

Synopses of Research Articles

Assessing the Risks of Twin Pregnancies

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As mothers get older and assisted conception becomes more common in developed countries, the incidence of multiple births—primarily of nonidentical siblings, but also of identical ones—has dramatically increased. Multiple pregnancies are high-risk pregnancies, with preterm delivery and monochorionicity (shared placenta) the major problems. Consequently, efforts are underway to optimize the management of these pregnancies.

While identical (monozygotic) twins are much less common than dizygotic ones, monozygotic twinning events are increased after induced ovulation and in vitro fertilization. Monozygotic twins can be diamniotic dichorionic (two amniotic sacs, two placentas), monoamniotic monochorionic (one amniotic sac, one placenta), or diamniotic monochorionic (two amniotic sacs, one placenta). The last type accounts for approximately two-thirds of all monozygotic twins.

Monochorionic twins are at higher risk because they share a common placenta; they are primarily at risk from circulation abnormalities like twin–twin

transfusion syndrome (the smaller twin [donor] does not get enough blood while the larger twin [recipient] becomes volume overloaded) and intrauterine growth restriction. However, the majority of diamniotic monochorionic twin pregnancies do not develop such complications.

Nicholas Fisk and colleagues have studied records of 151 seemingly uncomplicated diamniotic monochorionic pregnancies and found a surprisingly high rate of fetal death: ten unexpected intrauterine deaths occurred in seven of the 151 pregnancies with no prior signs of complications. All deaths occurred within two weeks of a normal scan, at a median gestational age of 34 weeks and 1 day.

The authors conclude that “despite intensive fetal surveillance, structurally normal monochorionic diamniotic twin pregnancies without twin–twin transfusion syndrome and intrauterine growth restriction are complicated by a high rate of intrauterine death.” As the deaths occurred predominantly after 32 weeks’ gestation, the authors suggest

that the prospective risk for fetal death in these pregnancies might be eliminated by elective preterm delivery after 32 weeks.

In an accompanying Perspective (DOI: 10.1371/journal.pmed.0020180), Jane Cleary-Goldman and Mary D’Alton agree that, despite the limitations of the study (its retrospective nature, small numbers, and lack of dichorionic controls), it highlights a critical question for obstetricians, namely, “when is the ideal gestational age to deliver apparently uncomplicated monochorionic twins?” As Cleary-Goldman and D’Alton discuss, at 32 weeks of gestation many of the risks associated with prematurity have abated, but the remaining ones are not negligible. Until larger prospective observational studies have been conducted, balancing these risks remains challenging.

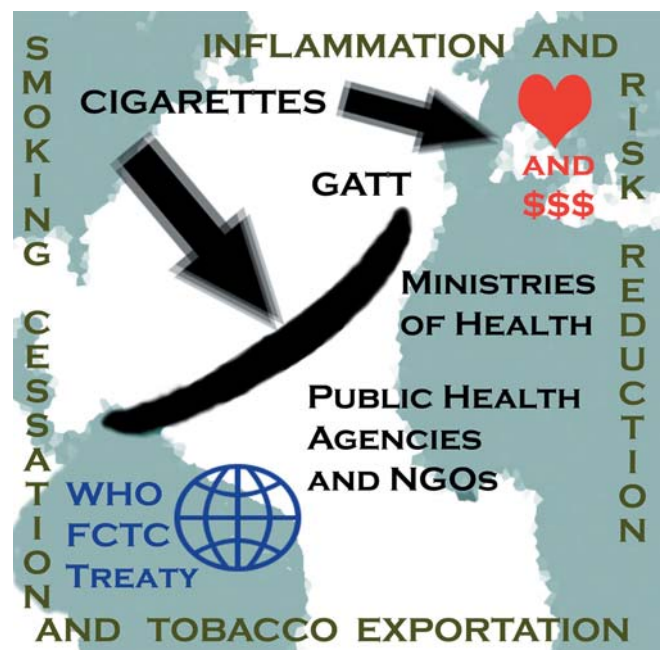
Barigye O, Pasquini L, Galea P, Chambers H, Chappell L, et al. (2005) High risk of unexpected late fetal death in monochorionic twins despite intensive ultrasound surveillance: A cohort study. DOI: 10.1371/journal.pmed.0020172

Smoking and Inflammation

DOI: 10.1371/journal.pmed.0020198

Smoking is the single largest preventable cause of disease and premature death, according to the World Health Organization. Smoking-related diseases kill one in ten adults globally, i.e., 4 million deaths annually; by 2030, if current trends continue, smoking will kill one in six people. Smoking is a prime factor in heart disease, stroke, and chronic lung disease, which cost the United States more than \$150 billion a year. The relationship between smoking and cardiovascular disease is well documented, as is the association of smoking with increased levels of inflammatory markers and accelerated atherosclerosis. It is also well known that when smokers quit, their risk of mortality and future cardiac events declines, but there is little data quantifying the rate of this risk reduction.

