The non-flavonoid polyphenol resveratrol induces anti-obesity effects via SIRT1 dependent improved mitochondrial functioning

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Abstract

There is an urgent need for novel preventive and therapeutic strategies for the dramatically growing problem of obesity and related metabolic diseases. In this context, the non-flavonoid polyphenol resveratrol is of increasing interest for its suggested mimetic effect of calorie restriction (CR). This thesis will discuss the underlying mechanisms by which resveratrol induces anti-obesity effects, with the focus on sirtuin1 (SIRT1) dependent effects on mitochondrial functioning. Several studies indicate that resveratrol activates SIRT1, either directly or indirectly via AMPK, and subsequently increases PGC-1α protein levels. This pathway influences several metabolic processes in various tissues. The key themes discussed in this thesis are fatty acid oxidation, thermogenesis and mitochondrial biogenesis in rodents as well as in humans. In rodents, resveratrol treatment stimulates the enzymes CPT1, MCAD and LCAD leading to increased fatty acid oxidation. In addition, it increases energy dissipation via uncoupling proteins (UCPs) and induces mitochondrial biogenesis via increased levels of mTFA. However, human studies have shown contradictory results, which might be due to the use of sub-optimal doses. This indicates a need to understand the absorption pathway of resveratrol and gives abundant room for further research. Although this thesis focuses on a limited number of pathways affected by resveratrol, the findings already suggest a promising role of resveratrol in the treatment or prevention of obesity and related metabolic diseases.

**Keywords:** resveratrol, obesity, French paradox, sirtuin1, AMPK, PGC-1α, fatty acid oxidation, thermogenesis, mitochondrial biogenesis.
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Introduction

The problem of obesity

In recent decades, there has been a dramatic increase in the prevalence of obesity and related metabolic diseases. The number of people suffering from those diseases is still growing progressively and the World Health Organisation (WHO) predicts a fast increase in the number of countries with epidemic proportions of obesity (Timmers et al., 2013). Therefore, there is an urgent need to address this problem with novel strategies, including lifestyle changes as well as therapeutics.

The major cause of the growing obesity problem is an unhealthy lifestyle: intake of excess of sugar and fat and the lack of physical activity. As a consequence, energy intake exceeds energy expenditure resulting in fat accumulation in non-adipose tissues (Lowell & Spiegelman, 2000; Timmers et al., 2013). This leads in turn to mitochondrial dysfunction, which normally plays a critical role in the balance between energy storage and expenditure (Bournat & Brown, 2010).

Although it is known that calorie restriction (CR) and exercise contribute to advantageous metabolic health, most patients have difficulties in adhering to strict exercise schedules and diets (Timmer et al., 2011). In addition, CR is associated with decreased rest energy expenditure, which leads to poor long-term efficacy of this treatment for obesity (Lowell & Spiegelman, 2000). Therefore, other strategies such as therapeutics which mimic the effects of CR and exercise are of increased interest nowadays.

The effects of resveratrol

Resveratrol is a non-flavonoid polyphenol, which is found in red wine (Burns et al., 2002). The rising interest originates from the ‘French paradox’ revealed by epidemiological studies. The French paradox refers to the situation in France where rates of cardiovascular disease are low despite a general diet containing high amounts of saturated fat. A possible explanation for this disconnection might be resveratrol in red wine. Red wine is relatively enriched with resveratrol due to the process of crushing and mashing grapes, which contain low amounts of resveratrol (Malhotra et al., 2014).

Resveratrol contributes to a wide range of health benefits, resulting in a prolonged lifespan of different organisms (Baur et al., 2006). It has been indicated to influence various pathways in several organ systems and prevent or reduce the severity of several disorders such as cardiovascular diseases, cancer and inflammatory diseases (Meydani & Hasan, 2010). More recently, resveratrol has been revealed to affect the development of obesity (Timmers et al., 2011).

