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In vitro investigations related to the hypothesis that *Lipoatrophia semicircularis* finds its origin in electro-stimulation

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ABSTRACT: *Lipoatrophia semicircularis* (L.s.) is an idiopathic condition characterized by semicircular impressions of the skin, usually at the front and sides of both thighs. It is characterized by atrophy of the subcutaneous adipocytes whereas the skin and muscles remain normal. L.s. was originally considered to be very rare but over the past 13 years an outburst of L.s. has been observed in companies in Belgium and later on also in companies in other countries (e.g., Spain) where several hundred individuals were diagnosed with L.s. All these subjects belonged to the administrative personnel and worked in renovated or new offices. Different hypotheses have been put forward to explain the appearance of *Lipoatrophia semicircularis* but the hypothesis of an 'electric' origin of L.s. is probably the best documented and plausible. The present study was aimed to further investigate the likelihood of this hypothesis. The alkaline comet assay was used to investigate DNA damage in cells of different origin following exposure to a (strong) electric current. It was found that adipocytes showed more DNA damage than the other cells and hence that they are more vulnerable to such a current than macrophages and white blood cells. It was also found that DNA damage is significantly induced by an electric current in the blood from L.s. subjects whereas this is not so in blood from subjects without L.s. This may indicate that L.s. subjects are more responsive to an induced electric current and supports the hypothesis of an 'electric' origin of L.s. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: adipocytes; blood; comet assay; electric current; lipoatrophia semicircularis; macrophage; electro-sensitivity

Introduction

The medical literature at first described *Lipoatrophia semicircularis* (L.s.) as a rare, idiopathic condition characterized by semicircular impressions of the skin, at the front and sides of both thighs (Fig. 1). It is characterized by atrophy of the subcutaneous fatty tissue at the location of the impressions. Skin and underlying muscles remain intact (for an overview, see Maes *et al*., 2003). Some 10 years ago, L.s. was diagnosed in hundreds of persons, mainly women, among the administrative personnel of two large companies in Belgium. Many other companies followed, also in other countries including the Netherlands, Spain, France, the UK, Germany and Italy. L.s. is thus not as uncommon as previously thought and apparently became an important job-related illness or condition. In some instances, especially just before L.s. becomes apparent, the afflicted subjects complain about extreme fatigue, a feeling of heaviness and/or a tingling or burning sensation. The most striking fact is that the impressions on the thighs always occur at the height of the desk top at which the individuals sit. Women are much more afflicted than men. This was ascribed to a difference in volume of subcutaneous fat or differences in cosmetic concerns (Gruber and Fuller, 2001) or to a difference in the structure of fat tissue (Maes *et al*., 2003). Several hypotheses have been put forward to explain the occurrence of L.s. It was for example hypothesized that it is due to pressure as a result of frequent leaning against the sharp ends of a desk top (e.g. De Groot 1994; Gruber and Fuller, 2001) or due to the wearing of tight trousers (Mascaro and Ferrando, 1983; Herane *et al*., 2007). A more substantiated hypothesis is that L.s. finds its origin in pressure exerted by the seat surface of office chairs (Hermans *et al*., 1999). Thermal energy losses by continuing contact between the leg(s) and a metal frame or cable duct of a desk was also hypothesized as an explanation for L.s. (Van Loock, 2006, 2007) as well as electrostatic discharges or other 'electric' phenomena (Maes *et al*., 2003; Verschaeve and Maes, 2009). The latter hypothesis was supported by a large number of observations. However, one may wonder why subcutaneous adipocytes are damaged (atrophy) and not skin or muscle cells in their vicinity, and it may be questioned why some individuals have L.s. why others, working in exactly the same office environment, do not contract L.s. We tried to find some explanation through *in vitro* investigations using the alkaline comet assay. If an 'electric phenomenon' is responsible for L.s., adipocytes might be more

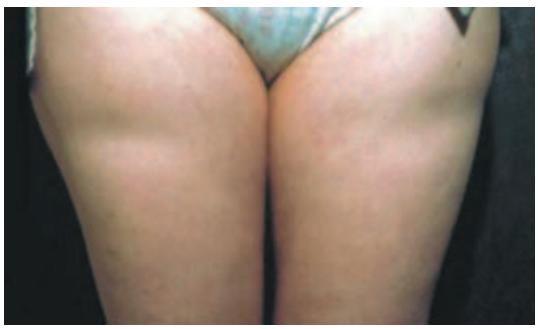

Figure 1. Typical example of *Lipoatrophia semicircularis*. This figure is available in colour online at www.interscience.wiley.com/journal/jat

