Chapter 9   Vitamin B12 and cancer prevention

Though I speak with the tongues of men and of angels, but have not love I am only a resounding gong or clanging cymbal.

And though I have the gift of prophecy, and can understand all mysteries and all knowledge, and though I have faith, that can move mountains, but have not love, I am nothing.

And though I give all I possess to the poor, and surrender my body to the flames, but have not love, I gain nothing.

Love suffers long and is kind; love does not envy; love does not parade itself, is not puffed up; does not behave rudely, does not seek its own, is not provoked, thinks no evil; does not rejoice in iniquity, but rejoices in the truth; bears all things, believes all things, hopes all things, endures all things.
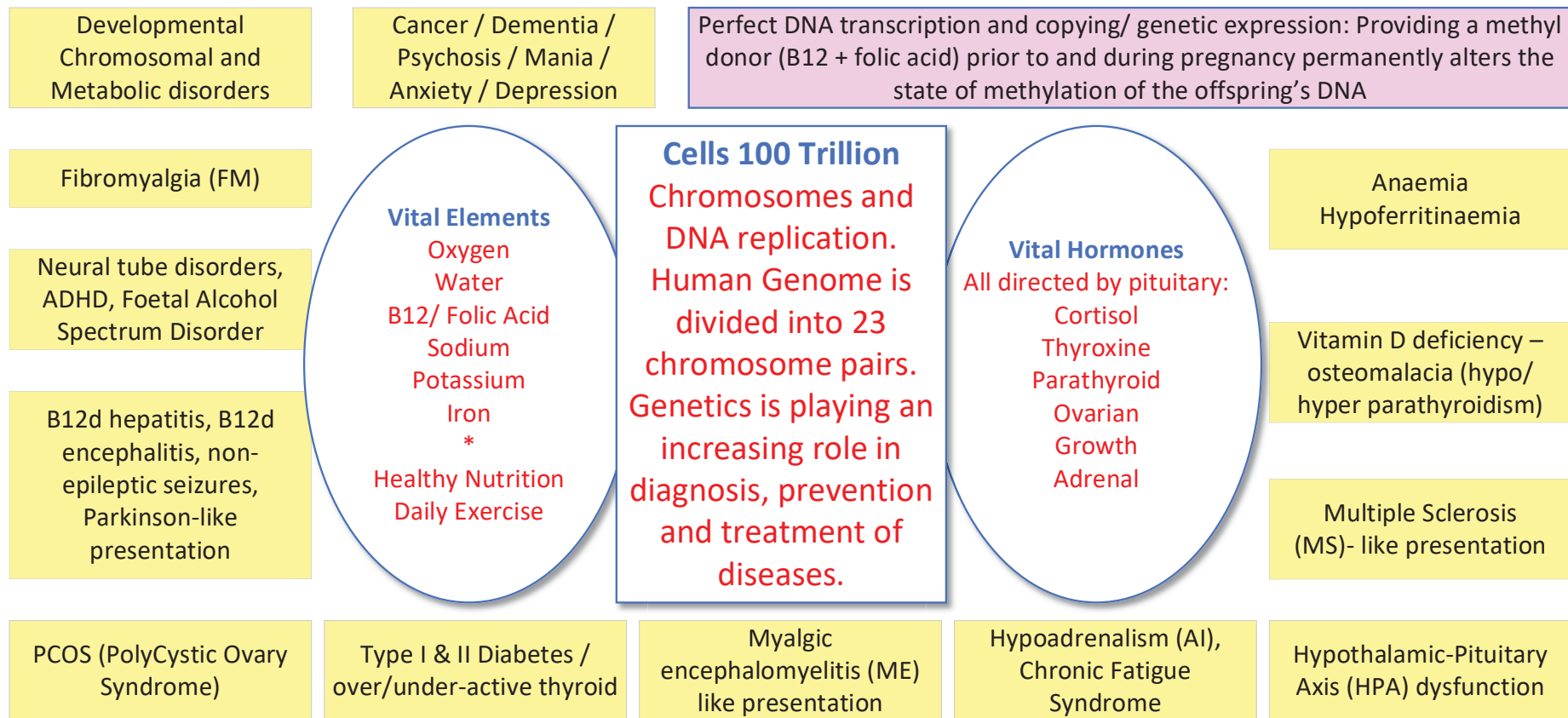
1 Corinthians 13:1-7 New King James Version

Figure 9-1 Prevention is better than cure

Prevention is better than cure (B12 and genetics)

Vitamin B12 deficiency with neuropsychiatric signs and symptoms Stages 1, 2, 3 and 4

Many, if not all, of the conditions listed below can be prevented or treated successfully by early diagnosis. Normal RNA and DNA activity are dependent on optimum B12 level

Developmental Chromosomal and Metabolic disorders	Cancer / Dementia / Psychosis / Mania / Anxiety / Depression	Perfect DNA transcription and copying/ genetic expression: Providing a methyl donor (B12 + folic acid) prior to and during pregnancy permanently alters the state of methylation of the offspring's DNA

Fibromyalgia (FM)

Neural tube disorders, ADHD, Foetal Alcohol Spectrum Disorder

B12d hepatitis, B12d encephalitis, non-epileptic seizures, Parkinson-like presentation

Vital Elements
Oxygen
Water
B12/ Folic Acid
Sodium
Potassium
Iron
*
Healthy Nutrition
Daily Exercise

Cells 100 Trillion
Chromosomes and DNA replication. Human Genome is divided into 23 chromosome pairs. Genetics is playing an increasing role in diagnosis, prevention and treatment of diseases.

