Physical activity as an exogenous risk factor in motor neuron disease (MND): A review of the evidence

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Abstract
Motor neuron disease (MND) is a devastating neurodegenerative condition associated with considerable disability and a poor prognosis. Despite improvements in symptomatic management in recent years, few therapies are available which modify survival. However, the challenge to find successful treatments would be greatly assisted by clearer elucidation of the underlying pathoaetiology. Many potential exogenous risk factors have been proposed as part of a gene-environment interaction in the aetiology of MND. A growing interest in the role of vigorous physical activity in the development of MND has followed reports of a higher than expected incidence of the disease in professional sports people. Such an association is also supported by current hypotheses concerning the cellular and genetic mechanisms of MND. However, evidence from epidemiological research remains conflicting and inconclusive. This article reviews the existing literature regarding physical activity as a risk factor for MND and the potential biological and genetic plausibility for this association.

Key words: Motor neuron disease, risk factors, epidemiology, physical activity, football, gene-environment interaction

Introduction
Motor neuron disease (MND) is a disabling neurodegenerative condition, usually fatal within three years of diagnosis (1). Typical cases present with progressive weakness of the limb muscles with mixed motor neuron signs on examination, although considerable phenotypic heterogeneity is now recognized, including the less common bulbar-onset disease and concurrent non-motor manifestations (2–5). Increasing age is a risk factor, with incidence greatest in those aged 60 to 75 years and disease onset unusual below the age of 40 years (6,7).

Population-based research suggests a relatively uniform annual incidence among populations of European extraction of 1.5–2.5 per 100,000 (6,8–12) and prevalence of 3–8 per 100,000 (8,13,14). Two geographical exceptions, the Pacific Island of Guam and the Japanese peninsula of Kii, have a notably higher incidence, although the reasons for these clusters remain uncertain (15,16). Mortality studies suggest the incidence of MND may be increasing, particularly in women, the elderly and in southern European countries (17–19). Whether this represents a true increase, or simply reflects improved diagnostic accuracy and disease reporting in an ageing population, is unclear (1,20). As mortality studies do not determine the true rate of new cases, but are dependent on other factors including disease survival, this may provide an alternative explanation, particularly when incidence studies, such as the MND Scottish Register, have not demonstrated such a change (6).

Aetiology of MND
In most patients, the causes of MND are not established with certainty and there are no treatments currently available which substantially protect motor neurons from injury. The challenge to find successful disease-modifying therapies would be greatly assisted by clearer elucidation of the underlying pathoaeiology. Similar to other neurodegenerative conditions, a multifactorial interaction between fixed genetic components and modifiable exogenous factors seems the most likely explanation.

Genetic factors
A strong genetic aetiology is apparent in 5–10% of MND patients who are diagnosed with familial
disease (7,21,22). In approximately 20% of these cases, mutations in the copper/zinc superoxide dismutase (SOD1) gene, encoding for an antioxidant defence protein, have been found, with over 100 different mutations in this gene now reported (23–25). Seven other genetic loci have also been described in adult and juvenile onset familial MND pedigrees (ALS types 2–8). Of these genotypes, five are inherited as an autosomal dominant trait, with two following a recessive pattern. However, some of the causative genes have been identified (including alsin, senataxin and vesicle-associated membrane protein B), the pedigrees sizes are small and disease phenotype is often atypical. Recently, a further disease-causing gene has been confirmed, following the discovery of mutations in the TAR-DNA binding protein (TDP-43) gene in individuals with familial MND.

A genetic basis for sporadic MND is also considered likely, although the evidence is less established. The ‘British Motor Neuron Disease Twin Study’ provided support for a genetic component, reporting four MND cases from 26 monozygotic twins compared to none from 51 dizygotic twins (26). Both novel and existing mutations in the SOD1 gene have also been identified in sporadic cases, although at a much lower frequency than familial disease (27–29). Several susceptibility genes, which may predispose to motor neuron injury, have been suggested following candidate association studies, including the angiogenin, survival motor neuron and haemochromatosis genes (30–32), although attempts to replicate such findings have been inconsistent (33–35). Recently, genome-wide analysis using single-nucleotide polymorphisms has been applied in the detection of gene–disease associations, providing improvements in some of the methodological limitations of candidate studies. However, to date, results have been inconclusive and no clear associations confirmed (36–39).

Exogenous factors

The emergence of rare geographical and occupational clusters of MND has stimulated the search for potentially modifiable environmental and lifestyle factors that may act as disease triggers or modifiers in inherently susceptible individuals. Factors previously considered include physical activity, occupation, mechanical and electrical injury, military service and toxin exposure, particularly heavy metals and pesticides (40–48). However, beyond the known risk associated with male gender, family history and increasing age, smoking is the only other likely risk factor identified to date (40,49,50), although this remains controversial (51,52).

