INTRODUCTION

Schistosomiasis is a chronic parasitic disease caused by a trematode blood fluke of the genus *Schistosoma* that belongs to the *Schistosomatidae* family.[1] It is a multifactorial disease that includes environmental, behavioral, parasitic, vector and host factors. It continues to be a significant cause of morbidity and mortality.[2] World Health Organization (WHO) considers schistosomiasis as the second only to malaria in socioeconomic importance worldwide and the third more frequent parasitic disease in public health importance.[3]

Epidemiology

There are five species of *Schistosoma* with a tendency to occur in restricted geographic patterns. *S. mansoni* is most prevalent in certain tropical and subtropical areas of sub-Saharan Africa, the Middle East, South America and the Caribbean. *S. haematobium* infection is acquired in North Africa, sub-Saharan Africa, the Middle East and India. *S. japonicum* occurs only in Asia. *S. intercalatum* occurs in Central and West Africa while *S. mekongi* is restricted to Laos and Cambodia.[4] Currently, the largest number of cases of schistosomiasis occurs in Egypt, Yemen, and Algeria.[5]

In Egypt, and following construction of the Aswan High Dam in 1960s, a striking change in the geographic distribution of the two species of *Schistosoma* (*S. mansoni and S. haematobium*) happened with an increasing prevalence of *S. mansoni* in the Nile Delta and concomitant decrease of *S. haematobium* prevalence spreading from the Nile Delta into Upper Egypt. This change was believed to be caused by less silt and by variability in the velocity and volume of water flow with a resultant shift in relative abundance of the corresponding snail vectors.[5–9] The largest and the latest epidemiological survey in Egypt mentioned the prevalence of *S. haematobium* in Upper Egypt (where it is endemic) to be around 7.8% while the prevalence of *S. mansoni* in Lower Egypt (where it is endemic) to be around 36.4% in year 2000 .[10]

Hepatic schistosomiasis

Hepatic schistosomiasis, or schistosomal hepatopathy, is the most common form of the chronic disease and usually results from heavy S. mansoni infection.[11]

Pathogenesis

Hepatic schistosomiasis results from the host's granulomatous cell-mediated immune response to the soluble egg antigen of *S. mansoni*, which progresses to irreversible fibrosis and, consequently, severe portal hypertension.[12] Eggs remain viable in the liver for about 3 weeks. Primarily, the eggs cause a moderate type 1 helper (Th1) response to egg antigens. However, this usually evolves to a dominant Th2 immune response to egg-derived antigens with later recruitment of eosinophils, granuloma formation and fibrogenesis of the liver.[13,14] Although granuloma formation is beneficial for the host because it blocks the hepatotoxic effects of antigen released from parasite eggs, this process may lead to fibrosis with excessive accumulation of collagen and extracellular matrix proteins in the periportal space.[15] Granuloma formation is a helper T cell-mediated delayed hypersensitivity reaction driven by cytokines such as interleukin-4 (IL-4) and IL-

13, whereas IL-10, IFN, and a subset of regulatory T cells can limit the schistosomal induced pathology. In addition, a variety of cell types have been implicated, including hepatic stellate cells, activated macrophages, and regulatory T cells.[16] The balance between Th1- and Th2-type cytokines influences the extent of the pathology and the development of fibrosis.[17] Eggs are detectable inside the granulomas with the subsequent formation of marked portal and peri lobular fibrosis, which is most pronounced with *S. mansoni* and *S. japonicum*. Added to fibrosis, angiogenesis is an important step in pathogenesis of schistosomal lesions. Its role is evident during periovular granuloma formation as well as in the genesis of schistosomal portal fibrosis.[18]

The final result of hepatic schistosomiasis with a heavy *S. mansoni* burden is severe portal fibrosis and greatly enlarged fibrotic portal tracts, which resemble clay pipe stems thrust through the liver.[19] Interestingly, normal liver architecture is preserved, lobular architecture is retained, nodular regenerative hyperplasia is not observed, and thus the fibrosis could be reversible. Moreover, angiogenesis in schistosomiasis seems to have a two-way mode of action, participating both in fibrogenesis and in fibrosis degradation.[18] Evidence from treated schistosomiasis of the mouse showed that hepatic schistosomal lesions can undergo considerable remodeling with time. Obstructive vascular lesions are partially or completely repaired with regression of the excess extracellular matrix.[18] With degradation of the long standing hepatic fibrosis and its removal, the main signs of portal hypertension can progressively disappear.[20]

This dynamic state of equilibrium between forces of synthesis and breakdown with a possibility to cure schistosomiasis and associated hepatosplenic disease doesn't happen with hepatic cirrhosis.[21] Co-infection with viral hepatitis, either hepatitis B virus (HBV) or hepatitis C virus (HCV) is very common since the regions with a high prevalence of schistosomiasis usually have a high endemicity of chronic viral hepatitis as well. An important cause of the high exposure to HCV was the establishment of a large reservoir of infection as a result of extensive schistosomiasis control programs that used intravenously administered tartar emetic 35–50 years ago.[22] The association between both schistosomiasis and HCV is known to cause earlier liver deterioration and more severe illness. The liver is the principal site for both HCV replication and egg deposition, which down-regulates the local immune responses in the liver[23] and results in suppression of the intrahepatic bystander immune response to HCV. This may also occur during inactive schistosomal infection since the ova remain in the hepatic portal tracts and their soluble antigens could influence the host's cell-mediated immunity for a considerable time. [24] In addition, this co-infection can also produce a unique clinical, virologic and histologic pattern manifested by viral persistence with high HCV RNA titers, higher necro-inflammatory and fibrosis scores in liver biopsy specimens in addition to poor response to interferon therapy, and accelerated progression of hepatic fibrosis.[25]

Clinical manifestations

Clinical presentation of hepatic schistosomiasis markedly differs from that of cirrhosis. Although the symptoms and signs of portal hypertension and hypersplenism are dominant in schistosomiasis, the counter part of hepatocellular failure is absent. However, some patients with schistosomiasis progress to an end stage of the disease by exhibiting muscle wasting, hypoalbuminemia, ascites and coma. These observations led to the concept of compensated and decompensated schistosomiasis to differentiate patients with

the sole manifestations of portal hypertension from those who, in addition, presented signs of hepatocellular failure.[26]

Intestinal schistosomiasis

Intestinal schistosomiasis represents another form of schistosomal affection. Among spectrum of intestinal lesions, polyps are the commonest.[27]