Vital Hormones
All directed by pituitary:
Cortisol
Thyroxine
Parathyroid
Ovarian
Growth
Adrenal

Anaemia Hypoferritinaemia

Vitamin D deficiency – osteomalacia (hypo/ hyper parathyroidism)

Multiple Sclerosis (MS)- like presentation

PCOS (PolyCystic Ovary Syndrome)

Type I & II Diabetes / over/under-active thyroid

Myalgic encephalomyelitis (ME) like presentation

Hypoadrenalism (AI), Chronic Fatigue Syndrome

Hypothalamic-Pituitary Axis (HPA) dysfunction

9.1 Cancer worldwide prevalence

Cancer is one of the most prevalent diseases of our time and its incidence[36] is increasing in many countries, including the UK (Jones, 2015). In the first nine months of 2018, there were 18 million new cases worldwide. The most common types of cancer (in descending order of new cases) were: lung, breast, colorectum, prostate, stomach, liver, oesophagus and cervix (IARC, 2018).

The US National Cancer Institute describes the illness thus:

> *"Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues. Cancer can start almost anywhere in the human body, which is made up of trillions of cells…Cancer is a genetic disease - that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide. Genetic changes that cause cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to deoxyribonucleic acid (DNA) caused by certain environmental exposures."*

> *(National Cancer Institute, 2015).*

The cause of cancer is not known. It is believed to result from a number of interacting factors, including hereditary and environmental factors which cause changes to DNA. Among these is nutrition: inadequate supply of many micronutrients is now understood to play an important role in the development of some cancers. Such micronutrients include vitamin B12 which is known to be crucial for the correct formation of DNA and for the biochemical process known as DNA methylation (see below).

In this chapter we present some aspects of the relationship of vitamin B12 status to cancer as so far known. New treatments and preventive measures for cancer are constantly being researched. Having ourselves observed the widespread curative effects of vitamin B12 on body systems over many decades of clinical practice, it is our firm conviction that vitamin B12 will play an important role in both cancer treatment and prevention strategies in future. This view is reinforced by the fact that over three decades, during which we treated more than 1,000 patients for vitamin B12 deficiency at the Shinwell Medical Practice, we had *no new cases of cancer whatsoever* among these particular patients. This chapter gives evidence for why vitamin B12 may play a preventive and curative role in cancer treatment.

9.2 Some causes of cancer

The contributing factors to cancer are thought to include (in addition to inherited factors) (from the American Cancer Society (2014)):

- Lifestyle factors (nutrition, tobacco use, physical activity, etc.)
- Naturally occurring exposures (ultraviolet light, radon gas, infectious agents, etc.)
- Medical treatments (radiation and medicines, including chemotherapy which can cause secondary cancers, hormone drugs, drugs that suppress the immune system, etc.)
- Workplace exposures
- Household exposures
- Pollution

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[36] A **cancer incidence rate** is the number of new cancers of a specific type occurring in a specified population during a year, usually expressed as the number of cancers per 100,000 population at risk.

In general, cancer-causing agents (carcinogens) are classified into three groups:

- Chemical carcinogens
- Radiation exposure
- Microbial carcinogens

Lists of carcinogens are drawn up by several agencies, including the International Agency for Research on Cancer (IARC) which is part of the World Health Organisation (WHO). Lists of *Known Human Carcinogens* can be found on the American Cancer Society website (American Cancer Society, 2014).

9.3   Cancer treatment

9.3.1   Standard treatments for cancer

One of the main types of treatment for cancer currently used is chemotherapy, the use of drugs to kill cancer cells (Cancer Research UK, 2017). There are many types of chemotherapy drugs which target cells at different stages of their cycle according to the type of cancer. Another common treatment is radiotherapy which uses high energy x-rays to damage the DNA within cancer cells (Cancer Research UK, 2018). The disadvantage of these treatments is that they damage healthy cells as well and are not always totally effective at killing cancer cells or preventing metastasis (the spread of cancer from the site where it originated to another site in the body).

9.3.2   A role for vitamin B12 in cancer treatment?

In 1997, Japanese researchers showed that large doses of methylcobalamin (an active form of vitamin B12) could inhibit the proliferation of some malignant cancer cells and suppressed tumour cells in laboratory animals. The researchers proposed that methylcobalamin could be useful in the treatment of some malignant tumours (Nishizawa et al., 1997).

To our knowledge, this important finding has not yet led to any vitamin B12-based treatment for cancer.

Ten years later, another researcher suggested that there were strong grounds for investigating vitamin B12 therapy for cancer (Volkov, 2008). He based his hypothesis on the fact that vitamin B12 is known to play an important role in many body organs and systems and that the list of these is growing, plus the fact that high levels of cobalamin have frequently been found in cancer patients. His interpretation was that these high levels were signs of the body's compensatory mechanism – in other words, the body was releasing B12 to deal with the invasion of cancer cells. He viewed vitamin B12 as a substance which "functions to keep body systems in balance, even under the stress of severe pathology".