Epidemiological evidence for physical activity as a risk factor for MND

The challenges of epidemiological research

In 1962, the neurologist Macdonald Critchley stated: “Nothing has been said about the possible role in aetiology of a previous habit of athleticism. I have the uncomfortable feeling that a past history of unnecessary muscular movement carried out for no obvious reason may be followed in later life by the development of motor neuron disease in a statistically significant number of cases” (53).

Since then, a growing volume of epidemiological research designed to establish any association between physical activity (PA) and MND has been published, fuelled by several high profile cases in professional sportsmen including the boxer Ezzard Charles and baseball player Lou Gehrig. However, the challenges faced when conducting epidemiological research, particularly regarding a condition of such rarity as MND, have limited the impact of the evidence produced. Many early studies were designed to investigate general risk factors for MND. Both positive and negative associations of MND with PA in leisure and work were reported (43,54,55). These studies, however, were plagued by methodological flaws, particularly small study numbers, multiple hypotheses testing, and inadequate consideration of confounders.

Recent studies have improved on several of these weaknesses. However, design errors are still encountered and results remain conflicting and inconclusive. Selection bias is common, with cases often recruited from specialist clinics, representing a subgroup of MND patients with atypical disease, rather than the general MND population. Valid, reproducible methods for PA quantification, to allow differences in case and control exposure to be accurately determined have seldom been employed. Control for potential confounders is often inadequate, though should be addressed when investigating a condition of unknown aetiology. Delineating the overlap between PA and other potential risk factors poses a particular challenge, illustrated by considering military service in the aetiology of MND (46). Military personnel may be exposed to many chemical, environmental and physical factors proposed as pathogenic, requiring accurate quantification of each factor in order to determine any individual or interacting associations. The relationship between mechanical injury and PA, both recreational and occupational, represents another example. The European MND registry, EURALS, will address some methodological limitations by prospectively assembling a large, population-based cohort, allowing collection of valuable descriptive epidemiological information, including basic risk factor data (56). However, substantial challenges would be met in conducting a detailed study to determine the association of PA with MND in view of
the wide geographic, cultural and language diversity encountered in such a cohort.

In a paper reviewing exogenous risk factors in MND in 2003, suggestions for appropriate study methodology in epidemiological MND research were made, including a classification system to grade evidence (44). The author proposed that in view of the huge resource demands posed by an MND cohort study, a well designed population-based case-control design would offer a suitable alternative, using strictly defined inclusion and exclusion criteria, appropriately matched controls, high recruitment and case ascertainment rates, valid assessment tools and robust statistical methods.

In the following sections, we review the recent evidence regarding general and sport-specific PA as risk factors in MND. An outline of each study design and findings will be followed by critique of the work. Table I provides a summary of these studies.

Methods

Appropriate articles were identified using the U.S. National Library of Medicine PubMed search engine. A literature search of all article types was conducted using the terms ‘ALS’ or ‘MND’ and ‘physical activity’ or ‘sport’ or ‘football’ or ‘exercise’. All relevant publications since 1995 were reviewed and reference lists searched to identify further pertinent work.

Evidence for general PA as a risk factor in MND

Strickland D, et al. Physical activity, trauma, and ALS: a case-control study. In 1996, a retrospective case-control study designed to assess an association of MND with both PA and trauma was published (57). The authors reported a positive association between MND and sweating at work (odds ratio (OR) 1.6, 95% CI 1.1–2.4), sweating in leisure activities (OR 1.6, 95% CI 1.1–2.5) and receiving recognition in organized school or college sports (OR 3.1, 95% CI 1.04–9.3).

However, the methodology in this study limits the conclusions that can be drawn. Only 25 prevalent rather than incident cases with poor case definition were recruited from a specialist university MND clinic. When studying aetiology using case-control studies, prevalent cases may introduce recall bias due to the variation in time from exposure to prevalence and changes in exposure pattern since diagnosis which may influence recall accuracy. Prevalent cases also reflect prognostic factors of the disease rather than just aetiology. Those with “severe mobility or communication limitations” were excluded without justification. The authors admit to using a “convenience” case sample so as to allow age- and gender-matched healthy controls to be recruited from the same residential community. A second control group of patients with other neuromuscular diagnoses was also enrolled from the muscle disease clinic of the same university, with the intention of increasing statistical power and adjusting for case selection bias. The author also proposed that disease controls may respond to questions about the cause of their disease in a similar manner to cases, who “ruminate over possible causes of their condition” resulting in recall bias, and may therefore help to address this issue. A trauma and PA questionnaire was sent to participants at home and later completed in a nurse-led interview. Although this may reduce recall and response bias, this approach may also result in a lack of standardization in data acquisition. The questionnaire used a structure of questions “among the simplest and best-validated of the standard PA measures”, although this was not referenced. The overall questionnaire validation and methods used to quantify PA were not stated. Multiple hypotheses were tested for and analysis by logistic regression was undertaken, although adjustments were not made for many of the comparisons without justification. Very few results were tabulated to allow independent interpretation of the data.