The major metabolic tissues targeted by resveratrol are shown in Figure 1 (Aguirre et al., 2014). However, there are inconsistencies and contradictions regarding the underlying molecular pathways by which resveratrol causes these health benefits. One of the possible pathways comes from Howitz et al. (2003). They suggested that resveratrol increases sirtuin1 (SIRT1) activity through an allosteric interaction. SIRT1 activity has been implicated in the regulation of energy homeostasis, in particular via its effect on mitochondrial functioning (Yu & Auwerx, 2009). Mitochondria provide the main source of energy in the form of adenosine triphosphate (ATP) and occupy almost all human cell types (Nsiah-Sefaa & McKenzie, 2016).
Interestingly, obese women are characterized by decreased SIRT1 expression levels (Pedersen et al., 2008). In addition, the positive metabolic health effects of CR are associated with SIRT1 activation (Hu et al., 2011). Taken these findings together, the promising therapeutic role in obesity for resveratrol engagement to regulate energy homeostasis might be due to its function to activate SIRT1.

The aim of the thesis

The aim of this thesis is to discuss potential molecular mechanisms involved in the anti-obesity effects of resveratrol. In particular, the focus will be on the modulation of mitochondrial functioning by resveratrol via its effect on SIRT1 activity. In the first part some background information on resveratrol and SIRT1 will be given. The second part analyzes research in animal models concerning effects of resveratrol on mitochondrial functioning, focusing on three key themes: fatty acid oxidation, thermogenesis and mitochondrial biogenesis. The third part is concerned with human studies that have analyzed the effects of resveratrol supplementation on mitochondrial functioning. In the last section, an overall discussion- and conclusion is presented and recommendations for further research are given.

Figure 1. The major metabolic tissues targeted by trans-resveratrol. BAT: Brown adipose tissue; WAT: White adipose tissue (Adapted from Aguirre et al., 2014).


Structure, absorption and metabolism of resveratrol

**Trans-resveratrol**

Several plants, including grapes, peanuts, cranberries, blueberries and Japanese knotweed, naturally synthesize resveratrol in response to injury or fungal attack (Leiherer *et al*., 2013). The non-flavonoid polyphenol belongs to the stilbene class and exists in two isoforms, a cis- and a relatively stable trans-form (Figure 2A and 2B). The trans-form is speculated to be responsible for the beneficial health effects and therefore the research described in this thesis mainly uses this form. High performance liquid chromatography analyses showed that red wine contains high concentrations of trans-resveratrol compared to grapes and peanuts (Burns *et al*., 2002).

**Absorption and metabolism of resveratrol**

Resveratrol induces beneficial health effects, but also exhibits a limitation with respect to therapeutic strategy. The hydrophobicity of resveratrol provides low bioavailability in animals and humans, which is a major problem that is shared by many other potential drugs (Aguirre *et al*., 2014; Malhotra *et al*., 2014). Resveratrol is quickly metabolized in the liver and intestine by phase II enzymes, resulting in the formation of several metabolites such as resveratrol sulfates and resveratrol glucuronides (Figure 2C and 2D) (Wenzel & Somoza, 2005). It is suggested that these metabolites get deconjugated in the intestine but are subsequently reabsorbed for recycling or excreted into the feces (Marier *et al*., 2002). This process results in an average half-life of 130-180 minutes of resveratrol metabolites in rats (Malhotra., 2014). In order to enhance the bioavailability and duration of action, several pharmaceutical formulation techniques are used to change the design and formulation of resveratrol (Neves *et al*., 2012). Importantly, high interindividual variation of resveratrol bioavailability is indicated in animals and humans. The main explanation for the variability in bioavailability is the role of microbiota in resveratrol metabolism, since microbiota are extremely diverse between individuals (Tomé-Carneiro *et al*., 2013). The rate and extent of resveratrol absorption also depends on food. Under fasting conditions the peak in resveratrol plasma concentration is delayed, whereas food containing high levels of fat decreases the peak (Walle *et al*., 2002; La Porte *et al*., 2010). In addition, other polyphenols could inhibit resveratrol metabolism, as a result from competitive metabolization via the same phase II enzymes (Wenzel & Somoza, 2005). In the context of the French paradox, it is interesting to note that resveratrol combined with alcohol does not influence resveratrol absorption (La Porte *et al*., 2010).

However, the exact processes involved in the absorption and elimination of resveratrol metabolites remain yet unclear, and only few studies have been carried out to investigate this important topic (Malhotra *et al*., 2014).