Explaining his hypothesis, he said: "As yet I have not been able to find another explanation for high level of vitamin B12 in oncology patients other than that it is a compensatory mechanism. **Perhaps following this body's ''warning sign'', we should start treatment with high doses of vitamin B12 to try to help the <u>stabilization of normal function</u> of the organs and systems. Laboratory researches should be continued to substantiate <u>introduction of cobalamin as preliminary treatment of particular diseases</u>**" (Volkov, 2008).

High levels of cobalamin in the plasma of cancer patients have been found with such frequency that researchers in Denmark decided to conduct a major study to investigate whether these levels could be used as a diagnostic marker of cancer (Arendt et al., 2016). Their key population-based study used data from Danish medical registries from 1998 to 2014 and covered more than 80,000 cancer patients.

Results showed that those with "high plasma cobalamin levels prior to [cancer] diagnosis had higher mortality, indicating more advanced and aggressive cancers". Contrary to sensational news reports implying that this somehow meant that vitamin B12 caused cancer (Bird, 2013), the researchers speculated that the results reflected changes in cobalamin metabolism *caused* by cancer, not that high cobalamin levels caused cancer. They quoted the suggestion by Geissbühler et al. (2000) that hepatic metastases (cancer having spread to the liver) in particular are linked to high B12 levels. Other evidence they gave for this explanation was that haptocorrin, to which circulating cobalamin is bound, is metabolised only in the liver. Another possible explanation was that the high levels of cobalamin might reflect a "pronounced inflammatory response" to the cancer.

9.3.3 Vitamin B12 as "master key"

Volkov hypothesised that vitamin B12 has a "master key effect" on the human body. He noted that several experimental laboratory studies had indicated that use of vitamin B12 inhibited the growth of malignant cells, that there were no experimental results indicating the opposite and that there was no data indicating a toxic effect of vitamin B12. He expressed surprise that studies demonstrating these associations of vitamin B12 had not been followed up, and put forward three reasons for this:

1. Preference for treatment with vitamin B12 to modern perspective medicines does not seem appropriate for oncology patients who do not have time for such kind of experiments (an ethical question).
2. The unconvincing or unequivocal results of the research, which could be a consequence of using not high enough doses of vitamin B12, do not encourage oncologists to try vitamin B12 treatment.
3. *The paradoxical dilemma, in which the solution is so close, well-known, accessible, and cheap, makes it hard to believe that vitamin B12 may be effective in the treatment of oncology diseases* [Our italics]. (Volkov, 2008)

Vitamin B12 is not currently a "proven" anti-cancer treatment but specialists acknowledge that it is "an important nutrient for genetic stability, DNA repair, carcinogenesis, and cancer therapy" (Donaldson, 2004).

9.3.4 Vitamin B12 cancer therapy with nanotechnology?

Vitamin B12 as an anti-cancer therapy is also being investigated in other fields of science, such as nanotechnology. Interestingly, in one potential application of this technology, a team of scientists has proposed that vitamin B12, which they note has "several characteristics that make it an attractive entity for cancer treatment and possible therapeutic applications", could be used as a cancer treatment if it were delivered to cells via solid lipid nanoparticles (SLNs). These improve drug bioavailability and enable precision drug targeting (Genc et al., 2015).

Other researchers are investigating the synthesis of novel variant forms of vitamin B12 for potential use in cancer treatments (University of Kent, 2012).

9.3.5 Vitamin B12 as protection in chemotherapy

Vitamin B12 has no known toxicity, even at very high doses (see Chapter 3) and is in fact already used as a supplement to reduce toxicity in some existing chemotherapy treatments, such as Pemetrexed therapy (Singh et al., 2015; Takagi et al., 2016).

Vitamin B12 is so effective as an antioxidant that some medical scientists have suggested it should be used with caution in chemotherapy because it can counteract the effects of cytotoxic chemotherapy on tumour DNA (Eren et al., 2014). Vitamin B12 (and folic acid) are given because if levels of these vitamins are insufficient in the body, Pemetrexed may affect noncancerous as well as cancerous cells, which leads to toxic effects. One research study into the dosage of these vitamins in Pemetrexed treatment for lung cancer concluded that they did not reduce the effectiveness of the chemotherapy. The researchers noted that patients receiving these vitamins had greater tolerance for the chemotherapy treatment and improved survival prospects (Yang, Chang, et al., 2013). Further trials on the timing and dosage of these vitamins to protect patients undergoing chemotherapy are ongoing (Baldi et al., 2016).

9.4 Cancer prevention

9.4.1 The role of nutrition

It is now well established that inadequate or unbalanced nutrition plays an important role in the development of cancer. Dietary deficiencies are estimated to account for as much as one-third of preventable cancers (Ames & Wakimoto, 2002; Donaldson, 2004). Such effects can occur not only in cases of acute deficiency but also when intake is only slightly below the recommended dietary allowance (Ames & Wakimoto, 2002).

It was once thought that the only relation of diet to cancer was through exposure to carcinogens, such as alcohol or heterocyclic amines in meat (Sugimura et al., 2004), but it is now known that micronutrients play a crucial role in maintaining the integrity of DNA and correct activity of genes which help to prevent the occurrence of cancer (WCRF & AICR, 2007).