Longstreth WT, et al. Risk of amyotrophic lateral sclerosis and history of physical activity: a population-based case-control study. In 1998, a population-based case-control study was published to determine the risk of MND according to previous PA (58). The authors reported no significant difference in PA performed in work and leisure between cases and controls, with the exception of organized school sports, where cases were more likely to have been participants (OR 1.52, 95% CI 1.03–2.25). They recruited 174 cases, with 97% recruitment rate, consisting of “all incident cases of ALS” over four years from three West Washington State counties. The method of case ascertainment was not described. Although eligibility criteria were clearly stated, they produced a heterogeneous case group which included four familial cases. Random sampling was used to identify age, gender and residentially-matched controls. However, due to inadequate age matching using a telephone dialling method (75% response rate), the sampling technique was changed during the recruitment process to random selection from Medicare eligibility lists (60% response rate). Both sources may introduce selection bias, particularly the use of Medicare, a health insurance programme with very restricted eligibility criteria. Using memory aids to minimize recall bias, participants were interviewed about PA at work, home and leisure, although no data for PA at home were published. Unspecified surrogates were used to replace 20 of the cases who died before interview and their corresponding controls, a source of considerable bias. Without justification, two different PA intensity weighting
Table I. Summary of the existing evidence that investigates physical activity and sport as risk factors for motor neuron disease.

<table>
<thead>
<tr>
<th>Author, year and study design</th>
<th>Research question/aims</th>
<th>Reported findings</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strickland D, et al. (1996). Case-control study.</td>
<td>Is MND associated with previous PA or trauma?</td>
<td>MND associated with sweating at work, sweating in leisure PA and receiving recognition in organized school or college sports.</td>
<td>Use of valid PA measurements within PA questionnaire; use of 2 control groups.</td>
<td>Small numbers (25 cases), poor case selection and definition; multiple hypotheses tested.</td>
</tr>
<tr>
<td>Longstreth WT, et al. (1998). Case-control study.</td>
<td>Is MND associated with previous PA?</td>
<td>No overall association of MND with PA; MND cases more likely to have participated in school sports.</td>
<td>Population-based; full case ascertainment; PA quantified using Compendium of PA.</td>
<td>Included familial MND; control bias from poor selection method; surrogates used for deceased cases.</td>
</tr>
<tr>
<td>Scarmeus N, et al. (2002). Case-control study.</td>
<td>Is MND associated with varsity athletics, weight and BMI?</td>
<td>MND associated with previous participation in varsity athletics and slim habitus.</td>
<td>Larger sample numbers (279 cases and 152 controls)</td>
<td>Case and control selection bias from eligibility criteria and recruitment source; poor PA variable definition.</td>
</tr>
<tr>
<td>Valenti M, et al. (2005). Case-control study.</td>
<td>Are sport and sport-related trauma risk factors for MND?</td>
<td>No association between sport or sport-related trauma and MND.</td>
<td>Multicentre population-based study; well defined cases.</td>
<td>Included familial MND; poor PA definition and quantification; limited analysis; little discussion of results.</td>
</tr>
<tr>
<td>Veldink JH, et al. (2005). Case-control study.</td>
<td>Is lifetime PA a risk factor for MND?</td>
<td>No association between PA and MND but MND onset earlier if more cumulative PA in the past.</td>
<td>Good case definition; PA quantified using Compendium; considers temporal relation of PA and phenotype outcome.</td>
<td>Bias from convenience selection of controls by cases; incomplete control recruitment and matching.</td>
</tr>
<tr>
<td>Belli S, Vanacore M (2005). Retrospective mortality study.</td>
<td>To identify the causes of death in Italian professional footballers.</td>
<td>↑ MND incidence (SMPR 1158) and earlier age of onset than expected.</td>
<td>Very large cohort size (24,000 players).</td>
<td>Non-specific mortality study; incomplete case ascertainment and PA data; incomplete follow-up.</td>
</tr>
<tr>
<td>Taioli E (2007). Follow-up mortality study.</td>
<td>To identify the causes of death in Italian professional footballers.</td>
<td>↑ MND incidence (SMR 18.18) and earlier age of onset than expected.</td>
<td>Complete cohort assembly (5389 players).</td>
<td>Non-specific mortality study; lack of confounding adjustment; American mortality data used as reference.</td>
</tr>
<tr>
<td>Chio A, et al. (2005). Retrospective cohort study.</td>
<td>Is MND associated with playing professional football?</td>
<td>↑ MND incidence (SMR 6.5), earlier age of onset, ↑ bulbar disease incidence. ↑ risk if played &gt;5 years and in a midfield position.</td>
<td>Complete cohort assembly (7325 players); well defined cases; consideration of some confounders.</td>
<td>Appropriateness of using general population-based reference data tested multiple hypotheses due to confounding exposures in football.</td>
</tr>
<tr>
<td>Abel EL (2007). Retrospective cohort.</td>
<td>To describe the rate of MND in NFL American footballers.</td>
<td>Significant ↑ prevalence of MND in American footballers.</td>
<td>American football not studied before.</td>
<td>Poor case ascertainment; limited analysis; lack confounder adjustment.</td>
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</table>
methods were used, with the Compendium of PA (59) applied to leisure activities and an unspecified weighting system for work related activities. However, by categorizing PA data, the activity patterns of cases could be compared using the controls as a reference. Consideration of the effects of subclinical disease on PA was included by omitting data from 10 years before diagnosis, although adjustments in analysis were made only for age and education.

