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

Influence of diet on IGF-I levels and cancer risk

Introduction and background to the review

1. Members have previously considered a number of papers concerning serum IGF-I levels and cancer risk. This topic arose from concerns regarding cattle treated with bovine somatotropin (BST) that were claimed to have higher levels of Insulin-like Growth Factor (IGF)-I in their milk, which was argued to increase the risk of cancer, notably breast cancer, in consumers of the milk.

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 the discussion, Members made a number of points and suggested that a systematic review would be useful and suggested a number of topics that could be included. These are attached at Annex A to this paper. For reasons of resource, a narrative review was conducted.

3. In April 2012, Members considered an introductory paper covering topics such as physiological control of levels of IGF-I, human physiological levels of IGF-1 and its binding proteins, IGF-I in foods and tissues use as a human medicine and toxicological studies on IGF-I as well as the available data on IGF-I and breast cancer (CC/2012/06). In November 2012, this was followed by a paper on prostate cancer (CC/2012/16) and in March 2016 a paper on IGF-I and lung and colorectal cancer. The minutes of these meetings are also attached at Annex A.

4. Members considered the associations between IGF-I and breast cancer and noted that, although there was evidence for an association between higher levels of serum/plasma IGF-I and breast cancer, there was insufficient evidence to link dietary IGF-I and breast cancer; the conclusions regarding IGF-I and prostate cancer were similar. When considering lung and colorectal cancer and plasma levels of IGF-I, it was noted that the results were inconsistent and difficult to interpret in the absence of good exposure data, especially since the majority of IGF-I measurements were taken only at baseline, many years before the cancers occurred.

5. Following the discussions at the March 2016 COC meeting, when it was noted that few of the available studies linked dietary intake with both serum IGF-I and with cancer risk, the aim of this paper is to bring together any data linking diet, IGF-I levels and cancer risk. Some of the data presented, which have been included for background and context, have been presented in previous papers but for ease of

reading have been included again in the current paper but updated as necessary. Similarly, some of the epidemiology studies considered have been included in previous papers.

Cancer and diet

6. It is well-known that the risk of some cancers can be affected by diet. It has been suggested by some authors that, in humans, different serum levels of IGF-I arising from different diets might explain the differences in the rates of certain cancers between Asian and Western countries (Allen, *et al*., 2002; Plant, 2007). As noted above, it has been further suggested that the elevated levels of IGF-I thought to occur in the milk of BST treated cattle would increase the risk of cancer.

7. Data on diet and lifestyle factors and cancer risk is compiled by the World Cancer Research Fund (WCRF) in a series of expert reports and a continuous update project. The WCRF/AIC Second Expert Report (2007) noted that there was limited suggestive evidence that milk decreased the risk of bladder and colorectal cancer and limited suggestive evidence that milk and dairy products increased the risk of prostate cancer; a specific mechanism was not identified, but IGF-I was discussed in the context of milk increasing IGF-I levels and this being potentially involved in prostate cancer, and, in the relationship between birth weight and breast cancer. In 2011, the continuous update program of the WCRF concluded that milk probably decreased the risk of colorectal cancer, thus strengthening their earlier conclusion (WCRF, 2011). Consideration of the studies assessing the association between milk consumption is beyond the scope of this paper, but is discussed in Chapter 4 of the WRCF report (WCRF, 2007 p129 onwards). The continuous update program of the WCRF also noted that being tall was strongly associated with an increased risk of prostate cancer (WCRF, 2014). As noted elsewhere height is also associated with IGF-I concentrations.

8. Milk has different properties to dairy products due to the processing involved and the latter have not been considered in this review. The associations between milk, milk protein, protein and other dietary exposures and circulating IGF-I levels are summarised below (paragraphs 26-32) and described in more detail in Annex B to this paper (see paragraphs 1-5, 6-31 and 32-58 of Annex B for animal, human observational and human intervention studies respectively).

9. The function of IGF-I in milk is unclear (Wiley, 2011). It may enhance the activity of lactase, or support the development of the neonatal GI tract. However, it is unknown whether it is absorbed intact and therefore able to have systemic effects (Burrin, 1997). Wiley (2011) discussed whether the consumption of milk outside the neonatal period could result in a longer growth period and considered that this might have an association with cancer since, for example, height and early menarche are associated with cancers. IGF-I levels in children rise in response to milk consumption and are higher in milk drinking children, but not in adults – so the effect of IGF-I may vary with life stage. IGF-I could have the greatest effect in adolescence (where IGF-I levels are highest) where consumption of it would be an anomaly.

Background on IGF-I

IGF-I function, structure and regulation

10. IGF-I (also known as somatomedin C) is a peptide growth factor with a structure similar to insulin. It has an important role in growth, particularly in childhood, with the IGF-I and growth hormone axis being involved in cell proliferation and inhibition of apoptosis. IGF-I deficiency as a result of a failure to make or respond to IGF-I results in growth failure, whereas acromegaly caused by excess growth hormone is associated with elevated levels of IGF-I. These conditions are associated with markedly lower and higher rates of certain cancers.

11. The mechanism of IGF-I action and regulation was considered in detail in CC/2009/08 and CC/2012/06. It is largely produced in the liver in response to stimulation by growth hormone (GH). In circulation, IGF-I is largely bound to six binding proteins, most usually (>90%) IGFBP-3. The IGFBP-3 is cleaved by proteases which increase the availability of IGF-I. IGF-I acts via the IGF-I receptor (IGFIR). Levels of IGF-I are controlled via a feedback loop with free IGF-I inhibiting the secretion of growth hormone, which in turn reduces the levels of IGF-I production. Free IGF-I binds to IGFBP-2 and IGFBP-5 with a greater affinity than it binds to the IGF-I receptor so increases in the levels of these binding proteins will reduce IGF-I activity.

IGF-I concentrations in food

12. With the exception of milk, there are few data available on the concentrations of IGF-I in foods derived from animals (see CC/2012/06 for further details). No data have been identified on the levels of IGF-I in meat, offal or eggs from food-producing animals, however, some data are available on the concentration of IGF-I in laboratory animals.

13. 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 (e.g. breed of cow) and external factors (e.g. 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 consumed by humans. The highest concentration of IGF-I in milk commonly consumed by humans is unlikely to be greater than 100 ng/ml.

14. 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.

15. Assuming a concentration of 101 ng/g in lean meat, poultry, sausage and milk[1], the dietary exposure of mean and high level consumers (97.5%) 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

[1] This value was the highest reported IGF-I level in the fifth post-partum milking of Ayrshire cows. Tissue IGF-I levels in laboratory animals are lower than 100 ng/g.

bw/day in adults. Production of IGF-I by humans has been estimated to be 10,000 µg/day (VPC, 1999) suggesting that dietary IGF-I is less than 1% of endogenous production, assuming a 70 kg adult and thus exposure of 1.83 x 70 = 128 µg. The VPC (1999) estimated exposure of 10 µg /day IGF-I from milk (assuming consumption of 1L milk with IGF-I present at 10 ng/ml).

Pharmacokinetics

16. This topic is considered in detail in paper CC/2012/06.

17. There is some evidence that some dietary components, notably acidic proteins such as casein and to a lesser extent bovine serum albumin, might protect IGF-I from breakdown in the gut lumen and that some IGF-I might be available for absorption.

18. Some studies in suckling animals suggest that IGF-I can be absorbed but others suggest that the treatment related effects of IGF-I in new-born animals are a result of IGF-I or its truncated form reaching the lumen of the intestine and affecting intestinal tissue from the luminal side rather than from exposure through blood levels of IGF-I. There is less evidence that IGF-I is absorbed in 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 (see paragraph 15 above).

19. Any dietary IGF-I that was 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 (2007) noted that metabolism of IGF-I occurs in the liver and kidneys.

20. Experiments with ligated intestinal sections from laboratory animals suggest that rapid proteolysis occurs in adult animals, but that this may be incomplete in some individuals. IGF-I was not broken down in the stomach of neonatal animals.

21. In an *in vitro* study by Elmlinger *et al*. (2007) term and pre-term milk samples were incubated with neonatal gastric juice. It was reported that IGF-I, IGF-II and IGFBP-3 survived the gastric enzymes to a significant degree if they were complexed with each other. If not complexed with a binding protein, IGFBP-2 was completely digested into small peptides and uncomplexed IGF-I and IGF-II were also partially digested.

22. An additional study on oral dosing in humans has been identified since this topic was initially reviewed. Mero *et al*., (2002) gave 12 adult volunteers I^{123} labelled recombinant IGF-I; serum samples were taken 60 minutes after dosing and were subjected to gel electrophoresis. It was reported that 69% of the radioactivity was detected in a peak at 0.6 kDa and 4% at a peak of 40-90 kDa. It was concluded that the IGF-I was fragmented during circulation since no radioactive IGF-I was eluted at the positions of free IGF-I (7.5 kDa) or the IGF-I binding proteins (150 kDa -high affinity complex of IGFBP-3 and an acid labile subunit).

Truncated IGF-I

23. 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). Little information is available on the concentration of the truncated form of IGF-I in other foods.

24. No significant new data on this topic have been identified.

Diet and serum/plasma IGF-I concentrations: Summary

25. The studies are briefly summarised in this section and described in detail in Annex B.

Animal studies

26. The effect of dietary composition has been assessed in a number of species including rats, mice, pigs, horses and chickens. In general, increased protein intake was associated with a higher level of IGF-I but not necessarily with growth hormone. Although the increased permeability of the gut in newborns may mean that IGF-I is more likely to be absorbed intact, higher IGF-I levels were not found in foals who had been fed colostrum from their dams rather than milk replacer.

Human epidemiology studies (largely cross sectional)

27. IGF-I levels are generally reported to be lower in breast fed babies.

28. A number of studies have investigated the association between dietary patterns and IGF-I levels. The results are not consistent but, in general, total energy, protein, fats, milk, fish, and calcium have been associated with increased IGF-I levels. Conversely, malnutrition is associated with lower levels of IGF-I

Human intervention studies

29. A variety of intervention studies have also been conducted, assessing the effects of supplementing the diet with protein, milk or other components.

Protein

30. Numerous studies have shown that protein supplementation (meat, vegetable, milk, soy) increases serum IGF-I levels.

Milk

31. In general and as noted above, formula fed babies have higher levels of IGF-I than breast fed babies, and where the formula has a higher protein content, the levels of IGF-I are higher still. Supplementation of the diet with whole milk has been shown to increase IGF-I in both children and adults; this was also observed in a

small study where adult volunteers were supplemented with colostrum. In a small number of studies where milk protein has been compared to other proteins it has been reported that milk protein increased IGF-I more than meat protein but less than soy protein. However, it should be noted that there are few studies available which do a direct comparison. In other studies, calcium, soy and a low fat/high fibre diet interventions were not shown to significantly affect IGF-I levels.

Studies that link IGF-I, diet and cancer risk

Animal studies:

32. Dietary restriction can affect the levels of circulating IGF-I which may then affect potentially cancerous events such as cell proliferation. Hursting, *et al*. (1993) found that a 40% dietary restriction for a group of 20 male Fischer rats resulted in a reduction in serum IGF-I to 44% of the levels in a group of 20 rats with *ad libitum* access to diet; serum growth hormone levels were 30% of levels in the *ad libitum* group. Hursting, *et al*. (1993) also investigated the effects of dietary restriction on growth of mononuclear cell leukaemia (MNCL) cells in groups of 35 male Fischer rats. The incidence of tumours in dietary restricted rats was 54%, as compared with 77% in rats with *ad libitum* access to feed. The tumours in dietary restricted rats had an increased latency period and a lower histological grade. The dietary restricted rats also had lower spleen weights. Serum taken from the dietary restricted rats caused less *in vitro* cell proliferation in CRNK-16 cells than serum from the control rats. When the dietary restricted rats were infused with rat growth hormone (resulting in serum concentrations of 50 ng/ml) their serum then induced a similar degree of proliferation of CRNK-16 cells as observed with serum from *ad libitum* fed rats. Hursting, *et al*. (1993) also studied the effect of dietary restriction on cell proliferation *in situ* by using groups of 15 male Fischer rats implanted diffusion chambers filled with CRNK-16 cells. The *in situ* proliferation index (ISPI), which is the diffusion chamber cell count, was significantly lower in dietary restricted rats than in those with free access to diet. When the dietary restricted rats were treated with growth hormone or IGF-I (respectively, 6.25 µg/h x 5 days or 10 µg/h x 5 days, administered by osmotic mini-pumps) the ISPI increased to that seen with the *ad libitum* fed rats.