Pathogenesis

Intestinal schistosomiasis is essentially due to *S. mansoni* infection[28] and it has been reported as well in some *S. haematobium* cases.[29] Egg-laying worms are present in the intestinal micro-vasculature especially in the distribution of the inferior mesenteric venous plexus. In the large intestine, ova are mainly distributed in the loose submucosa, and to a lesser extent in the subserosa where infrequently multiple granulomas are formed. Subsequently, the muscularis mucosa becomes involved and the overlying mucosa is either denuded forming small superficial ulcers or undergoes hyperplastic changes. Sandy patches develop when the submucosa becomes densely thickened by fibrous tissue containing immense numbers of calcified eggs; the overlying mucosa becomes atrophic and acquires a granular dirty yellowish appearance.[30]

The pathogenesis of polyp formation starts by deposition of schistosomal eggs in the superficial layers of submucosa where the connective tissue is loose and not bounded superficially by firmer tissue. This allows the accumulation of large amounts of reactive cellular debris and vascular granulation tissue. In the submucosa, the eggs produce a cell mediated inflammatory response with granuloma formation and necrosis. As necrotic foci heal, fibrous connective tissue is formed and the adjacent muscularis mucosa becomes hypertrophied. The fibrous tissue in the submucosa and the hypertrophied muscularis mucosa form a barrier to the usual route of ova transit from the mesenteric veins to the gut lumen. This entrapment of ova leads to a foreign body reaction with progressive inflammation and fibrosis. As this process continues, a nodule is formed that elevates the hypertrophied muscularis mucosa and mucosa to form the earliest detectable polyp.[31] This mechanism can explain the main concentration of the *S. mansoni* ova in the polyps than in the adjacent mucosa and submucosa.[32]

Colonic mucosa of affected patients is usually edematous and congested with petechial hemorrhage in acute schistosomal colitis cases, while shows confused vascular net with flat chronic colitis patients. Acute and chronic inflammation could be observed in colon segments of chronic active schistosomal colitis patients. The most characteristic finding is the grayish yellow or yellowish white schistosomal nodules similar to those of pseudomembranous enterocolitis.[32] Polyps range in size from 2 to 20 mm and may be sessile, pedunculated or showing a cauliflower appearance. They are mainly concentrated in the distal colon, and they count from few to very numerous polyps. The covering mucosa of the polyps is usually redder than the surrounding mucosa due to severe congestion and due to focal hemorrhages. Ulceration is common in rectal polyps, the ulcerated areas appear dusky to blackish gray in color caused by superficial hemorrhage, and are frequently secondarily infected.[28,33] Histologically, the typical polyp is composed of a stalk of fibrous connective tissue projecting from the sub mucosa into the lumen and partially covered with mucosa. The overlying mucosa consists of distorted glands with varied degrees of mucoid activity, mucinous degeneration, and adenomatous hyperplasia.

Focal areas of ulceration frequently interrupt the surrounding mucosa. Larger areas of ulceration may be replaced by granulation tissue. Mononuclear cells, eosinophils, and few polymorphonuclear leukocytes infiltrate the mucosa. The supporting tissue is composed of fibrous connective tissue and muscle derived from the muscularis mucosa. Blood vessels may be present in large numbers but diminish as fibrosis progresses. Viable and nonviable eggs are present in all polyps.[34]

Clinical manifestations

Schistosomal colonic polyposis affects mainly adult males. This male predominance is related to greater employment in agricultural work and higher rates of contact with water.[35] The primary presenting symptoms are usually tenesmus and the rectal passage of blood and mucous. Diarrhea, abdominal pain, dyspepsia, and irreducible schistosomal papilloma protruding from the anus occur in some patients.[34,36] Malnutrition, weight loss, nail clubbing, pitting peripheral edema, and pericolic masses may also be present.[29,36,37] Other manifestations include iron deficiency anemia, hypoalbuminemia, protein-losing enteropathy, and rectal prolapsed.[37,38] The presence of polyposis does not appear to predispose patients to the development of large bowel cancer [39,40] and many investigators even have rejected any relationship between schistosomiasis and colorectal carcinoma, although this view is debatable if we consider *S. japonicum.*[41–43] However, there is a report on a patient with sigmoid cancer coexisting with schistosomiasis and the authors entailed a possible but inconclusive role for chronic schistosomiasis *mansoni* in promoting carcinogenesis of colorectal neoplasms.[44] Schistosomal appendicitis is a rare complication that can occur in 0.02–6.3% in endemic areas (representing 28.6% of chronic appendicitis in such region) and 0.32% in developed countries. Its main mechanism depends on mechanical appendiceal lumen obstruction by adult worms rather than being a complication from egg deposition.[45]

Diagnosis

Diagnosis of the disease depends on certain tools as microscopy and egg identification, serology and radiologic findings. Other non-specific findings include eosinophilia, thrombocytopenia and anemia. Liver biochemical profile is usually normal.[46] Demonstration of parasite eggs in stool is the most common method used for making the diagnosis of schistosomiasis and species identification. To assess intensity of infection, quantitative sampling of defined amounts of stools (Kato Katz technique) is applied[47]. Concentration techniques improve the sensitivity of egg detection. Moreover, further slide readings from the same stool sample using the Kato Katz technique associated with a serological test (three slides reading and the IgG anti-Schistosoma mansoni-ELISA technique) proved to be a useful procedure for increasing the diagnostic sensitivity.[47] Schistosomiasis can be diagnosed also by finding eggs in tissue biopsy specimens from rectal, intestinal and liver biopsies.[48] However, the sensitivity of these procedures is variable due to fluctuation of egg shedding.[49] Serologic tests can detect antischistosomal antibodies in serum samples. The main drawback is their inability to distinguish between past and current active infection. However, a negative test can rule out infection in endemic population. Another drawback is that they remain positive for prolonged periods following therapy making them unreliable for post treatment follow up.[46] To solve these defects, techniques to detect parasite antigens, in sera and stools, have been developed and can identify current infection and its intensity.[50]

Urine dipstick diagnostic tests can detect schistosome circulating cathodic antigen (CCA). They were tested in fieldbased surveys, certainly for preschool children due to the difficulty to obtain consecutive stool samples, and provided a more sensitive and rapid testing for intestinal schistosomiasis. This may help in future epidemiological screening studies.[51] Sensitive and specific diagnostic methods of schistosomiasis at an early stage of infection are important to avoid egg-induced irreversible pathological reactions. Detection of free circulating DNA by PCR can be used as a valuable test for early diagnosis of prepatent schistosomiasis infection.[52] Polymerase chain reaction (PCR) methods to improve the direct detection of *Schistosoma* antigens. These tests are done on urine, stool, or organ biopsy samples, and involve the preparation of DNA from eggs prior to PCR amplification.[53]

