7 Prevention of CFA

7.1 Meeting objectives

BOX M

Objectives of the WHO meeting on the prevention of CFA

- Identify environmental and behavioural factors with established associations with orofacial clefts and other craniofacial anomalies (CFA) and recommend global public health initiatives for the prevention of CFA caused by these factors.
- Review evidence regarding the role of specific maternal, nutritional factors in the etiology of orofacial clefts and other CFA.
- Reach a consensus regarding the role and importance of nutritional supplementation trials in evaluating the causal role for specific nutrients in the etiology of orofacial clefts and other CFA.
- Discuss aspects of the design of orofacial cleft and CFA-prevention trials and their ethical, legal, social and financial implications.
- Make recommendations regarding the resources needed to implement international collaborative studies of CFA prevention with common core protocols.

7.2 Environmental and behavioural factors and orofacial clefts

Craniofacial anomalies are among the most common birth defects and, of these, orofacial clefts are the most frequent. As described in the *World Atlas of Birth Defects* (World Health Organization, 1998) and in Section 2.1 of this report, there is a great deal of variation in the occurrence of orofacial clefts in different populations throughout the world. It is likely that this is due to both environmental and genetic factors. Poverty has been previously associated with an increased risk of neural tube defects and, more recently, with the occurrence of orofacial clefts (discussed in Section 4.1), providing evidence that environmental factors play an important role in both type of birth defects. Data from Brazil, China and the United States (Utah) presented at the WHO/Utah meeting support the view that the pattern of occurrence of neural tube defects is different from that of orofacial clefts across geographic areas and time periods, indicating that the environmental factors that cause these defects are not the same. The specific components of the environment of the poor, relating to orofacial clefts, are unclear but could include exposure to tobacco smoke, alcohol, occupational or residential exposures to teratogens, and poor nutritional status.

7.2.1. Tobacco and orofacial clefts

Maternal cigarette smoking is perhaps the best studied environmental risk factor for orofacial clefts. As summarized above in Section 4.5.1, maternal tobacco use during pregnancy has been consistently associated with a modest elevation in risk of orofacial clefts. Given the frequency of the habit among women in the United States, smoking may account for as much as 20% of orofacial clefts in the country's population. The risk of orofacial clefts attributable to smoking may be underestimated because exposure of pregnant women to passive smoking in the home and workplace has not usually been taken into account.

Over one billion people worldwide smoke and nearly three-quarters of these live in developing countries, often with relatively low levels of public and political support for effective tobacco control measures. (Aghi et al., 2002). Numerous reports have documented that smoking prevalence rates among women aged 15-25 years have steadily increased globally over the past decade (Windsor, 2002). It was estimated that in 1995, 12-14 million women worldwide smoked during their pregnancy and, when passive smoking was accounted for, 50 million pregnant women, out of a total of 130 million, were exposed to tobacco smoke during their pregnancy (Windsor, 2002). The second wave of the epidemic of tobacco-related

diseases is resulting from women being actively targeted by tobacco companies and taking up smoking in increasing numbers (Kaufman and Nichter, 2002). The traditional habit of chewing tobacco among women in many populations may also represent an under-studied source of tobacco exposure during pregnancy.

The association between maternal smoking and orofacial clefts may not be widely appreciated by international health organizations. The US Surgeon-General's *Report on Women and Smoking* notes that, while the overall risk of birth defects does not appear to be related to maternal smoking, certain specific birth defects have been including orofacial clefts, limb reduction defects, and urogenital defects (Office of the US Surgeon General, 2001). Orofacial clefts were not mentioned however in the most recent WHO report, *Women and the Tobacco Epidemic: Challenges for the 21st Century* (Samet and Yoon, 2002). The tobacco-related health effects of stillbirth, prematurity and intrauterine growth retardation are much more common and better studied than orofacial clefts, yet the topic of orofacial clefts may have powerful and persuasive effects if incorporated into public health campaigns on the consequences of maternal smoking. The images of faces of disfigured children have been used to establish some of the world's largest medical charity organizations that are devoted to providing free orofacial cleft surgeries in under-served populations. Similar images might prove effective in public health campaigns to protect pregnant women from tobacco smoke and other environmental teratogens.

The association between maternal smoking and orofacial clefts may not be widely appreciated by international health organizations

7.2.2 Maternal alcohol use and craniofacial anomalies

Maternal alcohol use during pregnancy is a well-known cause of the fetal alcohol syndrome. Delegates at the WHO/Utah meeting reported that the occurrence of the fetal alcohol syndrome ranges between 1 per 1000 births in western industrialized countries, 8-10 per 1000 in selected Native (North) American populations, and 108 per 1000 in selected South African populations. The populations at high risk for the fetal alcohol syndrome are almost always impoverished, have easy access to alcohol and, in many cases, have experienced rapid deterioration of their traditional culture and subsistence patterns. The fetal alcohol syndrome represents an extreme example of the effects of maternal alcohol consumption during pregnancy and the pattern of alcohol consumption usually involved – binge drinking – is also extreme. While the characteristic and severe features of the fetal alcohol syndrome are mainly neurologic, resulting in diminished cognitive and behavioural functions, animal and human studies have shown that midline craniofacial anomalies, including orofacial clefts may also occur (Kotch and Sulik, 1992; Johnson et al., 1996).