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vulnerable to an applied electric current than other cells and we examined whether this could be reflected by a more important induced DNA damage in these cells. We therefore investigated adipocytes from mouse and human origin, as well as human white blood and macrophage cells. Macrophages were chosen as we previously postulated that L.s. may be due to a direct effect on adipocytes, or an indirect effect following activation of macrophages (Maes *et al*., 2003). Blood cells were used as a third cell type that is not known to be particularly involved in L.s.

On the other hand, we thought that some subjects might contract L.s. because they are more sensitive (hypersensitive?) to an applied electric field than other subjects without L.s. and that this could eventually also be seen through an *in vitro* investigation with the comet assay on their white blood cells following exposure to a strong electric field.

Materials and Methods

Exposure System and Cells

Hinsenkamp *et al*. (1997) previously used a specially designed exposure unit to investigate *in vitro* biological effects of lowfrequency pulsed electrical current. We used the same unit for our experiments. It enables the delivery of a chosen electric current for a given period of time to cells in culture medium using platinum electrodes, as shown in Figs 2 and 3. We investigated four cell types: mouse 3T3-L1 cells (ATCC, CL173), human THP-1 macrophages (ATCC, TIB-202), human adipocytes (Zen-Bio, SP-F-2) and human white blood cells (freshly taken by venipuncture). Each experiment was performed twice (independent repeat experiment). 3T3-L1 cells are embryonic mouse cells that differentiate in culture from a fibroblast to an adipocyte phenotype (Russo *et al*., 1990). It was in the 'adipocyte' form that the cells were electro-stimulated. The same held true for the human adipocytes that were obtained as cryopreserved human preadipocytes and cultivated as indicated by the provider using pre-adipocyte medium, differentiation medium and adipocyte medium. THP-1 macrophages were of leukemic origin. Exposure was for 1 h per day up to 5 days for 3T3-L1 cells and THP-1 cells during cell cultivation. Human adipocytes were also exposed for 1 h per day but only during 3 days as longer cultivation times were not possible in the experimental setup used. Human white blood cells were kept and electro-stimulated for 1–5 h in culture medium but were not cultivated (not stimulated by phytohaemagglutinine). The current amplitude was 25 and 50 mA based on preliminary trials.

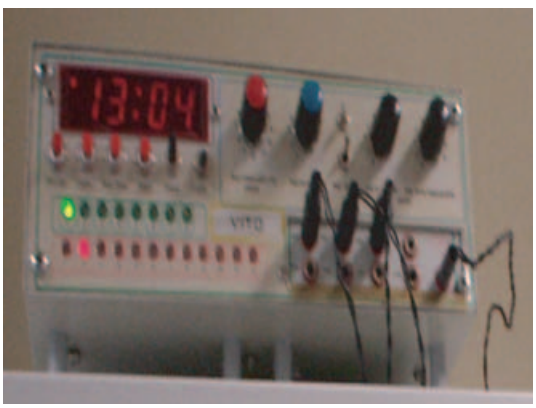

Figure 2. Exposure unit. This figure is available in colour online at www.interscience.wiley.com/journal/jat

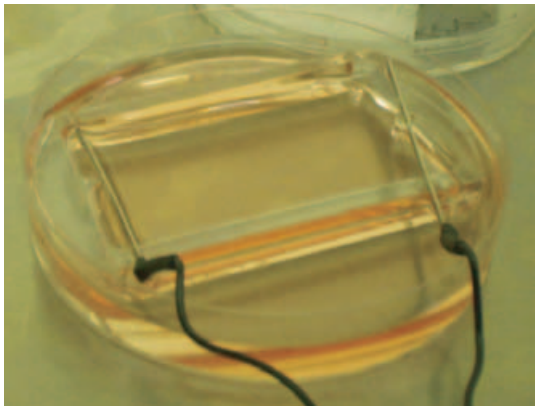

Figure 3. Cell cultures with Pt electrodes to be inserted on both sides of the culture for exposure to an electric current. This figure is available in colour online at www.interscience.wiley.com/journal/jat

In a second experiment whole blood from 21 female individuals with L.s. and 17 of their (female) colleagues without L.s. who worked in the same office environments were exposed to an electric current of 25 mA current amplitude. All subjects were between 20 and 40 years of age and both groups apparently did not differ from each other substantially (e.g. use of tight trousers was certainly not more common in the L.s. individuals compared with those who were not afflicted by L.s.).

