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Can resveratrol help to maintain metabolic health?

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The number of people suffering from metabolic diseases is dramatically increasing worldwide. This stresses the need for new therapeutic strategies to combat this growing epidemic of metabolic diseases. A reduced mitochondrial function is one of the characteristics of metabolic diseases and therefore a target for intervention. Here we review the evidence that mitochondrial function may act as a target to treat and prevent type 2 diabetes mellitus, and, if so, whether these effects are due to reduction in skeletal muscle fat accumulation. We describe how exercise may affect these parameters and can be beneficial for type 2 diabetes. We next focus on alternative ways to improve mitochondrial function in a non-exercise manner. Thus, in 2003, resveratrol (3,5,4′-trihydroxystilbene) was discovered to be a small molecule activator of sirtuin 1, an important molecular target regulating cellular energy metabolism and mitochondrial homoeostasis. Rodent studies have clearly demonstrated the potential of resveratrol to improve various metabolic health parameters. Here we review data in human subjects that is available on the effects of resveratrol on metabolism and mitochondrial function and discuss how resveratrol may serve as a new therapeutic strategy to preserve metabolic health. We also discuss whether the effects of resveratrol are similar to the effects of exercise training and therefore if resveratrol can be considered as an exercise mimetic.

Resveratrol: Ageing: Metabolic health: Mitochondria: Human subjects

Westernised society is confronted with major challenges to combat chronic metabolic diseases, whose prevalence are on the rise. Even though human subjects are living longer, this increased longevity is also accompanied by increased prevalence of chronic diseases, such as type 2 diabetes mellitus (T2DM) and CVD. Therefore, the challenge for the upcoming years will not only be to increase lifespan, but also to age healthily. In that respect, another major challenge will be to combat the prevalence of obesity, as nowadays ~50 % of the population is overweight or obese. In fact, obesity is well known to increase the risk of metabolic diseases such as T2DM and CVD.

We and others have shown that obesity is associated with the accumulation of fat not only in white adipose tissue, but excessive fat is also stored is so-called ectopic sites, such as skeletal muscle, liver and the heart[1–5]. In these tissues, accumulation of intracellular lipid is associated with cellular dysfunction, including cellular insulin resistance. The latter is one of the earliest hallmarks in the development of T2DM, and therefore ectopic fat storage has been thought to be a causal factor in the development of diabetes. Importantly, insulin sensitivity is also known to decline with ageing[6]. Petersen *et al.* showed that whole-body and muscle-specific insulin sensitivity was reduced in elderly compared with young subjects, and was accompanied by elevated intramyocellular lipid (IMCL) content[7]. Interestingly, Wijsman *et al.*[8] reported previously that offspring from long-lived siblings, participants in the Leiden Longevity study, were characterised by higher peripheral, specifically skeletal muscle, insulin sensitivity when compared with control subjects. Recently, this finding was extended by showing that IMCL content, measured by ^{1}H-magnetic resonance spectroscopy, was reduced in these offspring, further illustrating that maintaining insulin sensitivity and keeping ectopic fat accumulation low may be important factors that determine longevity[9].

Abbreviations: IMCL, intramyocellular lipid; PLIN, peripilin; SIRT1, sirtuin 1; T2DM, type 2 diabetes mellitus.

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Does a reduced mitochondrial function underlie ectopic fat storage?