Over 40 micronutrients, including vitamin B12, play crucial roles in body metabolism and contribute to metabolic harmony. Deficiencies in one micronutrient can cause disturbances in many systems (Ames & Wakimoto, 2002). For instance, it has been shown that there is an inverse relationship[37] between the consumption of fruits and vegetables and many types of cancers. Case-control studies quoted by Ames and Wakimoto (Ames & Wakimoto, 2002 Table 2) show that a reduced consumption of fruits and vegetables can double the risk of developing most types of cancer. It should also be noted that meat, the main food source of iron, zinc and B12, is another important source of micronutrients.

9.4.2 The EPIC project

This knowledge has prompted much new research into the effects of nutrition in cancer prevention and treatment. Such research includes the ongoing extensive European Prospective Investigation into Cancer and Nutrition (EPIC) study designed to investigate the relationships between diet, nutritional status, lifestyle and environmental factors, and the incidence of cancer and other chronic diseases. Using information on diet, lifestyle characteristics, body measurements and medical history collected between 1992 and 1999 from more than half a million participants recruited across 10 European countries and followed for almost 15 years, EPIC investigators researched many aspects of the contribution of diet and lifestyle to cancer. This research is still ongoing but a summary of the significant findings by 2010 (Gonzalez & Riboli, 2010) listed the following:

- gastric cancer risk was less likely to occur with high plasma vitamin C, some carotenoids, retinol and α-tocopherol, high intake of cereal fibre and high adhesion to Mediterranean diet;

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[37] An "inverse relationship" is a relationship between two numbers in which an increase in the value of one number results in a decrease in the value of the other number.

- red and processed meat were associated with increased gastric cancer risk;
- high intake of dietary fibre, fish, calcium, and plasma vitamin D were associated with a decreased risk of colorectal cancer;
- red and processed meat intake, alcohol intake, body mass index (BMI) and abdominal obesity were associated with an increased risk of colorectal cancer;
- high intake of fruit and vegetables in current smokers was associated with a decreased risk of lung cancer;
- an increased risk of breast cancer was associated with high saturated fat intake and alcohol intake.

The researchers noted: "These results contribute to scientific evidence for appropriate public health strategies and prevention activities aimed at reducing the global cancer burden".

9.4.3   DNA – a brief sketch

As stated above, cancer is a genetic disorder. In order to give some idea of the mechanisms by which vitamin B12 and other micronutrients may affect the activity of genes, we include this simplified sketch of the main features of DNA as so far known. Interested readers may find further detail on the US National Human Genome Research Institute website and in articles by the International Human Genome Sequencing Consortium (see bibliography in this book).

DNA is a long molecule that contains the instructions for life. It has frequently been called the "blueprint" for life but scientists see that as a limited metaphor for "something so intricate, complex, multilayered and dynamic" (Ainsworth, 2015). DNA is replicated in nearly every cell in a living organism. Most DNA is found in the nucleus of cells but a small amount is located in the mitochondria (structures within cells that convert the energy from food into a form that cells can use) (National Library of Medicine (US), 2018b).

All DNA is composed of the same chemical building blocks, called nucleotides (or bases), made of a phosphate molecule, a five-carbon sugar molecule (deoxyribose), and one of four nitrogen-based molecules: adenine, thymine, guanine and cytosine. What makes the differences between one person and another is the order in which these smaller molecules are arranged.

A person's complete set of DNA is known as the genome. Each genome contains all the information needed to build and maintain that person. A copy of the entire genome—more than 3 billion DNA base pairs — is contained in all cells that have a nucleus (National Library of Medicine (US), 2018a).

Genes are small sections of DNA that contain the instructions for building a specific molecule, usually a protein. There are estimated to be around 19-20,000 genes in humans, coding for around 120,000 proteins which are the production line which manufactures everything in the body, whether more permanent elements like bone or cartilage, or instantaneous chemicals such as noradrenaline used for a millisecond for nerve transmission.[38] Scientists trace genes by giving them names. Polymorphisms are multiple forms of genes which contribute to different outcomes in different people.

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[38] Protein-coding sequences account for only a very small fraction of the genome (approximately 1.5%), and the rest is associated with non-coding RNA molecules, regulatory DNA sequences, LINEs, SINEs, introns, and sequences for which as yet no function has been determined (International Human Genome Sequencing Consortium, 2001).

DNA strands are extremely long in relation to the size of a cell. If unravelled, the DNA strand in just one cell would be two metres long! (Ashworth, 2011). The DNA is tightly packed and coiled into structures called chromosomes. A human chromosome can have up to 500 million base pairs (two nucleobases bound to each other by hydrogen bonds – for example, adenine-thymine) of DNA with thousands of genes.

In order to make proteins, the information stored in a gene is transferred to a similar molecule called RNA (ribonucleic acid) in the cell nucleus in a process known as *transcription*. The type of RNA that contains the information for making a protein is called messenger RNA (mRNA) because it transmits information from the nucleus into the cell's cytoplasm. The next step, known as *translation*, involves interaction between the mRNA and a ribosome, which links amino acids (the building blocks of proteins) in an order specified by mRNA. A type of RNA called transfer RNA (tRNA) assembles the protein, one amino acid at a time. This whole process is known as gene expression.

All genes do not need to be expressed all of the time so cells have mechanisms to regulate gene activity by turning genes on and off at any point in the gene expression pathway. "In multicellular organisms gene regulation defines the cell, its structure and function, and ultimately the whole organism. Aberrant gene regulation results in cancer, birth defects, and even death" (Laybourn, 2001).

