IGF-I, IGFBP-1 and IGFBP-3. A sub-set of 66 samples were also analysed by Western ligand blots for various proteins, including IGFBP-2, IGFBP-3 and IGFBP-4. A retrospective case-control study was performed. Mean serum IGF-I levels were similar in cases (149 ng/mL) and controls (174 ng/mL). Levels of IGFBP-1, IGFBP-3 and IGFBP-4 were significantly ($p<0.05$) associated with risk of breast cancer: risk increased with high levels of IGFBP-1 and IGFBP-3, but risk decreased with high levels of IGFBP-4.

14. Del Giudice *et al*. (1998) reported on an American retrospective case-control study that was performed on 99 pre-menopausal women who had been diagnosed with node-negative invasive carcinoma of the breast (cases of mean age 42.6 years) and 99 age-matched controls with non-proliferative breast disease. Blood samples were taken post-operatively at an average of 16 weeks after surgery and were analysed for IGF-I, IGFBP-1, IGFBP-3 and insulin. After adjustment for age and weight,

elevated insulin levels were significantly ($p<0.05$) associated with breast cancer. There were no statistically significant ($p>0.05$) differences between cases and controls for IGF-I and IGFBP-1. A positive association approached significance for IGFBP-3 with the odds ratio (OR) for the highest quintile IGFBP-3 level versus the lowest quintile being 2.05 (95% CI, 0.93-4.53) $p=0.07$.

15. Bohlke, *et al*. (1998) measured IGF-I and IGFBP-3 in plasma from 94 premenopausal women, aged <40 to 49 years, who had ductal carcinoma *in situ* of the breast (identified from the Massachusetts Cancer Registry, 1 January 1993 to 30 August 1997). On average, blood was collected 18.5 months after diagnosis. Plasma from a control group of 76 women, matched for age and area of residence, was also analysed. The results showed an elevated risk of the cancer in women in the upper two tertiles of IGF-I levels (adjusted odds ratios = 2.4 and 1.8), as compared with the lowest tertile. Conversely, there was a decreased risk of the cancer in women in the upper two tertiles of IGFBP-3, as compared with those in the lowest tertile. Furthermore, women in the two highest tertiles of IGF-I and the lowest tertile of IGFBP-3 were at notably higher risk than women in the lowest tertile of IGF-I and highest two tertiles of IGFBP-3. The authors concluded that the combination of high serum levels of IGF-I and low IGFBP-3 may increase the risk of premenstrual ductal carcinoma *in situ* of the breast.

16. A case-control study was conducted in 438 women from the Rancho Bernado cohort (Jernström & Barrett-Connor., 1999). The cohort was initially recruited in 1972-74 and was free of cancer at that time, they were then followed annually. The purpose of the study was to investigate the association between breast cancer, weight, weight change, IGF-I, proinsulin, C-peptide and insulin in post menopausal women. The hormones were measured during 1992-4 with total IGF-I being measured by a specific radioimmunoassay (stated to not cross-react with other IGF components). 45 women aged 53-90 were diagnosed with incident breast cancer between 1972 and 1994; seven of the women had recurrent breast cancer. The control group were 393 women of the same age who did not have breast cancer. IGF-I levels were 120.22 ng/ml (range 25-245 ng/ml) in the cases and 126.96 (25-711 ng/ml) in the controls; this was not significantly different. It was noted that as only the survivors had their IGF-I measured in the 1992-4 follow up, women with more aggressive forms of breast cancer might have died before samples could be taken. By the time the samples were collected, the cohort were elderly and more had died, with breast cancer being listed somewhere on the death certificate, than had survived to participate in the study as cases.

17. Plasma IGF-I levels and serum IGFBP-3 levels were measured in 130 women undergoing treatment for breast cancer (Vadgama *et al*., 1999). The women were aged 31-80 and were largely African-American or Hispanic in origin. 42 hospital staff aged 22-74 served as control subjects. Plasma IGF-I levels (measured by RIA, using acid extraction) were 111.9

ng/ml in the patients and 92.1 ng/ml in the controls; this was statistically significant (p = 0.0119). There was no difference in the IGF-I levels of African-American and Hispanic patients. IGF-I levels were correlated with age in the controls but not in the breast cancer patients. There was no correlation between IGF-I levels and node positivity or negativity, but IGF-I levels did increase with tumour size. IGF-I was not affected by ER status but was higher in progesterone receptor positive patients. Pre-menopausal breast cancer patients had higher IGF-I levels than post-menopausal patients and pre-menopausal patients with recurring breast cancer had higher levels that those without recurrence. Patients given tamoxifen treatment had lower IGF-I levels than those without tamoxifen treatment; other treatment regime did not affect IGF-I levels. Survival times were also longer in patients with lower IGF-I. IGFBP-3 levels were higher stated to be higher in breast cancer patients, but full data are not provided and it is unclear whether the patients were compared against the study controls or the normal range. In the breast cancer patients, IGF-I was correlated with IGFBP-3 and the relationship with factors such as tumour size, receptor status and menopausal status was similar to that of IGF-I. IGFBP-3 was not affected by tamoxifen treatment.

18. Serum IGF-I, IGF-II and IGFBP-3 concentrations were compared in women with benign (n=12) or malignant (n=31) breast disease and their corresponding age-matched controls (Holdaway *et al*., 1999). Mean serum IGFBP-3 was higher in benign breast disease (3.6 ± 0.7 mg/L) than in controls (2.7±0.7 mg/L) or breast cancer patients (2.7 ± 0.5 mg/L) (p = 0.001) Serum IGF-I and IGF-II levels were not significantly different between the groups. IGF-I levels were 150, 142,128 and 126 µg/L in the benign breast disease group and their matched control group, and the cancer group and their matched controls respectively. For IGF-II the levels were 768, 777, 749 and 836 µg/L respectively. The index of free unbound IGF (measured as the molar ratio of IGF-I + IGF-II divided by IGFBP-3) was significantly lower in the women with benign breast disease compared to the other groups being 1.38, 1.8, 1.75 and 1.73 in the benign breast disease group and their matched control group, and the cancer group and their matched controls respectively. The authors proposed that either IGFBP-3 production or clearance was altered in benign breast disease such that less free IGF-I was available, potentially protecting against malignant transformation. IGF-I was measured by RIA following acid extraction, IGFBP-3 by a commercial kit and IGF-II by a RIA. The association between IGF-I, IGF-II and IGFBP-3 concentrations and factors such as histology, tumour size and were also analysed.