Smoking triggers an immunologic response to vascular injury, which is associated with increased levels of inflammatory markers, such as C-reactive protein and white blood cell count. Several studies have shown that such markers predict future cardiovascular events. Markers such as C-reactive protein are also increasingly implicated in the pathogenesis of atherosclerosis. There are, however, still some gaps in our knowledge of cardiovascular disease, smoking, and the predictive use of such markers. For example, few studies have examined the impact of smoking cessation on levels of inflammatory markers or on cardiovascular risk reduction; the level and rate at which the inflammatory response subsides following smoking cessation



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Short- and long-term health-care savings may be realized if smoking cessation is made a priority

is also uncertain. Furthermore, whether traditional risk factors can explain the decline in cardiovascular risk following smoking cessation is also unclear.

In this month's *PLoS Medicine*, Arvind Bakhru and Thomas Erlinger investigate the association between smoking and smoking cessation and levels of inflammatory markers and cardiovascular risk factors. Data were gathered on 15,489 US adults between 1988 and 1994 in the Third National Health and Nutrition Examination Survey. Of these, 7,665 were classified as never smokers, 3,459 were former smokers, and 4,365 were current smokers.

The investigators focused on changes in C-reactive protein, white blood cell count, albumin, and fibrinogen, and the traditional risk factors—total cholesterol, high-density lipoprotein cholesterol, triglycerides, systolic blood pressure, and diabetes—that occurred with decreased smoking intensity and increased time since smoking cessation. They found that inflammatory markers had a dose-dependent and temporal relationship to smoking and smoking cessation. They noted that both inflammatory and traditional risk factors improved with less smoking, but as the time since smokers quit increased, inflammatory markers resolved more slowly than traditional cardiovascular risk factors. Still, the smoking-associated inflammatory response returned to normal within five years after

smokers quit, suggesting that the vascular effects were reversible and that cardiovascular risk subsides gradually with reduced exposure.

The authors conclude that these findings support the hypothesis that cardiovascular risk falls as inflammatory response falls, and that inflammatory markers are good indicators of this risk reduction. Despite limitations of the study, including possible errors from self-reporting and lack of data on second-hand smoke and newer measures such as interleukin-6 and high-sensitivity C-reactive protein, the inflammatory markers studied here demonstrated a much clearer trend and longer-lasting effect after smoking cessation than traditional risk factors, and hence were more useful and accurate markers of disease.

As with related studies, these results suggest that smoking cessation should be a more prominent goal of public policy, and the authors conclude that policymakers must pursue smoking cessation plans as an opportunity to make savings on health care through cardiovascular risk reduction. Further research should explore the acute phase response in the months after smoking cessation, which this and other studies have not been able to study adequately.

Bakhru A, Erlinger TP (2005) Smoking cessation and cardiovascular disease risk factors: Results from the Third National Health and Nutrition Examination survey. DOI: 10.1371/journal.pmed.0020160

Weight Loss and Mortality

DOI: 10.1371/journal.pmed.0020200

If you are overweight, then losing weight is good for your health, surely? Unfortunately, the evidence on which an answer to this seemingly simple question might be based is at best equivocal, and at worst very controversial. Previous work has shown that weight loss in obese people improves risk factors associated with cardiovascular diseases and diabetes, but studies are conflicting on the long-term effects of weight loss on mortality. A study in this month's *PLoS Medicine* by Jaakko Kaprio and colleagues on a Finnish dataset adds more evidence to this debate, but experts are divided on what can be concluded from it.

The major difficulty in getting clear results on this question is that it is virtually impossible to do a controlled trial to answer it. Hence, the evidence accumulated has come mostly from epidemiological studies, but it is notoriously difficult to remove all confounding factors from these studies. Kaprio and colleagues' study is another epidemiological study, but we should not simply dismiss the data as unreliable just because of the problems inherent to such a study design. Instead, we should consider their study in the light of all the other evidence available.