Only a small volume of sample can be used for DNA extraction, and it is dependent on chance whether the processed sample contains ova or not. Similarly, PCR has the same limitations as microscopy and does not provide a significant clinical benefit.[49] Another study detected *S. haematobium*-specific DNA in urine with similar specificity to detection of parasite eggs but with improved sensitivity.[54] Hopefully, an updated PCR assay has been available for the detection of *Schistosoma mansoni* DNA in human stool samples using QIAamp_ DNA Stool Mini Kit. It allows the heating of the sample (until 95 C) to facilitate the rupture of the egg and cellular lysis. It also includes Inhibitex, which adsorbs DNA damaging substances and PCR inhibitors present in the fecal material. For amplification, the DNA samples are diluted only 5-fold with good reproducibility and the study can provide high sensitivity and specificity results.[55] Another novel diagnostic strategy is developed, following the rationale that Schistosoma DNA may be liberated as a result of parasite turnover and reaches the blood. Cell-free parasite DNA (CFPD) can be detected in plasma by PCR for any stage of schistosomiasis.[49]

Radiologically, abdominal ultrasonography plays an integral role in the diagnosis of hepatosplenic schistosomiasis. Imaging can show periportal fibrosis, splenomegaly, portal vein dimensions and the presence of collateral vessels. In addition, ultrasonography helps to assess degree of periportal fibrosis by measuring portal tract thickness: Grade I if thickness is 3–5 mm, Grade II if it is 5–7 mm and Grade III if it is more than 7 mm. This method reflects the hemodynamic changes and provides a good estimate of the clinical status of patients who have periportal fibrosis.[56] Portal hypertension is suspected when dilatation of one or more of the portal, mesenteric and splenic veins is detected. For the collateral vessels, the most commonly described are the left and right gastric, the short gastric, the par umbilical and the splenorenal veins.[57,58] Lastly, the hepatic veins in schistosomiasis can be assessed ultrasonographically. They remain patent with normal phasic flow as the disease evolves, which is different from liver cirrhosis. In advanced cirrhosis, hepatic venous outflow becomes monophasic.[57] Colonic affection can be diagnosed by endoscopy and biopsy from the abnormally apparent mucosa. Also, barium enema and double contrast enema may provide a diagnostic tool for colonic polyps.[28]

Parenteral Antischistosomal Therapy

Schistosomiasis was not treatable until 1918.[59] In that year, JB Christopherson discovered that injections with the antimony salt tartar emetic could induce a cure. Egypt had the world's greatest schistosomiasis problem, parenteral antischistosomal therapy was extensively adopted starting in the 1920s. The treatment became available in different

areas of the country at different times. In rural areas, health centers and traveling clinics practiced parenteral antischistosomal therapy in the form of mass treatment. Although a few antischistosomal drugs could be injected intramuscularly, tartar emetic was injected intravenously, and tartar emetic was the most widely used in Egypt. Beginning in the 1950s and continuing until the 1980s, the Egyptian Ministry of Health conducted large campaigns using the standard treatment at that time, tartar emetic, as community-wide therapy.[60] Between 1964 and 1982, more than 2 million injections were given annually to an average of 250,000 patients.[59]

Thus, in 18 years, approximately 36 million injections were administered, in addition to the numerous injections that had been administered earlier than 1964. Each human under treatment received, or was supposed to receive, a series of injections. In the 1960s, the average number of injections per patient was 9. After 1975, it dropped to 6 or fewer. The enormous scale of these campaigns is suggested by a 1964 WHO report, describing a clinic. "Patients are grouped according to weight and appropriate dose and are lined up in queues for admission. The skilful doctor began injecting at 9:20 am and completed 504 injections of men, women and children by 10:10 am. This remarkable performance is being repeated at various areas all over Egypt. The used syringe is placed in an 'out' tray, from which it is taken by the nurse, washed through and boiled for a minute or 2. As soon as the syringe is cold, it is filled with a volume of the drug solution. It is then placed in the 'in' tray. . . There are usually 20 to 30 syringes in rotation."[61]

The rapidity of the inoculations, 10 per minute on average, and the small supply of syringes allowed little time for sterilization in boiling water for the recommended 2 minutes. The nurse would have had to wash a syringe, place it boiling water, remove a freshly boiled syringe, allow it to cool, fill it with the drug solution, and bring it to the doctor every 6 seconds, while making sure that each syringe in the queue was boiled for an adequate time. The evidence that is examined shows that the syringes were not properly sterilized and thus transferred traces of blood and blood-borne pathogens from human to human. As a result, this massive effort to control one health problem resulted in the creation of another, as HCV was spread through the intravenous injections.[60] Indeed, this is estimated to be the largest known iatrogenic transmission of blood-borne infections in the history of the world.[59]

These mass injections transmitted blood-borne diseases because:

1) Humans received multiple injections over time

2) The injection equipment was insufficiently sterilized

3) Parenteral antischistosomal drugs were injected to humans of all age groups and treatment stages in a mass setting.[59] Compared with a children's vaccination program, where injection equipment would be reused among a group with a low prevalence of HCV, this program, which treated children and adults, probably treated many more HCV-infected humans.[59] Furthermore, a full course of tartar emetic required several injections. The recommended regimen was 12 to 16 injections, and when the program began, the injections were administered over 2 to 3 weeks, or at the rate of almost one per day. For the comfort of the patients, however, this dosing schedule was changed in the 1960s to once a week over the course of 9 to 16 weeks. This seemingly benign change, however, permitted greater

transmission of HCV. Those who were already infected with HCV before they started treatment for schistosomiasis were transmitters of the virus. In addition, those who became infected with HCV early in the treatment period who acquired it from their earlier injections became transmitters of HCV in just 2 to 4 weeks. Therefore, at some point in the middle of their therapy, these both previously infected and newly infected individuals were capable of passing the disease to others who chanced to be treated with the same glass syringes or needles. In this way, continuous cycles of infection could have developed within treatment facilities. Such epidemic outbreaks could easily have gone undetected because acute clinical symptoms are not present in about 80% of HCV infections and, in addition, the symptoms could have been confused with schistosomiasis itself or with the side effects of tartar emetic. Oral drugs for schistosomiasis were developed in the 1970s. The first such drugs, metrifonate and niridazole, were effective against *S. haematobium*, but not against *S. mansoni*, the largest source of schistosomiasis in Egypt.[59] By the mid-1980s, an effective oral drug for treating *S mansoni*, praziquantel, became available and gradually replaced tartar emetic as the standard treatment throughout Egypt. This not only reduced schistosomiasis; it also stopped the main engine of growth for the hidden HCV epidemic.[60]