19. Mantzaros *et al*., (1999) compared the levels of leptin as well as a range of hormonal variables in 83 women with pre-menopausal breast cancer and 69 healthy controls. The levels of IGF-I measured by a commercial immunoradiometric assay were161.52 ng/ml in the cases and 157.95 ng/ml in the controls. IGFBP-3 levels also measured by a commercial immunoradiometric assay were 3.32 and 3.34 µg/ml respectively. The differences were not statistically significant.

20. Petridou *et al*. (2000) performed a retrospective case-control study. Seventy-five cases of breast cancer (14 pre-menopausal and 61 post-menopausal) diagnosed in mammographic screening in Greece were matched for age and type of residence with 75 controls who screened negative. The youngest patients were aged less than 45 years and the oldest was older than 75 years. Blood samples were taken and analysed for leptin and IGF-I. There was no evidence of an association between IGF-I and either pre- or post-menopausal breast cancer. However, there was a strong association of leptin with breast cancer.

21. Toniolo *et al*. (2000) reported a study that was part of the New York University Women's Health Study, a prospective study of the role of hormones, diet and metabolism in the aetiology of cancer in women. The cohort included 14,275 women, aged 35 to 65 years. Blood samples were taken at the time of recruitment (1985-1991). Cohort members diagnosed with breast cancer six or more months after cohort recruitment and prior to 1995 were included in a case-control study nested within the cohort. The time between blood sampling and diagnosis varied between 7 and 120 months (median = 57 months). Blood samples were analysed for concentrations of IGF-I, IGFBP-3 and C peptide. The results for 115 case subjects were compared with those for 486 control subjects from the cohort, matched for age, menopausal status (pre- or post-menopausal), and day of menstrual cycle at the time of blood sampling. There were no significant (p>0.05) trends in breast cancer prevalence associated with plasma concentrations of IGFBP-3 or C peptide. For cases aged 50 years or less, there was a significant (p<0.05) positive association between RR of breast cancer (adjusted for benign breast disease and family history of breast cancer) and plasma concentration of IGF-I. There was no significant trend with unadjusted RR for these women. The OR; 95%CI for women aged 50 or less in the upper quartile of IGF-I levels (>265.5 ng/mL) as compared with the lower quartile (<180 ng/mL) was 1.91; 0.96-3.83 p = 0.09 (unadjusted) or 2.3; 1.07-4.94 p = 0.03 (adjusted). The OR; 95%CI for the upper quartile (>256 ng/mL) of pre-menopausal cases was 1.52;0.89-2.60 p = 0.14 (unadjusted) or 1.60; 0.91-2.81 p = 0.09 (adjusted) as compared with the lower quartile (<168 ng/mL). In post-menopausal women, breast cancer risk was unrelated to plasma IGF-I levels.

22. In an extension of this study, Rinaldi *et al*, 2005a explored the hypothesis that the variability in the risk estimates for the association between IGF-I and IGFBP-3 and the increased risk of pre-menopausal breast cancer might be to do with variation in the ability of different IGFBP-3 assays to specifically measure intact/functional forms of the protein. Measurements of IGFBP-3 and IGF-1 had previously been made using an in-house radioimmunoassay, but were repeated using commercially available ELISAs, one made by DSL and another which was stated to have a greater specificity for the active form of IGFBP-3. The absolute values of IGF-I and IGFBP-3 measured by the different assays varied. The correlations between the RIA and the ELISAs for IGF-I measurements were high, but were lower for IGFBP-3 measurements. IGF-I and IGFBP-3

measurements by the DSL ELISA showed significant association with risk (OR for top versus bottom quartiles were 1.93, (95%CI 1.00-3.72) *p*=0.02 and 2.03 (95%CI, 1.09-3.76) *p*=0.02 respectively). This was consistent with the findings of the previous study. In contrast, measurements of functional IGFBP-3 tended to be unrelated to risk. The association with IGF-I became weaker and lost statistical significance when corrected for IGFBP-3 measured by the DSL-ELISA, but increased in significance when adjusted using the functional IGBP-3 assay (OR for top versus bottom quartiles were 2.43, (95%CI 1.21-4.90) *p*=0.005) or when considering the molar ratio of IGF-I to IGFBP-3 (OR= 2.37(95%CI, 1.13-5.00) p=0.02). The results were consistent with an association of breast cancer risk in young women with elevated IGFBP-3 and IGF-I, but show that the strength of the association for IGFBP-3 could vary substantially depending on the assay used.

23. Rinaldi *et al*, (2005b) conducted a pooled re-analysis, using data on pre-menopausal women from three prospective case-control studies nested within 3 cohorts in New York, Sweden and Italy (see Toniolo *et al.,* 2000; Muti *et al.,* 2002; Kaaks *et al.,* 2002 respectively) plus 62 newly diagnosed breast cancer cases from the New York cohort. In all, a total of 220 cases and 434 matched controls were considered. IGF-I and IGFBP-3 were measured using the DSL ELISA with a positive but not statistically significant relationship between IGF-I levels and breast cancer risk being reported. Mean levels of IGF-I were 301.5 (174.1-444.2) ng/ml in the cases and 293.6 (165.6-450.1) ng/ml in the controls. The results, OR and 95%CIs (adjusted for age at menarche, BMI, family history and benign breast disease in addition to matching criteria) are given in Tables 3 and 4 below. The adjusted OR for IGF-1 (highest vs lowest) was 1.41(0.75-2.63; *p* for trend 0.015) but this was reduced to 1.14 (0.55-2.03) when adjusted for IGFBP-3. There was no relationship between risk and molar ratio of IGF-I and IGFBP-3, but the association of IGFBP-3 levels and risk reached borderline significance (adjusted OR 1.77 (0.97-3.24) for highest vs lowest, *p* for trend =0.07). It was noted that the results differed from those of the previous studies where a significant increase in risk was associated with increasing IGF-I in Toniolo *et al.,* 2000 and Muti *et al.,* 2002 but not in Kaaks *et al.,* 2000. It was suggested that the additional cases could have resulted in a regression towards the mean, or that the findings were due to the differences in the assays methods used. However, testing of a sub-set of the New York samples suggested a high degree of correlation when measured either by DSL ELISA or an in house method, though the correlations for IGFBP-3 levels were lower. For IGFBP-3 there was some tendency for an increase in breast cancer risk with increasing concentration, in contrast to finings reported elsewhere (Hankinson *et al*., 1998; Chan *et al*., 1998; Ma *et al*., 1999).