Growth and development depend on cells dividing and the DNA replicating. Every time a human cell divides it has to correctly replicate the same sequence of 3 billion nucleotides. In this process, mistakes (mutations) can occur. Many of these are corrected by DNA repair processes but some are not, particularly where the DNA repair enzymes themselves are damaged. This may then lead to illness. All cancers begin when one or more genes in a cell mutate (Cancer.Net, 2018). The first mutated gene associated with cancer was discovered in 1982 and since then several hundred "cancer genes" have been identified (Pray, 2008).

9.4.4   Epigenetics: environmental influences on genes

The study of the ways in which interactions from biomolecules in the environment (including diet) affect which genes will be expressed without changing the DNA sequence is a relatively new field of science called epigenetics. All the chemicals that have been added to a person's DNA are known as the epigenome and include three inter-acting mechanisms: DNA methylation, histone modifications and non-coding microRNAs (miRNA). "The combination of these marks and miRNAs is responsible for regulating gene expression not only during cellular differentiation in embryonic and foetal development (Reik, 2007) but also throughout the life-course" (McKay & Mathers, 2011). Some researchers argue that epigenetic influences on genes are as important as the structure of genes in determining a person's characteristics and health (Ainsworth, 2015).

Research into the effects of nutrition on gene expression is a sub-field known as Nutritional Epigenetics (Landecker, 2011). Research on animal nutrition has found that changing the diet can change the animal's physical characteristics (Zhang, 2015). In humans, the micronutrients folate, vitamin B12, methionine, choline, and betaine can affect DNA methylation and histone methylation through altering one-carbon metabolism – a series of biochemical exchanges[39] (Choi & Friso, 2010). The ways in which

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[39] One-carbon metabolism is the movement of biochemical groups containing a single carbon atom. There are three ways in which this can be done: through Tetrahydrofolate (THF) as a cofactor in enzymatic reactions; through

dietary deficiencies influence the development of cancer are highly complex but they are known to include gene mutations, DNA lesions (damaged bases or chromosome breaks), increased cell-division rates and other factors (Ames et al., 1995).

> *"Dietary exposures can have consequences for health years or decades later and this raises questions about the mechanisms through which such exposures are 'remembered' and how they result in altered disease risk. There is growing evidence that epigenetic mechanisms may mediate the effects of nutrition and may be causal for the development of common complex (or chronic) diseases"* (McKay & Mathers, 2011).

This has led to the *developmental origins of health and disease (DOHAD)* hypothesis which suggests that "environmental exposures during development increase susceptibility to cancer in adulthood, not by inducing genetic mutations, but by reprogramming the epigenome" (Walker & Ho, 2012).

Here it should be noted that the results of research into the effects of diet on cancer are often mixed or contradictory. One reason for this is that studies of dietary effects are extremely difficult to conduct. Because of the interactions of many nutrients, it is often difficult to separate the effects of one from another. Researchers must also decide over what length of time to conduct the research and at what dosages. There are also many variables (such as the lifestyle and ethnicity of participants) which have to be taken into account.

9.5   Vitamin B12, folate and correct formation of DNA

DNA damage is known to be an important risk factor for cancer (Basu, 2018; Esteller, 2003). Although much research remains to be done, there is significant evidence that several vitamins and minerals play essential roles in correct formation of DNA (Zhang, 2015). By implication, deficiencies of such micronutrients become a cancer risk. "Micronutrient deficiency can mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both. Those micronutrients whose deficiency appears to mimic radiation are folic acid, B12, B6, niacin, C, E, iron and zinc, with the laboratory evidence ranging from likely to compelling" (Ames, 1979).

Much of the research into the relationship of nutrition to cancer has focused on folate (and folic acid, the synthetic form used in supplements). This is partly because of the known importance of folate in preventing birth defects, and debates about folic acid fortification of food.

Low folate has been associated with chromosome breaks, a disorder which researchers explain as resulting from excessive uracil incorporation into DNA instead of thymine. "Both in vitro and in vivo studies with human cells clearly show that folate deficiency causes expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, micronucleus formation, DNA hypomethylation and mitochondrial DNA deletions" (Fenech, 2012). Vitamin B12 deficiency is expected to have the same effect because of the synergy between the two vitamins: "Vitamin B12 deficiency would be expected to cause chromosome breaks by the same uracil-misincorporation mechanism that is found with folate deficiency" (Ames & Wakimoto, 2002).

S-adenosylmethionine (SAM) as a methyl (-CH$_3$) donor and through Vitamin B12 (cobalamin) as a co-enzyme in methylation and rearrangement reactions.

Less research appears to have been done on the role of vitamin B12 in cancer prevention, perhaps because until recently it was assumed that vitamin B12 deficiency was less prevalent in the general population (Choi et al., 2004). However, folate cannot perform its functions in the human body correctly without vitamin B12 which is essential for many biochemical reactions. Findings which implicate low folate status with cancer development would therefore also apply in cases of vitamin B12 deficiency. This has been shown to be true in laboratory experiments (Choi et al., 2004).