Women are more commonly exposed to lower levels of alcohol intake during pregnancy than occurs during the binge drinking associated with fetal alcohol syndrome. Alcohol drinking takes place in a variety of social contexts that may include the modifying or confounding effects of diet, smoking and drug use; it is thus understandable why the association between maternal alcohol use and risk of isolated birth defects is not entirely consistent. Maternal alcohol use during pregnancy has been associated with an increased risk of isolated orofacial clefts in some, but not all, studies, as discussed in Section 4.5.2. An examination of the social and dietary context in which alcohol consumption takes place may help to clarify its relationship to orofacial clefts and other CFA. For example, the risk from alcohol consumed while drinking beer at a bar with non-nutritious snacks and exposure to active or passive smoking is not likely to be equivalent to that when the same amount of alcohol is consumed by drinking wine with a nutritious meal. Despite some remaining uncertainties about the relationship between patterns of alcohol consumption and the risk of isolated orofacial clefts, enough evidence exists of a firm causal relationship between maternal alcohol consumption and craniofacial anomalies and other adverse reproductive health effects to warrant strong, worldwide, public health measures to discourage maternal alcohol consumption near the time of conception and during pregnancy.

There is enough evidence of a firm causal relationship between maternal alcohol consumption and CFA

7.2.3 Other maternal exposures related to craniofacial anomalies

Maternal exposures to possible teratogenic medications and chemicals in the workplace and residence were reviewed above in Section 4.5. These teratogens may be critically important to women exposed to them but do not seem as widespread as nutritional deficiencies and tobacco and alcohol exposures; they do not, thus, seem to be ideal choices for broad, population-based, intervention studies. Birth-defect prevention efforts related to medications might ideally be focused on clinical approaches, and occupational exposures to teratogens might best be studied further, with prevention efforts targeted at specific occupational groups.

7.3 Maternal nutrition and orofacial clefts

Adequate nutrition of the mother at the time of conception and in the first trimester of pregnancy appears to be important for the normal development of the lip, palate and other craniofacial structures of the fetus. Much experimental evidence for this view has accumulated from studies of laboratory animals in which specific nutritional deficiencies were induced either by dietary manipulation or by the administration of specific nutrient antagonists. Observational studies of human populations are highly supportive of an important role for maternal nutrition in

normal craniofacial development but, with this approach, it has been difficult to identify the specific nutrients involved because of the high intercorrelation of the many nutrients in multivitamin preparations, fortified foods and healthy dietary patterns. A comprehensive review of laboratory animal and human epidemiologic studies of maternal nutrition and orofacial clefts is available (Munger, 2002). Taken together, the evidence from laboratory animal experiments and human observational studies point to folic acid and vitamin B-6 as leading candidate nutrients that may be useful in the prevention of orofacial clefts, and a lesser body of evidence implicates riboflavin (vitamin B-2) and vitamin A.

7.3.1 Folic acid

The role of maternal dietary folate intake in orofacial clefts has been difficult to determine in human case-control studies

Animal models for the study of folate deficiency as a cause of fetal death, orofacial clefts and other birth defects were first established in the 1940s by Nelson, using a combination of dietary folate deficiency and folate antagonists (Nelson and Evans, 1947; 1949; Nelson et al., 1950). Folate antagonists were eventually found to cause craniofacial and other birth defects in mice, rats and chickens, and folate supplementation was found to prevent orofacial clefts in a breeding line of dogs with a genetic predisposition to orofacial clefts (Elwood and Colquhoun, 1997). Medications that disrupt folate metabolism have been shown in human case-control studies to be associated with an increased risk of birth defects, including orofacial clefts (Hernandez-Diaz et al., 2000). The role of maternal dietary folate intake in orofacial clefts has been difficult to determine in human case-control studies because folates from food sources have a wide range of bioavailability and folic acid supplements are usually taken with other vitamins, minerals and trace elements that may also have protective effects against orofacial clefts. Studies of genetic variation of folate-dependent enzymes may yield clues about the role of folate in orofacial clefts, but to date genetic studies have not altered the current state of equipoise: the MTHFR C677T thermolabile genotype was found to be associated with an increased risk of orofacial clefts in Ireland (Mills et al., 1999) but not in the United States (California) – (Shaw et al., 1998; 1999).

7.3.2 Vitamin B-6

Vitamin B-6 (pyridoxine and closely related compounds) is known to protect against orofacial clefts induced in laboratory animals by teratogens including corticosteroids (Fraser and Fainstat, 1951; Kalter, 1957; Peer et al., 1958; Bonner and Slavkin, 1975; Melnick et al., 1981), vitamin A excess (Yamaguchi, 1968), cyclophosphamide (Dostal and Schubert, 1990), and beta-aminoproprionitrile (Jacobsson and Granstrom, 1997). Deoxypyridine, a vitamin B-6 antagonist, was shown to induce orofacial

clefts (Miller, 1972) and vitamin B-6 deficiency alone was sufficient to cause cleft palate and other birth defects in mice (Davis et al., 1970). Less information is available from human studies on the possible role of vitamin B-6 in orofacial clefts *(see Section 4.4.1)*.

In a case-control study in the Netherlands, mild maternal homocysteinemia was associated with an elevated risk of nonsyndromic orofacial clefts (Wong et al., 1999). Biochemical studies revealed that case-mothers had lower levels of whole blood vitamin B-6 (measured as pyridoxal-5'-phosphate) compared to controls; no differences were found in levels of serum vitamin B-12 and case-mothers had higher levels of serum and red-cell folate compared to controls. Thus, in the Netherlands poorer vitamin B-6 status was associated with a higher risk of orofacial clefts and one possible mechanism may have been elevated homocysteine levels in mothers with poorer vitamin B-6 status.