Table 3: OR per quintile of IGF-I (Rinaldi, *et al*., 2005b)

IGF-I	**OR**	**95% CI**
1	1	Referent
2	0.9	0.5-1.62
3	1.63	0.89-2.73
4	1.46	0.78-2.73
5	1.41	0.75-2.63

Table 4: OR per quintile of IGFBP-3 (Rinaldi, *et al*., 2005b)

IGF-I	**OR**	**95% CI**
1	1	Referent
2	0.98	0.54-1.75
3	1.06	0.59-1.91
4	1.04	0.58-1.87
5	1.77	0.97-3.24

24. Li, *et al*. (2001) measured free IGF-I, total IGF-I, IGF-II and total, intact and fragment IGFBP-3 in plasma from women with primary breast cancer. The plasma was sampled, prior to treatment, from 40 newly-diagnosed patients (23 African-Americans and 17 Caucasians) who were selected consecutively from a breast cancer clinic at Louisiana State University Health Sciences Center (LSUHSC), USA. The age of the patients ranged from 31 to 67 years (mean = 52 years). One age- and race-matched control was selected for each case. The controls were selected from LSUHSC employees and healthy women who had had check-ups at LSUHSC. 66.7% of the cases and 67.5% of the controls were pre-menopausal. The association between IGF-I levels and breast cancer was examined by conditional logistic regression analysis. The odds ratios (OR) for breast cancer patients having high plasma IGF-I (median or greater), after adjusting for menopausal status and IGFBP-3, were 2.00 (95% CI = 0.43-9.28; p=0.38) for total IGF-I and 6.31 (95% CI = 1.03-38.7; p= 0.047) for free IGF-I. A high IGF-I/IGFBP-3 ratio was also positively associated with breast cancer, with odds ratios of 3.25 (95% CI = 1.06-9.97; p=0.36) for total IGF-I/intact IGFBP-3 and 3.25 (95% CI = 1.06-9.97; p=0.039) for free IGF-I/fragment IGFBP-3 (for ratios greater than or equal to the median). No association was found for high levels of IGF-II or for total, intact or fragment IGFBP-3. The authors concluded that measurement of free-IGF-I in plasma was a better predictor of breast cancer than total IGF-I.

25. Muti, *et al*., (2002) reported a case-control study nested within the ORDET cohort (the hormones and diet in the aetiology of breast cancer prospective cohort) of 10,786 women aged 35-69 in the Varese province of northern Italy. Blood samples were taken on recruitment to the cohort between June 1987 and June 1992, and for pre-menstrual women the samples were taken during the luteal phase of the menstrual cycle. Cohort

members diagnosed with breast cancer after blood samples were taken and before June 1995 were included in a case-control study nested within the cohort. A total of 133 cases of invasive breast cancer were included (69 pre-menopausal and 64 post-menopausal at the time of recruitment). For each breast cancer case, 4 matched controls were chosen from the cohort. Concentrations of fasting glucose, insulin, total IGF-I, free IGF-I IGFBP-1, IGFBP-2 and IGFBP-3 were measured in serum. In pre-menstrual women, there were significant ($p<0.05$) positive trends associating risk of breast cancer (both unadjusted and adjusted for various social and physiological variables) with serum concentrations of fasting glucose and total IGF-I. Comparing the highest and lowest quartiles of concentrations of total IGF-I in serum, the unadjusted OR; 95%CI for premenopausal breast cancer risk was 2.75 (1.04-7.28) p_{trend} = 0.03 and 3.12 (1.13-8.60) p_{trend} = 0.01 when adjusted for age, BMI, social and economic status and reproductive variables. For free IGF-I the association was not significant, the ORs being 1.40 (0.58-3.36) p_{trend} = 0.22 and 3.12 (1.13-8.60) p_{trend} = 0.01 when adjusted. IGFBP-3 concentration was also associated with an increased risk in pre-menopausal women the unadjusted RR (highest vs lowest) was 2.30 (0.98-5.41) p_{trend} = 0.02, and 2.31 (0.97-5.53) p_{trend} = 0.02 when adjusted. In post-menstrual women there was no association between breast cancer risk and levels of any of the substances analysed in serum.

26. Yu, *et al*. (2002) performed a population-based case-control study to assess the associations of levels of circulating IGF-I, IGF-II and IGFBP-3 with breast cancer risk in Chinese women, aged 48.5 ± 8.3 years. The study included 300 female incident breast cancer patients (diagnosed in Shanghai between August 1996 and March 1998) and 300 age-and menopause-matched controls randomly selected from the general female population of Shanghai. Blood samples were collected from cancer patients after diagnosis but before any radiotherapy or chemotherapy. Breast cancer patients (all women) had higher plasma levels of IGF-I and IGFBP-3 than controls ($p<0.001$). The median plasma concentrations of IGF-I, IGF-II and IGFBP-3 were respectively 143, 860 and 4340 ng/mL in cases and 127, 856 and 4030 ng/mL in controls. There were also significant trends for increased breast cancer risk with increased plasma levels of IGF-I and IGFBP-3. These associations were more evident in pre-menopausal women or in women with a high body mass index or high waist-to-hip ratio. No significant association was found for IGF-II.

27. Krajcik, *et al*. (2002) reported the results of a nested case-control study conducted on women selected from a cohort of 95,000 women who underwent health check-ups, including collection of a blood sample, when enrolled in the Kaiser Permanente Medical Care Program between 1964 and 1971. Breast cancer cases investigated in this study were 66 women who were pre-menopausal (aged 19 to 46 years) at the time of diagnosis and 60 post-menopausal women (aged 29 to 73 years), who had been randomly selected from more than 2000 breast cancer cases that had diagnosed in the cohort by 1991 and at least 2 years after blood collection. One control was matched to each case on the basis of age,

date of examination, and length of follow-up. Serum from each participant was analysed for IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, insulin and glucose. Breast cancer risks were compared in relation to the substances measured in serum using conditional logistic regression analysis (2-sided). In pre-menopausal women, elevated serum IGF-I (p_{trend} = 0.051 when adjusted for insulin, glucose and BMI) and IGFBP-3 (p_{trend} = 0.033 when adjusted for insulin, glucose, BMI and IGF-I) were associated with increased risk of breast cancer. In post-menopausal women, elevated IGFBP-2 was associated with reduced risk of breast cancer (p_{trend} = 0.007 for unadjusted data; p_{trend} = 0.002 when adjusted for insulin, glucose and IGF-I). Serum IGFBP-1 levels were not associated with breast cancer in either pre- or post-menopausal women.