![Figure 2. Isoforms and metabolites of the non-flavonoid polyphenol resveratrol. A: trans-resveratrol B: cis-resveratrol C: Resveratrol sulfate D: Resveratrol glucuronide (A and B Adapted from Burns *et al*., 2002; C and D adapted from Ladurner *et al*., 2014).](image-url)
Pathway of sirtuin1 and its effect on energy homeostasis via mitochondria

Role of sirtuin1 deacetylation activity in energy homeostasis

SIRT1 belongs to a family of nicotinamide adenine dinucleotide (NAD)-dependent enzymes. Activated SIRT1 deacetylates numerous proteins involved in fundamental cellular processes, including metabolic regulation (Chaudhary & Pfluger, 2009). The deacetylase activity of SIRT1 is regulated by the ratio NAD$^+/NADH$. In response to low glucose concentrations, cells activate metabolic pathways which increase NAD$^+$ levels. Increases in NAD$^+$ levels are sensed by SIRT1 and activate its deacetylation activity, whereas increased NADH levels inhibit SIRT1 activity (Figure 3) (Gerhart-Hines et al., 2007). In this way, SIRT1 detects cellular energy availability and plays a key role in energy homeostasis. Dysfunction of SIRT1 is consequently associated with several metabolic diseases (Chaudhary & Pfluger, 2009).

Sirtuin1 activation by resveratrol

In addition to NAD$^+$, 21 different small molecule activators of SIRT1 have been identified by Howitz (2003), of which resveratrol was the most potent one. However, this is controversial since there are studies that question SIRT1 activation by resveratrol completely. Beher et al. (2009) showed that resveratrol did not activate Fluor de Lys-SIRT1 substrate that lacked a covalently attached fluorophore, which was initially used for labeling. Together with the observation that resveratrol extend lifespan independently of SIRT1, it is suggested that resveratrol activates alternative pathways to induce health benefits (Zhang, 2006). However, a dependent and independent SIRT1 mechanism of resveratrol could occur simultaneously and explain the wide range of effects mediated by resveratrol (Meydani & Hasan, 2010).

Debate continues about the mechanisms by which resveratrol activates SIRT1. Besides direct activation, there might be an indirect activation route via AMPK (Baur et al., 2006). Park et al. (2012) describes a competitive inhibition of cAMP-degrading phosphodiesterases by resveratrol, resulting in increased cAMP levels. AMPK senses elevated cAMP levels and subsequently increases NAD$^+$ concentration, followed by an increase in SIRT1 activity (Cantó et al., 2009). These finding are in line

![Figure 3. Model of glucose dependent SIRT1 deacetylation activity. Decreased glucose levels increase intracellular NAD$^+$ levels that will activate deacetylation activity of SIRT1 (Adapted from Gerhart-Hines et al., 2007; Revollo & Li, 2013).](image-url)
with the results of Um et al. (2010) in AMPK knockout mice. The authors found that in the absence of a catalytic subunit of AMPK, mice lost the metabolic benefits resulting from resveratrol treatment. Um et al. (2010) and Park et al. (2012) both suggest that AMPK might be a target of resveratrol, prior to SIRT1 activation. Interestingly, SIRT1 might use this AMPK route to induce a positive feedback cycle. Activated SIRT1 deacetylates LKB1, which activates AMPK by phosphorylation. The resulting increased NAD⁺ levels enhance the activity of SIRT1 in addition to the resveratrol stimulus (Figure 4)(Price et al., 2012).

Despite the fact that the underlying mechanisms are still unclear, resveratrol is a promising molecule to activate SIRT1, either directly or indirectly.

**Sirtuin1 effects on mitochondrial functioning**

The diverse biological effects of activated SIRT1 are provided by its ability to deacetylate transcription factors which is an important mechanism for the regulation of their activity in almost all metabolic tissues (Yu & Auwerx, 2009). Since obesity is characterized by dysregulated energy homeostasis, this thesis focuses on the main organelle of energy expenditure in humans; the mitochondria (Gerhart-Hines et al., 2007; Yu & Auwerx, 2009). It has been demonstrated that activated SIRT1 deacetylates proliferator-activated receptor-c coactivator 1alpha (PGC-1α) and consequently improves mitochondrial biogenesis and function, consistently demonstrated in in vivo and in vitro studies (Baur et al., 2006; Lagouge et al., 2006). In skeletal muscle activated PGC-1α induces an upregulation of genes linked to mitochondrial activity and fatty acid oxidation. In brown adipose tissue (BAT) it improves the thermogenic capacity (Gerhart-Hines et al., 2007; Yu & Auwerx, 2009).