The mechanism of uracil misincorporation into DNA is understood to result from abnormalities in the folate cycle which lead to low levels of methylene-THF (the folate cofactor for thymidylate synthase). This in turn decreases synthesis of thymidylate which is required for methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP). The proportion of dUMP increases, leading to excessive uracil accumulation in DNA (Ames & Wakimoto, 2002; Blount et al., 1997; Wickramasinghe & Fida, 1994).

The result is disordered composition of DNA and chromosome breaks. The effects are described thus: "Excessive amounts [of uracil] are incorporated when thymidylate synthesis is inadequate to meet the cellular requirements for DNA synthesis and repair, leading to inappropriate nucleotide sequences, and the increased likelihood of chromosomal breakage, deletions and mutations" (Choi et al., 2004). Such damage, which can occur with either folate or vitamin B12 deficiency, could "contribute to the increased risk of cancer and cognitive defects" in humans (Blount et al., 1997). The same researchers note that folate *therapy* can reduce high uracil levels and chromosome breaks.

Abnormalities in the folate cycle may result from deficient folate intake from diet or from disruption of folate metabolism by lack of vitamin B12. As described in Chapter 1, vitamin B12 is required for the cofactor methionine synthase (MS) which is necessary for biochemical reactions in both the folate and methionine cycles. If there is not enough vitamin B12, 5-methyl tetrahydrofolate cannot be converted to tetrahydrofolate which is necessary for the next series of biochemical reactions. It is effectively "trapped" in cells as 5-MTHF which the body cannot use.

9.6   Aberrant DNA methylation in cancer

The vitamin B12-dependent cofactor MS also affects DNA methylation (the addition of one-carbon groups to DNA which affects gene expression) through the methionine cycle. When MS activity is reduced, methionine cannot be adequately metabolised to S-Adenosylmethionine (SAMe) (Zhang, 2015) which acts as the methyl donor in >80 reactions, including the methylation of DNA, histones and other proteins, neurotransmitters, and phospholipids and the synthesis of creatine – reactions which play important roles in development, gene expression, and genomic stability" (Choi et al., 2004; Shane, 2008).

DNA methylation is a "crucial epigenetic modification of the genome that is involved in regulating many cellular processes. These include embryonic development, transcription, chromatin structure, X chromosome inactivation, genomic imprinting and chromosome stability". It has been found that errors in DNA methylation processes are linked to many human diseases, including cancer (Robertson, 2005).

9.7   Dual role of folate and folic acid in cancer development

There is considerable evidence that healthy folate status plays a significant role in cancer prevention. Research has shown links between higher intakes of dietary folate and a reduced risk of cancer of the colon, of other parts of the gastrointestinal tract, and also of the pancreas (Ulrich, 2007). The picture is more complex than first thought, however, and the role of synthetic folic acid taken as a supplement and as used in food fortification may be different from that of dietary folate. Folate may "play a dual role in

cancer development: it may provide protection early in carcinogenesis and in individuals with a low folate status, yet it may promote carcinogenesis if administered later and potentially at very high intakes" (Ulrich, 2007). (The difference between folate and folic acid and the issues this raises are discussed by Kresser (2012)).

The debate centres largely around the role of folate and folic acid in DNA methylation and the question of whether high intakes promote growth and replication of all cells, including cancer cells. In an article concerning the European reluctance to fortify flour with folic acid (compared with the US where this has been practised since the 1990s) because of an assumed possible cancer risk, one specialist has pointed out that folate/folic acid is only <u>one</u> of the components of the methylation cycle so "linking only folate intakes ⁄ status to DNA methylation is a considerable simplification, as folate is just one of several actors supplying C1 [one-carbon] groups to the SAM pool" (Jägerstad, 2012).

The relationship of folate deficiency to the development of cancer is thus controversial and research has produced conflicting results. A recent review of research on the relationship of folate status to colorectal carcinogenosis concluded that the balance of evidence was that there is an inverse relationship between folate status and colorectal cancer risk (i.e. higher folate means less risk). However, it was noted that the relationship is complex and folate appears to have a dual role, depending on the timing and dose of folate intervention and whether the cancer is already established (Kim, 2007). Similar effects were found in relation to breast cancer: adverse effects were related to supplementation with folic acid rather than high intake of natural-source folate (Kim, 2006).

Others have similarly found that higher folate intake reduces the risk of colorectal and breast cancer, but they emphasise that the "optimal dose, duration and stage of carcinogenesis" for folate intervention are not yet known. They also state that the protective effects of folate may be due to other protective factors in fruits and vegetables not yet known (Eichholzer et al., 2001).

In contrast, a study conducted in Norway (Ebbing et al., 2009) from 1998 to 2005 (and followed up to December 2007), in the context of debates about food fortification with folic acid, found that folic acid and vitamin B12 taken together raised cancer incidence. The study concluded that the higher incidence of cancer was likely to have resulted from the folic acid supplementation, not the B12: "However, the observed associations between the primary end points and vitamin concentration measured during study treatment were confined to serum folate, suggesting that the adverse effects were mediated by folic acid." The suggestion is that excess folic acid, or unmetabolized folic acid, may "impair cancer immune defence".

From the above it is evident that more research is needed to establish the exact mechanisms by which folate, folic acid and vitamin B12 may impact on cancer prevention or development.