28. Kaaks *et al*., (2002) reported a case control study nested within two prospective cohorts in Umeå and Malmö, Sweden. The Umeå cohort was based on 3 concurrent projects: the Västerbotten Intervention project (VIP) (30,425 women, 27,756 men), the Northern Swedish Component of the WHO multinational study for the Monitoring of Trends in Cardiovascular Disease study (MONICA) (2540 women and 2507 men) and a population based mammary screening project (MSP) (18,750 women). The Malmö cohort (17,035 women and 11,063 men) were recruited for a prospective study, the Malmö Diet and Cancer Study (MDCS). From these, a total of 513 incident cases were identified and matched with 987 controls. For the whole group, risk was not associated with levels of IGF-I, IGFBP-3 or IGF-I adjusted for IGFBP-3. However, when broken down by sub-group, an increase in risk was observed for increasing levels of IGF-I in women aged 50 or older at the time of recruitment in the Umeå cohort only (ORs of 1.00, 1.73, 1.76, 1.90 *p* trend =0.04); this ORs for this trend became stronger when restricted to breast cancer cases detected after at least 1 year of follow up (ORs of 1.00, 1.82, 1.94, 2.85 p_{trend} =0.06) although the *p* trend value decreased slightly. The CIs were not presented for these analyses. The effect was weakened when the analysis was restricted to subjects who did not use exogenous hormones for the treatment of menopausal symptoms. Levels of IGF-I and IGFBP-3 were not related to risk in younger women. In a sub-cohort analysis, where samples were taken after 4 hours fasting, breast cancer risk was not associated with levels of insulin, IGFBP-1 or IGFBP-2. It was noted that the background information supplied varied at the time of recruitment and information on reproductive history and use of exogenous hormones had to be obtained at a later date; subjects with unknown use were excluded from the analysis. It was also noted that there were some differences in blood sampling procedures between the cohorts.

29. Keinan-Boker *et al* ., (2003) reported a case-control study nested within 2 Dutch cohorts; Prospect-EPIC (17,357 women recruited through a breast-cancer screening programme 1993-1997 as part of the EPIC cohort) and PPHV (a Dutch project aimed at monitoring risk factors for cardiovascular disease consisting of 35,491 participants (half-women) aged 20-59 at recruitment). From these subjects, 149 breast cancer cases were identified through linkage with cancer registries; these were matched with

333 controls. Plasma levels of IGF-I, IGFBP-1,-2, and -3 and C-peptide were measured by immunoradiometric assay. For IGF-I, the adjusted OR (95%CI) was 1.1 (0.6-2.1) for the top versus bottom quartile, for IGFBP-I it was 0.7 (0.3-1.3), IGFBP-2, 1.1 (0.5-2.4), IGFBP-3, 1.6 (0.7-3.5) and for IGF-I/IGFBP-3 ratio 1.0 (0.5-1.8). The authors concluded there was no relationship between circulating levels of IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, c-peptide and post-menopausal breast cancer. It was noted that mean levels of almost all of the peptides were lower in the subjects from the EPIC cohort, but this was thought more likely to result from an experimental artefact rather than being a significant difference between the two populations.

30. Grønbæk *et al*., (2004*)* conducted a case-cohort study which examined the association between total IGF-I, IGF-II, IGFBP-2, IGFBP-3 and IGFBP-3 protease (reported to increase following Tamoxifen treatment which decreases IGF-I levels) activity and breast cancer taking into account oestrogen receptor (ER) status, within a Danish Follow up study "Diet, Cancer and Health". The participants were aged 50-64 years at recruitment and had no previous history of cancer. 411 cases were identified who were matched with a group of 397 controls who were cohort members (more controls than case were subsequently excluded due to missing information). 285 and 80 tumours respectively were ER positive and negative. The associations were analysed using a Cox proportional hazards model (NB. *p* values not given). No association was found between IGF-I levels and breast cancer risk but an association was found between IGFBP-3 and breast cancer risk. Per 500 units higher levels of IGFBP-3, an incidence rate ratio (IRR) of 1.14 (95%CI 1.00-1.30) was estimated. The latter value is given in the abstract with slightly different values being given in the summary tables 1.12 (1.02-1.24 for IGFBP-3 adjusted for IGF-I and 1.13 (1.02-1.26 for IGFBP-3 adjusted for parity, age of first birth, benign tumours, BMI, education, alcohol and HRT duration). For ER positive breast cancer the IRR for IGFBP-3 was 1.18 (95%CI 1.05-1.33); adjusted for both IGF-I and for other criteria as above. IGFBP-3 protease activity was not associated with breast cancer risk. Per 275 units higher levels of IGF-II, an IRR of 1.33, 95%CI of 1.10-1.66 was observed for ER positive tumours whereas IGFBP-2 was not associated with breast cancer risk. Adjustment for potential confounders did not affect the risk. There was no association between IGF-I, IGF-II, IGFBP-2, or IGFBP-3 and the risk of ER-negative breast cancer. The peptides were measured by non-competitive time resolved immunofluorometric assay.