Accumulation of fat in ectopic fat depots is in essence due to a disbalance between fat uptake and oxidation. Research over the past two decades has revealed that both obese and insulin-resistant subjects as well as T2DM patients are characterised by a decreased capacity to oxidise fatty acids[10,11]. Initially, this reduced fat oxidative capacity was pinpointed to be the result of reduced activity by oxidative enzymes in skeletal muscle in patients with T2DM[12,13]. In the past decade, focus has shifted towards mitochondrial function as the underlying cause for reduced fat oxidative capacity. In that respect, Kelley *et al.*[14] were the first to suggest that mitochondrial function may be reduced in T2DM patients. Shortly after, two independent investigations, using DNA microarrays, showed a general reduction in genes involved in mitochondrial function, both in T2DM as well as in family history positive insulin-resistant subjects[15,16]. On a more functional level, using ^{13}C and ^{31}P (magnetic resonance spectroscopy), Petersen *et al.*[7] showed a 40 % reduction in resting muscle TCA cycle flux in elderly compared with young controls. The latter was accompanied by a reduced saturation transfer between inorganic phosphate and ATP, which is a reflection of ATP synthesis rate, suggesting a reduced *in vivo* mitochondrial function upon ageing. We have used a slightly different magnetic resonance spectroscopy technique to determine *in vivo* mitochondrial function and examined phosphocreatine recovery rate after exercise, assessed by ^{31}P magnetic resonance spectroscopy[17]. Post-exercise recovery of phosphocreatine is an almost entirely aerobic-driven process[18], rendering an adequate measure of *in vivo* mitochondrial function[19]. Using this technique we found that *in vivo* mitochondrial function was reduced in T2DM patients compared with BMI-matched obese control subjects[17]. In addition, we could show, using high-resolution respirometry, that the reduced *in vivo* mitochondrial function was paralleled by a reduction in intrinsic mitochondrial capacity as evidenced by lower state 3 and state U respiration in type 2 diabetic muscle fibres[20]. Together, these findings do suggest that mitochondrial function is affected in T2DM and insulin resistance, and may underlie the accumulation of fat in skeletal muscle. However, it should be noted that these kinds of studies do not allow us to unravel if a reduced mitochondrial function causes the development of diabetes in human subjects. At present the question if mitochondrial dysfunction is cause or consequence of diabetes is still an ongoing debate and reviewed elsewhere (see also[21,22]).

Mitochondria as a target to improve insulin sensitivity

The focus on mitochondrial dysfunction as a potential cause of insulin resistance and T2DM has also resulted in a renewed interest of mitochondria as a target to treat diabetes. It is well known and well described that physical exercise training is perhaps the single best option for the prevention and treatment of T2DM. Nielsen *et al.*[23] showed that a 10-week aerobic cycling training in obese, male T2DM patients and age- and BMI-matched controls indeed improved mitochondrial content (by ~40 %) and that this was accompanied by an increase in insulin sensitivity in both groups. In 2010, we performed an exercise-training intervention in T2DM patients and BMI- and age-matched obese controls, and examined mitochondrial function in further detail. We used a 12-week progressive training programme, consisting of twice weekly endurance exercise training combined with once weekly resistance training session. We showed that all aspects of mitochondrial function, including *in vivo* mitochondrial function using phosphocreatine recovery rate, intrinsic mitochondrial function using high-resolution respirometry, and mitochondrial content, increased after the 12 weeks training[24,25], and restored T2DM patients towards control values. We performed hyperinsulinaemic euglycaemic clamps to investigate if the training also resulted in improved insulin sensitivity, and indeed found an improvement in skeletal muscle insulin sensitivity, which was most pronounced in T2DM. In a subset of patients, we also confirmed the reduced intrinsic mitochondrial capacity in T2DM patients, which we reported earlier[20], and showed that mitochondrial respiration in permeabilised muscle fibres was significantly improved and restored to control levels upon the 12-week training programme. These findings are consistent with findings by Hey-Mogensen *et al.*[26], and show that exercise training improves mitochondrial function in parallel with improvements in insulin sensitivity. However, again, such studies do not show a causal relationship between mitochondrial function and insulin sensitivity. We therefore investigated if a higher mitochondrial function indeed could prevent the development of insulin resistance. To this end, we compared endurance-trained athletes with sedentary, age- and BMI-matched controls. First, we confirmed that endurance trained athletes indeed had higher mitochondrial oxidative capacity, as determined by state 3 and state U respiration in permeabilised muscle fibres. We also confirmed that endurance trained athletes have a higher (muscle) insulin sensitivity. Next, we tested if the enhanced mitochondrial function in endurance-trained athletes would also protect them from the development of lipid-induced insulin resistance. To this end, we infused subjects, during a hyperinsulinaemic euglycaemic clamp, with high levels of a TAG emulsion together with heparin, which results in elevated circulating fatty acids and the development of insulin resistance within 2–3 h after initiating the lipid infusion[4]. Interestingly, whereas lipid infusion resulted in a ~70 % reduction in insulin sensitivity in sedentary controls, only a ~30 % reduction in insulin sensitivity was observed in endurance trained athletes, suggesting that endurance training indeed partly protects against the development of lipid-induced insulin resistance[27].