In both experiments the applied currents were rather high and could not be compared with normal electric currents to which an organism may be exposed to in real life. However, this is not important for the purpose of this study, which only aimed at detecting differences in electro-sensitivity between cells or individuals, whatever the methods used or currents applied.

The Alkaline Comet Assay

The alkaline comet assay was conducted according to standard procedures (Singh *et al*., 1988). Briefly, cells were exposed in culture medium as indicated above. Whole blood from L.s. subjects and their colleagues without L.s. was exposed for only 1 h. After exposure cells were embedded in agarose on a microscope slide, lysed and their DNA unwound. The DNA was then subjected to a gel electrophoresis procedure. DNA 'comets' are formed due to the migration of (negatively charged) DNA fragments or DNA loops towards the positive pole. DNA comets were visualized with a fluorescent microscope after staining with ethidium bromide. The comet tail DNA content (%) was used to measure the extent of DNA damage. Measurements were done with the image analysis system Komet 3.1. from Kinetic Imaging Ltd (UK) on 50 cells per individual or exposure regime. The Mann–Whitney *U*-test was used to determine statistical differences at $P < 0.05$.

Results

Figures 4–7 show the results of two independent experiments on the comet assay in adipocytes, macrophages and white blood cells. A time- and dose-dependent increase in DNA damage is found in all cell types. From the figures it appears that mouse adipocytes were more damaged than macrophages and human white blood cells (e.g. up to approximately 25% of tail DNA in

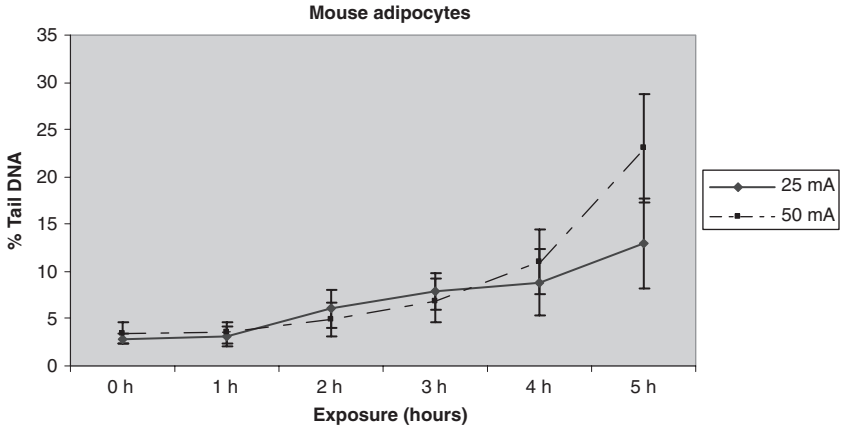

Figure 4. Results from the alkaline comet assay in mouse adipocytes following electro-stimulation for 1 h per day up to 5 days (results from two independent experiments; bars = standard deviation).

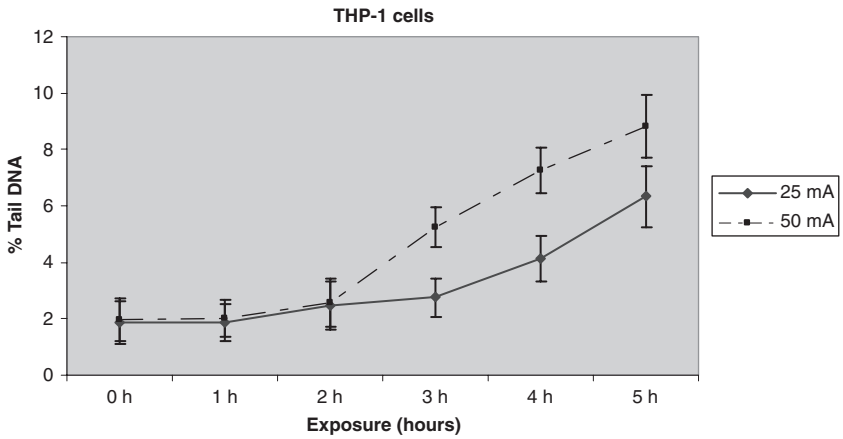

Figure 5. Results from the alkaline comet assay in THP-1 macrophage cells following electro-stimulation for one hour per day up to 5 days (results from two independent experiments; bars = standard deviation).