31. Renehan, *et al*. (2004) performed a meta-analysis as part of a systematic review of the associations of circulating concentrations of IGF-I and of IGFBP-3 with the risks of cancers of the prostate, colorectum, pre-menopausal breast, post-menopausal breast and lung. Databases were searched for epidemiological studies published between January 1996 and December 2002. For the association between IGF-I and pre-menopausal breast cancer risk in five studies (Del Giudice, *et al*., 1998; Toniolo, *et al*., 2000; Muti, *et al*., 2002, Krajcik, *et al*., 2002; Yu, *et al*., 2002), the OR; 95%CI was 1.96 (1.28-2.99). For the association between

IGF-I and post-menopausal breast cancer risk in four studies (Toniolo, *et al*., 2000; Muti, *et al*., 2002, Krajcik, *et al*., 2002; Yu, *et al*., 2002), the OR was 0.97 (0.53-1.77). When data from four studies (Hankinson, *et al*., 1998; Toniolo, *et al.,* 2000; Muti, *et al*., 2002, Yu, *et al*., 2002) were scaled to an increase in blood levels of IGF-I from 25th to 75th percentiles, there was a positive trend ($p < 0.001$) for pre-menopausal breast cancer but no significant trend ($p = 0.63$) for post-menopausal breast cancer. These data indicated odds ratios for IGF-I with pre- and post-menopausal cancers of 1.65 (1.26-2.08) and 0.95 (0.77-1.17), respectively. When data from three studies (Toniolo, *et al*., 2000; Muti, *et al*., 2002; Yu, *et al.,* 2002) were scaled to an increase in blood levels of IGFBP-3 from 25th to 75th percentiles, there were no significant dose-related trends associated with pre- or post-menopausal breast cancers and ORs were, respectively 1.51 (1.01-2.27) and 1.01 (0.74-1.38). The authors concluded that high circulating levels of IGF-I and of IGFBP-3 were associated with increased risk of pre-menopausal breast cancer, but not of post-menopausal breast cancer.

32. In a meta-analysis of 16 studies conducted by Shi *et al* (2004) Hedges' standardised mean differences (HSMD) and odds ratios were calculated to estimate the effect of IGF-I and IGFBP-3. Data analysis showed that circulating IGF-1 levels were not higher in breast cancer patients than in controls for all women or for post-menopausal women but were significantly higher in pre- menopausal women. For all and post-menopausal women, the HSMD were 0.024 and 0.035 respectively; p= >0.4 but were significantly higher for pre-menopausal women (HSMD = 0.170, p<0.001) with the ORs (95%CI) for breast cancer risk being 1.05 (0.94-1.17), 0.93 (0.80-1.10) and 1.39 (1.16-1.66) for all, post and pre-menopausal women respectively. The studies included in the analysis were those by Peyrat *et al*., 1993; Bruning *et al*., 1995; Hankinson *et al*., 1998; Holdaway *et al*., 1999; Mantzoros *et al*., 1999; Vadgama *et al*., 1999; Li *et al*., 2001; Petridou *et al*., 2000; Toniolo *et al*., 2000; Jernström *et al*., 1999; Agurs-Collins *et al*., 200; Krajcik *et al*., 2002; Muti *et al*., 2002; Kaaks *et al*., 2002; Yu *et al*., 2002; and, Keinan-Boker *et al*., 2003. Two of the studies (Kaaks *et al*., 2002 and Keinan-Boker *et al*., 2003) which compared different populations were considered as 2 studies each in the analysis. The HSMD of IGBP-3 was 0.18 (p <0.001) and the OR (95%CI) 1.42 (1.15-1.74) for pre-menopausal women. The authors concluded that their results supported the suggested association between high IGF-I and IGFBP-3 levels and an increased risk of breast cancer in pre-menopausal women.

33. In a meta-analysis conducted by Sugumar *et al* (2004). The literature was searched for studies published between January 1990 and March 2003 with ORs reported for a highest versus lowest measure of association and with a nested or population based case control design. 31 studies were initially identified with seven of them being used in the analysis; these were Bohlke *et al* (1998), Hankinson *et al* (1998), Toniolo *et al*., 2000, Kaaks *et al*, 2002, Krajik *et al*, 2002, Muti *et al*., (2002) and Yu *et al.,* (2002). It was reported that a marginally significant increased risk of pre-

menopausal breast cancer was associated with higher circulating levels of IGF-1 with an OR (95% CI) of 1.74 (0.97-3.13) *p*= 0.06. There was no association with IGFBP-3 and pre-menopausal breast cancer, OR 1.60 (0.84-3.02) *p* =0.15 (six of the seven studies were included in this part of the meta-analysis). The authors recommended that a measure of free IGF-I as well as IGF-I/ IGFBP-3 should be included in future studies.

34. A nested case-control study investigating the association between breast cancer risk and IGF-I, IGF- II and IGFBP-3 was performed on a cohort of women from Guernsey, UK (Allen *et al*., 2005). This cohort consisted of 6127 women aged 35 years or older, recruited between 1977 and 1991. Follow up was through pathology reports, Guernsey death certificates and the Wessex Cancer Registry; cases were identified up to November 2000. A total of 117 cases (70 pre-menopausal and 47 post-menopausal at the time of blood collection) were identified and matched to 350 controls; women taking exogenous hormones were excluded. For the women who were premenopausal at recruitment, the mean age at diagnosis was 57 and although the information was not available, it was thought that the majority of women would have been postmenopausal at that time. IGF-I was measured by ELISA and IGFBP-3 by an in-house RIA. Pre-menopausal women in the top vs bottom third of serum IGF-I concentration had a non-significantly increased risk for breast cancer after adjustment for IGFBP-3 (OR 1.71, 95%CI, 0.74-3.95; test for linear trend *p* = 0.21. Serum IGBP-3 was associated with a reduction in risk in pre-menopausal women after adjustment for IGF-I (top vs bottom third OR 0.49, 95%CI, 0.21-1.12; *p* for trend = 0.07). Neither IGF-I nor IGBFP-3 was associated with risk in postmenopausal women and serum IGF-II was not associated with risk in pre- or postmenopausal women. The authors concluded that the data were compatible with the hypothesis that women with relatively high circulating levels of IGF-I and low IGFBP-3 levels were at an increased risk of developing breast cancer.

35. Rollison *et al*., (2006) reported a nested case-control study conducted among women in the CLUE I and CLUE II cohorts in Maryland, US. The participants were enrolled in 1974 (n=15,192) and 1989 (n=18,724) when blood samples were taken. Breast cancer cases were identified via the Maryland Cancer Registry. Of the cases identified, 152 were pre-menopausal at the time of blood sampling, while 91 were post-menopausal. Levels of IGF-I and IGFBP-3 were measured by ELISA. Analysis was restricted to women aged 55 y or more to exclude perimenopausal cases. Prior to age stratification, IGF-I levels were not associated with post-menopausal breast cancer, whether taken before or after the menopause. After age-stratification, associations between pre-menopausal IGF-I adjusted for IGBP3 and post-menopausal breast cancer risk were suggested in the youngest pre-menopausal women (upper vs lowest third OR 5.31, 95%CI 0.85-33.13, *p* trend =0.006) and oldest post-menopausal women (upper vs lowest third OR 3.41, 95%CI 0.66-17.71, *p* trend =0.13). There was no overall association between IGF-I and breast cancer risk whether adjusted or not for IGFBP-3.