Scarmeas N, et al. Premorbid weight, body mass, and varsity athletics in ALS. A retrospective case-control study published in 2002 investigated the association of MND with varsity athletics, premorbid weight and body mass (60). This study reported that MND patients were more likely to have participated in varsity athletics (OR 1.89, 95% CI 1.05–3.4) and to have been of slim body habitus (OR 2.1, 95% CI 1.08–4.07).

A major source of bias in this study is the definition and selection of cases and controls. Two hundred and seventy-nine cases, defined by the authors’ own diagnostic criteria, and 152 controls with ‘other neurologic diseases’ were recruited from successive patients attending the clinic of a single neuromuscular neurologist over an eight-year period. The diagnostic criteria would construct a very heterogeneous case sample, possibly including diseases with different pathoetiology. Controls were adequately matched from the same source, but without clarifying severity and duration of their diagnoses it is not unreasonable to suspect that their “neurologic disease“ may have impacted on their activity levels. This control group is unlikely to be representative of the general population and therefore conclusions drawn from this study would not translate to the whole population at risk from MND. The exclusion of some participants for ‘age-related decline in performance’ was neither defined nor justified. An unspecified questionnaire was used to gather self-reports of three variables (previous body habitus, participation in varsity athletics and premorbid weight and height). These variables were poorly defined with little attempt to quantify them or establish a temporal relationship. The results section focuses on unadjusted ORs (all results significant) rather than adjusted ORs (only slim habitus and varsity athletics participation significant). However, these three variables are unlikely to be truly independent and therefore interpretation of the results should be made with caution, as highlighted by the authors. The authors provide a thorough review of the potential mechanisms by which PA may pose an underlying susceptibility in the discussion, including a common genetic trait.

Valenti M et al. Amyotrophic lateral sclerosis and sports: a case-control study. In 2005, a multicentre retrospective population-based case-control study was published in response to concerns from the Italian National Olympic Committee regarding suggestions of an association between MND and competitive sports (61). They concluded that neither participation in sport nor sport-related trauma was associated with MND.

Case recruitment from 10 Italian MND centres may provide a sample representative of multiple Italian regions, although these cannot be considered truly population-based data. Referral criteria to these centres were not stated. Three hundred incident cases defined by El-Escorial criteria and specified exclusion criteria were recruited over 17 months. However, the inclusion of nine familial cases, three with SOD1 mutations, introduces an unadjusted confounder. Healthy controls were matched 1:1 by gender, age and place of residence, although the recruitment method was not described and therefore the appropriateness of these controls is unclear. A standardized interview-delivered questionnaire collected self-reported data on sports participation and related trauma, although its validity was not discussed. Although sport was classified by discipline and professional status, definitions were unclear with little attempt to quantify the activity or timing of participation. The manuscript reports no significant association between MND and any of the exposure variables. However, only a limited selection of the results was displayed in a single table and no other analyses were described in the text. Most of the tabulated ORs were significantly less than 1, suggesting a negative association, although this is not discussed at all by the authors. It is also not clear whether adjusted analysis was performed or which confounders were considered in the model.