9.8   Tissue-specific cancers and vitamin B12

Less research appears to have been done specifically on the relationship of vitamin B12 deficiency to cancer. Much is not known about this relationship and research, particularly where it looks at vitamin B12 and folate together, has produced mixed results. The inconsistent results can be attributed partly to the dual roles of folate and folic acid in carcigenosis described above. Another reason may be misinterpretation of high levels of plasma cobalamin as a cause of cancer rather than as the body's response and attempt to restore balance which we described in the opening paragraphs of this chapter.

DNA methylation is known to be tissue-specific so the effects of specific micronutrients are likely to differ depending on the part of the body affected. This might explain why some types of cancer are specifically associated with B12 deficiency or with variations in the body's ability to metabolise B12.

9.8.1 Gastric cancer

The strongest known link of vitamin B12 deficiency with specific cancers is with gastric cancer and has been known for decades (Chanarin, 1990, p. 86) Patients suffering from pernicious anaemia (PA), which causes vitamin B12 deficiency (and may also be a consequence of this deficiency – see Chapter 4), have an increased risk of developing gastric cancer. Some researchers have found the relative risk rate to be seven times higher than in the general population (Vannella et al., 2013).

The link between PA and cancer is apparent when it is understood that both involve DNA damage. For example, Shane notes that megaloblastic anaemia is a "condition reflecting deranged DNA synthesis in erythropoietic cells" and that in this condition megaloblastic changes occur in all fast-growing tissues, such as the marrow and gut epithelia. He continues, "Megaloblastic cells contain close to twice the normal DNA content and the DNA is partially fragmented…The defect in DNA synthesis has been ascribed to defective thymidylate synthesis under these conditions with a resulting increase in uracil misincorporation into DNA. Removal of uracil by the repair enzyme uracil DNA glycosylase, and a decreased repair of the gaps produced by this enzyme, would lead to an increase in double-stranded DNA breaks under these conditions (Blount et al., 1997)" (Shane, 2008).

The authors of a study conducted in 1993 observed that there was not only an increased incidence of stomach cancer among PA patients but that these patients also had a high incidence of other cancers of the digestive tract and process, such as esophageal, pancreatic and rectal cancer. They also observed increases in myeloid leukaemia and multiple myeloma among PA patients (Hsing et al., 1993).

More recently, Chinese scientists noted a direct link between vitamin B12 metabolism and the occurrence of gastric cancer. In contrast to many studies which have concentrated on the one-carbon pathway described above, their study investigated possible links between gene variants which affect the uptake of B12 in the body and the incidence of gastric cancer. Their study group included 492 patients with gastric cancer and a control group of 550 non-sufferers. The results showed that a significant number of patients with gastric cancer were carriers of a risky variant of the TCN1 gene (which affects how B12 is bound to haptocorrin) or of a CUBN haplotype (which encodes the intrinsic factor (IF)-vitamin B12 receptor, cubilin). They concluded: "The circulating vitamin B12 concentration-related variants were associated with the occurrence of gastric cancer. This finding shed light on the unexpected role of vitamin B12 metabolism genes in gastric carcinogenesis and highlighted the interplay of diet, genetics, and human cancers" (Zhao et al., 2016).

9.8.2 Colon cancer

Other parts of the digestive tract also appear to be particularly affected by vitamin B12 status. For instance, recent research has shown a connection between vitamin B12 intake and colorectal cancer (CRC) risk (Sun et al., 2016). Noting the importance of diet in CRC risk, and that previous research on the relationship between vitamin B12 and/or folate and colon cancer was controversial, the researchers aimed to "quantitatively and comprehensively summarize whether vitamin B12 intake or blood vitamin B12 level is related to CRC risk". They extracted data from 14 studies involving 9,693 patients. The results showed that there was no reduction of cancer risk where only small amounts of B12 (dietary intake of <7 mcg/day) were taken, but when larger amounts were ingested (>12 mcg/day) the risk was significantly reduced. They did not find any correlation between blood B12 levels and risk, however. They suggested

that further research was needed on the interaction of dietary and supplemental B12 intakes and the differing effects of the active and inactive forms of B12.

9.8.3   Pancreatic cancer

Less research has been done into the effects of micronutrients on pancreatic cancer risk. The results of one major recent research project, for example, were inconclusive. The researchers found no overall association between plasma concentrations of vitamin B12, vitamin B6, folate and homocysteine and pancreatic cancer risk, but did find a possible inverse relationship between circulating levels of vitamins B12, B6 and folate in those who obtained these micronutrients exclusively from diet (Schernhammer et al., 2007).

9.8.4   Lung cancer

Lung cancer (mostly caused by cigarette smoking) is the leading cause of cancer deaths worldwide (Lu, 2011). It is clinically divided into two broad groups, that is, small cell lung cancer (SCLC) and nonsmall cell lung cancer (NSCLC). Researchers have found close links between faulty DNA methylation and the development of lung cancer (Lu & Zhang, 2011; Piyathilake et al., 2000). Some have proposed that DNA methylation markers could be used in early diagnosis of this illness, and expressed the hope that new methylation-based therapies "will clinically cure lung cancer one day" (Lu & Zhang, 2011).

Piyathilake et al. specifically investigated the relationship between DNA hypomethylation and deficiencies of folate and vitamin B12 in patients suffering from squamous cell lung cancer. They found lower concentrations of both vitamins in SCCs than in uninvolved tissues. However, other results from this research suggested that folate, rather than vitamin B12, might be the "limiting vitamin for proper DNA methylation".