36. The association between total IGF-I and IGFBP-3 and breast cancer risk was examined in a large prospective case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Rinaldi, *et al*., 2006). There were 1081 incident cases and 2098 matched controls. Of the incident cases 370 were identified as pre-menopausal, 614 as post-menopausal and 97 unknown. Increasing IGF-I and IGFBP-3 concentrations were associated with a significant increase in breast cancer risk in women who developed the disease after 50y of age (highest vs lowest quintile OR (95%CI) 1.38 (1.02-1.86), p=0.01 and 1.44 (1.04-1.98), p=0.01 for IGF-I and IGFBP-3 respectively but no relationship was observed in younger women. There was significant heterogeneity in the relationship between breast cancer and serum IGF-I and IGFBP-3 levels depending on the time between blood donation and tumour diagnosis. A reduction in breast cancer risk with increasing IGF-I concentrations was observed in cases with a diagnosis of cancer less than 2 years after blood donation OR (95%CI) 1.38 (1.02-1.86), p=0.01 while an increase in risk was observed for women with a later diagnosis (2 years or greater, OR 1.51 (1.19-1.91); a similar pattern was observed for IGFBP-3. The EPIC cohort consists of 366,251 woman and 153,457 men recruited in 10 Western European countries.

37. Lukanova *et al*., 2006 conducted a nested case control study in women from the Northern Sweden Maternity Cohort an ongoing prospective study of > 83,000 women. The authors proposed that exposure to elevated IGF-I during early pregnancy, a period characterised by intense cell proliferation in breast tissue and the presence of high concentrations of sex steroids, could be associated with an increased maternal risk of a breast malignancy. The study compared 212 cases and 369 controls from cohort members who had delivered singleton babies. RIA was used to measure IGF-I and IGF-II levels during weeks 7-18 of pregnancy. Cases were identified through linkages with the Swedish Cancer Registry. Breast cancer risk was found to increase with increasing IGF-I levels (top tertile OR (95%) 1.7 (1.1-2.7). The association was stronger among primiparous women OR = 2.2 (1.1-4.4) than non-primiparous women OR, 1.4 (0.7-2.8). The risks appeared to decrease in the older women. There was no association of breast cancer with IGF-II levels. The authors concluded that IGF-I was important in breast cancer and that the adverse effect of IGF-I on the breast might be stronger before the remodelling of the gland induced by a first pregnancy.

38. In a follow up to the study by Lukanova *et al*., (2006) above, Chen *et al*., 2010 conducted a further study in women from the same cohort, in which study subjects were limited to women who had donated a blood sample during their first full term pregnancy to allow for any effect due to parity. The study consisted of 244 women and 453 matched controls. IGF-I was measured by an Immulite 2000 analyzer (chemiluminescence based). A significant association of breast cancer risk and IGF-I level was observed, with OR (95%CI) of 1.73 (1.14-2.63) for the top tertile (p_{trend} =0.009). Sub-group analyses indicated that the effect was stronger in women aged ≤ 25 (OR= 1.99 (0.74-5.35) (p_{trend} =0.014) and > 30 than in women aged 25-30

at index pregnancy (OR= 2.69(1.23-5.91) (p_{trend} =0.232). A stronger association of IGF-I with risk was also observed in cases diagnosed within 15 years of blood donation OR =2.46(1.02-5.91) p_{trend} =0.038). The authors noted that IGF-I levels were fairly constant during the first half of pregnancy and similar to levels measured before pregnancy, suggesting that the levels were indicative of non-pregnant exposure. Thus the elevation of risk in this young population was consistent with the hypothesis that immature glands which had not undergone a differentiation cycle associated with the completion of a pregnancy were most vulnerable to mitogenic and anti-apoptotic hormonal effects.

39. The association described above was explored in more detail by Toriola *et al*., (2011) in a further nested case-control study in the same cohort. This consisted of primiparous women aged <40 who had donated blood in the first twelve weeks of pregnancy and delivered a single child at term. 719 cases were compared to 1434 matched controls. No significant associations were observed between serum IGF-I levels and breast cancer risk overall, OR (95% CI)= 1.08 (0.80-1.47), or in any analyses stratified by histological subtype lag time to diagnosis, age at pregnancy or age at diagnosis. IGF-I was measured by an immunometric assay on an Immulite 2000 analyzer. The study did not measure, or correct for, IGFBP-3. The authors noted that the ER status of the tumours was unknown but that as the diagnosis was before less than 50 years of age the tumours were likely to be ER negative, and pointed out previous work (Key *et al*., 2010) suggesting that the association between IGF-I and breast cancer was confined to ER positive tumours.

40. Baglietto *et al*., (2007) conducted a case-cohort study within the Melbourne Collaborative Cohort Study which included a random sample of 1,901 women (sub-cohort) and 423 breast cancer cases diagnosed during a mean 9.1years of follow up; cases were diagnosed between baseline and June 2002. Cases were ascertained via a cancer registry. The Melbourne Collaborative Cohort Study is a prospective short study of 41,528 individuals (24,479 women) aged 27-75 at recruitment although 99.3% were aged 40-69. Recruitment took place between 1990 and 1994. Women taking hormone replacement therapy at baseline were excluded. Overall, there was no significant increase in breast cancer risk associated with higher levels of IGF-I or IGFBP-3; adjusted for known and potential confounders, the hazard ratio (95%CI) for breast cancer comparing the fourth with the first quartile was 1.2 (0.87-1.65) for IGF-I and 1.09 (0.78-1.53) for IGFBP-3. The associations between IGF-I and IGFBP-3 and breast cancer status varied according to menopausal status at baseline with no association for premenopausal women but a positive association for postmenopausal women. The HRs (95%CI) associated with a doubling of hormone concentrations were1.47 (1.00-2.14) and1.88 (0.96-3.67) for IGF-I and IGFBP-3 respectively. Consistent with this, the associations of IGF-I and IGFBP-3 with breast cancer risk were heterogeneous according to attained age during follow up: for IGF-I, the HRs for breast cancer comparing the fourth with the first quartile were 0.60 (0.25-1.45) before age 50 and 1.61 (1.04-2.51) after age 60 (test for

the log-linear trend of HR according to age, $p = 0.05$); for IGFBP-3, the HRs were 0.79 (0.34-1.83) before age 50 and 1.62 (1.03-2.55) after age 60 (test for the log-linear trend, $p = 0.08$). The authors concluded that IGF-I was positively associated with breast cancer risk in older but not younger women.