Figure 4 represents an overview of the SIRT1 pathway, from activation and feedback mechanism to finally its effect on mitochondria.

![Figure 4](image_url)

*Figure 4. Model for improved mitochondrial functioning induced by resveratrol in a SIRT1 dependent manner. Resveratrol induces direct or indirect activation of SIRT1, which leads to deacetylation of LKB1. This results in a positive feedback cycle of SIRT1. In addition, SIRT1 deacetylates PGC-1α and consequently improved mitochondrial functioning (Adapted from Price et al., 2012)*
Sirtuin1 dependent effect of resveratrol on mitochondrial fatty acid oxidation

To understand the balance of lipid levels between oxidation and storage, the processes and regulation of lipid oxidation need to be discussed. The primary function of mitochondria is the production of energy in the form of ATP and occurs mainly in skeletal muscle and liver (Galgani & Racussin, 2008). The production of energy is mediated by three enzymatic pathways: the tricarboxylic acid (TCA) cycle, oxidative phosphorylation (OXPHOS) and fatty acid beta-oxidation (FAO) (Figure 5). Acetyl-CoA, derived from sugar, fats and amino acids, is oxidized to generate NADH and flavin adenine dinucleotide (FADH$_2$) via the TCA cycle. Electrons from NADH and FADH$_2$ are used by the OXPHOS pathway to generate ATP. The metabolism of fatty acids in mitochondria by FAO is crucial in times of fasting or endurance exercise, leading to degradation of fat stores (Nsiah-Sefaa & McKenzie, 2016). Moreover, downregulation of glycolysis results from CR and is proposed as a functional mechanism to improve metabolic health caused by CR (Hipkiss, 2006). Therefore, stimulating the switch from glucose to fatty acid metabolism is an interesting target for obesity therapies.

FAO is a complex pathway in which at least 20 different transport proteins and enzymes are required (Figure 6) (Houten et al., 2016). Carnitine palmitoyltransferase-1 (CPT1) is a rate limiting enzyme for conversion of fatty acids prior to transport into the mitochondrial matrix. Depending on the length of the fatty acid, MCAD or LCAD is required for the initial step of the acyl-CoA dehydrogenase reaction in FAO (Nsiah-Sefaa & McKenzie, 2016). Resveratrol increases the activity of these three enzymes, CPT1, MCAD and LCAD, in skeletal muscle of mice receiving a high fat diet (HFD). These increases were in such a way that the activities, which were decreased by HFD, exceeded the normal FAO activities. Similarly, resveratrol normalized the altered SIRT1 and PGC-1α expression in skeletal muscle of those mice. This suggests a regulatory role of SIRT1 and PGC-1α in the switch from glucose to fatty acid metabolism (Chen et al., 2011).

Figure 5. Mitochondrial metabolism to generate energy in the form of ATP. Three enzymatic pathways are involved: Light blue: Fatty acid beta-oxidation (FAO) dark blue: Tricarboxylic acid (TCA) green: Oxidative phosphorylation (OXPHOS) (Nsiah-Sefaa & McKenzie, 2016).
Gerhart-Hines et al. (2007) found comparable results in skeletal muscle of rats. They showed that deacetylated PGC-1α by SIRT1 increases CPT1 and MCAD levels and consequently raised the rate of FAO. In order to gain more insight into the mechanism of the switch from glucose to fatty acid metabolism of cells, the effect of SIRT1 and PGC-1α activation on PKD4 was determined. Because PKD4 prevents pyruvate entry into the TCA cycle, it is considered a key enzyme in the switch from glucose to fatty acid metabolism (Sugden et al., 1993). PGC-1α is known as a common transcriptional coactivator of the transcription factors FOXO1, ERRα and PPAR. These transcription factor are activators of PDK4 gene expression (Wende et al., 2005). In agreement, Gerhart-Hines et al. (2007) showed that PGC-1α induces PDK4 mRNA expression, supporting the regulatory role of PGC-1α in the upregulation of FAO. In the context of resveratrol, these findings must be interpreted with caution, since Gerhart-Hines et al. (2007) investigated the role of resveratrol on function of SIRT1 but instead used other interventions, including fasting conditions and adenoviruses with small hairpin RNA to silence SIRT1 gene expression.

