Protection against muscle damage exerted by oestrogen: hormonal or antioxidant action?

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Introduction

In the early 1980s it was felt necessary to develop diagnostic tests for a population of patients who attended the Neuromuscular Diseases Clinic of the university hospital. Their complaints were often vague and difficult to objectify, and for a large group the complaints were often triggered by some form of exercise. Exercise, in this context, is to be taken quite broadly, and varied from going up the stairs, vacuum cleaning the house, cycling or walking home with heavy groceries, to extremes such as an army sports instructor, who only experienced complaints after strenuous physical (sports) exercise [1]. At rest, these patients as a rule showed no signs of weakness or indeed any abnormality, but the most common complaint was 'fatigue', i.e. not being able to complete a physical activity, be it vacuum cleaning the room or a half marathon. Biochemically, these patients hardly ever showed any abnormalities, except sometimes a high creatine kinase (CK) activity or a slightly raised lactate concentration in plasma. Increased CK activity in plasma is usually interpreted as a sign of muscle damage, but this interpretation should be made with care, as there is no clear relationship between CK activity and the 'gold standard' for muscle damage, histological assessment. Strictly speaking, increased vulnerability or increased permeability would be better terms, but muscle damage is commonly used. This aspect is discussed in more detail in [2]. Most patients did not show any sign of their complaints at rest, and we therefore decided to develop tests that might trigger the complaints in a laboratory setting during or after carefully controlled exercise and with biochemical monitoring of blood variables. In doing so, we hoped to be able to detect possible metabolic disorders caused by enzyme deficiencies, such as mitochondrial myopathies, McArdle's disease, carnitine palmitoyltransferase deficiency and others [3].

Abbreviations used: CK, creatine kinase; Mb, myoglobin; E2, oestradiol; OVX, ovariectomized; ERT, oestrogen-replacement therapy.

Diagnostic exercise tests

The first test that we set up was a long-term exercise consisting of 2 h of cycling on an ergometer [4]. Before, during and after the test several indices were measured: (1) general variables, such as Na⁺, K⁺, Ca²⁺; (2) parameters of metabolic adaptation to exercise, such as concentrations of glucose, lactate, non-esterified fatty acids and ketone bodies; (3) indications of muscle damage, such as lactate dehydrogenase, aspartate aminotransferase, CK and, as an addition to the original test, myoglobin (Mb), which we have found to be a sensitive marker of muscle damage [5]. Also, a fasting test was introduced, which consisted of fasting for 72 h after which the same variables were measured. These tests yielded very little extra information, and only rarely did patients reproduce their complaints while cycling. The most interesting indices, from a diagnostic point of view, were the measures of muscle damage or increased muscle permeability: CK and Mb. These were clearly higher in patients with a variety of metabolic disorders, who had been included in the tests as positive controls. A separate study with patients with chronic progressive external ophthalmoplegia, a mitochondrial disorder affecting oxidative phosphorylation, showed a striking correlation between the patient's post-exercise CK and Mb increases and their residual mitochondrial oxidative phosphorylation [6]. The idea that the muscle membrane may leak Mb and CK after exercise even during subclinical disease was underlined in yet another test that we used to detect carriers of Duchenne's muscular dystrophy. Mothers and sisters of patients with this X-linked recessive disease, which leads to muscle weakness and degeneration and to respiratory insufficiency and death at the age of about 20 years, are often carriers (about 30% of cases of Duchenne's muscular dystrophy are spontaneous mutations), and, after diagnosis of a boy with the disease, want to know their risk of having more affected babies. The standard test for carrier detection in the 1980s consisted of measuring CK activity three times at rest on different occasions, the idea being that skeletal muscles of
carriers are affected and leak more than normal amounts of CK; only rarely are carriers clinically symptomatic. If all three measurements were higher than normal, the woman was considered to be a carrier. This simple test, however, has a low specificity and sensitivity and therefore missed real carriers as well as falsely diagnosing others. We decided to improve the muscle provocation test [7], in which subjects performed short but intense exercise to provoke the subclinically affected muscle of carriers, by measuring Mb next to CK. Indeed, the sensitivity and specificity of this test were vastly improved over the repeated CK determination and the original provocation test, but it was never introduced for routine use as, at the same time, the gene for Duchenne's muscular dystrophy was discovered, and with that knowledge DNA testing became possible. Collectively, however, these results taught us that CK and Mb have different qualities as muscle-damage indicators, and can be used to detect acute muscle damage (Mb) or to monitor the course of a disease such as polymyositis [8].

At that time it was known, but not entirely understood, that men and women had different resting concentrations of CK, and also that they had different exercise-induced increases. Explanations such as different muscle mass, fibre recruitment, specific CK activity in muscle and differences in muscle metabolism had been forwarded. One explanation that we thought was particularly interesting but unproven was that oestrogens accounted for the difference between men and women in this respect [9]. As oestrogens, and sex hormones in general, indeed make the difference between males and females in most aspects, this explanation was a gift from nature rather than a proven fact. Therefore we decided to study this gender difference with the idea that, once it was understood, we might be able to enhance the resistance of muscle to damage, e.g. for patients with muscle disorders or even for athletes that also suffer from muscle damage after strenuous exercise.