41. A nested case control study was conducted among 35,105 Norwegian women who were part of a health screening survey (Vatten *et al*., 2008). The women were aged 40-42 years and were followed for a mean period of 4.3 years, during this time, 325 women were diagnosed with breast cancer and these were matched with 647 controls. Serum concentrations of IGF-I and IGFBP-3 were measured by radioimmunoassay. The median IGF-I level did not differ between cases and controls; when analysed by category the relative risk for women in the highest versus the lowest quintile was 1.46 (95%CI 0.93-2.32; *p* trend =0.015) after adjusting for serum IGBP-3, age and year of blood collection. The results were not affected by exclusion of cases that were diagnosed within 2 years. The authors concluded that they had found only a modest positive association between serum IGF-I levels and risk of breast cancer in women younger than 50 years of age. When analysed by quintile, a weak inverse association was apparent. The highest risk of breast cancer were seen among women in the highest tertile of IGF-I and the lowest tertile of IGFBP-3 (OR 2.00; (95%CI, 1.01-3.96) but patterns of association were inconsistent and there was no evidence that the association of IGF-I with breast cancer risk differed at differing levels of IGFBP-3. It was noted that information on risk factors or on the use of oral contraceptives was incomplete, but it was thought that this would not have a large confounding effect.

42. A case-cohort study of incident breast cancer was conducted in non-diabetic women who were part of the Women's Health Initiative Observational Study (WHI-OS) a cohort of 93, 676 postmenopausal women (Gunter *et al*, 2009). Fasting serum samples obtained at study entry from 835 incident breast cancer case and 816 randomly chosen WHI-OS participants were tested for levels of insulin, glucose, total IGF-I, free IGF-I, IGFBP-3 and oestrodiol. The cases included women who developed breast cancer after at least 12 months of follow up. Multivariate Cox proportional hazards models were used to estimate associations between serologic factors and baseline characteristics. Insulin and obesity were associated with an increase in the risk of postmenopausal breast cancer but there was no association between total IGF-I and breast cancer risk, HR (95%CI) 1.21 (0.85-1.72) $p_{trend} = 0.92$ for top vs lowest quartile or for IGFBP-3, HR 0.77 (0.55-1.08) $p_{trend} = 0.26$ for top vs lowest quartile. However, there was a modest positive association between the level of free IGF-I and the risk of breast cancer among non-users of hormone therapy in the second and third quartiles HR =1.83 (1.11-3.02) for quartile 2 vs quartile 1, and 1.82 (1.09-3.04) for quartile 3 vs quartile 1 but not for the highest quartile HR 1.24 (0.73-2.1) suggesting a possible curvilinear association between free IGF-I levels and breast cancer. The

association was reduced by the inclusion of insulin in the multivariate model.

43. In a case control study of Taiwanese women, 297 incident cases of breast cancer were compared with 593 controls (Wu *et al*, 2009). Plasma levels of IGF-1 and IGFBP3 were measured at the time of diagnosis (between 2004 and 2006) by immunoradiometric assay. The controls were women admitted for health examinations at the same hospital. Conditional logistic regression was used to calculate odds ratios and confidence intervals. After adjustment for matching factors and IGFBP-3, the data indicated that the breast cancer risk related to IGF-1 differed with menopausal status, with high IGF-1 increasing risk of pre but not post menopausal breast cancer (top vs bottom tertile, adjusted OR (95% CI) = 1.86 (1.01-3.44). Elevated IGF-1 levels in conjunction with a prolonged interval of critical period of oestrogen exposure (as estimated by the interval between age at menarche and age at first full term pregnancy) significantly increased risk of breast cancer, particularly among oestrogen-positive cases (adjusted OR, 2.42 (1.33-4.38). For oestrogen negative cancers the adjusted OR, was 1.61 (0.70-3.70). The authors proposed that a synergistic effect could occur, noting that evidence from the literature suggested that oestrogens could potentially modify the responsiveness of breast endothelial cells to IGF-I and that IGF-I and oestrogen synergistically enhanced the transcriptional activity of the oestrogen receptor.

44. Rollison *et al*., 2010 measured IGF-I and IGFBP-3 in a subset of women from a population based case control study. Women currently taking Tamoxifen or undergoing chemotherapy were excluded. It was noted that Hispanic women had lower IGF-1 levels than non-Hispanic white women (WNH) but the association between IGF-I, IGFBP-3 and breast cancer had not been assessed in this group. The study consisted of 184 cases (129 WNH and 55 Hispanic and/or native American) and 522 controls (296 WNH and 226 Hispanic and/or native American) women. IGF-I was measured by an IGFBP-3 blocked commercial radioimmmunassay and IGFBP-3 by an immunoradiometric kit which directly measured “active” non-glycosylated IGFBP-3. Both IGF-I and IGFP-3 were significantly associated with breast cancer overall. The OR IGF-I (highest vs lowest quartile) (95%CI) was 3.04 (1.63-5.67) for IGF-I and 1.92 (1.07-3.43) for IGF-I and positive associations were observed for both pre and post-menopausal breast cancer. IGF-I was associated with breast cancer in NHW women, the OR (95%CI) for highest vs lowest quartile was 2.82 (1.36-5.83) but not in Hispanic women 0.81 (0.29-2.27) whereas IGFBP-3 was associated with breast cancer in both ethnic groups OR= 3.32 (1.45-7.6) for Hispanic women and 2.15 (0.76-6.04). It was concluded that the association between IGF-I and breast cancer varied by ethnicity, while no ethnic differences were observed in the association between IGFBP-3 and breast cancer.