Starting from a group of 19,993 twins from Finland who have been studied since 1975, the authors gathered data from the 2,957 overweight participants who remained after they had excluded people with pre-existing disease, and those with missing data. These twins had been asked in 1975 if they intended to lose weight, and then had information on weight collected in 1981. Information on mortality was then collected over the next 18 years; the authors then analyzed mortality in relation to intention to lose weight and actual weight change.

In total, 268 people died. When the results were analyzed, the surprising finding was that people who intended to lose weight,

and who did so, had a somewhat higher mortality than those who intended to lose weight but whose weight remained stable, or went up. People who intended to lose weight, and who did so, also had a slightly higher mortality than those who did not intend to lose weight and whose weight was stable.

The problems with such a study are outlined in an accompanying Perspective (DOI: 10.1371/journal.pmed.002018) by Meir Stampfer from Harvard School of Public Health, and there is no doubt that these results seem counterintuitive. Some readers may take away the idea from this paper that overweight people should not be advised to lose weight, but Stampfer cautions against that interpretation. Perhaps the safest interpretation of these results is that by the time adults are overweight, the health benefits of losing weight are not clear-cut. If there is one message therefore that should be taken from the paper it is this: in order to prevent the associated health effects of obesity, preventing obesity, especially in childhood, should be an overriding public health priority.

The study leaves us with the question of how intentional weight loss could lead to excess mortality. The authors suggest that this could be due to the unavoidable loss of lean body mass, which according to several other studies may increase mortality, and which may outweigh the beneficial effects of losing fat mass in healthy individuals. The authors therefore conclude that "the long-term effects of weight loss are complex, and they may be composed of oppositely operating effects with net results reflecting the balance between these effects."

Sørensen TIA, Rissanen A, Korkeila M, Kaprio J (2005) Intention to lose weight, weight changes, and 18-y mortality in overweight individuals without co-morbidities. DOI: 10.1371/journal.pmed.0020171

A Promising Candidate for a Lassa Fever Vaccine

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Lassa fever, a viral hemorrhagic fever caused by the Lassa virus and commonly transmitted by its rodent host, is endemic in certain areas of West Africa, where several hundred thousand people are estimated to be infected each year. The disease is asymptomatic or mild in approximately 80% of infected patients, but the remaining 20% have severe multisystem disease. Estimated overall mortality is 1%–2%.

Death rates are particularly high for women in the third trimester of pregnancy, and for fetuses, about 95% of which die in the uterus of infected pregnant mothers. The most common complication of Lassa fever is deafness. Various degrees of deafness occur in approximately one-third of cases, and in many cases hearing loss is permanent. Disease severity does not seem to affect this complication: deafness may develop in mild as well as in severe cases.

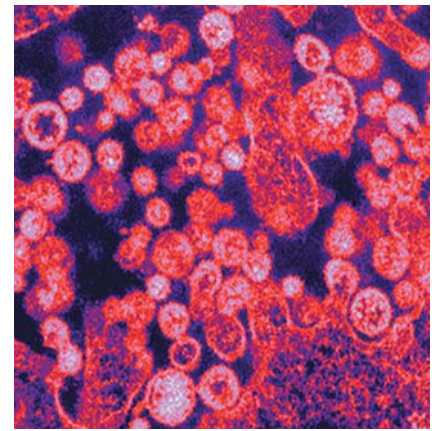
Lassa fever remains a serious challenge to public health in West Africa, threatening both local residents in rural areas and those who serve them, particularly medical care providers. Ribavirin, an antiviral drug, has been used successfully in Lassa fever patients, but it needs to be given early and is not readily available in the infected areas. Given the ecology of the rodent host and conditions in the endemic area, a vaccine is mandatory for control. Lassa vaccine initiatives have suffered from a lack of funding in the past, but bioterrorism and recent importation of the disease to the

United States and Europe have brought new resources to Lassa virus science.

Early attempts to develop a Lassa fever vaccine in the 1980s focused on killed pathogens, which caused a strong humoral response but failed to protect nonhuman primate test animals. Subsequently, recombinant vaccines used vaccinia vectors carrying different combinations of structural Lassa proteins. Some of these protected 90% of nonhuman primates from a lethal challenge in the absence of a strong humoral response, suggesting that cellular responses are important for protection.

Use of vaccinia vectors in humans is problematic, especially in areas where HIV infection is common—immune-suppressed individuals can develop serious skin lesions—and several alternative vaccines based on other vectors as well as harmless vaccinia ones are under development. Thomas Geisbert and colleagues now report promising results with a replication-competent vaccine based on attenuated recombinant vesicular stomatitis virus vectors expressing the Lassa viral glycoprotein. A single intramuscular vaccination protected all four vaccinated cynomolgus macaques against a lethal challenge of a particular Lassa strain, while two control monkeys that had received empty vector died after injection with the same dose of virus.

