Endothelin-1 synthesis reduced by red wine

Red wines confer extra benefit when it comes to preventing coronary heart disease.

Statistical evidence of reduced coronary heart disease in areas of high wine consumption has led to the widespread belief that wine affords a protective effect[1,2]. Although moderate drinking of any alcohol helps to reduce the incidence of coronary heart disease[3,4], there is no clear evidence that red wine confers an additional benefit[5]. Here we show that red wines strongly inhibit the synthesis of endothelin-1, a vasoactive peptide that is crucial in the development of coronary atherosclerosis[6]. Our findings indicate that components specific to red wine may help to prevent coronary heart disease.

The concept of the 'French paradox' has arisen from reports that deaths from coronary heart disease are much lower in France than in the United Kingdom, despite a comparable dietary intake of saturated fats by these populations[1,2] — this has been attributed to the higher consumption of alcohol in France, particularly of wine[1,2]. Mechanisms implicated in this phenomenon include increases in high-density lipoproteins (HDLs) and fibrinolytic activity, and decreased platelet aggregation[2–4], but these changes are modest and can also be caused by ethanol consumption *per se*[3,4]. Indeed, the very existence of the French paradox has been questioned as it may simply reflect a time lag in dietary cholesterol intake[4]. Identification of a specific property of red wine that accounts for reductions in coronary heart disease could resolve this controversy, as well as providing insight into the health benefits of a Mediterranean diet.

Endothelin-1 (ET-1) was originally described as a highly potent vasoconstrictor peptide[7], and its overproduction is seen as a key factor in the development of vascular disease and atherosclerosis[6]. Experimental models of atherosclerosis indicate that endothelin antagonists prevent manifestation of the early stages of the disease, such as endothelial dysfunction or fatty-streak formation[6], and reduce myocardial infarction in established disease[8]. The coronary blood supply of patients with coronary heart disease is also severely perturbed by local ET-1 production[9]. We investigated whether red wine could inhibit the synthesis of ET-1, as this might explain its cardioprotective properties.

We found that polyphenols from red wine made from Cabernet Sauvignon grapes decreased ET-1 synthesis in cultured bovine aortic endothelial cells (BAECs) by suppressing transcription of the ET-1 gene (Fig. 1a). To test whether this property is peculiar to red wine, we prepared ethanol-free extracts from 23 red wines, four white wines, one rosé wine and one red-grape juice (see supplementary information for details). These extracts were tested on BAECs to determine the concentration of each wine that causes a 50% reduction in basal ET-1 synthesis (IC_{50}) (Fig. 1b).