33. Dunn, *et al*. (1997) studied the effects of diet restriction on the effect of a bladder carcinogen, *p*-cresidine, in heterozygous p53-deficient male mice (TGS-p53 strain). Groups of 10 mice were given diet *ad libitum* (control group), placed on a 20% dietary restriction (DR group) or placed on 20% dietary restriction and given IGF-I via a mini-osmotic pump to match the serum IGF-I levels of the control group (DR/IGF-I group). The serum levels of IGF-I in the DR group (271 ± 34 ng/ml) were on average 74% of the levels in the control group (367 ± 31 ng/ml) and the measured levels in the DR/IGF-I group were (390 ± 25 ng/ml). All animals had received a potentially carcinogenic dose of *p*-cresidine in their diet (1 week at 0.25% in the diet followed by 15 weeks at 5%) prior to allocation to their groups. Interim sacrifices at this time showed preneoplastic changes (focal and atypical hyperplasia) in the bladders but no cancers had so far developed. At termination, after 35 days of treatment with IGF-I, the number of animals with bladder tumours were 4 in the controls, 2 in the DR group and 6 in the DR/IGF-I group. The control and DR/IGF-I groups included animals with multiple tumours, whereas none of the DR group animals had multiple tumours. Furthermore the tumours in the control and DR/IGF-I

groups tended to be of a high histological grade than in the DR group. Cell proliferation, as measured by BrdUrd labelling, was 6-fold higher in the control and DR/IGF-I groups than in the DR group and these groups also had a 10-fold lower rate of apoptosis. It was concluded that the effects of dietary restriction on the prevalence and severity of tumours was mediated by reduced serum levels of IGF-I.

Human Studies

34. Mucci, *et al*. (2001) reported the results of a study of 112 men who had been admitted to hospitals in Athens, Greece, for disorders other than cancer. Participants were controls in a study of the aetiology of liver cancer. They were interviewed, filled in a food frequency questionnaire and had blood taken at the time of recruitment between January 1995 and December 1998. The blood samples were analysed for serum levels of IGF-I and IGFBP-3. There were negative associations between IGF-I levels and age and between IGF-I levels and intake of alcohol. There was no effect of smoking or coffee consumption on IGF-I levels. Among foods, the consumption of cooked tomatoes was substantially and significantly inversely associated with IGF-I levels, with a mean change of -31.5% (95% CI = -49.1% to -7.9%; p=0.014) for each serving per day. The IGF-I/IGFBP-3 ratio was also inversely associated with consumption of tomatoes, with a mean change of -27.7% (95% CI = -54.9% to -0.5%; p=0.047) for each serving per day.

35. Ma, *et al*. (1999 & 2001) performed a nested case-control study within the Physicians' Health Study cohort (a total of 22,071 healthy men aged 40 to 84 years in 1982 with blood samples available from 14,916 of the men), using prospectively collected plasma from 193 men within the cohort who had developed colorectal cancer in the following 13 years and 318 age- and smoking-matched control men. Intakes of skimmed milk, low fat milk, calcium from milk and calcium from dairy produce were associated with modest increases in plasma IGF-I, but intakes of red meat, poultry and fish were not associated with plasma IGF-I levels – see Table 1 below. Non-drinkers of milk who had the highest tertile ratio[2] of IGF-I to IGFBP-3 had an increased risk of colorectal cancer (relative risk = 3.05), but no significantly increased risk was seen in frequent drinkers of low fat milk with the highest tertile IGF-I/IGFBP-3 ratio (relative risk = 1.05). The authors concluded that there was a protective effect of dietary calcium on colorectal cancer incidence among men with a high IGF-I/IGFBP-3 ratio, despite a moderate positive influence of milk or dairy food on circulating IGF-I levels.

[2] A high molar ratio suggests higher circulating concentrations of free (i.e. active IGF-I).

Table 1: Relative risks (RR)[¥] of colorectal cancer according to IGF-I/IGFBP-3 ratio in plasma and intakes of various foods (Ma, *et al*., 1999 & 2001)

	IGF-I/IGFBP-3 molar ratio					
	Tertile 1		**Tertile 2**		**Tertile 3**	
	No Case subjects/No control subjects	**RR (95% CI)**	**No Case subjects/No control subjects**	**RR (95% CI)**	**No Case subjects/No control subjects**	**RR (95% CI)**
Skim/low-fat milk						
Tertile 1	15/37	1 (Referent)	27/35	1.96 (0.83-4.62)	31/25	3.05 (1.29-7.24)
Tertile 2	22/44	1.18 (0.48-2.93)	11/36	0.84 (0.33-2.16)	30/34	2.24 (0.97-5.18)
Tertile 3	13/17	1.59 (0.55-4.64)	16/29	1.43 (0.59-3.51)	16/39	1.05 (0.41-2.69)
				$P_{interaction}$ = 0.03 *		
Calcium from total milk						
Tertile 1	18/38	1 (Referent)	23/36	1.48 (0.65-3.39)	28/28	2.24 (1.00-5.02)
Tertile 2	22/40	1.02 (0.44-2.40)	18/35	1.14 (0.48-2.69)	31/29	2.49 (1.09-5.68)
Tertile 3	14/25	1.04 (0.41-2.64)	15/34	0.99 (0.43-2.28)	21/46	1.00 (0.43-2.36)
				$P_{interaction}$ = 0.18 *		
Calcium from dairy food						
Tertile 1	21/37	1 (Referent)	18/40	0.80 (0.34-1.91)	27/29	2.05 (0.93-4.55)
Tertile 2	22/45	0.81 (0.36-1.84)	22/32	1.23 (0.54-2.77)	37/29	2.78 (1.23-6.27)
Tertile 3	12/24	0.75 (0.29-1.93)	16/34	0.89 (0.39-2.03)	18/48	0.72 (0.31-1.67)
				$P_{interaction}$ = 0.14 *		
Red meat						
Tertile 1	13/29	1 (Referent)	19/31	1.83 (0.72-4.61)	22/31	2.38 (0.93-6.07)
Tertile 2	21/26	2.12 (0.84-5.36)	21/35	1.61 (0.66-3.92)	24/43	1.91 (0.76-4.80)
Tertile 3	21/49	1.14 (0.48-2.71)	14/39	0.99 (0.38-2.61)	35/30	3.12 (1.30-7.49)
				$P_{interaction}$ = 0.38 *		

	IGF-I/IGFBP-3 molar ratio					
	Tertile 1		Tertile 2		Tertile 3	
	No Case subjects/No control subjects	RR (95% CI)	No Case subjects/No control subjects	RR (95% CI)	No Case subjects/No control subjects	RR (95% CI)
Poultry						
Tertile 1	10/18	1 (Referent)	11/13	1.86 (0.50-6.93)	8/9	1.71 (0.46-6.32)
Tertile 2	17/47	0.63 (0.23-1.73)	20/41	0.94 (0.35-2.55)	33/48	1.61 (0.62-4.16)
Tertile 3	28/38	1.45 (0.57-3.67)	22/52	0.93 (0.38-2.28)	41/47	2.06 (0.81-5.19)
				$P_{interaction}$ = 0.50 *		
Fish						
Tertile 1	16/34	1 (Referent)	13/32	1.04 (0.41-2.68)	25/28	2.63 (1.08-6.39)
Tertile 2	26/40	1.63 (0.70-3.78)	24/43	1.46 (0.63-3.37)	30/32	2.24 (0.98-5.12)
Tertile 3	13/30	0.86 (0.33-2.26)	17/31	1.34 (0.53-3.39)	27/44	1.90 (0.81-4.44)
				$P_{interaction}$ = 0.93 *		

¥ Adjusted for age, smoking, body mass index, alcohol intake, multivitamin use, aspirin use and exercise.
* All P-values were two-sided.

36. The association between colorectal cancer risk with serum IGF-I, total IGFBP-3 and intact IGFBP-3 was investigated in a large case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (Rinaldi, *et al*., 2010). Between 1992 and 1998, blood samples were taken prospectively from participants from eight European countries. Those who developed cancer by December 2002 were identified from national cancer registries. Investigators compared 1,121 cases of colorectal cancer with 1,121 matched controls. Relative risks (RR) for colon and rectal cancers and 95% confidence intervals (CI) were calculated in relation to quintile categories serum IGF-I concentrations by conditional logistic regression. Possible confounders that were considered for to use for adjustment included body mass index, ratio of waist to hip circumference, height, smoking status, education, physical activity, alcohol intake and dietary intakes of red meat, processed meat, dairy products, fruit, vegetables and fibre. The results showed no associations with risk of colorectal cancer overall. Sub-group analyses showed some moderate positive associations of IGF-I levels with risk: in younger participants (less than 55 years-old) for colon cancer only (RR per quintile increase = 1.18; 95% CI = 1.00-1.39) and among participants whose milk intake was in the lowest tertile of the population distribution (RR for an increase in serum IGF-I of 100 ng/mL = 1.43; 95% CI = 1.13-1.93). There were no statistically significant ($p>0.05$) increases in colorectal cancer risk for an increase of 100 ng/ml of serum IGF-I associated with dietary intakes of dairy calcium, non-dairy calcium, dairy proteins, non-dairy proteins, red and processed meat, red and processed meat plus poultry and fish, fruit and vegetables, and fibre. Neither total IGFBP-3 nor intact IGFBP-3

were associated with risk of colorectal cancer with colon or rectal cancers separately.

37. In a study by Lin *et al*. (2012) the effects of smoking, green tea consumption and *IGF1*, *IGF2* and *IGFBP3* genotype on lung cancer risk were assessed in 170 primary lung cancer patients and 340 healthy controls, in a Taiwanese population. Green tea is stated to contain polyphenols which are strong antioxidants and the study suggested that risk was higher in smokers who did not consume green tea. The majority of the participants were aged >60 and were about 40% female. Fruit and vegetable intake, family history of lung cancer and exposure to cooking fumes were considered as confounding factors. A number of interactions were assessed but among green tea drinkers who drank more than one cup per day, *IGF1* $(CA)_{19}/(CA)_{19}$ and $(CA)_{19}$/X genotype carriers had a significantly reduced risk (OR = 0.06, 95%CI = 0.01-0.44) of lung cancer compared with *IGF1* X/X carriers. It was concluded that smoking-induced carcinogenesis could be modulated by green tea consumption and their growth factor environment.

38. Aronson *et al*. (2011) conducted a phase 2 intervention trial in men undergoing radical prostatectomy. The subjects were randomly assigned a diet with 5 g of fish oil which was given to decrease fat and to decrease the ratio of omega 6 to omega 3, or a control Western diet for 4-6 weeks, to assess the effects of the intervention on prostate cancer biomarkers. No effect on serum IGF-I levels was seen, although decreased prostate cancer proliferation was reported.