These are encouraging results, but future larger studies will need to assess the duration of protection



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Color-enhanced transmission electron micrograph of Lassa virus particles

and demonstrate the safety of this replication-competent vaccine. Another crucial question is how quickly vaccinated individuals acquire protection, and thus whether the vaccine would be suitable for creating a ring of vaccination around an outbreak zone, the most likely early application of a promising candidate vaccine. In addition, there are at least four different strains of the Lassa virus, and an ideal vaccine should provide protection across all strains. Finally, conducting trials in endemic areas, many of which lack political stability, remains a serious challenge.

Geisbert TW, Jones S, Fritz EA, Shurtleff AC, Geisbert JB, et al. (2005) Development of a new vaccine for the prevention of Lassa fever. DOI: 10.1371/journal.pmed.0020183

Brain Activity and Tinnitus

DOI: 10.1371/journal.pmed.0020194

Exposure to short periods of very loud noise can cause tinnitus—a persistent ringing or buzzing in the ears that cannot be blocked out. Tinnitus may affect around 10%–15% of the population; severe tinnitus is very debilitating (1%–2% of the population). Previous work has shown that tinnitus has a neurophysiological basis, but precisely which parts of the brain and the auditory circuits are involved is not yet understood.

The human ear is essentially a very sensitive vibration sensor, one that is able to receive the minute longitudinal vibrations in air that make up sound waves. It can detect sounds from 20 Hertz (Hz) (very low pitch) to 20,000 Hz (very high pitch) but is particularly sensitive to sounds in the range of 500–5,000 Hz—the so-called speech frequencies. However, the ear, and in particular the cochlea, or inner ear, can be damaged by exposure to excess noise, leading to permanent damage to the ear, i.e., deafness.

Some studies in both animals and humans have suggested that tinnitus and hearing loss may be related. These studies have

found that neurons in regions of the auditory cortex that have been deprived of stimuli because of hearing loss change their receptive field and may develop enhanced spontaneous activity. Other studies, such as some involving neuroimaging using positron emission tomography, have suggested that parts of the brain involved in attention and emotional regulation might be involved in the production of tinnitus.

One of the key research targets in tinnitus has been investigation of cortical activity, especially in animal models of tinnitus, but studies in humans have been rare. Previous studies have identified temporal and frontal temporal changes in individuals whose tinnitus is severely disabling; however, there have been no group studies comparing abnormalities of ongoing, spontaneous neuronal activity in people with and without tinnitus.

In this month's *PLoS Medicine*, Nathan Weisz and colleagues studied 17 patients with chronic tinnitus and hearing loss and 16

control individuals with normal hearing. Patients were asked to fill in a questionnaire about the impact of tinnitus on their lives and had their levels of tinnitus assessed.

The team's methods differed from previous work in that the team chose to examine the power spectrum of neuromagnetic oscillatory activity during rest, whereas previous studies had focused on measuring neurophysiological responses following sounds.

Normally in awake and healthy subjects a certain rhythm of brain activity at 8–12 Hz—the so-called alpha rhythm—is dominant. Finding enhanced slow-wave, or delta, activity (<4 Hz) in awake subjects is usually a sign of a dysfunctional neuronal network, as these waves can be observed in various neurological and psychiatric disorders. Weisz and colleagues' analysis of the frequency spectrum of recorded magnetic fields revealed that the energy in the alpha band was strongly reduced and that of the delta band enhanced in the group with tinnitus compared with the individuals with normal hearing. This pattern was particularly pronounced in the temporal regions, and overall the effects were stronger for the alpha than for the delta frequency band.

This is the first study to show these changes in delta and alpha spontaneous cortical activity, say the authors. But they concede it is still unclear whether the enhancement of delta activity compared with alpha is the abnormal activity perceived

as tinnitus. However, the fact that regions that show slow-wave activity during slow-wave sleep are also regions of low alpha activity supports the idea that changes in cortical activity might be mediated by sensory deprivation, in this case that partial hearing loss might be involved in producing tinnitus.

Tinnitus-related distress as assessed by the questionnaire was strongly associated with this abnormal spontaneous activity, especially in the right temporal and left frontal areas, thus pinpointing a possible tinnitus-related cortical network.

A limitation of this study was that the tinnitus group also had high-frequency hearing loss, whereas the control group did not; the ideal control group would have been patients with the same sort of hearing loss but no tinnitus.