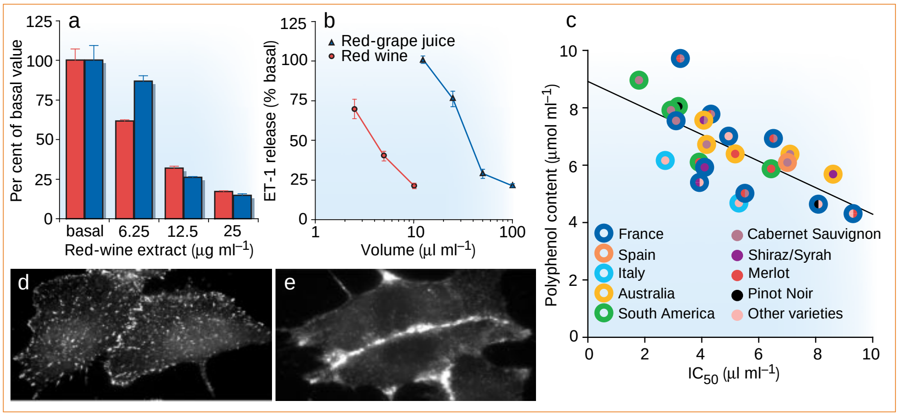

Figure 1 Red wine inhibits endothelin-1 (ET-1) synthesis by bovine aortic endothelial cells and alters the distribution of phosphotyrosine immunofluorescence. **a**, Red wine extract suppresses ET-1 release (red bars) and transcription of the prepro-ET-1 gene (ET-1 reporter gene activity; blue bars). **b**, ET-1 release over 6 h during incubation with extracts of red-grape juice (triangles) and a representative red wine (circles) (plotted as equivalent dilutions to unextracted samples). **c**, Correlation of total polyphenol content of red wine (measured with Folin and Ciocalteu's reagent and expressed as catechin equivalents in µmol ml^{-1}) with the concentration of red-wine extract causing a 50% reduction in basal ET-1 synthesis (IC_{50}, expressed as µl-equivalents of unextracted wine per ml culture medium) (see supplementary information for details). **d**, **e**, After 1 h treatment of bovine aortic endothelial cells with either medium or red-wine extract (50 µg ml^{-1}), phosphotyrosine (PY)-containing proteins in permeabilized cells were detected as brightly staining regions by using anti-PY monoclonal antibody 4G10 and FITC-labelled goat anti-mouse IgG. **d**, Control cells incubated with medium; **e**, cells after treatment with red-wine extract. Original magnification, ×100.

For the red wines, the degree of inhibition of ET-1 synthesis was correlated with the total polyphenol content (Fig. 1c; $r^2 = 0.46$, mean $IC_{50} = 5.0 \pm 0.4$ µl ml^{-1}). Red-grape juice also inhibits ET-1 synthesis, but is markedly less potent than red wine ($IC_{50} = 35$ µl ml^{-1}). The white and rosé wines had no effect on ET-1 synthesis (<5% inhibition at 100 µl ml^{-1}). As the rosé wine was from Cabernet Sauvignon grapes, this indicates that the active principle in red wine must derive from red-grape skins or other grape components during the vinification process.

Although polyphenols in red wines are known to have antioxidant properties[10], it is unlikely that this accounts for the effect on ET-1 synthesis. For instance, quercetin (10 µM) totally inhibits the oxidation of low-density lipoproteins (LDLs)[10]; however, at this concentration neither red-wine polyphenols (quercetin, resveratrol, D,L-catechin, D,L-epicatechin) nor anthocyanins (delphinidin, pelargonidin, cyanidin, peonidin, petunidin, malvidin) affect ET-1 production.

Inhibitors of the cellular tyrosine-kinase family of phosphorylating enzymes that share structural similarity to red-wine polyphenols also suppress ET-1 synthesis[6]. We therefore investigated whether this action of red wine might be explained by an inhibitory effect on this same family of enzymes by using immunocytochemistry to visualize tyrosine phosphorylation in endothelial cells. Compared with control cells, red-wine extract causes a marked change in cell morphology and a redistribution of phosphotyrosine staining (Fig. 1d, e). These effects on tyrosine phosphorylation are presumably due to modified tyrosine-kinase signalling in endothelial cells.

Red-wine extract is also known to elicit endothelium-dependent vasodilation and lower blood pressure[11], which may provide further protection against coronary heart disease. Our findings indicate that remarkably small amounts of red-wine extract can suppress ET-1 synthesis: assuming adequate absorption of the active component, they support assertions that a moderate intake of red wine can prevent coronary heart disease. Characterization of the vascular mechanisms underlying red wine's beneficial effects should help in the design of strategies to prevent atherosclerosis.

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Image processing

Fractals in pixellated video feedback

Video feedback occurs whenever a video camera is directed at a screen displaying the image currently being recorded by the camera. It can be observed in everyday situations, for example at sporting events when a stadium's display screen comes into the camera's view. Here we consider how this simple physical process is affected by the fact that monitors are pixel-based, and show that it can result in stationary fractal patterns such as von-Koch snowflakes and Sierpinski gaskets.

Video feedback is a popular scientific phenomenon[1,2], mainly because of its 'beautiful and mesmerizing' images[3]. It was scientifically investigated in the days of scanline-based cameras and monitors[4,5] and is often discussed in the context of fractals as an example of a simple feedback process (see ref. 6, for example).

The best known video-feedback phenomenon is perhaps the 'monitor-inside-a-monitor' effect[7], which occurs when the overall magnification, M, of the camera–monitor combination is less than one ($M < 1$), causing the monitor to display a tunnel-like, non-fractal pattern consisting of nested images of itself. Video-feedback set-ups can be modified, for example by using multiple monitors[8] or multiple lenses[9], in order to produce stationary fractal patterns.