The worldwide occurrence of vitamin B-6 deficiency is not well described although it is known to be a regional problem in poorer populations of Asia where highly polished rice is the dietary staple and few other dietary sources of vitamin B-6 are available (Bamji et al., 1979). These populations also appear to have elevated rates of orofacial clefts. Vitamin B-6 deficiency is also induced by use of certain medications, including isoniazid for the treatment of tuberculosis, and oral contraceptives (Sauberlich et al., 1972).

7.3.3 Riboflavin (vitamin B-2)

Riboflavin (vitamin B-2) deficiency was found by Warkany in the 1940s to cause skeletal malformations and orofacial clefts in laboratory rats (Warkany and Nelson, 1940). In further studies of the timing of deficiencies during gestation, Warkany found that riboflavin supplementation before Day 13 prevented the malformations but later supplementation did not, thus establishing the principle of a critical period in embryonic development for the susceptibility to nutritionally-induced birth defects (Warkany, 1954). Further studies by others confirmed that riboflavin deficiency caused birth defects in rats (Noback and Kupperman, 1944; Giroud and Boisselot, 1947; Leimbach, 1949; Piccioni and Bologna, 1949; Giroud and Boisselot, 1951), mice (Kalter and Warkany, 1957), and fowl (Lepkovsky et al., 1938; Romanoff and Bauernfeind, 1942).

Despite the findings that riboflavin deficiency caused orofacial clefts and other birth defects in laboratory animals, it does not seem to have been the subject of research in studies of human orofacial clefts. This is an important gap in current knowledge because riboflavin deficiency is one of the most common vitamin deficiencies worldwide (Sauberlich, 1984); it commonly co-occurs with vitamin B-6 deficiency (Bamji et al., 1979) and is closely interrelated with vitamin B-6 metabolism (Sauberlich, 1999).

7.3.4 Vitamin A

Both excessively high and low levels of vitamin A intake during pregnancy have been associated with an increased risk of orofacial clefts and other craniofacial anomalies. Hale was the first to report that maternal vitamin A deficiency caused eye defects, orofacial clefts and other birth defects in experiments with pigs (Hale, 1933; 1935). Human vitamin A deficiency is widespread, especially in developing countries around the world (West et al., 1999). Birth defects related to vitamin A deficiency may be unnoticed in impoverished populations because of the larger burden of other health problems. In a case-control study in Japan maternal consumption of vegetables rich in the plant form of vitamin A, ß-carotene, was associated with a reduced risk of CL/P (Natsume et al., 1999).

Most subsequent research on vitamin A-related compounds and craniofacial anomalies in laboratory animals has involved excess exposure to retinoic acid and other retinoids (Kochhar et al., 1984; Abbott and Pratt, 1988; Abbott and Birnbaum, 1990; Whitby et al., 1994; Soprano and Soprano, 1995; Ross, 1999). Human clinical studies have revealed that fetal exposure to retinoid compounds may result in severe craniofacial anomalies (Lammer et al., 1985) and dietary exposures to high levels of vitamin A may also be important. In a prospective study of more than 22 000 births to women in the United States, craniofacial anomalies and other malformations were more common in women who consumed more than 10 000 IU of vitamin A in the peri-conceptional period (Rothman et al., 1995).

7.4 Nutritional supplementation

BOX N

Trials of maternal nutritional supplementation and orofacial clefts

Several attempts have been made to conduct human trials to evaluate maternal vitamin supplementation during pregnancy as a means of preventing orofacial clefts; these were first motivated by the seemingly promising results of experiments in laboratory animals. The first published reports appeared in 1958 and described attempts in the United States to give mothers supplementary multivitamins but the studies were very small; few methods and no statistical analyses were reported (Conway, 1958; Douglas 1958; Briggs, 1976). Other attempts at vitamin supplementation trials for the prevention of orofacial clefts were attempted in Europe (von Krebig and Stoeckenius, 1978; Schubert et al., 1990) and these authors made claims for the effectiveness of the treatments, yet each of these studies also had insufficient data to allow an evaluation of the results.

7.4.1 The Czech orofacial-cleft prevention trial

Tolorova et al. began a trial of vitamin supplementation for the prevention of orofacial clefts in high-risk Czech women in 1976 (Tolarova, 1982). High-risk mothers were defined as those who had given birth to a child with a cleft or who had a cleft themselves. Participating mothers were advised to take a multivitamin preparation daily, during the period three months before conception until the end of the first trimester. The daily multivitamin dose included:

vitamin A (6000 IU),
vitamins B-1 (3 mg), B-2 (3 mg), B-6 (3 mg),
vitamin C (150 mg),
vitamin D (300 IU),
vitamin E (6 mg),
nicotinamide (30 mg),
calcium pantothenate (3 mg), and
folic acid (10 mg).

The exclusion of non-compliant participants in a clinical trial may seriously bias the results

The "treated" mothers were those who accepted supplements and the "controls" were those who refused or failed to comply. Results reported in 1982 revealed that 1 of 85 "supplemented" pregnancies and 10 of 212 "unsupplemented" pregnancies were affected with orofacial clefts (Tolarova, 1982). Later updates (Tolarova, 1987; Tolarova and Harris, 1995) revealed that 3 of 211 "supplemented" pregnancies and 77 of 1824 "un-supplemented" pregnancies were affected with orofacial clefts (Fisher exact p-value, one-sided test, $p = 0.03$; two-sided test, $p= 0.058$). Important limitations of the Czech study include lack of random assignment of mothers to the treatment and control groups and exclusion of non-compliant participants from the analyses. The mothers in the supplement-treated group received additional interventions that the control group did not receive, including advice to conceive in the late spring and summer months because of the greater availability of fresh fruit and green vegetables and a lesser risk of respiratory tract infections. The exclusion of non-compliant participants in a clinical trial may seriously bias the results, even if the trial begins with random assignment; this is the basis for "intention-to-treat" analyses in the design of modern clinical trials (Meinert, 1986). Because of these design limitations and the lack of statistical significance, the results of the Czech trial are not interpretable.