Veldink JH, et al. Physical activity and the association with sporadic ALS. In 2005, a population-based case-control study was published investigating lifetime PA as a risk factor for MND (62). Although no significant association between MND and PA was found, the authors reported that disease onset was seven years earlier in those who participated in more cumulative leisure PA before the age of 25 years (HR 1.7, 95% CI 1.3–2.4), and three years earlier in those with greater cumulative leisure PA in the 10 years before reference age (HR 1.6, 95% CI 1.2–2.2). Two hundred and nineteen incidence cases, defined using El-Escorial criteria and excluding familial disease, were recruited from two national referral centres, a source which the authors admit may introduce bias. Each MND case was requested to identify two age- and sex-matched controls. In contrast to random control identification, this convenience sample may unintentionally match for work and recreational activity, making analysis of PA as a risk factor inaccurate and resulting in a bias towards
the null hypothesis. Recruitment was incomplete (254 controls), with inadequate matching. An unspecified postal questionnaire collected qualitative and quantitative data on PA in work and leisure only. Postal questionnaires may reduce response bias, where participants provide answers that they believe the interviewer wants to hear, but can introduce bias through incorrect interpretation or misunderstanding of the questions, which may explain the missing values referred to by the authors in one of their results tables. The Compendium of PA was used to quantify PA and multivariate analysis was performed. Strengths of this study include the analysis of a temporal relationship between exposure and disease by categorizing PA into early life, late life and total. They also examine whether PA is independently associated with certain clinical features of the disease. This paper included a systematic review of research into the association between PA and MND published between 1966 and 2003. By applying the previously suggested evidence grading system (44), they concluded that the number of studies supporting a positive association decreased as the level of evidence improved.

Evidence for an association of MND with soccer

In recent years, attention has focused on a possible association between MND and soccer. Interest arose from an unexpected outcome of a mortality enquiry of Italian professional soccer players in 1999, ordered to address concerns regarding the health consequences of suspected widespread illicit drug use in this group. Early media reports of the results suggested that professional soccer players may have a higher than expected incidence of MND, a concept reinforced by reports of individual cases (63). Most publications since continue to suggest a positive association between MND and soccer, although this association and its nature have not been confirmed. However, increased rates of bulbar and young-onset disease are fairly consistently described, suggesting that disease phenotype may be modified in this cohort. As many potential pathogenic exposures are associated with playing soccer, clarification of the role of PA alone to MND is challenging and has not yet been achieved.

Belli S, Vanacore M. Proportionate mortality of Italian soccer players: is amyotrophic lateral sclerosis an occupational disease? In 2005, a retrospective cohort mortality study carried out in the frame of the Italian enquiry was published (64). All causes of death in soccer players playing between 1960 and 1996 in the three highest Italian professional leagues were investigated. Eight out of the 375 deaths identified were from MND, giving a standardized proportionate mortality ratio (SPMR) of 1158 (95% CI 672–1998). Age of onset was earlier than expected (three cases under 39 years, six cases under 59 years).

Despite the substantially increased MND incidence reported, these results should be interpreted with caution. This study was not designed to investigate PA as a risk factor for MND. Therefore, measures to control for the multiple confounding exposures in soccer, such as drug use and injury, will not have been undertaken and a cause-effect relationship should not be interpreted. The cohort consisted of ‘about 24,000 soccer players’ without stating the completeness of this cohort, although sources from which it was assembled were described. The authors acknowledged that PA exposure data were incomplete and therefore a relative measure of risk, SPMR, rather than standardized mortality ratios (SMR), was calculated. As SPMR describes the mortality from a specific disease as a proportion of the mortality from all diseases, the ratio can be influenced by changes solely in the denominator, thus introducing inaccuracies in this method. Case ascertainment may not have been comprehensive, as the cause of death was undetermined in 25 individuals. Rigid diagnostic criteria were not applied to confirm those MND cases identified.

Matched national mortality rates were used to calculate the cause-specific expected number of deaths (0.69 for MND). This calculation and the appropriateness of the reference group have been criticized by Armon, suggesting that an inappropriately low number of expected cases was used (65). However, establishing an accurate denominator to estimate lifetime risk for MND is challenging. The alternative method proposed by Armon may also be questioned as it gives a figure higher than might be expected based on published epidemiological studies of MND. Although national data may not provide a truly matched comparison group, the chosen reference data were matched for age, gender and calendar period of death.