39. Tsilidis *et al*. (2013) investigated whether an association between 16 Single Nucleotide Polymorphisms (SNPs) associated with circulating IGF-I and IGFBP-3 concentration existed within sub-groups defined by protein intake. The study was conducted in 5,253 cases and 4,963 controls of European ancestry within the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (BPC3). The SNPs included rs1520220 and rs10735380 for *IGF-I*, rs3751380, rs197056, rs 197057 and rs174643 for *SSTR5* (somatostatin receptor 5), rs2270628, rs3110697, rs2854746, rs2854744 and rs2960436 for *IGFBP-3* rs1178463, rs17559, rs344352 and rs11865665 for IGFALS (insulin-like growth factor binding protein acid labile subunit). Per allele odds ratios for prostate cancer for the SNPs were compared across tertiles of protein intake, which was expressed as the percentage of energy derived from total, animal, dairy or plant protein sources using conditional logistic regression models (adjusted for age at blood draw, BMI, case control status, cohort, country (within EOC) and energy consumption. Total, animal, dairy and plant intakes were significantly associated with blood IGF-I ($p<0.01$) but not IGFBP-3 levels ($p>0.20$) or with risk of prostate cancer ($p>0.02$). After adjusting for multiple testing, the SNP-prostate cancer associations did not differ by intakes of protein, although two interactions by intake of plant protein were of marginal statistical significance. It was concluded that there was no evidence that the associations between 16 IGF-I pathway SNPs and prostate cancer differed by intakes of dietary protein. The weak association between dairy foods and prostate cancer risk observed in earlier studies was noted, with a further study (Allen *et al*., 2007) reporting an increased risk of prostate cancer associated with dairy protein.

Summary and discussion

40. COC members have considered a sequence of papers examining the possible association between circulating IGF-I and the risk of certain cancers. The topic originally arose as a result of concerns that cattle treated with the hormone BST might have increased levels of IGF-I in their milk and since this was a known growth factor, this could increase the risk of cancers in consumers.

41. IGF-I is present in milk, notably colostrum, and other animal tissues, though there are no data on levels in the tissues of food producing animals making dietary intakes difficult to estimate; though they are likely to be very low compared to endogenous IGF-I. The levels of IGF-I in milk vary depending on the breed and diet of the cows, but the levels decline rapidly as the number of milkings post-parturition increases.

42. As a peptide, it is likely that, following ingestion, IGF-I is broken down in the gut, although limited data suggest that dietary components such as casein may protect it from degradation. In a small study of oral absorption in adult volunteers, neither free nor bound IGF-I appeared to be absorbed intact. However, the intact IGF-I may act on the luminal wall rather than being absorbed. If IGF-I is absorbed, it is unclear whether it would behave any differently to endogenously produced IGF-I. It has been suggested that a truncated form of IGF-I might be more potent, but no recent data have been identified and it is unclear whether a truncated from would be absorbed.

43. A variety of studies have considered the association between circulating IGF-I and the risk of cancers-previously, members have concluded that these many of these data are inconclusive with respect to dietary IGF-I due to the absence of good exposure data, and since the majority of IGF-I measurements were taken only at baseline. Others have reported associations between dietary consumption of particular foods and cancer risk. In particular, the association between milk and dairy products has been considered. The WCRF considered that there was limited, suggestive evidence that milk might be associated with prostate cancer and dairy products and cheese with colorectal cancer but also limited, suggestive evidence that milk could be protective against bladder and colorectal cancer.

44. There are very few studies which have attempted to link both dietary exposure and IGF-I concentration, with cancer risk. Where these data are available, if anything, the effect of milk is protective against colorectal cancer, though this may be due to confounding by calcium.

45. Interpretation is complicated by the observation that proteins (including milk protein) intake can increase IGF-I levels; therefore any increase may be not indicate absorption of IGF-I.

46. A number of items remain outstanding from the COC's original discussion. These include potential mechanism(s) and comparative potency. In addition a number of cancers and their associations with IGF-I have not been reviewed (such as ovarian, thyroid and endometrial cancers). However, in the absence of data linking dietary IGF-I, circulating IGF-I and cancer risk, members are asked to

consider whether further progress is possible and if so, what would be the most useful topics to consider.

Questions for the Committee

47. Members are asked to consider:

a) The data from the studies by Ma and Rinaldi, investigating diet, IGF-I and cancer risk.
b) The association between circulating IGF-I and dietary exposure to milk or protein.
c) Any other comments they may have on the available data.
d) Whether this issue can be taken forward, and if so, what the next steps might be?
e) If members feel that further progress is possible, what would be the most useful topics to consider?

Secretariat
October 2016

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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

Influence of diet on IGF-I levels and cancer risk:

Outcomes of discussions and minutes of previous meetings

Secretariat
October 2016

July 2009

f) 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."*

g) 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.

h) 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).
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.
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.

April 2012

ITEM 5: Possible Carcinogenic Hazard to Consumers from Insulin-like Growth Factor-I (IGF-I) in the Diet (CC/2012/06)

15. Dr N Wallis declared a personal specific interest as she is involved in research on IGF-I. Dr Wallis was excluded from participating in the discussion on this item. Professor A Boobis declared a lapsed personal specific interest having been member of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) during the assessment of bovine somatotropin which, when used in cows, is said to increase the amount of IGF-I in milk. This was noted but not considered to preclude full participation in the discussion.

16. This topic had been considered previously as part of the November 2008 horizon scanning discussion (CC/2008/17) and further in paper CC/2009/08 which summarised the claims about the health effects of IGF-I presented in Dr Plant's book "Your Life in Your Hands". The paper prepared for the current meeting presented 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 and the association between blood levels of IGF-I and breast cancer.

17. Measurement of IGF-I was discussed and it was noted that differences were found between results from studies using plasma measurements compared to those using serum measurements. It was also highlighted that the use of different anticoagulants for plasma sample collection affected results. There was little information on differences arising from the techniques used for sample measurement as both in house and commercial methods had been used, and the possibility of a lack of specificity in commercial approaches was noted. Finally, it was not clear from some studies whether total IGF-I (i.e. bound and unbound IGF-I) or free IGF-I was being measured in the samples.

18. With respect to physiological levels of IGF-I, there were some suggestions of discrepancy between the plasma levels in animals and those in humans, as far higher plasma levels were reported in a study in Wistar rats than in humans. However, it was not clear what the plasma level had been in the Sprague Dawley rats used for the two year bioassay, and therefore how this would compare with humans. For humans it was agreed that the IGF-I would reach a maximum during puberty and then decline with age.

19. In considering the levels found in food, it was queried whether there were numerous or only one truncated form of IGF-I found in milk. Though there was little information, it was considered that there was only one form. Members were informed that human and bovine IGF-I were indistinguishable. The effect of dietary exposure to IGF-I was discussed. It was apparent that dietary IGF-I increased serum concentrations of IGF-I. However it was not clear that this was necessarily a direct effect or may have been mediated by increased protein intake. Members noted that IGF-I would probably be broken down in the stomach and need to diffuse or be taken up from the gut against a concentration gradient and that it was possible that the a Plant J, 2007 “Your Life in Your Hands”, updated edition, Virgin Books Ltd, London. ISBN 978 0 7535 1204 3 6 effect of dietary IGF-I on serum IGF-I was due to protein load or an effect of intake of dairy foods.

20. The findings of the two-year carcinogenicity study were considered and it was noted that the dose at which mammary tumours occurred was higher than that associated with increased mortality and effects such as hypoglycaemia. It was also noted that IGF-I had been administered subcutaneously and there would have been 100% bioavailability, while bioavailability from dietary IGF-I would be much lower. It was suggested that, while route to route extrapolation would be difficult, a worst case screen could be used assuming 100% oral bioavailability and using a margin-of exposure approach between rat and human plasma levels.

21. It was queried whether there had been any follow up to the clinical studies or from medicinal use. While nothing had been identified at the last literature search, given that IGF-I had been used as a medicine in the US since 1995 it was considered likely that there would be data available.

22. The physiological importance of early life exposure to high dietary IGF-I was highlighted and the need to bear this in mind when interpreting studies where neonates receive high doses.

23. In considering Annex 2 to this paper, the Committee agreed with the conclusions drawn in paragraphs 104 to 109, with some minor changes suggested for paragraph 109 to clarify that high parenteral doses cause a slight increase in malignant mammary tumours in mice, the effect of maturation of the gut in neonatal animals and the assumptions regarding absorption from the gut.

24. The epidemiological database on the association between IGF-I and breast cancer included studies of different designs and with conflicting findings. A number of aspects were discussed. Differences were noted between results for pre- and post-menopausal women and it was highlighted that only one study had excluded peri-menopausal women. A difficulty in assessing and comparing the studies was that it was not always clear what adjustments had been made for confounding variables; it was agreed that this information should be included in the summary tables produced by the Secretariat where possible. Other points to take account of were which studies had measured IGF-I before diagnosis of cancer and how long the time period between measurement and diagnosis of cancer had been, and what associations other risks or confounders had with breast cancer in the studies.

25. It was noted that the three meta-analyses differed in their aims and findings. Only one had looked at the dose-response relationship and, while this meant there was less power, it was considered indicative of the quality of the individual studies as this meta-analysis excluded papers where there was not enough information to determine a dose-response relationship between serum IGF-I and breast cancer.

26. In conclusion, the epidemiology papers indicated a tendency for association between dairy protein and circulating IGF-I levels and also between IGF-I and breast cancer in premenopausal women. It was considered that it should be possible to assess the association between dietary protein and breast cancer through IGF-I in 7 appropriate studies. Overall, the database was deemed insufficient to link dietary IGF-I directly with breast cancer

27. A further paper on other cancers would be prepared for a future meeting and a statement prepared in due course.

November 2012

ITEM 7: Possible Carcinogenic Hazard to Consumers from Insulin-like Growth Factor-1 (IGF-1) in the Diet: IGF-1 and Prostate Cancer (CC/2012/16) 342

36. This topic had been considered previously in 2008 and 2009 and, most recently, at the April 2012 meeting. At that meeting the Committee was presented with a review of the identity and physiological control of IGF-1, human physiological levels of IGF-1, IGF-1 in food and tissues, the use of IGF-1 as a human medicine, toxicology and other safety studies, and information on the association between blood levels of IGF-1 and breast cancer. The paper presented at this meeting provided information on the potential association between blood levels of IGF-1 and the risk of prostate cancer.

37. Overall the studies on IGF-1 and prostate cancer were considered inconsistent in terms of design, conduct and the results obtained. It was noted that many studies had been designed with a view to improving screening. There was also difficulty because some studies considered the stage of prostate cancer, but in others this was not reported. It was also noted that elevated IGF-1 may be a disease marker rather than a risk factor.

38. It was considered that while there was variability in the results of individual 359 studies, the meta-analyses were more representative, and indicated an association between IGF-1 levels and the risk of prostate cancer. It was queried how the heterogeneity in the studies had been accounted for in the meta-analyses, for example, had meta-regression been used. In addition, information on how the meta-analyses varied, e.g. in exclusion of papers, would be helpful in interpreting the differences. It was considered that the later meta-analyses were of better quality than the older ones.

39. While all of the studies reported were on endogenous levels of IGF-1, it was noted that there was no information on the possible effects of exogenous/dietary IGF-1 nor on the influence of exogenous factors on circulating IGF-1 levels. Hence, it is very difficult to interpret the implications of the findings for environmental IGF-1.

40. With respect to the conclusions of the review, it was noted that there were genome-wide association studies for prostate cancer, indicating the involvement of BRCA-1 and BRCA-2. A member proposed that SNPs were a more important element. It was also highlighted that the results were not necessarily inconsistent where they show the same trend, even if not all are statistically significant.

41. A final paper on lung, colo-rectal, ovarian and endometrial cancers would be prepared for a future meeting and this would also consider mechanistic elements. The outcomes from all three discussions would then be drawn together to form a final view on the carcinogenic hazard from IGF-1 in the diet.

March 2016

ITEM 4: Possible carcinogenic hazard to consumers from insulin-like growth factor-1 (IGF-I) in the diet. Part 3: The potential association of IGF-I with colorectal cancer risk and with lung cancer risk (CC/2016/01)

16. This paper was a third part of the evaluation of the possible carcinogenic hazard to consumers from IGF-I in the diet. The first and second parts had been considered at the March and November 2012 meetings, and covered: identity and physiological control of IGF-I, human physiological levels of IGF-I, IGF-I in food and tissues, its use as a human medicine, toxicology and safety studies, association between blood levels of IGF-I and breast cancer, and association with prostate cancer. This paper presented data on potential associations between blood levels of IGF-I and colorectal cancer and lung cancer.