7.4.2 The Hungarian birth-defects prevention trial

The Hungarian Family Planning Program (HFPP) was the setting used by Czeizel and colleagues for a clinical trial to test the efficacy of peri-conceptional multivitamin supplementation in the primary prevention of birth defects (Czeizel and Dudas, 1992; Czeizel, 1993a, b; Czeizel et al.,

1994; Czeizel and Hirschberg, 1997; Czeizel, 1998; Czeizel et al., 1999). Participating women were given genetic counselling, and health advice regarding nutrition, smoking and alcohol use. The inclusion of health education on known reproductive hazards for all participants in the trial is laudable and is an early example of the provision of minimum local standards of care in a trial, an ethical issue that has emerged in more recent discussions. Participating women were randomly assigned to receive either a multivitamin or a trace-element tablet daily for the period one month before conception, until the third month of gestation. The trial was double-blind. The multivitamin contained:

vitamins A (6000 IU until 1989 and 4000 IU thereafter),
B-1 (1.6 mg), B-2 (1.8 mg), B-6 (2.6 mg), B-12 (4 ug),
C (100 mg), D (500 IU), E (15 mg);
folic acid (15 mg);
nicotinamide (19 mg);
calcium pantothenate (10 mg);
biotin (0.2 mg);
four minerals, including calcium (125 mg), phosphorus (125 mg), magnesium (100 mg) and iron (60 mg); and
three trace elements, including copper (1 mg), manganese (1 mg) and zinc (7.5 mg).

A trial of primary prevention must have a larger sample size than a recurrence-prevention trial to demonstrate a given treatment effect

The trace-element control group took a tablet with the same amounts of copper, manganese and zinc, with the addition of vitamin C (7.3 mg) and lactose (736 mg). Based on an "intention-to-treat" analysis, there was a significant reduction in NTDs (0 in 2471 vitamin-supplemented pregnancies versus 6 in 2391 trace-element-only treated pregnancies; $p = 0.02$), but no significant difference between the treatment groups was observed in the occurrence of a small number of orofacial clefts (4 among the vitamin-supplemented group and 5 in the trace-element-only supplemented pregnancies; $p = 0.57$) (Czeizel, 1998; Czeizel et al., 1999). Thus, the Hungarian trial showed a significant protective effect of multivitamins in reducing the primary occurrence of NTDs, but the trial was too small to determine whether or not multivitamin use prevented orofacial clefts. The Hungarian trial underscores the point that a trial of primary prevention must have a larger sample size than a recurrence-prevention trial to demonstrate a given treatment effect. Another difficulty in interpreting the lack of a treatment effect for orofacial clefts in the Hungarian trial is that the control group received trace elements, including copper and zinc, that may have lowered the risk of orofacial clefts, thus possibly obscuring a treatment effect in the multivitamin group.

7.4.3 Prevention trials

BOX 0

Future directions for orofacial-cleft prevention trials

The trials of maternal nutritional supplementation for the prevention of orofacial clefts conducted to date have been uninformative because of inadequate sample sizes and methodologic flaws. Further understanding of maternal nutrition and orofacial clefts will require that specific nutritional hypotheses and state-of-the-art trial design be applied in appropriate high-risk populations. Investigators interested in birth defects prevention would benefit from collaboration with others involved in prevention trials in different areas of reproductive health. Professor Keith West spoke at the WHO/Utah meeting about his experience in conducting large-scale nutritional intervention studies related to maternal and child health in Bangladesh, Indonesia, Nepal, the Philippines and Thailand. His most recent trial assessed the effect of vitamin A supplementation in reducing mortality related to pregnancy in women of reproductive age in a rural and undernourished population in Nepal. Nearly 45 000 women participated in the double-blind, cluster-randomized, placebo-controlled trial and over 22 000 pregnancies were followed. The results of the trial showed that supplementation to women of reproductive age with either preformed vitamin A or beta carotene in recommended dietary amounts significantly lowered mortality related to pregnancy (West et al., 1999). The Nepalese trial and others like it have studied reproductive outcomes such as maternal and infant death, prematurity and low birth weight – factors that are far more common than birth defects, in general, or orofacial clefts in particular.

One of the most difficult challenges in future orofacial-cleft prevention trials will be in recruiting many thousands of high-risk women in their reproductive years. These efforts will lead investigators to high-risk populations in culturally and economically diverse settings. This important research must be done according to current ethical standards – and this is not a straightforward issue because ethical standards continue to evolve and no single set of ethical standards is applicable in every setting around the globe. The lively discussion at the WHO/Utah meeting on appropriate ethical standards for prevention trials for human orofacial clefts reflected the larger sphere of international debate on ethical standards for human experimental trials.