Prevalence of HCV in Egypt

In rural areas where the parenteral antischistosomal therapy program was active, Egypt's population has a high prevalence of hepatitis C.[62] A cross-sectional study of serum samples from 1,945 Egyptians in 1996 estimated the overall rate of HCV seropositivity was 15.6% for Egyptians aged 15 to 65.[62] Nationwide, this suggests that in 1996 over 5 million Egyptians were HCV seropositive and that 3.5 million had chronic hepatitis of varying degrees. A more recent estimate is that 8 to 10 million Egyptians have hepatitis C and that 5 to 7 million have active infections.[63] The prevalence of HCV tends to be highest among humans who live in rural areas and who are old enough to have received parenteral antischistosomal therapy. Children under 5 years old were not treated, and parenteral antischistosomal therapy wa stopped between 1982 and 1986, so humans born after 1981 were not injected.[64] A random sample of 270 rural Egyptians in 1994 found that the prevalence of antibodies to HCV ranged from zero in children between 5 and 10 to 41% in adults over 50 who had the highest likelihood of receiving parenteral antischistosomal therapy.[65] Similarly, a 2001 community-based study of 801 humans who lived in the Nile River delta, an area targeted for antischistosomal therapy, estimated the seroprevalence of HCV among community residents 30 years old or older at 60%.[66] Lower rates have been reported in Upper Egypt. One study of 6,031 participants in this region found that 8.7% had HCV antibodies.[67] For comparison, in the United States, the National Health and Nutrition Examination Survey, which covered 15,079 participants between 1999 and 2002, found that the prevalence of HCV in this country was 1.6%.[68] For an African comparison, in a maternity hospital in Zimbabwe, antibodies to HCV were detected in 1.6% of indigent women. This group presumably does not have access to better health care than residents of Egypt, and yet has HCV prevalence similar to those of European and North American women and about 10% of the average prevalence in Egypt.[69] Another comparison comes from the Seychelles Islands off the east coast of Africa. Here, the age-adjusted seroprevalence of anti-HCV was even lower than among the indigent, pregnant women of Zimbabwe, 0.34% in a random sex- and age-adjusted sample of 1,006 humans of 25 to 64 years.[70] In short, the prevalence of HCV in Egypt is 10 to

more than 100 times higher than in the industrialized countries in Europe and the Americas or in some other countries in Africa. A brief look at the progression of hepatitis C and its medical consequences will suggest why the unintentional transmission of HCV was so harmful to Egypt's humans.

Coinfection

With high prevalence rates for both HCV and schistosomiasis, it is inevitable that Egypt has a large number of humans with both diseases. Having both is more damaging to the liver and is associated with higher mortality rates than having just one. A prospective, long-term study of 126 Egyptian patients with schistosomiasis, HCV, or both followed these 3 groups for 40 to 85 months.[71] Of the group with both diseases, 48% had liver cirrhosis, compared with only 15% in the group with HCV alone, and none in the group with schistosomiasis. Hepatocellular cancer was found only in the group with concomitant HCV and schistosomiasis. In addition, this group had more advanced liver disease, higher measures of HCV RNA, higher incidence of cirrhosis, and by far the highest mortality rate during follow-up, 48%.

Two other coinfection states need to be considered: HCV with HIV and HCV with hepatitis B virus (HBV). Although in the United States, HCV often appears as a coinfection with HIV/AIDS, in Egypt, the prevalence of HIV/AIDS in 2001 was <0.1% for adults.[72] Thus, the comorbidity of HCV and HIV is not a critical health issue for this country. In contrast, hepatitis B virus (HBV) has a high prevalence in Egypt. Seroprevalence rates for HBV in one Nile delta village, reported in a 1996 study, were 24% in the general population and 66% in the group aged 40 to 67 years.[73] Beyond the scope of this thesis although a full examination of the effects of HBV, coinfection of HCV with HBV may lead to aggravated symptoms and a faster progression to hepatocellular carcinoma.[74] In addition, the combination of HCV and HBV has a significant additive effect on the risk of developing hepatocellular carcinoma (OR = 42.9).[73] Egypt initiated a universal hepatitis B virus immunization of infants in 1991.[75] A study of acute viral hepatitis at a major urban referral center in Egypt determined that HBV decreased as a cause of symptomatic hepatitis between 1982 and 2002, dropping from 43.3 to 28.5% ($P < 0.01$).[75]

To clarify, this is not the general prevalence of hepatitis B in Egypt, but the prevalence in a symptomatic population that was referred to a specialty hospital. Its significance is that it shows a dramatic drop in patients with HBV. Another study also suggests that HBV is not nearly the problem that HCV is. It involved 20,000 Egyptian rural villagers, of whom 1,715 subjects were symptomatic for hepatitis and screened for ALT (Alanine aminotransferase)[76] of this group, 47 who had ALT at least twice normal level were tested for various forms of hepatitis. None of the tested individuals had serological evidence of either acute HBV or HCV. However, 33 had active HCV infection, as indicated by both anti-HCV and HCV RNA. Only 2 subjects were positive for hepatitis B surface antigen and had chronic HCV infection. As in the other study, the infection rate was much lower for HCB than for HCV. Concomitant HBV and HCV infections are less common than either infection alone, and they seem to be associated with more severe liver disease.[77]

Malnutrition in Patients with Liver Disease

Nutrition status is recognized as a predictor of morbidity and mortality in patients with advanced liver disease.[78-80] The liver is an important regulator of metabolism, storage, synthesis, and absorption of nutrients. Accordingly, the severity of malnutrition increases with decreases in liver function.[81]

Prevalence of Malnutrition in chronic liver disease patients

Patients with chronic diseases frequently become malnourished; they have an inability to meet macronutrient and micronutrient requirements through oral intake.[82] Inadequate intake and/or associated malabsorption alters body composition and diminishes biological functions. Parameters used to assess malnutrition in patients with liver disease include anthropometric and serum measurements and qualitative data on weight history and food intake.[83,84] Malnutrition is common in patients with advanced liver disease; the prevalence is reported to be 50%–90% among cirrhotic patients.[84-88] In a study of 300 patients, more than 75% of those with advanced liver disease presented with some degree of malnutrition, and almost 40% presented with moderate or severe malnutrition, based on anthropometric and serum measurements. [84] In the same study, 95% of patients of Child–Pugh(score of liver disease severity) class C presented with malnutrition, compared with 84% and 46% of classes B and A, respectively.[84] The prevalence of malnutrition among patients with even early-stage cirrhosis is concerning, given that nutrition status is associated with mortality and complications.[89,90] In a large nationwide analysis of hospitalized patients with cirrhosis and portal hypertension, patients with protein calorie malnutrition had greater incidences of complications such as ascites (65%, compared with 48% without malnutrition) and hepatorenal syndrome (5% vs 3%).[89] Malnourished patients also had longer hospital stays and had a 2-fold increase in in-hospital mortality, compared with well-nourished patients.[89]