17. It was noted that these papers considered blood levels of IGF-I rather than dietary exposure. The next paper would discuss dietary intake and contribution to

blood levels, as well as looking at the potential associations between blood levels of IGF-I and other cancers, including ovarian and endometrial cancer. Previously, the Committee had noted the importance of distinguishing between free and bound IGF-I in blood measures.

18. For colorectal cancer, there was general agreement to the summary and discussion on pages 38-39 of Annex B (paragraphs 61-67), noting that overall the findings of the studies are inconsistent, but the meta-analyses tend to show a positive association.

19. There was concern that many of the case-control and retrospective studies had a cross-sectional design with blood IGF-I levels measured at the same time as classification to case or control group, resulting in difficulty of interpreting these studies. For the prospective studies, only a few studies had longitudinal measures of blood IGF-I levels, and these did not describe any changes between the time points when measurements were taken. It was difficult to interpret studies where measurements of serum IGF-I had been taken at the start of the study and cancers occurred many years later. Without further information on other exposures during this time, it would be difficult to determine the biological plausibility of an association between IGF-I and cancer.

20. In addition, most studies did not adjust for dietary exposure, though one study that did, suggested the highest risk was associated with the lowest intake of IGF-I. The Committee queried whether there was any information on variations in IGF-I with a ‘normal’ diet or indication of daily variation. Recent papers on circadian rhythm were noted. Based on available animal studies, neonatal animals show substantial absorption of IGF-I, but there is variation across ages and the link with the diet has been explored more recently, and will be addressed in future Committee papers on IGF-I. If absorption was generally low, it would be difficult to perceive how a small change in circulating IGF-I could have a great effect on cancer risk. When this issue was initially raised it was suggested that partially digested (truncated) IGF-I was potentially absorbed, but few new data have been identified on this topic.

21. The Committee noted that a number of statements were made in the papers without the necessary supporting information provided. Some of this was as a result of considering the topic over a prolonged period, however often the supporting information was not available in the reviewed research papers.

22. In response to a question, Members were informed that some of the studies reviewed investigated IGF-I specifically while others considered the effects of binding proteins or growth hormones, in general.

23. The difficulty of drawing the available data together in a meta-analysis was noted, along with the need to choose which data to analyse. It was agreed that two Members would consider further the meta-analyses to review the studies selected, the data included from the studies, and to get a clearer impression of the data with respect to the size of the effect and range of the available estimates.

24. In considering lung cancer, a number of similar comments were made as for colorectal cancer, in terms of study design and the need to consider the meta-

analyses further. It was noted that some studies reported high level of IGF-I within lung tumours, but blood levels were not reported. The presence of high IGF-I within a tumour was not unexpected because the cancer cells grow more quickly than the surrounding tissue. The Committee was also unclear as to whether IGF-I in serum could be influenced by smoking.

25. It was agreed that the consideration of the meta-analyses for colorectal and lung cancer would support the drafting of a statement. In addition, the previous COC considerations would be checked to ensure consistency of interpretation with the new studies. A check would also be made of whether the book which prompted the COC's consideration had been updated.

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

Influence of diet on IGF-I levels and cancer risk – Detail

Secretariat
October 2016

Influence of diet on IGF-I levels and cancer risk – Detail

Animal studies

1. Rats aged 16 months old were fed a starch diet (13% sucrose and 49% wheat starch) or a sucrose diet (62% sucrose and 0% wheat starch) with or without supplementation with rutin, vitamin E, vitamin A, vitamin D, selenium or zinc for five months (Gatineau *et al*., 2015). The evolution of body composition and inflammation, plasma IGF-I concentration, antioxidant status, insulin sensitivity, muscle weight, superoxide dismutase activity, glutathione concentration and *in vivo* protein synthesis were assessed. Sucrose-fed rats lost significantly more lean body mass that the starched fed rats. Plasma IGF-I was unaffected by the different diet, but insulin sensitivity was reduced in the sucrose fed rats. The mean IGF-I ± SE levels across the groups was 6862 ± 0.17 ng/ml. Meal–induced stimulation of muscle protein synthesis was significantly lower in the sucrose fed rats. It was concluded that high chronic sucrose intake accelerated sarcopenia in older male rats through an alteration of post-prandial stimulation of muscle protein synthesis. This effect could be explained by a decrease of insulin sensitivity rather than by changes in IGF-I, inflammation or oxidative stress.

2. In 5 male Large White pigs, plasma levels of IGF-I rose after feeding, from a mean fasting level of 10 nM (76 ng/ml) reaching peak levels of about 13.5 nM (103 ng/mL) at 8 to 12 hours after a large meal (Morovat, *et al*., 1994). In groups of 4 piglets fasting levels of 5.9 ± 1.9 nM (45 ± 15 ng/ml) rose to a peak of 11.2 ±1.5 nM (86 ±11 ng/ml) at 8 hours after feeding.

3. In dogs, the effect of dietary protein and minerals on levels of growth hormone (GH) and IGF-I were studied (Hazewinkel, *et al*., 1996). Groups of six Great Danes were fed diets containing 13, 21 or 30% protein or mineral supplements (1.1% Ca + 0.9% P, 3.3% Ca + 0.9% P, or 3.3% Ca + 3.0% P), for 17 weeks (mineral diets) or 26 weeks (protein investigation). All the diets had similar energy contents. The low protein diet and the high Ca /low P diet were associated with decreased IGF-I, but unchanged GH. Six Great Danes given control diet had significantly higher plasma levels of GH and IGF-I than six miniature poodles given the same diet.

4. Kita, *et al*. (1996) examined the influence of nutrition on plasma IGF-I, IGF-II and IGF-binding protein (IGFBP) levels and on hepatic IGF-I gene expression in young chickens (Cobb broiler sire line). Food restriction for either 4 or 7 days of chickens fed diet containing 200 g/kg dietary protein decreased plasma IGF-I by 30% from the initial mean level of 15 ng/ml. When these chickens were re-fed *ad libitum* for 3 days after 4 days of restricted feeding, plasma IGF-I levels recovered to those of the control birds fed *ad libitum*. In chickens eating a low protein diet (100 g/kg protein), the plasma IGF-I tended to be lower but the decrease was not significant. The nutritional treatments had no effect on plasma IGF-II concentrations. IGFBP levels were lower in chickens fed the low protein diet than in those fed the high protein diet.

5. Serum IGF-I levels did not differ between new born foals given colostrum from their dams for the first 24 hours of life compared to those given a milk replacer, both

initially and in the 14 days following birth (Palm *et al*., 2013). The levels of IGF-I increased from approximately 125 ng/ml 24 h after birth to approximately 350 ng/ml at 14 days. It was noted that, for adequate immunoglobulin supply, all foals received 1L of equine plasma *i.v*. at a time corresponding to first suckling. The IGF-I levels in the equine plasma were 117.6 ± 11.5 ng/ml and 17.5 ± 1.6 ng/ml in the milk replacer. The IGF-I levels in the milk of the mares decreased from approximately 250 ng/ml within 8 hours of birth and remained low thereafter (approximately 10 ng/ml). The initial "dose" of IGF-I was 14 times higher in the colostrum fed foals for the first few hours and comparable thereafter, but did not affect levels in the two groups.

Human studies - observational.

Infants/breast feeding

6. Median IGF-I levels were higher in infants not being breast fed at 9 months compared to those still being breast fed (51.6 vs 44.2 ng/ml, p = 0.0005) (Madsen *et al*., 2011- abstract only available). IGF-I concentration was negatively associated with birthweight and positively associated with increase in weight, length and BMI between birth and 9 months of age.

7. Using data from the prospective ALSPAC cohort, serum IGF-I levels at age 7/8 years were 6.1 and 13.8 ng/ml higher in 488 children who had been partially or exclusively breastfed compared to those who were never breast fed (Martin *et al*., 2005)[3]. The increase in IGF-I levels per category of breast feeding exclusivity was 7.1 ng/ml; 95%CI : 0.3-13.9, p= 0.04). However when the model was adjusted for birthweight, gestational age, mother's age, socioeconomic and dietary factors, the association weakened and was no longer significant. Adjustment for IGFBP-3 made little difference and there was no evidence for any association between breast feeding and IGFBP-3 levels. The authors noted the results could possibly be due to residual confounding or chance but considered a larger study was warranted.

Children and adults

8. Serum levels of IGF-I were positively associated with consumption of red meats, fats and oils in 115 healthy subjects who completed a food frequency questionnaire (Kaklamani *et al*., 1999). In addition, serum IGF-I levels were independently and positively associated with energy intakes from lipids and negatively associated with energy intakes from carbohydrates. IGFBP-3 levels were independently associated with energy intake from saturated fat.

9. Allen, *et al*. (2000) found that mean serum IGF-I levels were similar in 226 meat eaters (153.4 ng/ml) and 237 vegetarians (153.4 ng/ml), but that the levels were 9% lower (statistically significant: *p*=0.002) in 233 vegans (141.2 ng/ml). The subjects were white men selected from the Oxford component of the EPIC cohort who had donated blood samples prior to 1998. Total serum testosterone was also raised in vegans, but free testosterone, androsterone, glucuronide and luteinising hormone were unaffected.

[3] The calorie and protein content of infant formula tends to be higher than human milk.

10. Allen, *et al*. (2002) conducted a cross-sectional study to determine whether a plant-based diet was associated with lower circulating levels of IGF-I. Participants in the study were selected from women recruited into the Oxford component of the EPIC cohort during 1994-1997, with roughly equal numbers in each of five 10-year age groups from ages 20 to 70 years. Three groups were chosen: 92 vegans, 101 lacto-ovo-vegetarians and 99 meat eaters. Blood samples were collected on average 4 months after recruitment into the EPIC cohort. Serum concentrations of IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, C-peptide and sex hormone-binding globulin (SHBG) were measured. The mean serum IGF-I levels were 13% lower in vegans than in meat-eaters or vegetarians (p= 0.0006). Mean concentrations of IGFBP-1 and IGFBP-2 were statistically significantly ($p \leq 0.005$) higher by 20-40% in vegans than in meat eaters or vegetarians. There were no significant differences in IGFBP-3, C-peptide or SHBG between the diet groups. The authors concluded that a vegan diet is associated with lower circulating levels of IGF-I and higher levels of IGFBP-1 and IGFBP-2 as compared with vegetarian or meat eating diets.

11. Holmes, *et al*. (2002) performed a cross-sectional study looking into the associations between diet and plasma levels of IGF-I and IGFBP-3 in 1037 healthy women who were the controls in a case control study of breast cancer risk (Hankinson, *et al*., 1998) nested within the prospective Nurse's Health Study cohort. The results were adjusted for various non-dietary factors (age, menopausal status, hormone replacement use, smoking, physical activity, BMI, parity, and history of breast feeding) known to be associated with IGF levels. Total energy intake was positively associated with IGF-I levels, with adjusted mean plasma concentrations of IGF-I across increasing quintiles being 181, 185, 191, 199 and 195 ng/mL (P_{trend} = 0.006). There were also positive associations between protein intake and plasma IGF-I (174, 188, 201, 192 and 196 ng/mL across the quintiles of protein intake, P_{trend} = 0.002) and between milk intake and plasma IGF-I (183, 189, 188 and 200 ng/mL across quartiles of milk intake, P_{trend} = 0.01). Higher intakes of fat, particularly saturated fat, were associated with lower plasma levels of IGFBP-3.

12. Giovannucci, *et al*. (2003) studied the influence of diet on 753 well-nourished men, aged 40 to 85 years. The subjects were participants in the Health Professionals Follow-up Study who had supplied a blood sample and completed a food frequency questionnaire. Men with relatively high intakes of protein (top quintile) and minerals (top quintile for the sum of K, Zn, Mg, Ca and P) had an approximately 25% higher mean plasma level of IGF-I than those in the lowest quintiles. High intakes of milk and fish (but not poultry or red meat) were associated with increases in plasma IGF-I and IGFBP-3 An increased IGF-I/IGFBP-3 ratio was positively associated with a high intake of poultry. Energy intake was positively associated with plasma IGF-I, but only in men with a body mass index of less than 25 kg/m^2. There were positive dose-related trends in levels of IGF-I, IGFBP-3 and IGF-I/IGFBP-3 ratio associated with quintiles of intake of protein, animal protein and vegetable protein (and carbohydrate for IGF-I/IGFBP-3 ratio).