**Exercise-induced muscle damage in the rat**

We developed a model to test our hypothesis that females and males differ with respect to muscle damage after exercise and that sex hormones are involved. Rats were trained to run on a level treadmill. It appeared that a gender difference also existed in rats [10], and that we could influence the results with hormone treatment: males could be protected from damage with high doses of oestradiol (E2), whereas ovariectomized (OVX) female rats showed damage similar to males, dependent on the time of ovariectomy [11]. Immature OVX rats responded to exercise like males, whereas rats that had undergone ovariectomy shortly after reaching sexual maturity had CK values between those of male and intact female rats. In other words, the longer that animals were exposed to E2, the less damage they developed. OVX female rats could again be protected by hormone-replacement therapy with E2. The results of these studies are summarized in Table 1. In separate experiments we had established that we were really looking at muscle damage by studying the morphology of the muscle and by measuring the muscle isoenzyme of CK, CK-MM [12].

The next step was to understand the mechanism by which this steroid effect is mediated. At that time the opinion was that effects were either

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<td><strong>Percentage increase in CK efflux immediately after 2 h of exercise in male and female rats</strong></td>
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<td>Controls are adult untreated rats. OVX means ovariectomized before (early) or after (late) reaching sexual maturity. Oestrogen (E2) treatment consisted of implantation of a pellet that slowly released E2, resulting in high plasma concentrations. In male rats, the duration of the treatment varied from 1 h to 21 days before exercise. Vitamin-E-deficient rats had been fed on a vitamin-E-deficient diet for 6 weeks before exercise. CK activity was measured in plasma obtained via an indwelling cannula. The percentages are the difference between values obtained immediately after compared with immediately before, as percentage of the starting value. Data are combined from refs. [10–12].</td>
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expressed via the E2 receptor or were non-genomic [13]. We found that E2 had to be present for at least 24 h to have an effect [11], and concluded that acute non-genomic direct membrane effects were less likely and that presumably the effect was mediated via the receptor. We explored this further by using the E2 antagonist tamoxifen. We tested its effect indirectly by measuring leakage of CK from muscles in vitro. Earlier experiments, in which rats had been previously treated with E2, had shown the validity of this approach [14]. It appeared, much to our surprise, that tamoxifen protected muscle, rather than leaving it unprotected by inhibiting E2 action [15].

Oestrogen and muscle damage: hormonal or antioxidant action?

Until that time, a possible scavenging or antioxidant effect of E2 had not been considered, despite studies describing such effects [16,17]. We did hypothesize, on the basis of our results and reports that lipid peroxidation of muscle in vitro differed between male and female mice, that this could be the result of an as yet unknown effect of E2 on lipid peroxidation [18]. In 1994 Wiseman [19] suggested that tamoxifen could protect membranes and lipoprotein particles against oxidative damage, and we considered this the most logical explanation for our earlier observations [20]; the non-acute nature of the effect is very much in line with an antioxidant effect, as lipophilic antioxidants are known to need to reach a certain tissue level before they are effective, the best example being vitamin E. Our experiments with vitamin-E-deficient rats had shown that (1) vitamin E, and thus probably lipid peroxidation, plays a role in muscle damage, and (2) it takes a very long time to decrease (or increase) vitamin E levels [21]. Experiments with dantrolene sodium, which blocks intracellular Ca<sup>2+</sup> release from the sarcoplasmic reticulum, had shown that Ca<sup>2+</sup> ions play an important role in exercise-induced muscle damage [22]. One of the ways by which Ca<sup>2+</sup> causes cellular damage, in brain, heart, liver and muscle, is by stimulating (via phospholipase A<sub>2</sub> degradation of phospholipids and prostaglandin metabolism) the formation of free radicals. It had also been suggested that exercise-induced muscle damage is probably partially caused by the action of free radicals [23]. Thus, in retrospect, there were indications that the protective effect of oestrogen, a potent antioxidant, might be based on protection against the devastating effects of oxygen radicals. It just needed the catalytic effect of one article [19] to make the case for an antioxidant effect very strong.