The incidence of malnutrition was 6% among patients with cirrhosis, compared with 2% of general medical patients—rates of malnutrition were significantly lower compared with those reported in other studies.[89] The impact of malnutrition on mortality and complications might have been larger in magnitude if a more sensitive measure of malnutrition was used. A study of patients of Child–Pugh class A demonstrated that malnutrition, even in early stages of cirrhosis, had large effects on patient outcomes. Among a cohort of patients that were primarily Child–Pugh class A, those that were malnourished had a 1-year mortality rate of about 20%, whereas none of the patients that received sufficient amounts of nutrients died within the 1-year period.[90]

Complications such as infections, hepatic encephalopathy, ascites, and hepatorenal syndrome also increased with malnutrition; in the same study, 65% of malnourished patients developed complications compared with 11% of well-nourished patients.[90] After liver transplantation, malnutrition has been associated with higher rates of infectious complications, longer stays in the intensive care unit, and higher mortality.[83,85] Additionally, patients with more severe malnutrition have longer postoperative hospital stays.[83]

Etiology of Malnutrition in chronic liver disease

The etiology of malnutrition is multifactorial and primarily related to reduced liver function; poor oral intake and complications of cirrhosis such as ascites and hepatic encephalopathy also contribute.

1-Hypermetabolism

Resting energy expenditure (REE) is the amount of energy an individual uses to perform vital organ functions, free of activity and digestion.[91] A commonly used predictive equation for REE is the Harris Benedict Equation, which factors weight, height, and sex in the calculation. Whereas most cirrhotic patients have a REE that is similar to predicted values, 15%–30% of patients are hypermetabolic.[93,94]Hypermetabolism is defined as REE_120% compared with the predicted value.[93] The causes of hypermetabolism are unclear; a recent study of 268 patients did not associate hypermetabolism with sex, etiology, severity of disease, protein depletion, presence of ascites, or tumors.[93] This finding is inconsistent with results from older studies that reported that energy expenditure increased among patients with ascites or hepatocellular carcinoma.[94,95] The increase in REE among patients with cirrhosis might result from infections or immune compromise. Plasma concentrations of catecholamines are increased in cirrhotic patients, indicating activation of the sympathetic nervous system.[96] Sympathetic overactivity could induce systemic responses such as tachycardia and increases in cardiac output and blood glucose levels,[97] which could all increase energy expenditure.[92] Proposed causes for the increased levels of catecholamine include gastrointestinal bacterial translocation, an inflammatory phenotype of chronic liver failure, or central neural dysregulation of the circulation.[98,99]

2-Malabsorption

There are multiple mechanisms that can lead to malabsorption of nutrients particularly of fat—in cirrhotic patients. One complication that affects nutrient absorption in patients with cirrhosis is portosystemic shunting. As cirrhosis progresses, portosystemic shunting causes nutrients to bypass the liver, without metabolic processing.[100] In addition, many patients with cirrhosis that is secondary to alcohol abuse have chronic pancreatitis, which contributes to malabsorption. An analysis of autopsy results found that 18% of cirrhotic patients also had chronic pancreatitis.[101]

Another factor that leads to fat malabsorption in patients with cirrhosis is intraluminal bile acid deficiency, which results from the decreased capacity for bile production and portosystemic shunting; intraluminal bile acid deficiency impairs formation of micelles and absorption of long chain fatty acids through the usual lymphatic route.[102] Portal absorption of long chain fatty acids might also occur in patients with cirrhosis; Cabre *et al* showed that the incorporation of radiolabeled fatty acid in chylomicron and very-low-density lipoprotein (VLDL)- associated plasma tricylglycerols was lower and less sustained in cirrhotic patients compared with healthy controls.[103] This finding is consistent with reports of impaired lipoprotein export in cirrhotic patients, probably from decreased synthesis of triaclyglycerols. [104,105] The findings of Cabre *et al* indicate an alternate route for fat absorption in cirrhotic patients, which bypasses standard lymphatic transport. A portal route for fat absorption has pathophysiologic implications; it could

result in excess hepatic storage of fat, which can reduce liver function and the systemic availability of fat for organic functions.

3-Altered Macronutrient Metabolism

Glucose metabolism has been well studied in patients with liver disease. Those with cirrhosis have increased levels of gluconeogenesis (generation of glucose from non-carbohydrate) and protein catabolism and decreased levels of glycogenlysis, compared with healthy individuals.[106,107] The altered rates of metabolism reflect a significant depletion in protein and fat reserves, reported in about 50% of cirrhotic patients.[83,93] Patients with chronic liver disease have increased rates of gluconeogenesis-a number of factors contribute to this. First, cirrhosis reduces the ability of hepatocytes to store, synthesize, and break down glycogen. These defects promote gluconeogenesis from fats and protein as alternate fuel sources. Following a short overnight fast, the rate of fat and protein catabolism in patients with cirrhosis is similar to that of healthy subjects who underwent 2 to 3 days of starvation.[108] Second, cirrhosis and insulin resistance are related; patients with cirrhosis have high serum levels of insulin after fasting and postprandial levels of glucose.[109] Fasting plasma levels of insulin, among 31 patients with cirrhosis, were 3-fold higher than those of healthy individuals.[109] Insulin resistance decreases peripheral glucose utilization and contributes to decreased hepatic glucose production and hepatic glycogen reserves.[110] Increased serum levels of glucagon, which result from impaired degradation by the liver, increases the rate of gluconeogenesis. Third, infection can increase rates of protein catabolism. The production of cytokines and other infection mediators activate proteolysis and increase oxidation of branched chain aromatic acids (BCAAs). This can promote the breakdown of muscle cells for substrates, if dietary protein intake is insufficient. In patients with cirrhosis, the utilization of oxidative fuels is associated with an increased rate of lipid oxidation particularly in the fasting state.[111]

4-Anorexia

As in other chronic illnesses, anorexia makes a significant contribution to malnutrition. Anorexia can be caused by physical symptoms of discomfort such as nausea, bloating, fatigue, and vomiting. Patients with ascites often experience early satiety resulting from the mechanical effects of ascetic fluid, which compress the stomach.[112] Additionally, loss of appetite can be related to the up-regulation of inflammation and appetite mediators.[93,113] Levels of tumor necrosis factor (TNF) and leptin correlate with satiety and energy expenditure; patients with cirrhosis have increased serum levels of this cytokine.[114] Tumor necrosis factor might affect appetite and metabolism by acting on the central nervous system, altering the release and function of neurotransmitters.[115] Leptin is an appetite-regulating hormone that is secreted by adipose tissue.[116] Cirrhotic patients had a 2-fold increase in fasting levels of leptin compared with healthy individuals; this might contribute to anorexia in these patients.[109]