Taken together, a high mitochondrial function in human subjects is, at least, associated with improved insulin sensitivity, and therefore improving mitochondrial

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function may be a useful target to prevent and T2DM and related metabolic disorders.

Non-exercise mimetics to improve mitochondrial function

Although there is little doubt that exercise training and regular physical activity is key for the prevention of diabetes, most people experience difficulties to strictly follow a regular exercise programme. Another, but perhaps even less attractive option to stimulate mitochondrial function is energy restriction. In human subjects, a study has been conducted in young overweight volunteers that reduced energy intake by ~25 % for 6 months. Subjects were studied before and after the intervention, and it was found that indices of obesity and ectopic fat storage were reduced after energy restriction. Furthermore, markers of insulin sensitivity also improved[28,29]. The authors then showed that skeletal muscle markers of mitochondrial functions such as the transcriptional factor PGC 1α, mitochondrial transcription factor A and sirtuin 1 (SIRT1) were all increased after energy restriction and this was accompanied by an increase in mitochondrial content (as determined by level of mtDNA content)[30].

This human data show that also in human subjects energy restriction is very effective in improving metabolic health, and thereby confirms the wealth of earlier rodent data. In fact, from animals studies it was concluded that SIRT1, the founding member of sirtuin protein family of NAD^+-dependent deacetylases, may be involved in the underlying mechanisms of energy-restriction induced improvements in insulin sensitivity and mitochondrial function[31]. Both exercise training and energy restriction may mechanistically converge and stimulate the AMPK–SIRT1–PGC1α axis to enhance mitochondrial oxidative capacity in muscle. Therefore, a search for novel stimulators of this pathway was initiated, which among others resulted in the discovery of resveratrol (3,5,4′-trihydroxystilbene) as a small molecule activator of SIRT1[32], most likely via activation of AMPK[33,34].

Can resveratrol improve metabolic health?

Resveratrol was first isolated from the roots of the poisonous medicinal plant white hellebore (*Veratum grandiflorum O. Loes*)[35]. In 1992, resveratrol attracted the attention when the compound was suggested to explain part of the cardioprotective effects of red wine, also referred to as the French paradox[36]. In follow-up studies, resveratrol was also shown to have anti-inflammatory and anti-oxidant properties (reviewed in[37,38]). In 2003, Howitz *et al.*[32] identified resveratrol as a potent SIRT1 activator[39,40], which boosted the interest in the compound as an energy restriction mimetic. They and others also showed that resveratrol could positively affect longevity in yeast[32], worms[41], flies[42,43] and in short-lived fish[44].

More recently, interest in resveratrol shifted towards its potential to affect metabolic health. In 2006, Lagouge *et al.*,[40] showed that a high dose of resveratrol (400 mg/kg per d) resulted in, among others, improvements in insulin sensitivity, and a reduction in body weight. The latter effect might be dose-dependent, as a lower dose of resveratrol (~22·5 mg/kg per d) was insufficient to result in weight loss, but it still improved glucose tolerance[39]. The reason for the effect of body weight is not clear, but it has been shown that at higher dose resveratrol results in an increase in energy expenditure, despite a reduction in voluntary exercise, and could underlie the effects on body weight[40]. Work by Dal-Pan *et al.*[45,46] confirmed that supplementing resveratrol for 1 year at a dose of 200 mg/kg per d to non-human primate *Microcebus murinus* resulted in an increase in BMR and average daily energy requirements as well as improvements in metabolic health[45,46]. Together, these and many other rodent data have suggested a potential for resveratrol to affect metabolic health and urged for studies in human subjects.