Both studies indicate that activation of SIRT1 and PGC-1α is necessary to increase FAO and as such may be implicated in the body fat lowering effects of resveratrol (Gerhart-Hines et al., 2007; Chen et al., 2011). However, there might be other factors that influence body fat mass, such as apoptosis of adipocytes and lipogenesis (Aguirre et al., 2014). Important to note is that Chen et al. (2011) showed that blood lipid levels were not affected by resveratrol, consistent with previous research (Baur et al., 2006; Lagouge et al., 2006). However, high blood lipid level is a consequence of HFD and related to several metabolic diseases (Baur et al., 2006). Resveratrol might reduce fat stores, but it is unlikely for a direct reducing effect on blood lipid levels to be a function of resveratrol.

Figure 6. Pathway of mitochondrial fatty acid oxidation (FAO). The required transport proteins and enzymes for different lengths of fatty acids are presented (Nsiah-Sefaa & McKenzie, 2016).
Sirtuin1 dependent effect of resveratrol on thermogenesis

Adaptive thermogenesis, also called nonshivering thermogenesis, functions to maintain basic body temperature (Andrade et al., 2014). The major contributor to heat production is brown adipose tissue (BAT) whereas a minor part takes place in skeletal muscle (Lagouge et al., 2006). Mitochondria are fundamental in this process, since uncoupling proteins (UCPs) are located in the inner mitochondrial membrane. UCPs mediate thermogenesis by dissipating the proton gradient, which results from the OXPHOS pathway, across the membrane. Instead of using this proton gradient to drive the synthesis of ATP, heat is produced (Figure 7) (Alberdi et al., 2013). In this manner, UCPs can dissipate excess of energy intake or energy stores and subsequently play an important role in the lowering of body-fat.

It has been reported that PGC-1α activates different transcriptional factors, such as PPARγ, retinoic acid receptor (RAR) and thyroid receptor (TR) (Aguirre et al., 2014). These factors are assembled on the UCP1 enhancer (figure 8) (Lowell et al., 2000). In rats treated with resveratrol significant increases in SIRT1 and PGC-1α were observed in intercapsular BAT, whereas the amounts of SIRT1 and PGC-1α in gastrocnemius muscle were not increased. Nevertheless, besides expression levels, the overall activity of PGC-1α is also determined by its acetylation status. The ratio between acetylated PGC-1α and total PGC-1α was decreased in resveratrol-treated rats, indicating increased activation of PGC-1α. Moreover, the rats showed a significant increase of UCP1 protein expression in intercapsular BAT, as well as an increased amount of UCP3 in gastrocnemius muscle (Alberdi et al., 2013). In addition, Lagouge et al. (2006) reported increased protein levels of SIRT1 as well as increased mRNA levels of PGC-1α and UCP3 in skeletal muscle of resveratrol-treated mice. The results of Lagouge et al. (2006) regarding intercapsular BAT are consistent with those of Andrade et al. (2014), who showed that mice treated with resveratrol display an increase in SIRT1 and UCP1 gene expression. However, in contrast to the study by Aguirre et al. (2014) and Lagouge et al. (2006), these mice were fed a standard diet instead of a high fat diet. These results indicate that the amount of adipose tissue does not affect the molecular mechanisms underlying the metabolic health effects of resveratrol. Taken all findings together, a model emerges in which resveratrol increases the level of UCPs that is likely related to increased SIRT1 and PGC-1α activity (Aguirre et al., 2014). Elevated thermogenesis resulting from the increased level of UCPs, can increase the dissipating energy and consequently lowers body fat.