Ghrelin, which stimulates appetite, is produced primarily by the stomach. Although some cirrhotic patients have been observed to have abnormal fasting levels of this hormone, the relationship between ghrelin and anorexia is unclear; some studies have reported increases and others reported decreases.[109,117] Changes in ghrelin levels might be related to the systemic response to liver disease and state of anorexia or a consequence of the liver's role in hormone regulation. [109,117] Aside from hormonal influences and

physical discomfort, disinterest in food can result from dietary restrictions and taste alterations. Dietary limitations, such as sodium restriction for ascites management, preoperative fasting, and limitation of protein intake for severe hepatic encephalopathy can reduce food variety; many patients do not accept the allowable foods. Although taste alterations have been commonly attributed to micronutrient deficiencies, researchers have questioned whether they are a consequence of cirrhosis itself.[118,119]

It is also important to consider alcohol-related anorexia. According to the American Liver Foundation, 10%–20% of chronic users of alcohol develop cirrhosis. Poor and irregular feeding is common among patients with alcoholic cirrhosis. Before hospital admission, 53% of alcoholic patients reported anorexia, 40% reported irregular feeding, and 36% ate only 1 meal per day.[120] In a study of middle-income patients with alcoholic cirrhosis, although their energy intake was similar to that of nonalcoholics, their overall intake of nutrients was lower, because they acquired most of their energy from alcohol rather than nutrient-rich foods.[121] The socioeconomic status of patients can also affect oral intake; patients who have alcoholic cirrhosis and low socioeconomic status are prone to poor and irregular feeding. As a result, they develop nutrient deficiencies, such as low serum levels of folate, B12, and B6, and macronutrient deficiencies.[122]

5-Micronutrient Status

Patients with advanced liver disease have an increased risk of micronutrient deficiencies (vitamins and minerals) that arise from anorexia, diuretic use, fat malabsorption, and hepatitis C. Patients with ascites have restricted intake of animal protein and are treated with diuretics, they commonly acquire zinc deficiency.[123] Similarly, magnesium deficiency can result from decreased oral intake of nutrients and use of diuretics. Although rates of deficiencies in fat-soluble vitamins vary among studies, vitamin A and vitamin D deficiencies are most commonly reported.[124] More than 90% of patients with cirrhosis have some level of vitamin D deficiency and 29% have severe vitamin D deficiency (17.5 nmol/L).[125] Low serum levels of fat-soluble vitamins can impair absorption of other nutrients, such as vitamin D and calcium. In patients with primary biliary cirrhosis (PBC), reduced concentrations of intraluminal bile increase the risk of malabsorption and deficiencies in fat and fat-soluble vitamins (A, D, E, and K).[124-126]

In a study of 180 patients with PBC, 33%, 13%, 2%, and 8% had deficiencies in vitamins A, D, E, or K, respectively.[124] Although these differences were not statistically significant (likely due to the small sample size), the authors associated the stage of PBC with the degree of vitamin deficiency. [124] Hepatitis C virus (HCV) infection has been associated with decreased levels of vitamin B6 and folate; therapy with pegylated IFN and ribavirin further decreased the levels of vitamin B6 and reduced plasma levels of vitamins B1 and B2. Many B-complex vitamins are cofactors for enzymatic reactions, so standard antiviral therapy for hepatitis C might impair physiological functions and cause complications.[127] In their study, dietary intake of B-complex vitamins did not differ between patients with hepatitis C and healthy individuals, indicating that hepatitis C virus might compete with human cells for vitamins; therapy might therefore affect nutrient utilization.

Nutrition Assessment in liver patients

Nutrition is correlated with the outcome of patients with liver disease, it is important to accurately assess nutritional status and provide timely nutritional support. This task is challenging, due to the complications of altered rates of protein metabolism and presence of ascites and edema. The European Society of Clinical Nutrition and Metabolism (ESPEN) 2006 guideline recommended the use of the subjective global assessment (SGA), anthropometry analysis, or the handgrip strength test to identify patients with cirrhosis who are at risk of malnutrition.[128] SGA is a bedside assessment tool used to collect information on dietary intake, weight change, and GI symptoms; it includes an examination for subcutaneous fat loss, muscle wasting, edema, and ascites.[91] The SGA is commonly used to assess patients with liver disease because it is simple and cost-effective.[91] Although traditional anthropometric measures such as weight, midarm circumference, and triceps skin-fold thickness are considered to be adequate for determination of nutritional status of cirrhotic patients, efforts to document these parameters in patients with advanced liver disease should be made on a regular basis. [128]

Albumin levels are poor nutritional markers because they are typically reduced in patients with advanced liver disease and fluctuate during periods of inflammation.[91] The handgrip strength test measures the strength of hand and forearm muscles. Subjects are classified as malnourished if their grip strength is less than 2 standard deviations from the mean of the age and sex groups.[90] This is a simple and quick tool to assess nutritional status, though its use as a sole assessment technique is not widespread. The handgrip test has been compared with the SGA in patients with cirrhosis and found to be a superior predictor of clinical complications such as uncontrolled ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, and hepatorenal syndrome.[90] Complications developed in 65% of patients who were classified as malnourished using the handgrip strength test, compared with 35.7% of patients classified as malnourished using the SGA.[90] Dual-energy x-ray absorptiometry, *in vivo* neutron activation analysis, and isotope dilution are other methods used to measure nutritional status.[128] Though they provide relevant and accurate information, their widespread application has been limited by cost and technical complexity.[129]

So, the SGA, anthropometric measures, and the handgrip test are most commonly used in routine nutritional assessments. Although the SGA is adequate as a stand-alone nutrition assessment tool, some studies have shown it can underestimate the frequency and severity of malnutrition of patients in the initial stages of the disease.[129,130] Figueiredo *et al* [129] suggested that nutritional intervention should be automatically initiated in patients with cirrhosis of Child–Pugh class B or C, due to the prevalence of malnutrition in these groups, with more extensive nutritional assessment for patients of class A, to provide timely support. Therefore, combination of subjective and objective data indicates the need for a comprehensive analysis of patients' nutritional status.[129,131] Diabetes is associated with poor prognosis for cirrhotic patients; because of the prevalence of impaired glucose tolerance among these patients, physicians should consider screening them for glucose intolerance.[132] Often, diabetes presents in patients with subclinical cirrhosis who have normal fasting glucose levels; the 75 g oral glucose tolerance test might be a better diagnostic tool.[132]