Taioli E. All causes mortality in male professional soccer players. Subsequently, a follow-up mortality study of Italian professional soccer players between 1975 and 2003 was funded by the Italian Ministry of Health (66). From 63 identified as deceased, four cases of MND were reported, yielding an SMR of 18.18 (95% CI 5.00–46.55). The mean age at death from MND was 40.6 years. A complete cohort of 5389 players was assembled and vital status established for all but 180 players. Causes of death were determined for all 63 deceased, predominantly using death certificates. The reliability of this case ascertainment method has been questioned, although mortality reporting for MND is thought to be more reliable than for other diseases (67,68). American mortality rates were used to calculate the SMR, as corresponding Italian population data did not include age, gender and calendar period specific rates to allow
matching, which the authors acknowledge is a limitation of the study. Although only four cases were identified (reflected by a wide but significant 95% CI), low case numbers are an inherent problem in cohort studies of rare conditions. Importantly, the authors acknowledge that they could not establish many of the confounding soccer-related exposures including drug use, injuries and toxin exposure and therefore could not adjust for them. However, the finding of an earlier than expected age at death from MND is interesting and is supported by other studies. It is plausible that the age of onset in soccer players developing MND is comparable to that seen in familial cases, supporting the concept of an underlying genetic susceptibility (21).

Chio A, et al. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. Beyond mortality studies, a retrospective cohort study designed to determine an association between MND and soccer was published in 2005 (69). Five MND cases were identified from a well defined cohort of 7325 Italian professional players, giving a standardized morbidity ratio of 6.5 (95% CI 2.1–15.1). The mean age of onset was 43.4 years with 60% of cases classified as bulbar onset. Subgroup analysis suggested that the risk increase was predominantly in those with onset under 49 years (SMR 7.5, 95% CI 2.0–19.2), those playing longer than five years (SMR 15.2, 95% CI 3.1–44.4), and those playing a midfield position (SMR 12.2, 95% CI 3.3–31.2).

The cohort consisted of ‘all male professional football players’ from the top two Italian soccer divisions between 1970 and 2002. Foreign players were excluded to achieve complete follow-up and case ascertainment of an exclusively Italian cohort. The authors speculate that case ascertainment may be incomplete as they failed to identify any MND cases during the 1970s, although this may be plausible in a relatively rare condition. All cases were defined using El-Escorial criteria and confirmed with medical records and interviews with patients and carers. Several important genetic and environmental confounders were considered, although others, such as smoking, were not.

Matched incidence rates from two Italian MND registers covering 15% of the country gave 0.77 as the expected number of cases. Similar to the earlier paper, this approach has been criticized (65). It has also been suggested that the use of population-based reference data may not be appropriate, as soccer players are likely to represent a unique genotypic sub-population to account for their sporting ability (70). However, if the data are interpreted with this in mind, valid conclusions can still be drawn, although causal inferences specific to soccer rather than sport in general would be hard to elucidate. This criticism does not alter the observation that disease onset was earlier than expected in this cohort. The findings also suggest a dose-response relationship, quantifying exposure by the sum of years played, although the number of matches played may have provided a more accurate calculation.

Vanacore N. Amyotrophic lateral sclerosis in an Italian professional soccer player. In 2006, a case report of an Italian professional soccer player outside the previous cohorts was published (71). In support of previous findings, the patient developed early-onset bulbar MND following a 17-year career playing in a midfield position. However, in addition to exposure to vigorous PA and chemicals on soccer pitches, this player had experienced significant sport-related trauma and consumed multiple dietary supplements, illustrating many of the potential confounders in soccer. Illicit and prescription drug use and microtrauma were not commented on, but should be considered. Although adjustments can be made during analysis, this is often not achieved, possibly due to incomplete collection of these sensitive data in retrospective studies. However, without considering such confounders, it is hard to determine any specific cause-effect relationship of a positive association between MND and soccer.

Wicks P. Three soccer playing friends with simultaneous amyotrophic lateral sclerosis. Although the majority of soccer studies have used Italian populations, several British professional players have also been diagnosed with MND, including Don Revie, Willie Maddren and Jimmy Johnstone. In 2007, a case series described three friends from the same area in England, all of whom played amateur soccer and subsequently developed MND (72). Although all three were probably exposed to other risk factors, including potentially unidentified mutual environmental exposures due to their geographical proximity, this represents an unusual cluster of MND.