We demonstrate that pixellated, but otherwise unmodified, video feedback with $M > 1$ can lead to fractal patterns (we dub this the 'monitor-outside-a-monitor' effect). Previous experiments with $M > 1$ produced non-stationary complex patterns — for example, rapidly rotating planet-like, fractal-looking structures suspected of being connected to pixels[10]. Pixels were also described as acting like the 'cells' of a cellular automaton, a class of abstract machine capable of producing fractal patterns[11], and simulations of video feedback on a matrix model — in which the matrix elements acted like square pixels — produced stationary fractal spirals[12].

We predicted that stationary self-similar fractal patterns would be created in pixellated video feedback by analogy with fractal laser modes[13]. These patterns result from iterated magnification and pixellation of the image: the former successively stretches any structure in the image to M, M^2, M^3, … times its original size, whereas the latter continuously adds small-scale structure in the form of the pixel mask. The result is a pattern consisting of the pixel-mask pattern in various sizes. This hallmark of self-similarity is seen in Fig. 1a: the pattern on the monitor is a large rosette consisting of small rosettes; the small rosettes, each comprising seven bright pixels, are magnified and pixellated images of individual pixels, and the large rosette is a magnified and pixellated image of the central small rosette.

The detailed shape of the stationary pattern depends on the shape and size of the individual pixels, the geometry of the pixel array, the magnification and the position within the pixel array of the centre of magnification. For example, a magnification $M = 2$, combined with a centre of magnification midway between three nearest-neighbour pixels in a hexagonal array of circular pixels, can result in a Sierpinski gasket pattern (Fig. 1b).

Another parameter is the rotation angle of the camera with respect to the monitor. Figure 1c shows a pattern recorded with a camera rotated by 45° and a magnification of $M \approx 1.29$. The experiment was greatly facilitated by our camera's ability to remove flicker-related effects by averaging over a number of frames. To our knowledge, this pattern, which arises directly from the pixellation of the display monitor, is the first published example of a stationary fractal created by unmodified video feedback.

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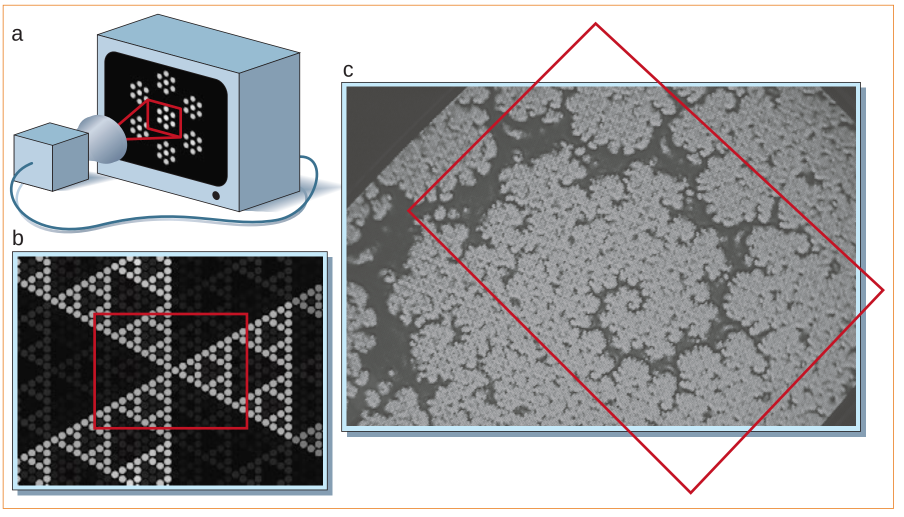

Figure 1 Stationary fractals resulting from pixellated video feedback. **a**, Basic set-up; the monitor displays an example of a self-similar pattern. **b**, Simulated video-feedback pattern consisting of pixel-limited Sierpinski gaskets. **c**, Pattern obtained with a rotated camera pointing at a colour monitor. In all three examples, the red rectangle marks the camera's respective field of view. Additional details can be obtained from the authors.