Serum Leptin

Leptin, the adipocyte-derived protein product of the *ob* gene, is involved in appetite regulation and obesity through central effects at the hypothalamus.[133] Leptin is related to amount of body fat.[134] Leptin is also associated with increased heart rate[134],blood pressure[135], and sympathetic neural activity[136] and may contribute to platelet aggregation.[137,138] The importance of leptin in the regulation of energy balance, body composition, and food intake has been demonstrated in both animal and human studies.[139,140]. Among liver patients, a high prevalence disease is represented by liver cirrhosis. The identification of obese patients who may progress from steatosis to non-alcoholic steatosis hepatitis (NASH) to fibrosis/cirrhosis is an important clinical challenge. It has recently been reported that most cases of obesity in humans are associated with high leptin levels. Thus, in humans, obesity may represent a state of leptin resistance. According to Tungtrongchitr *et al* obesity is found in 11% of elderly and moderate to severe obesity is increasingly found.[142] Obesity might be associated with clear health risks, including hypertension, diabetes and dyslipidemia and liver diseases. Moreover, another study has also indicated that obesity may play a more important role than alcohol intake in the development of steatosis in chronic HCV infection.[143] This overlap in host risk factors for the development of steatosis in chronic hepatitis C and non-alcoholic fatty liver disease (NAFLD) suggests a common pathogenesis, although several questions remain unanswered. There are common links between the host, viral, and other environmental factors that predispose to steatosis. One such common link may be leptin, which is increased in most cases of obesity, indicating leptin insensitivity or resistance. It is also required for hepatic fibrogenesis in toxic and metabolic forms of chronic liver injury. Several studies have shown that serum leptin levels are increased in patients with cirrhosis.[144]

Role of Leptin in fat metabolism

A 21-amino acid signal peptide is cleaved before release of mature leptin into the circulation.[145] Leptin, the protein product of the *ob* gene, is primarily an adipocyte-secreted hormone, exerts its influence on food intake, energy expenditure, body weight and neuroendocrine function through actions on neuronal targets in the hypothalamus. Leptin levels increase exponentially with increasing fat mass and leptin production is higher in subcutaneous than in visceral fat depots.[146]

Leptin levels reflect not only the amount of fat stored but also energy imbalance; prolonged fasting substantially decreases leptin levels, whereas overfeeding greatly increases them. The tertiary crystalline structure of leptin was originally reported by Zhang *et al.*.[147] The leptin molecule is thought to have similar structural features to members of the long chain cytokines family that includes growth hormone (GH), IL-6, leukemia inhibitory factor and ciliary neurotrophic factor (CNTF).[148] Human leptin is 84% identical to mouse leptin, and 83% identical to rat leptin.[149] The 4-α helix bundle structure consist of 146 amino acids having single disulfide bond that are located at C-terminal end between cystein residues 96 and 146 that is responsible for maintaining protein stability and biological activity.[147] The importance of leptin as an adiposity signal to the brain is supported further by the phenotype of animals that either do not synthesize it (*ob*/*ob* mice that have a mutation in the leptin gene) (Zhang *et al.*) or that have genetic mutations that compromise functioning of the leptin receptor (*db*/*db* mice and fatty Zucker *fa*/*fa* rats).

These animals are characterized by hyperphagia and extreme obesity. Administering small amounts of leptin into the brains of *ob*/*ob* mice reverses this syndrome. Mutation of the mouse ob gene results in a syndrome that includes obesity, increased body fat deposition, hyperglycemia, hyperinsulinemia, hypothermia and impaired thyroid and reproductive function in both male and female homozygous *ob*/*ob* obese mice.[150] Two distinct mutations of the ob gene have been identified. One mutant, SM/Ckc + Dacob2J/ob2J, expresses no leptin mRNA.[151] The other, C57BL/6J, over expresses by 20-fold an mRNA species resulting from a single base mutation at codon 105.[152] This mutation converts the coding sequence for arginine (Arg105) in leptin to a premature stop codon, resulting in the production of a truncated mRNA for leptin, which is translated into a protein that appears to be degraded in the adipocyte.

Leptin receptor

The leptin receptor (LR) gene was first cloned from mouse choroid plexus cDNA. LR mRNA was also found in a wide range of peripheral tissues e.g. heart, liver, skeletal muscle, pancreas ovaries, testes, spleen, adipose tissue as well as the hypothalamus. The closest relatives of LR encoded as gp130[153], the G-CSF receptor[154] and the leukemia inhibitory factor receptor.[155] Multiple transcripts of the leptin receptor, resulting from alternative splicing of *Ob*-R mRNA, encode at least six *Ob*-R isoforms.[156,157] All isoforms of the receptor share an identical extracellular domain at the amino terminus, but have cytoplasmic domains of different lengths arising from alternative RNA splicing at the most C-terminal coding axon. Five of the known receptor isoforms, LRa, LRb, LRc, LRd and LRf, contain transmembrane domains. LRe, lacking both transmembrane and cytoplasmic domains, circulates as a soluble receptor.[156,158] The roles of the short intracellular domain forms of *Ob*-R remain to be defined. It is tempting to speculate that the high levels of the short intracellular domain form in the choroid plexus play a role in transporting leptin from the blood into the CSF by a specific and saturable transport mechanism [159], where it can then move by diffusion to the brain centers that regulate body weight. The short isoform (LRa) can transducer signals through insulin receptor substrates and JAK-dependent singling to mitogen-activated protein kinase pathways. Short LR forms may play a role not only in transport but also in clearance or as a source of soluble receptor; it is assumed that proteolytic mechanisms exist for releasing the extracellular domain from the cell surface.[160]

The long receptor isoform LRb contains motifs within its intracellular domain that are required for signal transduction. LRb is a single membrane-spanning receptor that belongs to the class I family of cytokine receptors .[160] The homology of *Ob*-R to class I cytokine receptors immediately provided important clues as to possible intracellular mediators of leptin receptor activation. Leptin binding activates the Janus kinase (JAK)-signal transduction and activator of transcription (STAT) signaling cascade.[161] Typically, JAK proteins are associated constitutively with membrane-proximal sequences of the receptor intracellular domain (ICD) and phosphorylate the receptor ICD upon ligand binding. The phosphorylated ICD then provides a binding site for a STAT protein, which is activated upon binding the phosphorylated receptor ICD. The activated STAT proteins then translocate to the nucleus and stimulate transcription. It is possible that Ob-R's ability to control body weight may depend upon these signals as well. [161]