Abel EL. Football increases the risk for Lou Gehrig’s disease, amyotrophic lateral sclerosis. A retrospective cohort study published in 2007 investigated MND cases in American footballers (73). Since 1960 eight cases were identified from 3891 players, giving a prevalence rate of 206 per 100,000. When compared to US population rates, quoted as 5 per 100,000, the difference was calculated as significant using binomial testing. However, no further statistical analysis was performed and several methodological flaws were apparent including poor recruitment and case ascertainment, lack of confounder consideration and multiple hypothesis testing. Therefore, no firm conclusions can be reliably drawn from this study.
Biological plausibility for PA as a risk factor for MND

Considerable advances have been achieved in understanding the pathophysiological mechanisms involved in MND. Unrelated research has also demonstrated that PA modifies several of these mechanisms, providing a possible biological explanation to link MND and PA (Figure 1).

Oxidative stress

Oxidative stress is a pathological process representing an imbalance of normal metabolism. Detrimental effects of reactive oxygen species (ROS) occur due to excessive free radical production and/or failure of protective detoxifying or repair mechanisms. Abnormally elevated levels of oxidative injury biomarkers have been demonstrated within tissues of the nervous system in MND from both post mortem and in vivo studies (74–76). A body of evidence now exists that strongly implicates oxidative stress as a significant contributory factor to motor neuron injury in MND, supported by dysfunction of the antioxidant enzyme, SOD1 in familial MND (24,77).

Although there is some debate about oxidative stress during PA, it is conceivable that the elevated oxygen consumption and tissue metabolism required to perform vigorous exercise would augment ROS production. Evidence to support this was first published in 1982, when an exercise-induced increase in free radical concentration was demonstrated in muscle and liver, and has continued to grow since (78,79). Many mechanisms for this increase have been proposed, including altered mitochondrial activity and induction of an inflammatory response. A systematic compensatory increase in antioxidant capacity following exercise, which includes enhanced antioxidant enzyme activity and changes in tissue redox status, further supports this theory (80). The evidence for this exercise-induced modification in oxidative stress response has now been reviewed several times (81–83). However, during vigorous PA the normal adaptive processes seen in regular moderate exercise may not be achieved, resulting in deleterious effects of ROS. These effects may be compounded in individuals who, due to an inherent susceptibility, are unable to mount the normal physiological responses to exercise.

Glutamate excitotoxicity

Despite being a major excitatory neurotransmitter, glutamate in higher than physiologically normal
concentrations can over-stimulate receptors, causing abnormal calcium ion influx into neural cells and ultimately neuronal cell death, a process known as glutamate excitotoxicity. Evidence points towards derangement in several areas of glutamate handling in the nervous system of MND patients (84–86). This is supported by the modest but significant MND survival benefits of riluzole, which inhibits glutamate release from pre-synaptic terminals (87). It has been proposed that in performing vigorous exercise, intense motor neuron firing is required, causing excessive glutamate stimulation with toxic consequences in susceptible individuals (88).

Despite these explanations, there is little basic science research published which directly links MND and PA. In a review article, Longstreth et al. postulate several mechanisms by which PA may alter the risk of MND (88). Beyond suggesting that intense motor neuron firing during vigorous activity may cause glutamate excitotoxicity, these authors hypothesize that handling of and susceptibility to environmental, dietary or medicinal motor neuron toxins may be altered by the physiological mechanisms of PA. Beretta et al. proposed that in soccer players, oxidative stress may be augmented by concurrent mechanisms, including high levels of PA, dietary supplementation, prescription and illicit drugs, exercise-induced tissue ischaemia and sports-related injuries (63). They considered that neuroinflammation, another proposed pathogenic mechanism in MND (89,90), may also contribute to an association between MND and football, though predominantly through chronic anti-inflammatory drug use. Another editorial suggested that “chronic exhaustion of the body’s antioxidant capacity” may link PA, athleticism and neurotrauma as risk factors for MND (91).

Genetic plausibility for PA as a risk factor for MND
As interest in the health consequences of PA grows, genetic factors underlying the recognized heterogeneous response to exercise are being sought. Attention has focused on the effects of PA on physiological gene regulation and genotypic variations that may be associated with sporting ability and health-related fitness (92,93). Several genes also associated with the development of MND are now known to be up-regulated or their expression altered by PA, providing a potential genetic explanation to link MND and PA. Genes conveying angiogenic and neurotrophic properties provide the strongest evidence. However, to date there has been no research published which investigates specifically gene-environment interactions which may link PA with MND.