13. Gunnell, *et al*. (2003) reported a cross-sectional analysis of the association between diet and serum levels of IGF-I and IGFBP-3 in 344 healthy middle-aged men from the ProtecT cohort (which investigated early detection and management of prostate cancer). The IGF-I was analysed by ELISA and IGFBP-3 using in an "in house" radioimmunoassay. Food intake was assessed by a validated food frequency

questionnaire and standard food tables. Raised levels of IGF-I were associated with higher intakes of dairy products (P_{trend} = 0.09), calcium (P_{trend} = 0.035), carbohydrate (P_{trend} = 0.08) and polyunsaturated fat (P_{trend} = 0.017). High levels of IGFBP-3 were associated with high intakes of polyunsaturated fat (P_{trend} = 0.05). High levels of IGF-I/IGFBP-3 molar ratio were associated with lower levels of vegetable consumption (P_{trend} = 0.045), with the association with tomato ketchup and tomato juice being particularly strong. The authors concluded that associations had been found with several aspects of diet previously linked to prostate cancer with elevated IGF-I levels being associated with dairy product, milk and calcium intake, while high intakes of vegetables and tomatoes or tomato containing products were associated with lower levels of IGF-I or its molar ration. The association with calcium, milk and dairy products could provide a pathway linking dietary intake of these factors with prostate cancer, a link noted by Chan and Giovannucci, (2001). It was noted that such an association went in the opposite direction to the association reported for colorectal cancer (e.g. Ma *et al*., 2001: Wu *et al*., 2002) another neoplasm associated with elevated IGF-I (Ma *et al*., 1999).

14. Probst-Hensch *et al*. (2003) investigated the association between diet and levels of IGF-I and IGFBP-3 in 638 individuals (312 men and 326 post-menopausal women) from the prospective Singapore Chinese Health study of 63,257 Chinese man and women aged 45-74 years. Data on food intake was obtained at recruitment by questionnaire. The study began to take blood samples, 1 year after commencement of the study from a random sample of 3% of the cohort. IGF-I levels were significantly higher in men (144 and 121 ng/ml respectively) while IGFBP-3 levels were higher in women (3710 and 4147 ng/ml respectively). Intake of soy was positively associated with IGF-I and IGF-I:IGFBP-3 molar ratio, but only in men. Calcium intakes from food and supplements were associated positively with circulating IGF-I, IGFBP-3 and IGF-I:IGFBP-3 molar ratio. Consumption of saturated fat was found to decrease, and intake of ω-3 polyunsaturated fatty acids and of dietary fibre were found to increase circulating IGFBP-3 levels. Due to the nature of the diet in this population, intake of soy foods was assessed in detail, this has not been considered further.

15. In a cross sectional study of 261 pre-menopausal Japanese women, it was found that there was no significant correlation between soy product, soy isoflavone intake and serum IGF-I or IGFBP-3 after controlling for age, total energy, percent, body fat and education level (Nagata *et al*., 2003 - abstract only available). Total fat intake was significantly inversely correlated with serum IGFBP-3; correlations of saturated and monounsaturated fats with serum IGFBP-3 were of borderline significance.

16. A cross-sectional study was conducted in 224 pre-menopausal and 162 post-menopausal women participating in the Prospect EPIC study in the Netherlands (Vrieling *et al*., 2004). Diet was assessed with a food frequency questionnaire and was considered to have limited variability. In the study it was found that total energy, protein, phytoestrogens and lycopene were not associated with IGF-I and IGFBP-3 levels. Alcohol was inversely and some measures of phytoestrogen were positively associated with plasma IGFBP-1 or 2. Milk and/or dairy products were not considered as a category.

17. Colangelo, *et al*. (2005) studied the associations of dietary factors with serum levels of IGF-I and IGFBP-3 in 459 black and 682 white male subjects of the Coronary Artery Risk Development in Young Adults study at the Year 7 (1992-1993) examination. The mean age of the subjects at Year 7 was 32.5 ± 0.13 years in white subjects and 31.5 ± 0.17 years in black subjects. Dietary history was assessed by interviewer-administered food frequency questionnaires at Year 0 and Year 7, and the Year 7 data were used in this study. Fasted blood samples taken at Year 7 were used in the study. Mean serum levels of IGF-I were higher in white subjects (184 ± 4 ng/ml) than in black subjects (164 ± 4 ng/ml) and IGFBP-3 was also higher in white subjects (3439 ± 35 ng/ml) than in black subjects (3056 ± 43 ng/ml). Analysis of covariance and multivariable linear regression were used to assess associations of IGF-I with dietary factors and race. Serum IGF-I levels were positively associated with dietary intakes of magnesium in black men (p=0.008) and white men (p=0.05). It was also associated with dietary calcium in black men (p=0.04) and non-significantly so in white men (p=0.09). In black men, IGFBP-3 levels were positively associated with dietary magnesium (p=0.04). Also in black men, one serving of milk per day was associated with an increase in serum IGF-I of 8.23 ng/ml ($P_{trend.}$= 0.05). There were no other significant associations between IGF-I or IGFBP-3 and intakes of milk or dairy products. Tests for interaction revealed no differences between black and white subjects in the associations of nutrients with IGF-I or IGFBP-3.

18. Serum IGF-I was measured in 226 free-living healthy Swedish men aged 42-76 years (Larsson *et al*., 2005). An average of 14 24h dietary telephone interviews performed over 1 year were used to estimate long term dietary intake. The IGF-I was measured by radioimmunoassay following acid-ethanol extraction to remove the binding proteins. There were significant positive associations between serum IGF-I and protein (p for trend = 0.001) and zinc (p for trend = 0.002) after adjusting for age. The difference in mean IGF-I concentration was approximately 17% (162 ng/ml compared with139 ng/ml) between the highest and lowest quintiles for protein and approximately 16% (166 µg/L compared with143 ng/ml) between the highest and lowest quintiles for zinc. Consumption of red meat (p for trend = 0.07) and fish and seafood was modestly positively associated with IGF-I concentrations. This was assessed by using a regression model which determined the differences in IGF-I concentration which corresponded to 2-SD differences in dietary intake. Other dietary factors (including milk) were not associated.

19. Ben-Shlomo *et al* (2005) reported data from the Barry Caerphilly Growth study, the follow up of an original randomised trial conducted between 1972-74 in which pregnant mothers were randomised to receive milk tokens, entitling them to additional free milk throughout pregnancy and until the child reached the age of 5 years. Milk intake was assessed by questionnaire during pregnancy and anthropometric measurements of the baby were taken regularly. Between 1997and 1999, 676 of the 952 original participants were traced, of these individuals, 633 provided a blood sample, anthropometric data and completed a questionnaire. The levels of IGF-I and IGFBP-3 were measured by radioimmunoassay. Subjects in the intervention group had lower mean levels of IGF-I than the controls (139 and 146.2 ng/ml respectively) but IGFBP-3 levels did not differ (6.43 and 6.47 g/ml respectively[4]). The model used adjusted for maternal systolic blood pressure, maternal smoking, birth weight, birth length and gestational age as well as adult

[4] The units are given as g/ml but mg/ml seems more plausible.

smoking, alcohol consumption and BMI. The authors proposed that early life exposure could influence the IGF axis in the long term, with potential implications for disease risk. The differing results compared to those of Hoppe *et al*. (2004) were noted.

20. The associations between early diet and IGF-I levels and childhood growth were investigated in children participating in the Avon Longitudinal study of Parents and Children (Rogers, *et al*., 2006). Data on diet (3-day unweighed food records) and height were available for 744 children (404 boys) and on diet and IGF-I for 538 children (295 boys). Measurement of height, leg length, diet and levels of serum IGF-I and IGFBNP-3 were made when the children were 7-8 years-old. After adjusting for energy intakes, IGF-I levels were positively associated with intakes of cows' milk (p=0.040) and of dairy products (p=0.027), as shown in Table 1 below. IGFBP-3 levels were also positively associated with these parameters (p=0.082 & 0.067, respectively). These associations were abolished on controlling for protein intake.

Table 1: Geometric mean levels (and 95% CI) of IGF-I and IGFBP-3 in serum of boys and girls according to quartiles of intakes of milk and dairy products (Rogers*, et al*., 2006)

	Quartile of intake				***p* value after adjustment for energy intake**
	1 (low)	**2**	**3**	**4 (high)**	
Cows' milk					
Number of children	143	135	136	124	
Median intake (g)	67	187	301	462	
IGF-I (ng/ml)	134 (125-142)	139 (131-148)	141 (133-149)	142 (134-150)	0.040
IGFBP-3 (ng/ml)	4467 (4230-4683)	4661 (4434-4899)	4709 (4477-4953)	4749 (4503-5009)	0.082
Dairy products					
Number of children	76	72	75	72	
Median intake (g)	113	257	401	631	
IGF-I (ng/mL)	134 (125-143)	139 (130-148)	138 (130-146)	144 (137-152)	0.027
IGFBP-3 (ng/ml)	4451 (4230-4683)	4599 (4389-4819)	4688 (4457-4937)	4831 (4588-5088)	0.067

21. The above paper followed an earlier and similar study of the ALSPAC cohort which reported that IGF-I was positively associated with intakes of protein, magnesium, zinc, calcium, potassium, and phosphorus and IGFBP-3 was positively associated with energy (Rogers *et al*., 2005). The IGF-I/IGFBP-3 ratio was positively associated with intakes of protein, zinc and phosphorus. None of the foods examined (notably red meat and vegetables) were associated with IGF-I levels. Cheese consumption was assessed but that of other dairy products were not.

22. McGreevy, *et al.* (2006) performed a cross-sectional analysis of the impact of nutritional factors measured by a dietary questionnaire on plasma levels of IGF-I, IGFBP-3 and their molar ratio in 95 African American men and 138 white American men, aged 33-83 years. The data were analysed by multiple regression analysis. Comparing quintiles of intakes of various dietary components by African Americans, serum IGF-I levels were positively associated (p<0.05) with the amount of dietary calcium and the number of daily food servings and negatively associated with alcohol intake, but there were no significant effects on these parameters in white men. Also in African Americans only, there were significant positive associations of IGFBP-3 with intakes of polyunsaturated fat and fibre and with the daily number of servings of vegetables. The molar ratio of IGF-/IGFBP-3 was positively associated with fat (total fat, saturated fat, monounsaturated fat and percentage of the diet from fat) in white men but not in African American men. The dietary amount of fibre from fruit and vegetables was negatively associated with the IGF-I/IGFBP-3 ratio in African American men but not in white men.

23. Martin *et al*. (2007) investigated the effect of childhood diet on the serum levels of IGF-I and IGFBP-3 in adulthood in 728 individuals in the Boyd Orr cohort. The participants in this study were originally surveyed in 1937-39 (median age 5.8 years). They were subsequently traced and flagged on the NHS central Register. In 1997-8, the surviving 3182 traced members of the cohort were send a food frequency questionnaire; 1648 individuals responded and further surveys and follow up were conducted. Blood samples were taken in 2002-3 from 728 participants. Dietary exposure in childhood was assessed by 7 day household food inventories. IGF-I, IGF-II and IGFBP-3 were measured by an in house double antibody RIA. Outcomes were expressed as regression coefficients for the change in IGF-I per standard deviation increased childhood nutrient or food intake as derived from levels of household consumption. In fully adjusted models, energy rich family diets in childhood were not associated with IGF-I, IGF-II, IGFBP-2 or IGFBP-3 in adulthood. IGF-I was inversely associated with childhood diets rich in milk and positively with diets rich in vegetables. IGF-I was not associated with family diets rich in protein, carbohydrate, fats, calcium meat or fruit. IGF-II, IGFBP-2 and IGFBP-3 were not associated with childhood family diets. It was concluded that childhood diets did not program the IGF-I system in adulthood. The association between IGF-I and milk or vegetable rich diets was noted to be a possible chance finding but could warrant further investigation.