Leptin action in the hypothalamus and clearance

LRb is expressed at many sites in body, the highest levels of LRb expression in the body is found in neurons of the nuclei of the basomedial hypothalamus-including the arcuate (ARC), dorsomedial hypothalamic (DMH) and ventromedial hypothalamic (VMH) nuclei. Chemical or physical ablation of these nuclei results in increased feeding and neuroendocrine abnormalities that are similar to the phenotypes of *db/db* or *ob/ob* mice, suggesting that these hypothalamic nuclei (which is so-called "satiety center") are critical sites of leptin action [162,163] Within the nuclei of the basomedial hypothalamus, LRb is expressed at its highest levels in the ARC. Within the ARC, LRb is found in at least two distinct populations of neurons:

1) Neurons that coexpress neuropeptide Y (NPY) and agouti-related peptide (AgRP) and

2) Neurons that express pro-opiomelanocortin (POMC) [162,163]

POMC is processed to alpha melanocyte-stimulating hormone (α-MSH) in the LRb/ POMC neuron. α-MSH mediates a powerful anorectic (appetite-suppressing) signal; LRb stimulates the expression of POMC and activates the LRb/POMC neuron.[163,164] AgRP is an antagonist of α-MSH signaling and NPY is itself an orexigenic (appetite-stimulating) hormone that also acts to suppress the central LRb growth and reproductive axes.[165-168] Leptin acts via LRb to inhibit the NPY/AgRP neurons and to suppress expression of these neuropeptides. Thus, LRb signaling stimulates the production of anorectic neuropeptides and suppresses levels of orexigenic peptides. Conversely, when leptin action is decreased or deficient (e.g., starvation, *ob/ob* or *db/db* mice), appetite is stimulated via the suppression of anorectic neuropeptides e.g., POMC) and by increased expression of orexigenic peptides (e.g., NPY, AgRP) [169] LRb-expressing ARC NPY/AgRP and/or POMC neurons also regulate energy expenditure and other elements of neuroendocrine function.[170] Other distinct neurochemical properties of LRb-expressing neurons in the DMH, VHM and populations of LRb-expressing neurons may be found in the ARC.[171] The elsewhere (including the brainstem) are poorly defined. The relation of leptin to other hypothalamic neuropeptides, such as orexin, the tubby transcript[172,173], melanin-concentrating hormone, neurotensin, and cholecystokinin, has only recently begun to be deciphered.[174-176]

Leptin receptors are also expressed in peripheral tissues, including lung, kidney, liver, pancreas, adrenals, ovaries, hematopoietic stem cells and skeletal muscle, whereas the soluble leptin receptor isoform that circulates in the serum functions as a leptin-binding protein.[177] Although this wide expression may imply that the role of leptin is much broader than that of a circulating satiety factor, the full array of leptin's actions through activation of these receptors has not been fully clarified. However, it seems that short receptor isoforms present in the kidney may mediate leptin clearance[177-179], whereas those in the brain capillary endothelium[177] and the choroid plexus[178] transport leptin from blood into the brain interstitium and the cerebrospinal fluid by way of a saturable system[180,181]. There is a threshold level of serum leptin (about 25 to 30 ng/mL) above which increases in serum levels are not translated into proportional increases in cerebrospinal or brain leptin levels [180]; this, in turn, may result in an apparent leptin resistance and obesity.

Bioelectrical impedance analysis (BIA)

Historical background

Electrical properties of tissues have been described since 1871.[185] These properties were further described for a wider range of frequencies on larger range of tissues, including those that were damaged or undergoing change after death. Thomasset[186,187] conducted the original studies using electrical impedance measurements as an index of total body water (TBW), using two subcutaneously inserted needles. Hoffer *et al.*[188] and Nyboer[189] first introduced the four-surface electrode BIA technique. A disadvantage of surface electrodes is that a high current (800 mA) and high voltage must be utilized to decrease the instability of injected current related to cutaneous impedance (10 000 O/cm2).[190] By the 1970s the foundations of BIA were established, including those that underpinned the relationships between the impedance and the body water content of the body. A variety of single frequency BIA analyzers then became commercially available, and by the 1990s, the market included several multi-frequency analyzers. The use of BIA as a bedside method has increased because the equipment is portable and safe, the procedure is simple and noninvasive, and the results are reproducible and rapidly obtained. More recently, segmental BIA has been developed to overcome inconsistencies between resistance (R) and body mass of the trunk.

Principles of bioelectrical impedance

The resistance (R) of a length of homogeneous conductive material of uniform cross-sectional area is proportional to its length (L) and inversely proportional to its cross sectional area (A). Although the body is not a uniform cylinder and its conductivity is not constant, an empirical relationship can be established between the impedance quotient (Length2/R) and the volume of water, which contains electrolytes that conduct the electrical current through the body. In practice, it is easier to measure height than the conductive length, which is usually from wrist to ankle. Therefore, the empirical relationship is between lean body mass (typically 73% water) and height2/ R. Due to the inherent field inhomogeneity in the body, the term height2/R describes an equivalent cylinder, which must be matched to the real geometry by an appropriate coefficient. This coefficient depends on various factors, among them also the anatomy of the segments under investigation. Therefore, errors occur when there are alterations in resistivity of the conductive material, variations in the ratio height to conductive length, and variations in the shape of the body and body segments (body segments behave as if they are in series with each other, with shorter and thicker segments contributing less to the total R). Another complexity is that the body offers two types of R to an electrical current: capacitative R (reactance), and resistive R (simply called resistance). The capacitance arises from cell membranes, and the R from extra- and intracellular fluid. Impedance is the term used to describe the combination of the two. Several electrical circuits have been used to describe the behavior of biological tissues in vivo.[191]

One of them involves arranging R and capacitance in series, another in parallel, whilst others are more complex. A circuit that is commonly used to represent biological tissues in vivo is one in which the R of extracellular fluid is arranged in parallel to the second arm of the circuit, which consists of capacitance and R of intracellular fluid in series. R and capacitance can all be measured over a range of frequencies (most single-frequency BIA analyzers operate at 50-kHz). At zero (or low) frequency, the current does

not penetrate the cell membrane, which acts as an insulator, and therefore the current passes through the extracellular fluid, which is responsible for the measured R of the body R0. At infinite frequency (or very high frequency) the capacitor behaves as a perfect (or near perfect) capacitor, and therefore the total body R (RN) reflects the combined of both intracellular and extracellular fluid. Since practical constraints and the occurrence of multiple dispersions prevent the use of a direct current (zero frequency) or very high frequency AC currents, the R values at the ideal measurement frequencies are predicted using negative reactance versus R plot, [192] with R0 theoretically representing the R of the extracellular fluid (intracellular water) and RN representing the R of intra- and extracellular fluid (TBW) . At 50 kHz, the current passes through both intra and extracellular fluids, although the proportion varies from tissue to tissue. Another parallel model attempts to take into account the effect of 'mixing'. Mixing theory predicts that the R of conductive fluids increases as the amount of suspended non-conducting material increases.