In discussing their findings, the authors suggest that their study supports previous work indicating that the prefrontal cortex is a candidate region for integration of the sensory and emotional aspects of tinnitus. Further studies should focus on frontal areas, which could allow identification of interactions and modulating influences that higher-order psychological processes (e.g., emotions and thoughts) may have on the generation of tinnitus in the auditory cortex.

Weisz N, Moratti S, Meinzer M, Dohrmann K, Elbert T (2005) Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography. DOI: 10.1371/journal.pmed.0020153

Turning Stem Cells into Mesenchymal Tissues

DOI: 10.1371/journal.pmed.0020201

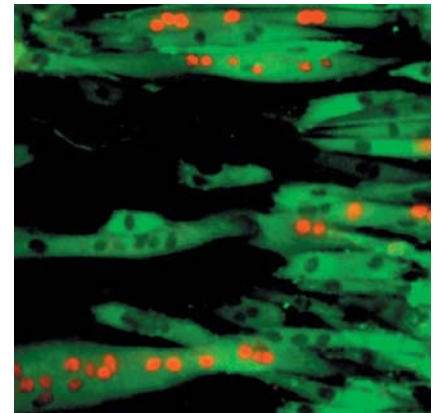
As cells specialize during development they pass through different levels of differentiation, from the earliest stem cells through to the highly specialized types that make up the body's organs. Hence, a number of different tissues may derive from common precursors. For example, muscle, fat, cartilage, and bone are all derived from a group of mesenchymal precursor cells that originate in the paraxial mesoderm. So pluripotent (i.e., able to differentiate into any cell type) human embryonic stem cells are potentially a starting point for the regeneration of all types of diseased or damaged organs (and already researchers have shown that it is possible to stimulate human embryonic stem cells to differentiate into specific cell types such as neural or hematopoietic cells). The isolation of intermediate multipotent stem cells (which can differentiate into a limited number of cell types) may also be valuable. For example, the production of an unlimited supply of mesenchymal precursors would be very useful, not only for the understanding of how cells differentiate, but also for eventual practical application.

In this month's *PLoS Medicine*, Lorenz Studer and colleagues from the Sloan-Kettering Institute in New York describe a protocol for deriving mesenchymal

precursors, which they then show are capable of differentiating into specialized cell types.

They used two undifferentiated stem cell lines—from the 22 lines that were approved in 2001 by President Bush for use in federally funded research in the United States. The specifications for approval for these lines are clear—see the guidelines at <http://stemcells.nih.gov/research/registry/eligibilityCriteria.asp>. The number of human embryonic stem cell lines available for researchers are strictly limited, making it necessary to develop protocols that expand these cells along various lineages.

In order to differentiate the cells into mesenchymal precursors, the stem cell lines were cocultured with mouse feeder cells to produce five different polyclonal lines. The authors then cultured these polyclonal precursors with appropriate tissue-specific stimulation in attempt to produce fat, bone, cartilage, or muscle cells. The evidence that the authors provide for these cells being differentiated includes analysis of gene expression, surface antigens, and immunocytochemistry typical of the mature tissues. For example, the authors were able to show the presence of fat granules in adipocytes, calcium in the matrix of osteogenic cells, and collagen



DOI: 10.1371/journal.pmed.0020201.g001

Myotubes formed in vitro from human ES-derived mesenchymal precursors upon cocultures with C2C12 myoblasts

in chondrocytes. It was harder to produce muscle cells, but even these types of cells could eventually be induced by specific culture conditions.

What are the possible concerns about these types of studies? One obvious one is the potential for residual undifferentiated cells to turn into tumors, but the authors tested the differentiated cell cultures for cell surface markers characteristic of undifferentiated cells and found no evidence of them. Another worry for

the use of these cells directly in humans is the need, at least at the beginning, to culture the cells with mouse feeder cells—obviously no human treatment could contain cells contaminated with mouse cells. Further development of protocols will be needed to address this issue. However, as the authors

comment, “the high purity, unlimited availability, and multipotentiality of hESMPCs [human embryonic stem cell–derived mesenchymal precursor cells] will provide the basis for future therapeutic efforts using these cells in preclinical animal models of disease.” In addition, the techniques described

here will provide a very useful resource for studying mesenchymal cell development.

Barberi T, Willis LM, Socci ND, Studer L (2005) Derivation of multipotent mesenchymal precursors from human embryonic stem cells. DOI: 10.1371/journal.pmed.0020161