24. Norat, *et al*. (2007) performed a cross-sectional study in 2109 women from various European countries who were controls in a case-control study of breast cancer nested in the European Prospective Investigation into Cancer and Nutrition (EPIC). Diet was estimated through questionnaires and concentrations of IGF-I and IGFBP-3 were measured in serum. The effect of different intakes of various nutrients and food types upon levels of IGF-I and IGFBP-3 were measured by comparing the levels in 1st quintile consumers (low intake) for each food/nutrient with the levels in the 5th quintile (high consumers). Levels of IGF-I were significantly (p<0.05) increased with increased intakes of protein, riboflavin, vitamin B_6, calcium, magnesium, phosphorus, potassium, milk and cheese; whereas IGF-I decreased with increased intakes of polyunsaturated fat, beta-carotene and vegetables. For IGFBP-3, levels increased with higher intakes of phosphorus and cheese and decreased with calcium and processed meat. The strongest associations (<0.001)

were for the increases in serum IGF-I with increased consumptions of protein, calcium and phosphorus. The authors of the study commented that they could not estimate the intake of protein by source, because this information had not been standardised across centres in EPIC. However, they noted that mean serum concentrations of IGF-I increased with increasing levels of consumption of red meat (p=0.27), poultry (p=0.18), fish (p=0.84) and eggs (p=0.56), all sources of animal protein, but none of the relationships were statistically significant.

25. In a study of bone health in 192 healthy adolescent girls (aged 12-22 years), milk consumption was associated with serum IGF-I levels but not any other calcium sources (Esterle *et al*., 2009) in post menarcheal participants only. Food intakes were estimated using a 7 day food recall. IGF-I was analysed using a commercial kit. In the two categories of post-menarcheal females milk intakes were 55-260 ml/day and >260 ml/day corresponding to mean ± SE IGF-I levels of 466 ± 29 and 493 ± 19 ng/ml respectively. This was statistically significant by multivariate analysis (p = 0.0019) adjusted for weight, years after menarche and vertebral area as well as the analysed nutrient from milk and other foods. The IGF-I levels were associated with calcium, phosphates, magnesium, protein and energy from milk but not from other foods. The authors proposed that the association was possibly due to an as yet unidentified component of milk.

26. The association between diet and serum concentrations of IGF-I and IGFBPs has been investigated (Crowe, *et al*., 2009) in a cross-sectional analysis of 1142 men and 3589 women taking part in the European Prospective Investigation into Cancer and Nutrition (EPIC). Diet was assessed using country-specific validated dietary questionnaires. Serum concentrations of IGF-I, IGFBP-1, IGFBP-2 and IGFBP-3 had been measured in blood collected from people from nine European countries who had been used as controls in various nested case-control studies of risk of various types of cancer. Almost all of the participants were of white European ethnicity. The results for serum levels were analysed by multiple linear regression after log transformation to approximate normal distributions. Adjustments were made for gender, age at blood collection, BMI, smoking status, alcohol intake and time since last consumption of food or beverage. Intake of protein was positively associated with serum IGF-I levels (p<0.001) and inversely associated with IGFBP-2 concentrations (p<0.001). There were similar associations for intake of animal protein and IGF-I and IGFBP-2 (p<0.001 for both), but not for the intake of protein of plant origin. When associations with animal proteins were analysed further, the results showed no significant associations between intakes of protein for meat & meat products, fish & shell fish and egg & egg products, and serum levels of IGF-I and IGFBP-2. The intake of dairy protein was however highly significantly positively associated with IGF-I and inversely associated with IGFBP-2. Each 1 SD increase in the intake of dairy protein was associated an increase in serum IGF-I of 2.4% (p<0.001) and a decrease in IGFBP-2 of 3.5% (p<0.001). There was also a highly significant positive association between calcium intake and concentrations of IGF-I and IGFBP-3 and an inverse relation with IGFBP-2. Each 1 SD increment increase in calcium corresponded to an increase in IGF-I of 3.3% (p<0.001) and IGFBP-3 of 0.8% (p<0.05) and a decrease in IGFBP-2 of 5.4% (p<0.01). The intake of monounsaturated fat was positively related to IGFBP-2 levels (p<0.05); however, there were no other statistically significant associations between the intake of total, saturated, and polyunsaturated fat and concentrations of IGF-I or IGFBPs. The

intake of starch was positively associated with concentrations of IGFBP-2; a 1 SD increment increase in starch corresponded to an increase in IGFBP-2 of 3.8% (p<0.05). Each SD increase in the percent of energy from sugar was associated with a decrease in IGFBP-1 concentrations of 5.0% (p<0.05). There was a positive association between the intake of fibre and IGF-I concentrations; each 1 SD increase in intake was associated with an increase in IGF-I of 1.9% (p<0.01). There was a significantly positive trend in IGF-I concentrations across quintiles of total and dairy protein intake; the differences in IGF-I between the lowest and highest quintiles were both 2.0 15.26 ng/ml. The overall test for trend for the inverse association between the intake of total and dairy protein and IGFBP-2 was significant and a lower IGFBP-2 concentration was clearly evident for participants in the 4th and 5th quintiles of protein intake. There was no significant association between calcium from non-dairy sources and concentrations of IGF-I (p=0.128) and neither was there evidence of significant heterogeneity of the trends for serum concentrations of IGF-I with protein, dairy protein, or calcium between men and women. The association between dietary intake and the ratio of IGF-I/IGFBP-3 was assessed but because there was little association between any of the dietary variables and IGFBP-3 concentrations, associations between dietary factors and the IGF-I/IGFBP-3 ratio were similar to those for IGF-I.

27. Tekle *et al.* (2010) measured the levels of IGF-I, IGFBP-I, coenzyme Q10 and vitamin E in 4 groups of 100 women from Poland, Sweden, Serbia I and Serbia II (the latter being an environmental emergency area). The results are presented as SD scores and have not been described as means. However, the distribution of levels were comparable for the Polish and Serbia I women, but the Swedish women had a sharper distribution. In the Serbia II women, the distribution was interpreted to indicate a shift towards higher IGF-I levels. IGFBP-I levels were lower in the Polish women compared to the Swedish women, and lower again in the Serbia I group. Levels were higher in the environmentally damaged Serbia II group. The lower levels of IGF-I in the Serbian and Polish women may have been due to lower nutrient intakes. In a similar study by the same group in Kenya (Theuri *et al*., 2013), levels of IGF-I were lower and IGFBP-1 higher in the nomadic Sombura population compared to individuals from the urbanised areas of Nairobi, this was interpreted to indicate malnutrition.

28. Bradbury *et al*., 2015 analysed plasma IGF-I in 1883 post-menopausal women from the Million Women Study a cohort of approximately 1.3 million UK women. At recruitment, the women completed a self-administered questionnaire which collected information on socio-demographic, lifestyle and reproductive factors. The women were then re-surveyed 3, 8 and 12 years later. The three year survey contained questions on the frequency of consumption and the types of foods consumed over a typical week. The blood samples were collected from a random sample of women who had completed both the 3 and 8 year surveys. Multivariate analysis was used to examine correlates of plasma IGF-I concentrations. The basic model for all analyses adjusted for age, BMI, smoking status, alcohol intake, HT use, log transformed total energy intake and total protein. A number of anthropometric factors were also assessed. The IGF-I was analysed by immunoassay and appears to be total, although this is not completely clear. Women in the highest quintile of total protein and total dairy protein intakes (expressed as percentage of energy) had plasma IGF-I levels which were 7.6 and 5.5% higher (112.21 and 111.45 ng/ml)

respectively than women in the lowest quintiles (58 and 106.11 ng/ml) (*p* trend <0.05 for both). Other factors which were significantly associated with reduced IGF-I were consuming ≥14 alcoholic drinks compared to 3-7 alcoholic drinks per week (8.8% lower), current vs non-current HT users (9.9 % lower), current use of oestrogen alone vs oestrogen + progestagen (16.9 % lower), obese vs overweight (6.8% lower) and women who reported wearing larger vs smaller clothes sizes at age 20 (4.9 % lower). The authors noted that although the positive associations between total protein and dairy protein intakes and circulating IGF-I concentrations were generally modest , measurement error associated with dietary assessment done almost 6 years before blood collection suggests that the true association might be larger than observed, although it was possible that non-random errors could have biased the findings away from null.

29. Günther *et al*. (2015) looked at dietary patterns in early life, adiposity rebound and puberty which were considered to be critical growth periods and the effect on the growth hormone IGF-I axis. IGF-I levels in adulthood were not associated with childhood dietary patterns but some were associated with IGFBP-3..

Human - intervention studies

Protein

30. Twelve insulin dependent diabetics aged 15-21 years were given diets containing 10 or 20% protein respectively for ten day periods (Rudberg *et al*., 1991).The level of IGF-I was increased after the high protein diet, but GH was unchanged (abstract only available).

31. In a study of renal function, nine normotensive, non proteinuric type 1 diabetic patients were fed in random order a diet containing animal protein (1.1 g/kg bw/day) or vegetable protein (0.95 g/kg bw/day) (Kontessis *et al*., 1995- abstract only available). Plasma levels of IGF-I (measured by radioimmunoassay) were slightly (but significantly) higher with the animal protein diet (1.1 ± 0.6 vs 0.9 ± 0.13 U/ml, $p < 0.05$).

32. In a 6-month randomised double-blind placebo-controlled trial with a 6-month post-treatment follow-up (Schürch, *et al.,* 1998), 82 elderly patients (aged 80.7 ± 7.4 years) with recent osteoporetic hip fracture were given 20 g/person/day of protein as dietary supplements (90% milk proteins). Controls received an isocaloric placebo. All patients (test and control) also received supplements of 550 mg/person/day of calcium and one dose of 200,000 IU of vitamin D_3. Serum levels of IGF-I increased in test and control groups, but the increase was greater in patients that received the protein supplement: 51.5% greater after 6 months of treatment and 23.7% after the 6-month post-treatment follow-up. The mean serum levels of IGF-I in controls and in the test group were, respectively, 24.5 & 45.1 ng/ml at the end of treatment and 23.1 & 34.5 ng/ml at the end of follow-up.

33. In a study by Roughead *et al*. (2003), 15 healthy post-menopausal women were given diets high or low in meat for 4 weeks each in a crossover study designed to assess the effect of meat on calcium retention or bone status. IGF-I levels were not affected by the amount of meat in the diet being 12.7 and 12.8 ng/ml in the low

and high meat diets respectively. The diets were designed to provide similar quantities of calcium and contained 45 or 297 g meat/day. Blood samples were taken at weeks 4 and 8 of the dietary period and IGF was measured by ELISA.

34. Twelve subjects with type 2 diabetes were included in a 5 week crossover trial of a high protein weight maintenance diet (Nuttall *et al*., 2003). The control diet was 55% carbohydrate, 15 % protein and 30% fat. A second diet consisted of 40% carbohydrate, 30% protein and 30% fat; the source of the protein was not stated. There was a 2-4 week washout period between the two phases. Mean fasting IGF-I and GH were elevated when the participants were on the high protein diet (149 ± 16 vs 205 ± 36 ng/ml, $p < 0.05$). IGFBP-3 was not measured.

35. Thirty two subjects aged ≥ 50 years with protein intakes less than 0.85 g/kg/day were given high (0.75 g/kg) or low (0.04 g/kg) meat protein supplements for 9 weeks (Dawson-Hughes *et al*., 2004). Isocaloric diets were maintained by advising subjects to reduce carbohydrate intake. Selected serum biochemistry was assessed at baseline and on days 35 and 49 or 63. IGF-I was not included at baseline, however the authors considered that as there was good agreement in the other clinical parameters, it was likely that baseline IGF-I would be comparable between groups. There were no changes in serum calcium excretion between the two groups, and this did not differ over the course of the study. The high protein group had higher levels of IGF-I ($p = 0.008$) and lower levels of N-telopeptide (a marker of bone resorption) than the low protein group. IGF-I was measured by radioimmunoassay but it is unclear whether this was free or bound (total). The levels were 8.09 ± 2.52 and 8.47± 2.21 ng/ml at days 35 and 49 or 63 in the low protein group and 11.52 ± 4.04 and 11.45 ± 1.9 ng/ml in the high protein group.