Other research has linked changes in DNA methylation to pollutants, such as tobacco smoke and chemicals in air pollution, both of which contribute to lung cancer (Jiang et al., 2017; Lee & Pausova, 2013).

9.8.5   Breast cancer

Research into the relationship of vitamin B12 intake to the occurrence of breast cancer has had mixed results. Data from the Shanghai Breast Cancer Study showed that women who consumed high levels of folate had reduced breast cancer risk and an even smaller risk if they also consumed high levels of folate cofactor nutrients such as vitamin B12, methionine and vitamin B6 (Shrubsole et al., 2001). Similarly, Yang et al. (2013) found that higher intake of folate, vitamin B12 and methionine were marginally associated with a lower risk of breast cancer. In contrast, Bassett et al. (2013) found no connection between vitamin B12 status and breast cancer, which again highlights the difficulties connected with these kinds of study.

9.8.6   Prostate cancer

A number of studies have been undertaken on the association of vitamin B12, folate or folic acid, and prostate cancer. These have also had mixed results. Some have indicated that high plasma levels of vitamin B12 and folate appear to be linked to increased prostate cancer risk (Hultdin et al., 2005) although they stress that further research is needed to confirm this relation. Collin et al. (2010), for example, noted that although their results might suggest a "possible causal relationship" between folic acid, vitamin B12 and prostate cancer risk, the limitations of research based on food frequency questionnaires are well known. A few years later, Collin suggested that the higher levels of B12 circulating in the blood of prostate cancer patients could be explained by "reverse causality" (i.e. the

cancer had caused the high levels) (Collin, 2013). This would be in accordance with Volkov's hypothesis described at the beginning of this chapter – that the body is somehow compensating for the proliferation of cancer cells by releasing B12 into the bloodstream.

A major recent pooled data study (Price et al., 2016) investigated the associations between circulating folate and vitamin B12 concentrations and risk of prostate cancer overall and by disease stage and grade. This study found some weak evidence of a link between higher folate and vitamin B12 levels and risk of prostate cancer. They also found that higher folate concentration was associated with high-grade disease risk but suggested that this might be due to folic acid supplementation rather than dietary folate intake. A response by others (Obeid & Pietrzik, 2016) discussed the limitations of this study which highlighted some of the difficulties of conducting research on individual nutrients and cancer.

9.9   Critical time-windows in cancer prevention

Research has shown that there appear to be critical time-windows when diet and other epigenetic influences have a particularly significant impact on predisposition to disease. It is well known that poor nutrition in a pregnant mother affects the child but the discoveries in epigenetics suggest that these effects may be more far-reaching than previously thought. McKay and Mathers (2011) comment: "dietary exposures can have long-term consequences for health and raise questions about the mechanisms through which early life exposures are 'remembered' over long-time periods and how they result in altered disease risk. Poor nutrition in utero may result in inadequate development of specific cells and tissues."

According to Neitzel and Trimborn: "Chromosomal disorders (gene mutations) occur in an estimated 10-25% of all pregnancies. They are the leading cause of fetal loss and, among pregnancies surviving to term, the leading known cause of birth defects and mental retardation" (Neitzel & Trimborn, 2007).

Studies of the effects of restricting the amounts of vitamin B12, folate and methionine in the diets of animals prior to pregnancy resulted in adult offspring with altered immune responses, insulin-resistance, and elevated blood pressure (Sinclair et al., 2007). The researchers concluded: "The data provide the first evidence that clinically relevant reductions in specific dietary inputs to the methionine/folate cycles during the periconceptional period can lead to widespread epigenetic alterations to DNA methylation in offspring, and modify adult health-related phenotypes".

These findings have been further supported by research showing the importance of vitamin B12 status for one-carbon metabolism, influencing patterns of DNA methylation at birth which may have lifelong effects (McKay et al., 2012). Other research into maternal nutrition in humans has shown that maternal concentration of vitamin B12, vitamin B6 and homocysteine may play a significant role in the three-year weight gain of infants (McCullough et al., 2016).

However, the subject is controversial and others have pointed out that nutritional effects are complex. As it is not possible to target a single gene through this method, "indiscriminate and potentially irreversible methylation changes in a broader range of biomolecules and cells/tissues may result from such dietary treatment" (O'Neill et al., 2014). Many other factors need to be considered such as foetal sex, maternal/foetal genetic background, other maternal factors, as well as tissue/cell specificity and concentration and duration of exposure to these nutritional supplements.

Scientists agree that there is much more to be learnt from epigenetics-based studies concerning how diet affects human health over long time periods, which may lead to new ways of preventing and

treating cancer. Specific research priorities include identifying the life stages during which particular epigenetic loci are affected by diet.

"'Tuning-up' human metabolism, which varies with genetic constitution and changes with age, could prove to be a simple and inexpensive way to minimize DNA damage, prevent cancer, improve health and prolong a healthy lifespan" (Ames & Wakimoto, 2002).

In the light of these new findings on the importance of diet in cancer prevention, together with the effects of other preventable causes (such as tobacco smoke), we strongly advocate ensuring healthy vitamin B12 levels throughout life, starting with the disease prevention programme for mother and child given in Figure 5-1, as a way of ensuring good health throughout life.