A first study that reported effects of resveratrol on metabolism in human subjects was performed by Elliot *et al.*[47]. They reported that supplementing T2DM patients with high doses of resveratrol (2·5 and 5 g/d) for 28 d resulted in decreased levels of fasting and postprandial glucose and insulin. Brasnyo *et al.*[48] reported in 2011 positive effects of 4 weeks supplementation of a low dose of 5 mg resveratrol to T2DM patients on insulin sensitivity, as determined by homeostatic model assessment (HOMA)-index, effects that were accompanied by a reduction in markers of oxidative stress. Crandall *et al.*[49] examined in a small pilot study the effect of 4 weeks resveratrol treatment, with high doses of 1–2 g/d on glucose homoeostasis in subjects with an impaired glucose tolerance, and reported that resveratrol was able to reduce postprandial glucose levels. These effects could not be confirmed by Ghanim *et al.*[50], who reported no beneficial effects of a 6-week intervention with an extract containing 40 mg resveratrol on HOMA-index, performed in healthy volunteers.

Given these promising effects in first human trials, we decided to perform a detailed characterisation of the metabolic effects of resveratrol in obese human subjects. Thus, we supplemented healthy middle-aged, obese men with normal glucose tolerance with 150 mg resveratrol/d for 30 d. We did so in a placebo-controlled, randomised and double-blinded crossover design. After 30 d of resveratrol supplementation, we found that fasting plasma levels of glucose and insulin were slightly, but significantly reduced compared to after placebo supplementation, resulting in an improvement in HOMA-index[51]. We reported no adverse effects of resveratrol, and also found reductions in plasma TAG and alanine aminotransferase levels, the latter being a marker for liver function. Also, a significant decrease in blood pressure was observed. Because we were interested in the effects of resveratrol on energy and mitochondrial metabolism, we further studied these subjects in so-called whole-body room calorimeters. In these respiration chambers, we found that 30 d resveratrol had a significant effect on sleeping metabolic rate, resulting in a decrease in energy metabolism. This finding is in contrast with the increase in energy expenditure that is observed in rodents, but consistent with the effects of energy restriction that are reported in human subjects[28,29]. We

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next turned our focus on skeletal muscle and examined mitochondrial metabolism in skeletal muscle biopsies taken after the intervention. Gene-set enrichment analysis of DNA microarrays performed on muscle tissue revealed that resveratrol activated similar pathways in human subjects compared with mice, as mitochondrial pathways related to ATP production and oxidative phosphorylation were up-regulated and inflammatory pathways were down-regulated. Moreover, we could show that, as in rodents, resveratrol acts on the AMPK–SIRT1–PGC1 axis and had beneficial effects on mitochondrial respiration as determined by high-resolution respirometry. Given this increase in mitochondrial oxidative capacity and the hint towards improved insulin sensitivity, we tested if resveratrol supplementation also resulted in the expected decrease in ectopic fat storage. Indeed, liver fat content was reduced after 30 d resveratrol supplementation[51]. However, very intriguingly, we found a pronounced increase in IMCL content, both in type 1 and type 2 diabetic muscle fibres.

Taken together, we showed that resveratrol had beneficial effects on metabolic health in obese, middle-aged men but also increases IMCL. Whether this increase in IMCL is a detrimental effect of resveratrol needs further study (see later). Please note that since our study was published, at least two other resveratrol trials were published that did not confirm beneficial effects of resveratrol[52,53]. The reason for the discrepancies between studies may lie in methodological differences, and has been recently reviewed by us and is therefore out of the scope of this review[54].

Is resveratrol an exercise mimetic?