The role of oestrogens in protecting muscle is discussed in detail in a recent review [24]. On the basis of our and the authors' own animal studies, and of the antioxidant and membrane properties of E2 and tamoxifen, it is discussed whether E2 is protective for muscle membranes and lipoprotein particles. The latter process is related to the observation that the incidence of atherosclerosis in premenopausal women is low [25]. Apart from having direct antioxidative effects, E2 (and tamoxifen) may also act by affecting the membrane fluidity and stability, in a way comparable with the effect of cholesterol and possibly with that of synthetic lipophilic antioxidants such as the aminosteroids (lazaroids) [26]. Interestingly, cholesterol-lowering drugs may cause myopathy, depending on the lipophilicity of the drug [27]. It was indeed shown earlier that tamoxifen has an effect on membrane fluidity [28]. Several groups are now studying the protective effect of oestrogens. Most studies report protection identical with the original finding [10], although one suggested that the effect was probably limited to the leakage of CK and did not extend to structural damage [29]. These conclusions were based on two animals only, and it is known that morphometry of damage is difficult and variable. In cultured myoblasts it was found that E2 promotes baseline ATP levels, and that, during inhibition of glycolysis, ATP remains at baseline levels longer than in untreated cells [30]. A fall in ATP in any cell is usually the first event in a long cascade of biochemical reactions, via increases in intracellular Ca<sup>2+</sup> concentration, enhancing oxygen radical formation. If this effect of E2 occurs in intact cells in vitro also, it might well be part of the protective mechanism of E2. In one human study, non-weight-trained women (one group used oral oestrogen-based contraceptives, the other group did not and acted as controls) performed a stepping eccentric exercise protocol, leading to serious pain and CK leakage [31]. The group taking oral contraceptives reported significantly less pain. CK was not significantly different between the groups, but it is known to vary widely especially after eccentric exercise. Obviously, the size of the groups and the non-double-blind character of the design precludes firm conclusions, but there is undoubtedly a relationship between muscle damage and oestrogen status. Unfortunately, the
A study has shown that a different steroid, we cannot exclude the possibility that there is an mone and luteinizing hormone. Not only did his patient also report increased well being, higher possible protective effect of tamoxifen described testicular function, caused by a combination of pure antagonists could clarify this. It could effect via the oestrogen receptor, as tamoxifen is be questioned whether the dose of circulating E2 in the plasma of premenopausal women is high enough to achieve protection. The concentration of E2 is thus far unknown.

Conclusions
Collectively, the data presented above make a strong case for oestrogens (and tamoxifen) protecting muscle via antioxidant action, although we cannot exclude the possibility that there is an effect via the oestrogen receptor, as tamoxifen is a mixed antagonist/agonist. Future studies with pure antagonists could clarify this. It could be questioned whether the dose of circulating E2 is high enough to achieve protection. The concentration of E2 in the plasma of premenopausal women varies between 0.3 and 2 nM, while in men it is about 0.002 nM. The physiological E2 concentration is thus low compared with the circulating concentration of vitamin E (\( \alpha \)-tocopherol, 24 \( \mu \)M) [37]. However, binding to testosterone–oestrogen–binding globulin (for E2) and distribution in adipose tissue (for both) make it difficult to interpret these data. Testosterone has no appreciable scavenging activity [17], which may lead to the conclusion that women are effectively ‘treated’ during their reproductive life, and longer if they receive oestrogen-replacement therapy (ERT), with a potent antioxidant. Whether this indeed has measurable or provable effects remains to be shown but it is a fact that women, on average, live longer than men.

One apparently very different future use of ERT could be in treating dementia: there is growing evidence that ERT is effective in preventing cognitive and affective dysfunction, and E2 therapy for Alzheimer’s dementia was tested in an open trial as early as 1986. As many neurodegenerative diseases have at least a radical component in their pathogenesis, ERT may have benefit not only via its trophic effects but also via additional protective effects against oxygen radicals.

2 Bär, P. R., Rodenburg, J. B., Koot, R. W. and Amelink, G. J. (1994) Basic Appl. Myol. 4, 5–16
Oestrogens as antioxidant cardioprotectants

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Introduction

The potential importance of oestrogens as antioxidant cardioprotectants is currently attracting considerable attention. Oestrogen is recognized to have a protective effect against coronary heart disease and its antioxidant action could contribute to the cardiovascular benefits observed on oestrogen administration in postmenopausal women [1,2]. This antioxidant-mediated cardioprotective action is likely to be independent of the favourable influence of oestrogen on risk factors such as the plasma lipid profile [1-3]. Furthermore oestrogen improves lipid metabolism in the postprandial state: it makes this state less atherogenic by improving the clearance of chylomicron remnants and by attenuating the postprandial decrease in levels of beneficial high-density lipoprotein (HDL) cholesterol [4]. Other beneficial effects of oestrogen include the lowering of plasma homocysteine levels [5] and serum levels of angiotensin-converting enzyme [6], both of which are risk factors for cardiovascular disease. Oestrogen may be able to enhance beneficial relaxation of the vasculature by modulating the synthesis of vasodilators such as the free radical, NO [7,8], and smooth-muscle Ca2+ signalling [8]. However, the role of NO as a beneficial mediator of cardiovascular function is clearly one of balance. This is because it can also react with other free radicals such as superoxide to form potentially damaging species such as peroxynitrite, which is implicated in low-density lipoprotein (LDL) oxidation [9].

Antioxidant properties in vitro have been shown for endogenous oestrogens such as 17β-oestradiol [10-13], synthetic exogenous oestrogens such as 17α-ethynylestradiol (used in oral contraceptives) [14] and the dietary phytoestrogens (possess weak oestrogenic properties) such as the isoflavonoid genistein [15,16]. Genistein is found predominantly in soya products and has been suggested to have cardioprotective properties [17,18]. Genistein has a number of