adipocytes compared to only 8–9% in white blood cells and macrophages at 50 mA). Human adipocytes apparently behave as their mouse counterparts but DNA damage was after 3 h already more extensive (Fig. 7). Overall, our results indicate that adipocytes are more vulnerable than other cells to electric stimuli (based on induced DNA damage). It is interesting to note that experiments were also performed on cells that were exposed to a magnetic field of 100 μT (as before, 1 h per day up to 5 days consecutively or for 1 h in white blood cells from L.s. and non L.s. individuals), but that no difference at all was found in DNA damage compared with non-exposed cells. We therefore do not present these results here.

In a second series of experiments we investigated electrostimulated white blood cells from 21 L.s. subjects and 17 individuals who worked in the same office environment and were not afflicted by L.s. Table 1 shows that electro-stimulation of whole blood results in a statistically significant increase of DNA damage in the white blood cells of individuals with *lipoatrophia semicircularis* ($P < 0.04$), whereas no significant increase is found in subjects without L.s. ($P = 0.24$). This holds true for average but not median values of DNA damage. It is interesting to note that there is a rather important difference between average and median values (Table 1).

Discussion

The results of the *in vitro* investigation thus indicate that, based on induced DNA damage, adipocytes are more vulnerable than macrophages and white blood cells to electric stimuli. This may to a certain extend explain why adipocytes are the cellular target in the phenomenon of *Lipoatrophia semicircularis*. It may furthermore also be assumed that adipocytes are the final place where discharges shall occur as the conductive path of the discharge via hair follicles and sweat glands ends at the highly resistant fatty tissue. Yet, this preliminary experiment does not prove anything and a number of shortcomings exist that should be addressed in future investigations. It is, for example, unclear whether an adherent monolayer (the adipocytes) can be compared with cells in suspension (the white blood cells) in terms of electric current, and macrophages are not a particularly relevant cell type to compare with subcutaneous adipose tissue. Increased sensitivity of adipocytes compared with keratinocytes, fibroblasts or muscle cells would in this respect probably be more appropriate. However, the above results show that adipocytes are sensitive to electro-stimulation and are therefore to a certain extent supportive to our hypothesis that electro-stimulation is the origin of L.s. (Verschaeve and Maes, 2009).

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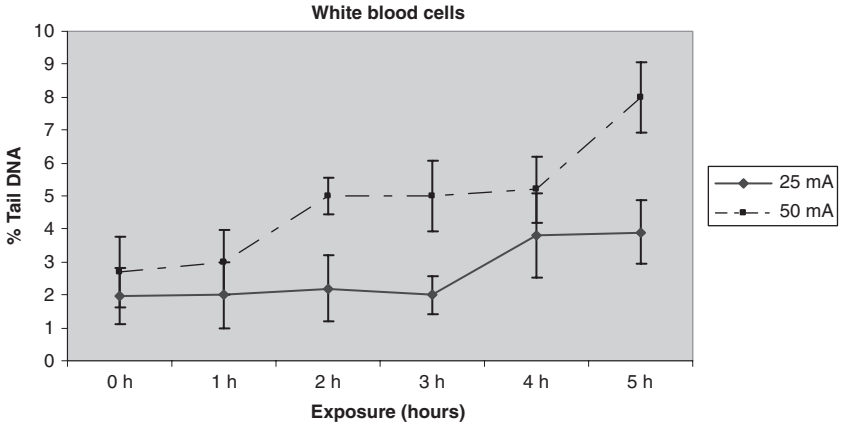

Figure 6. Results from the alkaline comet assay in human white blood cells following electro-stimulation for one hour up to 5 h (results from two independent experiments; bars = standard deviation).