One of the most intriguing findings of our resveratrol trial was the finding that IMCL was markedly increased after 30 d resveratrol. As outlined earlier, increased IMCL is found to negatively associate with insulin sensitivity, at least in sedentary and/or obese subjects. Given that all other markers of metabolic health, including circulating TAG improved, and that fat oxidative capacity was enhanced, it is not realistic to assume that this increase in IMCL is due to excessive fat storage as observed in the obese state. Interestingly, endurance-trained athletes, who are very insulin sensitive, are also characterised by elevated IMCL levels[55]. This dissociation between IMCL levels and insulin sensitivity is referred to as the athletes paradox[55] and suggests that with training lipid droplet may be stored in the muscle to serve as rapid available substrate fuel during exercise. Since resveratrol has many metabolic health benefits that are similar to those observed with endurance training, including reductions in blood pressure, energy metabolism, blood glucose, TAG and insulin levels and improvements in mitochondrial metabolism, it is tempting to speculate that resveratrol may actually have exercise-like effects. Recently, we showed that exercise not only resulted in an increase in mitochondrial metabolism but also resulted in the up-regulation of a programme of genes involved in lipid droplet storage in muscle[56]. We showed that these effects may be explained by exercise-induced activation of the transcriptional coactivator PGC1α, as transgenic PGC1α mice and experiments in primary myotubes in which PGC1α was overexpressed, showed that this transcription factor is not only involved in stimulating mitochondrial metabolism[57] but also in the activation of a lipid droplet gene programme. Furthermore, by comparing endurance-trained athletes with sedentary young human subjects, we could confirm that this lipid droplet gene programme was also enhanced in trained human subjects[56], illustrating that not only IMCL is increased in trained athletes, but that also the machinery to efficiently store and liberate lipids from these lipid droplets is enhanced by training. Interestingly, we have recently shown for two of the major players in the lipid droplet programme, perilipins (PLIN) 2 and 5, that they are essential for lipid storage in skeletal muscle cells[58,59]. Thus, both the overexpression of PLIN2 in C2C12 muscle cells as well as the overexpression of PLIN2 or PLIN5 in skeletal muscle of adult rats *in vivo* resulted in enhanced accumulation of intramyocellular lipids. Despite this fat accumulation PLIN2/5 overexpression did not result in the development of lipid-induced insulin resistance, suggesting that storage of lipid droplets in skeletal muscle does not need to hamper insulin sensitivity when also the machinery for proper lipid droplet dynamics is enhanced[58,59]. Consistently, it was previously shown that acute exercise is able to overcome lipid-induced insulin resistance by enhancing the capacity to store TAG in muscle[60]. Whether the effect of resveratrol on IMCL content in human subjects is also due to PGC1α-induced activation of the lipid droplet gene programme and results in efficient and safe storage of lipid in muscle is a tempting speculation, but needs further investigation. Also the longer-term effects of this increase in IMCL, and the question if resveratrol acutely improves skeletal muscle insulin sensitivity in human subjects, still needs to be determined, and such studies are currently underway.

Relevant to the question if resveratrol could be seen as an exercise mimetic, a recent paper was published in which it was found that resveratrol blunted the positive effects of exercise training on cardiovascular health in older men. That is, when high-intensity exercise training for 8 weeks was combined with 250 mg resveratrol/d training effects on blood lipids, blood pressure and maximal aerobic capacity were blunted when compared with the same training without resveratrol[61]. However, careful examination of the data reported shows that not all examined parameters were blunted by resveratrol and that actually several parameters were improved after resveratrol when compared with placebo, suggesting that the conclusion may be somewhat exaggerated.

Conclusions and perspective

There is ample evidence supporting the notion that improving mitochondrial function can have beneficial effects on metabolic health and may prevent some of the age-related metabolic disturbances that may

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ultimately lead to T2DM and CVD. From pre-clinical research, it is evident that resveratrol, and other compounds affecting the AMPK–SIRT1–PGC1 axis, have great potential to improve mitochondrial metabolism in a non-exercise manner. Whether such compounds also have therapeutic potential in human subjects is much too early to draw definitive conclusions, and many clinical trials will be needed to test this hypothesis. Designing such studies will not be easy as many factors that may determine the outcome of such trials are still unknown, but include dosing and timing of resveratrol, target population, selection of outcome parameters, etc. However, given that there are currently only very few successful non-exercise approaches to combat metabolic diseases, it is worth making the effort to investigate the true potential of resveratrol effects on metabolic health in human subjects.

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Conflicts of interest

None.

Authorship

Both authors contributed equally to the writing of this review.

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