7.5 Ethical issues

BOX P

Ethical issues related to studies of maternal nutrition and birth defects

Professor Richard Smithells, one of the founders of studies of the role of folic acid in human neural tube defects, gave a personal account at the WHO/Utah meeting of the early stages and evolution of his involvement in this area of research. Smithells faced many dilemmas because his personal convictions and dedication to patients collided at times with the mandates of ethical review boards, the opinion of colleagues, and the popular press. At an earlier stage he was not allowed to proceed with a correct randomized trial of folic acid for the prevention of neural tube defects. Later, however, when he was personally convinced that folic acid could prevent neural tube defects and had hence lost his state of equipoise, ethical review boards and health officials in the United Kingdom had become convinced that the time for a randomized, controlled clinical trial had arrived. Professor Smithells believed ethics were very personal and individual; relative rather than absolute. This view was echoed later by many of the meeting delegates.

Professor Smithells recognized the need for "someone else" to conduct the definitive trial of folic acid supplementation for the prevention of recurring neural tube defects and stepped aside. He listed several lessons he learned from this experience:

(1) What you judge to be ethical or unethical depends on what you believe – ethics are perhaps relative rather than absolute.

(2) If a thing is worth doing, it is worth doing properly – and that means getting it right the first time around if you can. If a randomized trial is possible – and it isn't always – it is to be preferred.

(3) The more circumstantial evidence there is that something works, especially from non-randomized or uncontrolled studies, the more difficult it is to launch a randomized study later. If you spend too long snapping at the heels of a problem, you may lose the opportunity to "go for the jugular and sort it out in one".

7.5.1 Ethical guidelines for research involving human subjects in orofacial-cleft prevention trials

Professor Robert J. Levine reviewed recent developments and current controversies in the international guidelines involving human subjects in research, with a focus on the recent revisions of the *Declaration of Helsinki* by the World Medical Association (WMA, 2000) and the 2001 draft revisions of the *International Ethical Guidelines for Biomedical Research Involving Human Subjects* by the Council for International Organizations of Medical Sciences (CIOMS, 2001). Professor Levine pointed out that problems inherent in the *Declaration of Helsinki* include an artificial distinction between therapeutic and non-therapeutic research and outdated views of contemporary ethical thinking, particularly in the area of placebo controls. This situation has led to widespread debate and has prompted the WMA and CIOMS to revise their recommendations (current drafts are available on the web sites of these groups). The discussions of placebo and control groups at the Utah/WHO meeting paralleled the broader international debates, with many divergent views being expressed on the basic definitions of placebo and control groups and their proper use.

A complete discussion of ethical issues related to biomedical research in general and to prevention trials in particular was beyond the scope of the WHO/Utah meeting and these topics are covered in detail in the references cited above. There was, however, detailed discussion on several ethical aspects of orofacial-cleft prevention trials, relating to the development of nutritional intervention trials for the prevention of orofacial clefts in industrialized and technologically developing countries and resource-poor populations. The following summary of ethical issues is a result of the presentations made by Professors Levine and Smithells, and discussions with the meeting delegates.

7.5.2 Equipoise

BOX Q

Equipoise

A fundamental requirement for the justification of a clinical or community-based intervention trial is a recognized state of uncertainty or unresolved dispute among expert clinicians and researchers regarding which therapeutic or preventive measures are superior. The term ***equipoise*** is often used to describe the state of equilibrium between view points. The requirement for equipoise before embarking on a trial should be most stringently applied when the treatments or interventions being tested are for lethal or disabling medical conditions (World Medical Association, 2000; Council for International Organizations of Medical Sciences, 2001).

Chalmers described the ideal conditions for an ethical clinical trial as a test of the perfect null hypothesis in which individual physicians have no idea as to whether a treatment is better than a placebo or if two alternative treatments differ in effectiveness (Chalmers, 1978; 1979). Freedman derided this view, labelling it ***theoretical equipoise,*** and proposed as a replacement the term ***clinical equipoise*** to describe the situation where both risks and benefits were considered as critical parts of the justification for a clinical trial (Freedman, 1987). Freedman allowed that individual clinicians may differ in their judgements about alternative treatments yet ideally join together in a trial to resolve the dispute; the situation described earlier by Professor Smithells regarding neural tube defects and folic acid supplementation is an example of this situation. The common purpose is to develop compelling evidence that one treatment is better than another (or better than placebo) so that other physicians and scientists who have not participated in the trial will be convinced of the results and change their pattern of practice. Unambiguous results are also needed to convince elected officials of the need to change public health policies through acts of legislation.

The information needed to establish a state of equipoise includes data from animal experiments, observations from human case-control and cohort studies, and evaluation of previous trials, if they exist. Professor Meinert pointed out that, in most of the important controversies in medicine and public health, there has been no single, definitive trial and the balance of equipoise is usually tipped by the accumulation of results from many separate studies.

The balance of equipoise is usually tipped by the accumulation of results from many separate studies

7.5.3 Appropriate study design and ethics

The Helsinki and CIOMS guidelines begin from the position that all studies involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of scientific literature, and employ the latest advances in study design and practice. Ethical review cannot be separated from review of study design and scientific methods. Research that is unsound or deficient because of lack of statistical power to detect treatment effects will not only result in a waste of the participants' time and the resources of sponsoring agencies but will also expose the participants to risk, even if slight, without the prospect of benefits. Further discussion of trial design, important in advancing knowledge of the prevention of orofacial clefts, appears in Section 7.6 below.