Hypoxia-response genes
In recent years, the role of hypoperfusion in MND pathogenesis has been considered due to mutations found in hypoxia-induced genes. Conceivably, the high oxygen requirements of motor neurons may increase their susceptibility to degeneration before other tissues if the normal response to hypoxia cannot occur. In support of this, a serendipitous discovery in 2001 revealed that deletion of the hypoxia response element in the promoter region of the vascular endothelial growth factor (VEGF) gene in mice causes the development of a condition resembling MND phenotypically and pathologically (94). Variations in the VEGF gene, lowered circulating serum VEGF and altered VEGF protein and receptor gene transcription have now been demonstrated in human MND patients (95,96). In addition, evidence for functional and neurotrophic properties of VEGF on motor neurons in vivo exists, possibly mediated via direct effects on neurons and indirect effects through blood vessel modification (94,97–99). Interestingly, it has also been demonstrated that strenuous exercise normally increases serum and muscle VEGF and VEGF receptor transcript expression (100–102). Polymorphisms and mutations in another hypoxia-response gene, angiogenin, which works in a similar way to and synergizes with VEGF and is also up-regulated by PA, have been identified in several MND populations (32,103–106). It is conceivable that individuals with defects in these genes may be unable to mount a normal physiological response to PA and be at increased risk of motor neuron degeneration.

Neurotrophic factors
Protective effects of neurotrophic factors on motor neurons in culture and animal models have been demonstrated (107,108), leading to clinical trials for insulin-like growth factor-1, ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), although no significant clinical benefits were demonstrated (104,109–111). It has also been suggested that genetic variations in the genes encoding these neurotrophic factors may convey pathogenic properties. A null mutation in the CNTF gene has been associated with early-onset disease in familial MND and mouse models, in the presence of a SOD1 mutation and also sporadic MND (112), although its presence in controls at a similar prevalence suggests a modifying rather than causative role (113). High serum levels and up-regulation of CNTF in motor neurons of MND spinal cord autopsy specimens have also been described (114,115). Regarding the response to PA, several neurotrophic factors are reported to be
up-regulated by PA in neural tissue and muscle, including BDNF and GDNF (116–119). Complementary to this, recent work from the authors’ laboratory, using microarray and laser-capture microdissection techniques, has demonstrated the transcriptional response of motor neurons and muscle to exercise in a mouse model (120). Compared to sedentary controls, motor neurons from mice which completed a 21-day voluntary exercise period demonstrated differential expression of 444 genes. Two hundred and three genes were up-regulated, including CNTF and leukaemia inhibitory factor receptor (LIFr) by at least two-fold, and 241 genes down-regulated. In muscle, 194 genes were up-regulated in response to exercise, including VEGF receptor 2, and 176 down-regulated.

Miscellaneous evidence

Dysfunction of NrF2, a transcription factor that regulates the expression of antioxidant response element genes, has been shown in motor neurons using a cellular model of mutant human SOD1 (121). Altered NrF2 levels in the lumbar spinal cord of the G93A-SOD1 rat model have also been reported (122,123). Although evidence of the effects of exercise directly on NrF2 has not been reported, proteins known to be induced by NrF2 are up-regulated by exercise, which may indicate an underlying up-regulation of NrF2 mediated transcription.

Conclusions

In recent years, the understanding of MND pathogenesis has advanced considerably, although the resultant health benefits for patients have been modest. The challenge to elucidate the causes of this devastating disease remains. A complex multifactorial etiology seems likely, supported by current epidemiological and basic science research and hypotheses regarding the etiology of other neurodegenerative disorders. With this in mind, the search for endogenous and modifiable exogenous pathogenic candidates continues.

Current evidence for PA as a risk factor in MND is limited, conflicting and not of sufficient calibre to allow undisputed conclusions. Many of the problems encountered in existing research are inherent to epidemiological studies and the nature and relatively low prevalence of MND. However, the positive association reported between MND and PA on several occasions has not been refuted beyond doubt and may represent an abnormal physiological response to an exogenous factor in genetically susceptible individuals (Figure 2). This warrants further investigation, particularly as developments in basic science research may reinforce such an association. An integrated epidemiological and basic science approach may provide the strongest evidence. Emphasis should be placed on optimizing case-control study design, particularly regarding the use of clear case definition and complete ascertainment, population-based case and control selection methods to minimize bias, and robust exposure quantification techniques. Power to detect a true difference of clinical significance should be ensured by using statistically determined sample sizes. Biological and genetic studies within the same cohort may be employed to determine the presence of susceptibility factors and pathogenic mechanisms known to be related to PA, thus working towards establishing the causal relationship.

Identifying risk factors for this devastating disease will provide valuable direction for future
research. Elucidating the complex genetic and environmental interactions at play in MND will help identify future therapeutic targets and may lend itself to the development of medical screening tools for those in ‘at-risk’ environmental and lifestyle situations.

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**References**


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