45. A nested case control study was conducted among participants in the prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) by Schairer *et al*., (2010). The study consisted of 389 breast cancer cases

and 470 controls aged 55-74. Mean follow up was 8.5 years and the mean time between serum collection was 4 years. IGF-I and IGFBP-3 were measured by ELISA. Multivariate HR(95% CI) s for IGF-I, IGFBP-3 and the molar ratio of IGF-I/IGFBP-3 comparing the highest quintile to the lowest were 1.28, 0.67-2.44, 1.12 (0.55-2.27) and 1.25 (0.72-2.15) respectively. Multivariate HRs per one quintile increase were 1.07 (0.92-1.25) for IGF-I, 1.01 (0.86-1.18) for IGFBP-3 and 1.10 (0.98-1.24) for the molar ratio. The associations for IGF-I and the molar ratio strengthened by adjustment for BMI and estradiol levels were positive but were not statistically significant; there was no association between risk and IGFBP-3 levels. The authors noted the inconsistency of results in this area and note that their assay (and that used in most other epidemiology studies) measures total IGF-I (both bound and unbound and total IGFBP-3 (both intact and some proteolytic fragments) but not necessarily the bioactive components. The study by Rinaldi *et al*., (2005) was noted in which the use of an assay for functional IGFBP-3 resulted in much stronger associations with breast cancer risk.

46. Pooled data on pre-diagnostic IGF-1and IGFBP3 concentrations from 17 prospective studies in 12 countries were analysed by the Endogenous Hormones and Breast Cancer Collaborative Group (Key *et al*., 2010). The 17 eligible studies were CLUE 1 and CLUE II, EPIC, the Guernsey study, the Janus biobank study, Danish Diet, Cancer and Health study (KKH), Kaiser-Permanente-Orentreich Foundation Study (KP-OFAS), Malmö and Northern Sweden Studies, Melbourne Collaborative Cohort study (MCCS), Nurses' Health Study, Nurses' Health Study II, New York University Women's Health Sudy (NYU WHS), Study of Hormones and Diet in the Etiology of Breast Tumours (ORDET) from Italy, Prostate, Lung Colorectal and Ovarian Cancer Screening trial (PLCO), monitoring Project on Cardiovascular Disease Risk factors (PPHV) and Prospect-EPIC; Study of Osteoporotic Fractures (SOF)[5] and the Women's Health Initiative, Observational Study (WHI-OS) (Gunter *et al* , 2009). IGF-I concentrations, adjusted for age were positively associated with age at menarche and years since menopause, and were higher in moderately overweight women and moderate alcohol consumers than in other women and moderate alcohol consumers than in other women. IGF-I was weakly positively associated with breast-cancer risk for premenopausal women (test for trend, p=0.050) and strongly positively associated with breast-cancer risk for post-menopausal women (test for trend p=0.0002) the test for heterogeneity by menopausal status at blood collection was not statistically significant. The OR for breast cancer in women in the highest versus the lowest fifth of IGF- 1 concentration was 1.28 (95% CI 1.14-1.44; p< 0.0001). This association was not altered by adjusting for IGFBP3, and did not vary significantly by menopausal status at blood collection. The ORs for a difference in IGF-1 concentration between the highest and lowest fifth were 1.38 (1.14-1.68) for oestrogen-positive tumours and 0.8 (0.57-1.13) for oestrogen-negative tumours (p for

[5] The reference provided for this cohort by Key et al., (2010) does not contain data on IGF-I. It does not appear to have been published separately and was possibly provided as a personal communication. The data are from 101 cases and 235 controls and are post-menopausal women.

heterogeneity =< 0.007). IGFBP3 was not associated with breast-cancer risk for pre-menopausal women but was associated with increased risk for post-menopausal women (OR in the highest fifth compared with the lowest, of 1.23 (1.04-1.45), test for trend *p*=0.012). In the combined analysis of pre and post-menopausal women, those in the highest fifth of IGBP3 had an OR of 1.13 (0.99-1.28) compared with women in the lowest fifth (test for trend *p*=0.062). Adjustment of the association between IGFBP3 and breast cancer risk for IGF-1 reduced the OR for linear trend from 1.12 (1.00-1.26) to 0.99 (0.87-1.14). The elimination of this association suggested that the initial association of IGFBP3 was due to its positive correlation with IGF-1.

Conclusions on IGF levels and risk of breast cancer

47. Studies comparing circulating blood IGF-I levels in women with breast cancer and control have reported both increased levels of IGF-I in cancer patients and no difference. Since cancers may produce their own growth factors, the results are difficult to interpret.

48. The results of the prospective studies investigating levels of IGF-I and breast cancer risk are also inconsistent. Some studies report an association between IGF-I and others report no association. Where women have been considered in terms of their menopausal status the associations reported for post and pre-menopausal women have also differed.

49. One explanation for this may be the wide range of different study designs and the range of potentially confounding factors that may influence the results. These may include issues such as age, classification of menopausal status (with one study only excluded peri-menopausal women from the analysis), use of exogenous oestrogens by the participants and time from sample collection to diagnosis. Data on ethnicity are often absent which may be important if particular polymorphisms are relevant to IGF-I levels. The choice of assay may also be important since it is unclear to what extent active IGF-I is measured by the different procedures. Rinaldi *et al*., (2005a) used an assay stated to be more specific for active forms and a stronger association was observed than in other studies. However, this has not been repeated by other workers. IGF-I concentrations may be measured and reported as total or free IGF-I or this may not be specified. Some studies adjust the IGF-I results for IGFBP-3 and vice versa, and others present information on the IGF-I/IGFBP-3 molar ratio.

50. Several meta-analyses have been performed. These also produced conflicting results, although were more generally positive. Renehan *et al*., (2004) reported a positive association between IGF-I and risk in pre but not post-menopausal women, Shi *et al*., (2004) in post-menopausal women only, Sugumar et al., 2004 a marginally positive association in pre-

menopausal women and Key *et al*., (2010) a weak positive association in pre-menopausal women and stronger ones in post-menopausal women as well as an association between IGF-I and oestrogen positivity in the cancer.

51. Results for an association with IGFBP-3 are also inconsistent. It has been suggested that high IGFBP-3 is protective by taking free IGF-I out of circulation, but the results from the studies are inconsistent.

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COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

POSSIBLE CARCINOGENIC HAZARD TO CONSUMERS FROM INSULIN-LIKE GROWTH FACTOR-1 (IGF-I) IN THE DIET

Annex 4. Key References

The following references are attached. For copyright reasons these cannot be made publicly available.

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