Ethical review cannot be separated from review of study design and scientific methods

7.5.4 Local health priorities and applications of findings

Sponsoring agencies and investigators should make every reasonable effort to ensure that a prevention trial is responsive to the health needs and priorities of the participating local populations and that the intervention can and will be made available to the local populations within a reasonable period of time. These considerations become especially important in populations or communities with limited resources. According to the CIOMS guidelines it is not sufficient to justify a prevention trial because of a high prevalence of the health condition of interest; it is also necessary that the intervention being studied, if found to be beneficial, could reasonably be introduced into the local population at the conclusion of the study. If the intervention being evaluated, such as nutritional supplementation, is too expensive or impractical to distribute in the population participating in the trial, and if the knowledge gained about the intervention is used to benefit other populations that have the resources to employ the intervention, then the study is exploitative and therefore unethical (CIOMS, 2001). Detailed baseline studies are needed to describe local health priorities, common maternal and child health problems, the birth prevalence of orofacial clefts and other important birth defects; dietary patterns and biochemical studies are needed as a baseline measure of maternal nutritional status. In most populations half – or more – pregnancies are not precisely planned, therefore nutritional interventions should have the potential to be introduced via dietary improvements and food fortification in the population at large to improve intake of vitamins in the peri-conceptional period. In most populations the more clinical approach of providing nutritional supplements in pill form will not reach a significant number of women in the peri-conceptional period; some notable exceptions however have included China and Hungary, and other areas where family planning and prenatal health care receive strong cultural and governmental support.

7.5.5 Selection of research subjects

The benefits and burdens of intervention trials and other research should be equitably distributed both within and between populations. According to CIOMS Guideline 12 "no group or class of persons should be required to bear more than its fair share of the burdens of participation in research; similarly, no group should be deprived of its fair share of the benefits of research" (CIOMS, 2001). In some areas it is possible that certain groups have been overused as study subjects where research institutions have had access to local patient populations. This is a particular concern when it is easy to recruit impoverished persons as research subjects because they are willing, due to their desperate condition, to participate – in exchange for a trivial (from the viewpoint of the sponsor) payment. This is a larger concern for pharmaceutical trials conducted among the poor – especially when the results are used to benefit wealthier populations, than for investigations of the specific conditions of the poor, as in studies of malnutrition and nutritional deficiencies in populations with a high risk of orofacial clefts.

7.5.6 Placebos and other control treatments

According to Article 29 of the *Declaration of Helsinki* (WMA, 2000):

> "the benefits, risks, burdens, and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists."

Professor Levine pointed out that a major weakness of the Helsinki guidelines is that trials appear to be ruled out in resource-poor countries if the standard of "best current method" is mandated as the control treatment yet is not locally available due to scarcity, high cost, or both (Levine 1999; 2000). According to Levine, this weakness in the Helsinki guidelines is the root of the most bitter controversy in research ethics over the past 30 years, precipitated by the trial of a short duration AZT regimen in the prevention of perinatal transmission of HIV-infected pregnant women. The medication that was the "best available method" at that time in industrialized countries cost 80 times the annual per capita health expenditure in sub-Saharan countries; and this cost did not take into account the advanced medical resources required to administer the medication. As early as 1993 this dilemma led to the recognition that an absolute standard of "best available treatment" could not be applied worldwide and that special arrangements had to be made for trials in low-resource countries. The CIOMS guidelines (CIOMS, 2001) now recognize that there are circumstances in which use of a control treatment other than the "best current method" is justified if:

(1) the scientific and ethical review committees in both the country of the sponsoring institution and the host country determine that use of the "best current method" as a control would be likely to invalidate the results of the research or make results inapplicable in the host country;

(2) plans to make the therapeutic product reasonably available in the host country or community are securely established; and

(3) a process of planning and negotiation, including justification of a study in regard to local health-care needs, has taken place with the health authorities in the host country before the research begins.

There is currently no nutritional intervention for women that is known to prevent orofacial clefts in their offspring

The three most important micronutrient deficiencies worldwide – iron, vitamin A and iodine – are causes of maternal and child illness and death, overwhelmingly greater in number than those affected by birth defects. Iron, vitamin A, and iodine are inexpensive in industrialized countries, yet scarce and difficult to distribute in resource-poor countries, underscoring the point that nutritional interventions face ethical dilemmas similar to those raised in the case of the AZT trials for the prevention of perinatal HIV transmission in Africa.

Folic acid supplementation for women in all populations appears to be the "best current method" of peri-conceptional care for the prevention of neural tube defects in industrialized countries but appears difficult to implement in many low-resource countries with health agendas crowded with a growing number of recommended health-related interventions.

There is currently no nutritional intervention for women that is known to prevent orofacial clefts in their offspring. At first glance this seems to be the ideal state of clinical equipoise, making the test of a nutritional intervention versus placebo timely. The issue becomes complicated quickly when folic acid supplementation, known to reduce the risk of neural tube defects in several populations, is proposed as a preventive intervention to reduce the occurrence of orofacial clefts. Many of the delegates at the WHO/Utah meeting felt that any study that did not provide 400 micrograms of folic acid per day to all mothers was unethical because folic acid would be "withheld" from mothers and they would be at higher risk of having a child with a neural tube defect. Some delegates extended the view that folic acid supplementation was mandatory for women participating in birth defect studies of any design, including observational cohort studies. Others felt that public health action to provide folic acid to women of reproductive age (and many other nutrients important to reproductive health) was well under way through public health campaigns to increase dietary intake of folates and folic acid-containing vitamins in the periconceptional period and through food fortification (in Chile, the United States and a growing number of other countries). Thus placebo-controlled

studies of higher levels of folic acid supplementation, as an "add-on" study to the increasing baseline intake of folic acid, was viewed by other delegates as ethical.