36. Twelve patients with type 1 diabetes were given isocaloric diets for 10 days with either high or low normal protein content in an open randomised cross-over study (Hedman *et al*., 2005). The protein came from meat and vegetable sources. There was an eleven days washout period between the two diets. There were no significant differences in IGF-I and II (free or circulating), IGFBP-3 and ghrelin measured after the low and high protein diets. Total IGF-I (mean ± SD) changed from 120 ± 33 to 121 ± 33 ng/ml and 131 ± 39 to 117 ± 28 ng/ml on the low and high normal protein diets respectively and free IGF-I from 138 ± 103 to 155 ± 88 ng/L and 257[5] ± 292 to 152 ± 88 ng/L on the low and high normal protein diets respectively. IGFBP-3 changed from 3670 ± 498 to 3710 ± 588 µg/L in the low normal protein diet and 3818 ± 616 to 3669 ± 577 µg/L in the high normal protein diet. IGFBP-2 was slightly higher in the low compared to the high protein group. It was concluded that dietary protein intake did not affect the circulating IGF-I system.

37. In a study by Ballard *et al*. (2005) 51 subjects aged 18-25 years were given a twice daily protein supplement (42 g protein and 24 g carbohydrate) (n= 29) or 70 g carbohydrate (n = 23) for 6 months accompanied by an exercise regime of running and resistance training. IGF-I, IGFBP-3, serum bone alkaline phosphatase and urinary *N* telopeptide collagen crosslink (NTx) were measured at baseline, 3 and 6 months. Three day diet records indicated that there were no differences in energy

[5] This SD value was caused by one very high reading of 1116 ng/L. If omitted, the mean would be 179 ± 118 ng/L.

intakes between the two groups but following supplementation, protein intakes were 2.2 ± 0.1 and 1.1 ± 0.1 g/kg in the protein and carbohydrate groups respectively. The increase in plasma IGF-I was greater in the protein group (time x supplement group interaction, p = 0.01), but there were no changes in IGFBP-3 by time or supplement group. Total IGF-I was measured by radio immunoassay with IGFBP-3 being extracted from serum by acid ethanol precipitation. IGF-I changed from a baseline of 135.9 ± 7.5 ng/ml and 131.8 ± 8.6 ng/ml in the protein and carbohydrate groups respectively; the final levels are not given in the text but can be estimated to be approximately 150 and 125 ng/ml from the figure provided. Both alkaline phosphatase and NTX increased over time and were greater in the carbohydrate group.

38. Hunt *et al*., (2009) investigated the effect of protein on calcium retention in 27 healthy post-menopausal women. In a 2 x 2 factorial cross-over design the women consumed either 675 or 1510 mg Ca/d with low or high protein (providing 10 or 20% energy) for 7 weeks each followed by a 3 week washout period; the additional protein came from meat. Isotopes were used to estimate calcium retention and a range of markers of calcium and bone health were measured, including IGF-I. High compared to low protein increased mean serum IGF-I by 27% from 129.8 to 145.0 ng/ml in the low calcium group and 160.3 to 190.8 ng/ml in the high calcium group.

39. A group of 71 postmenopausal women were randomised to receive 40g daily of either soy protein or milk protein for 3 months in a double blind placebo controlled study (Arjmandi *et al*., 2009). Of these 42 (20 soy and 22 milk) completed the study. Food frequency questionnaires were taken at baseline and at the end of the study period; there were some differences between groups but intakes were similar at baseline and at the end of the study. Both treatments improved IGF-I status but soy had a more pronounced effect increasing levels by 69% compared to 39%. The baseline IGF-I levels were 94.77 ± 14.57 ng/ml (*p* =< 0.0001) () increasing to 159.7 ± 15.11 (*p* =0.0045) in the soy group compared to 106.97 ± 14.27 ng/mlincreasing to 145.89 ± 14.27 in the milk group. When analysed by HRT status, the effect of soy was most marked in women who were not on HRT. The baseline IGF-I levels were 102.47 ± 19.08 ng/ml increasing to 151.46 ± 28.38 (*p* =0.002) in the soy group compared to 118.19 ± 14.12 increasing to 161.83 ± 19.53 ng/ml (*p* =0.001) in the milk group.

40. In a small study by Cao *et al*. (2011) the effect of a high protein and high potential renal acid load (PRAL) on calcium absorption and retention and markers of bone health were assessed. The design was a randomised crossover one with 16 postmenopausal women being given a low protein, low PRAL diet (LPLP) or a high protein, high PRAL (HPHP) diet for seven weeks. The protein contents of the diets were 61 or 118 g/day respectively. The protein content was largely increased by increasing the amount of meat consumed. IGF-I was measured by a commercial ELISA kit. Compared with the LPLP diet, the HPHP increased the serum IGF-I concentrations of the participants. The results for mean IGF-I were 132. 76 ng/ml in the LPLP group increasing to 137.34 ng/ml at 7 weeks, while in the HPHP group mean IGF-I increased from 132. 76 ng/ml to 186.94 (p < 0.0001 by ANCOVA).

Milk

Infant formula

41. Full and pre-term infants with 1 and 3 weeks of postnatal life were investigated in a study by Diaz-Gómez *et al*., 1997. In the first week after birth, IGF-I and IGFBP-3 levels were lower in the preterm infants, with levels being lower in the most premature of the babies. Pre-term infants fed with human milk supplemented with formula had higher serum IGF-I levels than those receiving milk formula alone (mean ± SEM 48.2 ± 9.5 ng/ml and 25.4 ± 4.4 ng/ml respectively). IGF-I and IGFBP-3 were correlated with each other and with energy and protein intake. Multiple regression analysis confirmed that energy intake and serum IGFBP-3 were the most predictable variables with regard to IGF-I levels in the neonatal period.

42. In a multicentre European study, 1138 healthy formula fed infants were randomly assigned to receive cows' milk based infant and follow on formula containing lower protein (1.77 and 2.2 g/100 kcal) or higher protein (2.9 and 4.4 g/100 kcal) for the first year; the calorie content was the same due to adjustment of the fat content (Socha *et al*., 2011). The intention of the study was to assess the effect of protein intake in infancy on serum amino acids and the IGF-I axis and its possible relationship to growth in the first two years of life. Biochemical variables were measured at 6 months in 339 infants receiving low protein formula, 333 infants receiving high protein formula and 237 breast fed infants. Essential amino acids and IGF-I were significantly higher in the high protein group, whereas IGFBP-2 was lower and IGFBP-3 did not differ significantly. Total IGF-I, free IGF-I and IGFBP-3 were all significantly lower (approximately ≤ 60%) in the breast fed infants than in the formula fed groups.

Table 2: Changes in the IGF-I axis following protein supplementation (Socha *et al*., 2011).

Variable ng/ml	**Low Protein***	**High Protein**	***P* value (LP compared with HP)**	**Breast Fed**
IGF-I free	0.43 (0.27, -0.77)	0.60 (0.34, 1.11)	< 0.001	0.31 (0.21, 0.48)
IGF-I total	34.7 (17.7, 57.5)	48.4 (27.2, 81.8)	<0.001	14.1 (5.1, 33.2)
IGFBP-2	1090 (865, 1438)	765 (575, 1013)	< 0.001	1370 (1055, 1740)
IGFBP-3	2908 (2449, 3440)	2696 (2538, 3483)	0.248	2454 (1984, 2794)

*All values median (interquartile range)

43. Total IGF-I at 6 months of age was associated with growth at 6 months but not thereafter. It was noted that because of the quality of cows' milk, formula fed infants had higher intakes of protein than breast fed infants and are reported to have higher levels of IGF-I along with different growth patterns.

44. In a European multi-centre, randomised clinical trial, 1090 term, formula fed infants were given infant formula and follow on formula (from 5 months) with either low or high protein contents for the first 12 months of life; a comparison group of 501 breast fed infants were also included (Rzehak *et al*., 2013). The low protein formulas contained 1.25 and 1.6 g/100ml protein in the infant formula and the follow on formula respectively whilst the high protein formulas 2.5 and 3.3 g /100ml. The

protein content of human milk was stated to be 1.2 ± 0.15 and 1.1 ± 0.15 g/100 ml at 3 and 6 months respectively. Eight single SNPs of the *IGF-I*-(rs6214, rs1496495, rs978458, rs7136446, rs10735380, rs2195239, rs35767 and rs35766) and two of the *IGFBP-3*- (rs1496495, rs6670) gene were analysed. When stratified by feeding group the concentrations of IGF-I, IGFBP-3 and the molar ratio were :

Table 3: Changes in the IGF-I axis following protein supplementation (Rzehak *et al*., 2013).

	Low protein formula		**High protein formula**		**Breastfed**	
	Mean	95%CI	Mean	95%CI	Mean	95%CI
IGF-I Total (ng/ml)	45.17	40.06-50.28	63.90	56.59-71.21	25.41	20.66-30.15
IGF-I Free (ng/ml)	0.60	0.52-0.67	0.81	0.71-0.9	0.45	0.37-0.53
IGFBP-3 (ng/ml)	2968.06	2866.33-3069.79	3006.02	2896.1-3115.82	2428.18	2313.24-2543.13
Molar ratio IGF-I/IGFBP-3	0.06	0.05-0.07	0.08	0.07-0.09	0.04	0.03-0.05

45. Serum levels of total and free IGF-I, IGFBP-3 and the molar ratio of IGF-I/IGFBP-3 at age 6 months were regressed on determined SNPs and feeding groups in 501 infants. IGF-I- SNPS rs1520220, rs978548 and rs2195239 significantly increased total IGF-I and molar ratio IGF-I/IGFBP-3 by approximately 1.3 ng/ml and 1.3 per allele respectively; compared to the low protein formula infants, concentration and molar-ratio were increased in high protein infants by approximately 1.3 ng/ml and 1.3 per allele respectively and decreased in breast fed infants by approximately 0.6 ng/ml and 0.6 per allele respectively. IGFBP-3 was only affected by the breast fed group with 450 ng/ml lower levels than the LP group. No gene feeding group interaction was detected for any SNP, even without correction for multiple testing. It was concluded that variants of the IGF-I gene play an important role in regulating serum levels of the IGF-I axis but there was no gene-protein interaction. The authors stated that the predominant nutritional regulation of IGF-I and IGFBP-3 gave further evidence that higher protein intakes contribute to metabolic programming of growth.

46. In a randomised study by Putet *et al*. (2015) infants received formula containing 1.8 (n= 74) or 2.7 (n=80) g protein/100 kcal for the first 4 months of life; a group of breast fed infants (n=84) were also followed as the reference comparison. The intention of the study was to establish whether growth velocity was mediated by IGF-I. Both formula fed groups had similar median levels of IGF-I at 4 months (66 and 63 ng/ml respectively) and both were significantly higher than the levels in the breast fed infants (46 ng/ml). At 9 months the median IGF-I levels were 62, 63.5 and 43 ng/ml respectively. IGFBP-3 levels were also similar in the two formula groups and lower in the breast fed group at 4 and 9 months (1.8, 1.7 and 1.5 ng/ml at 4 months and 1.9, 1.8 and 1.7 ng/ml at 9 months). It was concluded that in formula fed infants, the protein content did not affect growth but did affect length and head circumference, suggesting that factors other than IGF-I could be involved.

Milk supplementation

47. In an intervention study, Cadogan, *et al*. (1997) gave a pint per day (568 ml/person/day) of either whole milk or reduced fat milk to groups of 44 or 38 (respectively) white schoolgirls, aged 12.2 ± 0.3 years, from Sheffield for 18 months. The serum concentrations of IGF-I increased over the duration of the study in both sets of girls, with the increase being significantly greater in those given whole milk (35% *vs*. 25%, *p*=0.02). Initial serum of IGF-I levels were 390 ± 169 and 385 ±163 ng/ml in girls given whole milk and reduced fat milk, respectively and were 522± 104 and 448± 105 ng/ml after 18 months. No significant changes occurred in levels of bone alkaline phosphatase or in serum levels of osteocalcin, *N*-telopeptide, deoxypyridinoline, oestradiol and parathyroid hormone.