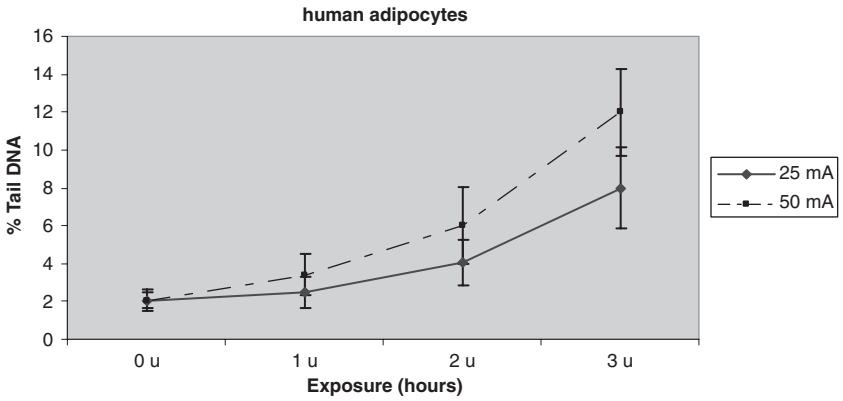

Figure 7. Results from the alkaline comet assay in human adipocytes following electro-stimulation for one hour up to 3 hours (results from two independent experiments; bars = standard deviation).

Table 1. Summary of the results of the alkaline comet assay on white blood cells from individuals with and without *Lipoatrophia semicircularis* working in the same office environment

	Unexposed blood		25 mA electro-stimulation		
	Average ± SE	Median	Average ± SE	Median	Statistics
Subjects without L.s.	2.5 ± 0.5	2.1	2.8 ± 0.9	2.0	$P = 0.24$
Subjects with L.s.	2.8 ± 0.4	2.6	3.6 ± 1.0	2.6	$P < 0.04$

SE = standard error.

Our investigation with L.s. and non-L.s. subjects points towards a greater electro-sensitivity of L.s. subjects compared with non-L.s. subjects. White blood cells of L.s. subjects have apparently also more 'spontaneous' DNA damage than white blood cells from colleagues without L.s. (Table 1), but this may be artifactual as the background DNA damage differs from one electrophoresis run to another and comparisons between different electrophoresis runs are therefore not as informative than comparisons within one electrophoresis run. It is also interesting to note that there is a rather important difference between average and median values (Table 1). This suggests a non-Gaussian distribution, as does the fact that only a sub-group of the affected persons demonstrates increasing DNA damage in response to current. This may at first sight question the hypothesis of electro-stimulation as the main cause of L.s., especially as differences between L.s. and non-L.s. subjects are apparently not very clear. However, one reason for this may be a difference in individual electro-sensitivity. In this case the alkaline comet assay can eventually become a tool to discriminate between electro-sensitive and non-sensitive individuals. One should also realize that L.s. should not necessarily be ascribed to one single cause only (in this case electrostimulation). It is more likely that

there is a multifactorial origin of L.s., as already postulated (Maes *et al*., 2003; Verschaeve and Maes, 2009). For example, repeated trauma and reduced perfusion, e.g. as a result of mechanical pressure by wearing trousers while sitting for long periods of time (Herane *et al*., 2007) or wearing a tight elastic girdle (Ogino *et al*., 2004) were also considered to be plausible explanations for L.s. However, in many cases no history of trauma could be found and many of the afflicted subjects do not wear trousers at all. According to our observations (Verschaeve and Maes, 2009), it is unlikely that pressure induced by tight trousers or a 'wrong sitting posture' is the major cause of L.s. but it cannot be excluded that these are contributing factors, amongst other possibilities. In other words, many factors may be important. This may explain why, besides individual electro-sensitivity, subtle differences in an individual's behavior (e.g. the sitting posture, choice of clothes) or even the characteristics of the furniture (e.g. type of chairs, materials of the desks) and environmental office conditions (e.g. humidity) may determine the occurrence or absence of L.s.

It may also be interesting to note that the indentations that are characteristic of L.s. improved in many cases after smooth barriers have been fitted along the edge of the desks (Gruber and Fuller, 2001). This was seen as an argument in favour of the 'trauma hypothesis', but it can also well be an argument in favor of the hypothesis of electro-stimulation as these materials also change the electric properties and charges between the desktop and the thighs. The same holds true for the hypothesis of thermal energy loss (Van Loock, 2006) where a barrier consisting of a thermo-insulating material was proposed as a way to avoid thermal energy loss. This again results in changes in electrical charges and potentials.

Overall, our results thus indicate that individuals with L.s. might be on average more electro-sensitive than individuals who do not have L.s. This is in favor of our hypothesis of electro-stimulation as the main cause of L.s., which has been extensively described elsewhere (Verschaeve and Maes, 2009).

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