The use of placebos is currently being debated by the WMA and CIOMS and the delegates at the WHO/Utah meeting were not successful in reaching a consensus either – indeed the basic definition of a placebo was not even widely agreed upon. Some investigators have added to their "placebo" other vitamins, minerals, trace elements, vaccinations, or treatments for parasites – each thought to be unrelated to the condition under study – as a way to provide some inducement for participation, even though the real benefits may have been difficult or impossible to quantify. This kind of comparison becomes difficult to interpret if later evidence arises that one of the additives to the "placebo" group indeed alters the risk of the outcome under study; if this is the case then the "placebo" is really an active control treatment. In a nutritional supplementation trial a strict placebo would include no active compounds and would be identical in appearance to the hypothesized active treatment, in most cases a pill or an injection. Anything else that is compared to a hypothesized active treatment should be referred to as an active control treatment (Meinert, 1996). In the Hungarian birth defects prevention trial the group actively treated with multivitamins was compared to a "trace element control" group that received a tablet with the same amounts of copper, manganese and zinc as the "active treatment" group received, but with the addition of vitamin C and lactose. The Hungarian study thus did not employ a true placebo-control group and concerns have been raised that, since zinc nutriture might be related to the risk of birth defects and zinc was provided to both groups, the occurrence of NTDs (and perhaps orofacial clefts and other birth defects) may have been reduced in both groups, obscuring the treatment effect of the other nutrients. The trial of the Medical Research Council (MRC) trial to prevent NTDs employed a control group that received tablets with iron and calcium (without the main "active" treatments compared, folic acid alone or folic acid plus multivitamins) rather than a true placebo control group. This was recently criticized by Turner (Turner et al., 2001) who speculated that exposure to high levels of iron and calcium (among control mothers who took more than one pill per day) may have interfered with zinc nutriture and raised the risk of NTDs. Turner's re-interpretation of the MRC results has been disputed by Moore (Moore, 2001). An important lesson from this experience is that investigators should rigorously define their control groups or risk endless re-interpretations of their study findings.

The use of control groups in nutritional intervention trials is thus complex and there is no global consensus on the precise guidelines for their use

The use of control groups in nutritional intervention trials is thus complex and there is no global consensus on the precise guidelines for their use. Investigators designing trials should follow the general principles regarding control treatments outlined in the *Declaration of Helsinki* and clarified by the CIOMS guidelines, but should decide on the appropriateness of control groups in consultation with the institutional review boards representing the sponsoring institutions and local populations participating in the study.

7.5.7 Standard of care

BOX R

Highest attainable and sustainable standard of care

In response to the deep divisions over the ethics of HIV-prevention trials among pregnant women in resource-poor countries and other similar dilemmas, a new standard of care for therapeutic methods in clinical trials has emerged in recent revisions of the Helsinki and CIOMS ethical guidelines: the "highest attainable and sustainable therapeutic method" (Lurie et al., 1994; Aaby et al., 1997; Levine, 1999; 2000; WMA, 2000; CIOMS, 2001). Professor Levine has recently published a detailed analysis of these developments (Levine, 1999; 2000) and discussed this at the WHO/Utah meeting.

"Highest attainable" therapy means that under the conditions of a clinical or community-based intervention trial, the level of therapy in the given location should be "the best one can do." The level of care available in a resource-poor population should define the minimum ethically-acceptable standard. "Sustainable" means the level of care, medical treatment, or nutritional supplementation that can be expected to be maintained by the local population after completion of the trial. These new standards are closely linked to the principles of addressing local health priorities in a research programme and ensure the application of the findings of the trial in the local population. The introduction of interventions of therapies that are not locally available and sustainable may undermine local health services and priorities. According to Levine, the main benefit of adhering to the standard of available and sustainable therapies "tends to facilitate the efforts of resource-poor countries to develop needed therapies and preventions that are within their financial reach. Until the imbalances in the distribution of wealth among nations of the world are corrected, this appears to be the best we can do" (Levine, 2000).

7.6 The design of orofacial-cleft prevention trials

Timing is the essence of an intervention trial because the state of equipoise may be a narrow window of opportunity. A feeling of urgency however should not lead investigators to start assigning treatments to participants until the infrastructure is in place and the study protocol is developed, data forms are established and tested, field staff are hired and trained for participant recruitment, data intake and analyses, and a mechanism has been established to independently monitor the trial (Meinert, 1986). No single trial is likely to be definitive and trials are needed in diverse populations in both industrialized and technologically developing countries.

7.6.1 Selection of the study population

Trials in high-risk populations are more likely to detect a treatment effect than trials in low-risk populations, and at lower cost and with greater speed. A recurrence-prevention trial of orofacial clefts in a high-risk population will still require that several thousand births are evaluated; a primary prevention trial would require tens of thousands of births. For planning a trial, baseline studies of cleft occurrence and recurrence are needed, as well as a good sense of whether the local population is willing to participate in a trial.

7.6.2 Specification of the test treatment or treatments

The choice of a specific nutrient intervention or interventions should be based on prior laboratory animal studies, observational studies of human populations, and detailed studies of biochemical indicators of nutritional status in the population of interest. The investigators must consider whether the goal of the study is to investigate dose levels of nutrients to correct inadequate dietary intake or higher pharmacological doses that might be necessary to overcome acquired or genetically-based metabolic problems. Well-targeted nutritional hypotheses will have greater public health benefits than the broad approach of multivitamin supplementation because knowledge of the specific nutrients involved could lead to food-based interventions that would ultimately reach a far greater number of women of reproductive age than programmes to encourage the use of supplements in the peri-conceptional period would. Factorial and dose-response study designs are highly efficient ways to answer several complex questions about multiple treatments and doses in a single trial.