48. In a randomised double-blind study by Mero *et al*. (2002), adult volunteers (n=19) were given 20 g/day of a bovine colostrum supplement or (n=11) a maltodextrin control during a two week training period. After the colostrum supplementation, serum free IGF-I and salivary IgA were increased. The numbers are not given, but from the figures presented IGF-I increased from 152.7 to approximately 183.2 ng/ml in the treated group, but was unchanged in the placebo group. A daily increase of 2.9 ng/ml was reported. This was lower that the change reported in an earlier study Mero *et al*. (1997) where nine male sprinters and jumpers were given 125 ml milk or 25 ml Bionervi colostrum supplement (in a 125 ml drink) or a 125 ml Bionervi colostrum supplement for 8 days with a 13 day gap between treatments. It was suggested (Mero *et al*., 2002), that the increase could be due to direct absorption of IGF-I or enhanced stimulus of IGF-I synthesis. The latter was thought to be more likely since studies with radiolabelled IGF-I by the same group detected only low molecular mass material circulating following dosing.

49. In a school milk intervention study by Zhu *et al*. (2005) Chinese girls aged 10 years were given 330 ml milk supplemented with calcium (n= 177) or supplemented with calcium and vitamin D (n =210) for 24 months and compared to 219 controls who did not receive milk. Milk was not consumed on weekends or holidays. The subjects were not randomised within the school for practical and ethical reasons, but the schools had a similar socio-economic status. IGF-I levels increased by 16.7- 23.3% which was significant in the individual analyses but not once the analyses had been adjusted for clustering by school. The reason for the high variability between schools was unclear but was not thought to be due to assay variation since the samples were assayed together. The results are given in table 4 below:

Table 4: Effect of fortified milk supplementation on IGF-I levels (Zhu *et al*., 2005)

IGF-I (ng/ml)	**Ca milk**	**Ca + Vitamin D Milk**	**Control**
Baseline	245.97 ± 85.77	258.56 ± 91.64	260.71 ± 88.15
12 month	269.47 ± 103.72	294.89 ± 100.21	264.03 ± 93.94
24 month	413.10 ± 126.54	442.21 ± 131.58	355.30 ± 120.80

50. In a pilot study to assess the effects of milk on somatotrophic hormones, 46 pre-pubertal children in Mongolia were given 710 ml whole milk daily for one month (Rich-Edwards *et al*., 2007). At the end of the study, the children had higher plasma

levels of IGF-I (the mean increasing from 290 ± 93.98 to 358.34 ± 125.62 ng/ml (p< 0.0001) and IGF-I/IGFBP-3 ratio (the mean increasing from 0.064 ± 0.016 to 0.073 ± 0.019 ng/ml (p< 0.0001)) and 75th percentile of growth hormone. In a separate study, 28 Boston schoolgirls were given 710 ml low fat milk or a macrobiotic milk substitute for 1 week in a crossover study. After drinking the milk for 1 week, the girls had small non-significant increases in IGF-I (mean IGF-I level 270.60 ± 89.46 ng/ml compared to 257.26 ± 69.12 ng/ml after the milk substitute) IGF-I/IGFBP-3 and GH. It was concluded that milk could raise GH levels. The children from Mongolia were included in the trial as a population of US children with very low dairy intake could not be identified.

51. A group of 193 overweight adolescents (aged 12-15 years) were randomised to drink 1L of skimmed milk, whey casein or water for 12 weeks (Larnkjær *et al*., 2014). A sub group of 32 individuals acted as a pre-test control for 12 weeks before the start of the study with no intervention occurring. The protein content of skimmed milk, whey or casein was comparable at 3.46-3.48%. Examinations included anthropometry, diet registration and blood samples which were analysed for IGF-I, IGFBP-3; the IGF-I/IGFBP-3 ratio was calculated to assess available IGF-I. IGF-I and IGFBP-3 were assessed by chemiluminescence methods. IGF-I increased compared to baseline in the skimmed milk group (p= 0.015) and tended to increase in the casein group but this was not significant. IGFBP-3 but not IGF-I increased in the skimmed milk compared to the water group. The data is not provided in detail, but based on the figures provided, IGF-I increased by approximately 5, 10 and 2 ng/ml in the casein, skimmed milk and whey groups respectively, whereas in the pre-test controls and the water group, levels declined by around 5 and 4 ng/ml. For IGFBP-3, levels increased by approximately 0.15, 0.10 and 0.2 µg/ml in the casein, skimmed milk and whey groups respectively compared to a 0.4 µg/ml increase in the pre-test controls and 0.18 µg/ml decrease in the water group. IGF-I availability was not affected by treatment. There was no difference in height or height Z score for any of the test groups compared to the water controls. However height Z score decreased within the whey group. It was concluded that skimmed milk and casein could have a stimulating effect on the IGF-I system.

Milk vs Protein

52. Healthy men were given a supplement of 40 g milk (n=22) protein or soy protein (n=24) daily for 3 month in a double blind study investigating the markers of bone metabolism (Khalil *et al*., 2002). The increase in serum IGF-I was higher in the men consuming soy rather than milk protein, increasing 11 and 3 nmol/L respectively (estimated from figure). The response to soy was similar in men aged up to 65 years and those aged 65 or over. Baseline IGF-I was higher in the milk protein group (21.7 ± 2.3 compared to 16.7± 2.3 nmol/L.

53. Hoppe, *et al*. (2004) measured serum levels of IGF-I and IGFBP-3 in groups of 12 eight-year-old boys given diets supplemented with 1.5 L/day of skimmed milk or 250 g/day of lean meat for 7 days. The milk and meat provided similar amounts of protein. The milk diet caused significant ($p \leq 0.05$) increases in serum IGFI (19% increase from 209 ± 55 ng/mL to 249 ± 67 ng/mL), IGFBP (increase from 3612 ± 409 ng/mL to 3806 ± 471 ng/mL) and IGF-I/IGFBP-3 ratio (13% increase from 0.23 ± 0.04 to 0.26 ± 0.04), but the meat supplemented diet did not affect any of these

parameters. In a further intervention study of eight-year-old boys, Hoppe, *et al*. (2008) gave groups of 13-15 boys daily drinks of 540 mL of one of four milk-based drinks for 7 days. The drinks were (1) whey with low milk mineral (calcium and phosphate) content (WHEY-LOW), (2) whey with high milk mineral content (WHEY-HIGH), (3) casein with low milk mineral content (CASE-LOW) and (4) casein with high milk mineral content (CASE-HIGH). The post-treatment serum IGFBP-3 levels for the groups were, respectively, 4312 ± 685, 3849 ± 542, 4262 ±533 and 4474 ± 579 ng/mL, with the difference between WHEY-HIGH and CASE-HIGH being significant ($p \leq 0.05$). There were no significant differences between any of the groups with regard to serum levels of IGF-I, IGF-/IGFBP-3 ratio, insulin or C-peptide. When the values for the boys given whey (WHEY-LOW plus WHEY-HIGH) and those for boys given casein (CASE-LOW plus CASE-HIGH) were examined, there were significant increases in serum levels of IGF-and IGFBP-3 in the casein group and increased serum insulin in the whey group. Furthermore, the increases in IGF-I in the casein groups were significantly greater than in the whey group.

Other interventions

54. Manios, *et al*. (2007) used an intervention study to investigate the effect of the use of calcium supplementation for prevention of osteoporosis in women. Volunteers were recruited from three districts of Athens, Greece, and were allocated to groups of 26-36 women given dairy products supplemented with calcium (1200 mg/person/day) and vitamin D_3 (7.5 µg/person/day), calcium supplements (1200 mg/person/day) or a control group. After 5 months, serum levels of IGF-I in the control, Ca supplemented and dairy ($Ca+D_3$) groups were 117±6, 95±13 and 133±7 ng/mL, respectively, with the difference between the control and dairy ($Ca+D_3$) groups being statistically significant ($p<0.05$). After 12 months there were no significant differences between any of the groups with regard to serum IGF-I levels.

55. In double-blind study (Mori, *et al.,* 2007), serum levels of IGF-I were increased in a group of 9 Japanese schoolgirls, aged 15-18 years, who took 300 mg/day of calcium as tablets for 8 months, as compared with a control group of 9 schoolgirls who were given placebos tablets. The chemical form of the calcium in the tablets was not reported. In the calcium treated group, serum IGF-I levels were 537 ± 109 ng/m at the start of the study and 523 ± 205 ng/m at the end of the study. In controls the initial level was 599 ± 154 ng/m and with 502 ± 156 ng/m at the end.

56. In a randomised trial by Gann *et al*. (2005) pre-menopausal women were given either an isocaloric low fat high fibre diet compared to their usual diet for 12 menstrual cycles, followed by a second 3 cycle phase in which the two diets were supplemented with soy with or without isoflavones. There was no difference in the levels of IGF-I, IGFBP-1 or IGFBP-3 between the two diets in the first phase of the experiment. In the second phase, there were no differences in any IGF protein in the normal diet group for either form of soy but there was a small but significant decrease in IGFBP-3 and an increase in the IGF-I/IGFBP-3 ratio among all the subjects consuming either type of soy supplement. The IGFBP-3 decreased by 48.4, 139.3 and 91.9 ng/ml in full, isoflavone free and both soy groups when the diets were combined. When analysed separately, the largest decreases were in the isoflavone free group. The findings were stated to be correlated with changes in the intakes of calcium, total vegetable and soy. This was noted to be comparable with

other studies in which increased dietary soy, protein or calcium resulted in an increase in free IGF-I.

57. Flood *et al*. (2008) investigated the effect of dietary intervention on serum IGF-I, IGFBP-3 and insulin. The study population were selected from the Polyp Prevention Trial and were aged ≥ 35 y, did not have a history of diabetes but who had at least one histologically confirmed adenoma removed 6 months before randomisation. In the intervention group (n= 248) a low fat, high fibre and high fruit and vegetable diet was consumed. After 4 years of follow up, IGF-I levels declined significantly from baseline levels in both groups, but there was no difference between the groups (133 ± 2.68 ng/ml decreased by 8.86 in the intervention group and 139 ± 1.98 decreasing by 7.74 in the controls). Concentrations of IGFBP-3, insulin and glucose were not statistically significant from baseline. When analysed by BMI, glucose concentrations in the intervention group were significantly decreased in the lean subjects only.

58. Young *et al*., (2013) investigated the effect of dietary fat and fatty acid content on the plasma levels of IGF-I, IGFBP-3, glucose, insulin and IGF-I/IGFBP-3 ratio in an 8 week crossover study in healthy post-menopausal women. The test diets were high (40% energy as fat), low (LF 20% energy as fat) and a low fat diet with high omega 3 fatty acid (LFn3). There was a washout period of 6-12 weeks between diets, when participants resumed their normal diets. Seventeen individuals completed all 3 dietary phases. Blood samples were taken at weeks 0 and 8 of the diet periods. IGF-I and IGFBP-3 were measured by ELISA. Eight weeks of the LFn3 diet increased circulating IGF-I from 17.8 ± 1.5 to 21.4 ± ng/ml ($p<0.01$), and IGFBP-3 from 183.5 ± 14.9 to 199.9 ± 16.0 ($p=0.01$) and the LF diet increased IGFBP-3 from 167.1 ± 14.9 to 179.6 ± 16.0 ($p=0.04$) resulting in trends towards an increased IGF-I/IGFBP-3 ratio with the LFn3 diet and a decreased ratio with the LF diet. There were no significant differences between the diets at baseline and at 8 weeks for IGF-I, IGFBP-3 or the ratio of IGF-I/IGFBP-3. The authors suggested that the LFn3 diet could be having a greater effect on growth hormone than the LF diet, which would then influence IGF-I and IGFBP-3 levels.

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