7.6.3 Specification of the placebo or other control treatment

Many investigators may be tempted to avoid the difficult issues regarding the use of placebo controls or active treatment controls discussed above by attempting to make comparisons between participants receiving the test treatment and so-called "historical controls" (untreated persons from an earlier time period in the same geographic area) or "geographic controls" (untreated persons from a different geographic area in the same time period). Use of historical or geographic controls almost always leads to unclear findings and confusion, thus should be avoided. The use of placebos and active treatment controls was discussed in detail previously.

7.6.4 Outcome measure for evaluating the study treatment

Orofacial clefts appear to be the only group of CFA to be common enough at present for a trial. Since cleft lip with or without cleft palate seems to be etiologically distinct from cleft palate alone, a trial should have its primary focus on one group or the other. The issue of detecting and evaluating early pregnancy losses should be carefully considered.

7.6.5 Bias-free method for assigning patients to the study treatments

Test and control treatments should be randomly assigned to participants. In the assignment of treatments, "haphazard" does not equal "random" thus formal mechanisms should be in place and monitored to assure true random assignment of treatments.

7.6.6 Double masking of treatment status

The treatment status should be concealed from participants and investigators to avoid bias in the attention given to each participant. Because curiosity seems to be a universal human trait, even the most dedicated co-investigators and field staff may be tempted to decipher the treatment allocations, thus much attention should be given to this issue.

7.6.7 Monitoring

An independent data, safety, and monitoring committee (DSMC) should be established to regularly review progress of a trial. This committee should have access to all information gathered in the trial, including the treatment allocations of participants. Side-effects and compliance of participants should be closely monitored by the trial field staff and study investigators and reported to the committee.

7.6.8 Analysis by assigned treatment

Investigators should analyse and report results according to the original treatment assignment of participants. This is the only analytical approach that is compatible with the randomized design and it avoids treatment-related selection bias in the composition of the treatment groups. Analysis by assigned treatment provides a conservative and realistic measure of the treatment effect that remains after losses due to participant or health-care provider rejection of the treatments.

7.7 Conclusions

7.7.1 Environmental and behavioural factors related to CFA

Craniofacial anomalies are among the most common birth defects and, of these, orofacial clefts are the most common. Most discussions in the WHO/Utah meeting focused on orofacial clefts but the points raised may be relevant for many other craniofacial anomalies. Orofacial clefts appear to have substantial environmental causes, thus the potential for primary prevention seems considerable. The pattern of occurrence of orofacial clefts is different from that for neural tube defects therefore their causes may also be different.

7.7.2 Tobacco

Maternal tobacco use has been consistently associated with risk of orofacial clefts. This association is modest, yet the attributable risk may be of public health importance because many women are exposed to passive smoking and tobacco use is rapidly increasing among women, especially in technologically developing countries. National health agencies and voluntary organizations may be unaware of the association between maternal tobacco use and orofacial clefts.

7.7.3 Alcohol

Maternal alcohol use has been associated with risk of orofacial clefts in some – but not all – studies. The type and context of alcohol consumption differs considerably across populations and more consistent methods are needed for the assessment of maternal alcohol intake.

7.7.4 Maternal nutrition and orofacial clefts

There is considerable circumstantial evidence that maternal nutritional factors may be related to the occurrence of orofacial clefts, the most common of CFA. The most promising candidate nutrients include folic acid and vitamin B-6 (pyridoxine) and a lesser body of evidence suggests roles for riboflavin (vitamin B-2) and vitamin A.

7.7.5 The need for nutritional supplementation trials

The current state of equipoise regarding maternal nutrition and orofacial clefts makes intervention trials of specific nutrients an urgent priority. Further understanding of the role of maternal nutrition in CFA will require well designed and expertly conducted trials. No single trial is likely to be definitive and trials are needed in diverse populations in industrialized and technologically-developing countries and resource-poor populations.

7.7.6 Ethics and design of orofacial-cleft prevention trials

Poorly conceived and conducted trials are unethical because they waste limited resources and further delay the discovery of effective interventions. Intervention trials should employ strict random assignment of participants to treatment groups, include either a placebo or other appropriate control group, include an adequate sample size, be double-masked, monitored by an independent data and safety committee, employ intention-to-treat analyses, and use appropriate procedures to obtain informed consent from each participant. Comparison of an active treatment group to "controls" from a different time period or geographic location is unlikely to yield an interpretable result. Trials in high-risk populations are not only more likely to detect a treatment effect than trials in low-risk populations, but also at lower cost and with greater speed. The choice of nutrient interventions should be based on prior detailed studies of biochemical indicators of nutritional status in the population of interest.

7.7.7 International cooperation

BOX 5

Role for WHO, governmental agencies and non-governmental organizations

An orofacial-cleft recurrence-prevention trial is far more feasible than a trial of prevention of primary occurrence, but will still require many thousands of high-risk mothers. Orofacial cleft surveillance systems and registries need to be further developed and linked to provide the critical infrastructure for orofacial-cleft prevention trials. A current and urgent need is linkage of existing birth defects registries, harmonization of methods of data collection and data management, and the development of these activities in technologically-developing countries and resource-poor populations. Public health action is needed on other fronts as research on the causes of CFA continues. The association between maternal smoking and alcohol use during pregnancy and the risk of orofacial clefts is strong enough to warrant inclusion of this information in public campaigns to reduce exposure to these teratogens in women of reproductive age.