![Figure 7](image-url) Mechanism of thermogenesis. Uncoupling proteins dissipate the proton gradient and produce heat, instead of using the proton gradient to drive the synthesis of ATP (Ledesma et al., 2002).
However, studies concerning thermogenesis are scarce and more evidence is needed to confirm the influence of resveratrol on thermogenesis. Another important note is the questionable relevance of thermogenesis in BAT for humans, since there is debate whether or not thermogenesis in BAT takes place in adult humans. Some studies indicate an important metabolic role in adults (Cypess et al., 2009), while others showed age-related impaired functioning of BAT (Graja & Schulz, 2015). As long as the function of BAT in humans is not clear, the results regarding the effects of resveratrol on thermogenesis in rodents cannot be easily translated to humans.

Figure 8. PGC-1α stimulates UCP-1 gene expression via co-activation of PPARγ, RAR and TR (Adapted from Lowell et al., 2000).
**Sirtuin1 dependent effect of resveratrol on mitochondrial biogenesis**

Since fatty acid oxidation and thermogenesis take place in mitochondria, the amount of mitochondrial mass indirectly influences the possible increase of these processes by resveratrol (Alberdi et al., 2013; Nsiah-Sefaa & McKenzie, 2016). Several studies suggest that resveratrol increases mitochondrial biogenesis via deacetylation of PGC-1α by activated SIRT1 (Baur et al., 2006; Price et al., 2012; Alberdi et al., 2013). As described by Lowell & Spiegelman (2000) mitochondrial biogenesis is dependent on PGC-1α binding and stimulation of nuclear respiratory factor-1 (NRF-1) localized on the mitochondrial transcription factor A (mTFA) promoter. Consequently, this leads to increased synthesis of mTFA, which in turn affects transcription and replication of the mitochondrial genome (Figure lower part). Activation of this pathway is essential for mitochondrial biogenesis (Lowell & Spiegelman, 2000).

Baur et al. (2006) showed mitochondrial biogenesis in HeLa cells in the presence of resveratrol. In addition, the livers of mice treated with resveratrol showed more mitochondria compared with mice without resveratrol treatment. The resveratrol treated mice also showed a threefold lower acetylated PGC-1α status, supporting the suggested role of activated PGC-1α in mitochondrial biogenesis.

In skeletal muscle mitochondrial biogenesis was determined by Price et al. (2012). Mitochondrial mass in wild type (WT) mice was increased after resveratrol treatment, whereas this was not observed in SIRT1 knock out (KO) mice. Moreover, transcript levels of PGC-1α, NRF-1 and mTFA were increased in the resveratrol-treated mice. These findings indicate mitochondrial biogenesis by resveratrol in a SIRT1-dependent manner (Price et al., 2012).

In addition to mitochondrial biogenesis in liver and skeletal muscle, Alberdi et al. (2013) focussed on BAT. They showed increased levels of mTFA as well as increased activation of PGC-1α in rats treated with resveratrol. The observation of increased expression of COX2, a crucial protein in the OXOP pathway, supports the contention that increased mitochondrial mass is related to increased mitochondrial activity (Alberdi et al., 2013).
Sirtuin1 dependent effects of resveratrol on mitochondrial functioning in humans

Despite the positive effects of resveratrol on mitochondrial functioning in rodents, results from studies with resveratrol supplementation in humans are less clear (Yoshino et al., 2012; Poulsen et al., 2013). For example, Poulsen et al. (2013) found no effects on either resting energy expenditure or rates of lipid oxidation after resveratrol administration to obese men. In addition, in this study levels of phosphorylated AMPK and PGC-1α gene expression were not significantly altered in skeletal muscle by resveratrol. Moreover, resveratrol supplementation to non-obese women did not result in changes in SIRT1, PGC-1α, and UCP3 gene expression in both muscle and adipose tissue and did not alter phosphorylation levels of AMPK in skeletal muscle (Yoshino et al., 2012). Both studies therefore suggest neither a direct nor an indirect role of resveratrol on mitochondrial functioning via SIRT1.

In contrast, there are results from human studies which appear consistent with those obtained by rodent studies (Timmers et al., 2011; Konings et al., 2014). Timmers et al. (2011) observed increased levels of AMPK phosphorylation, SIRT1 and PGC-1α in skeletal muscle of resveratrol-treated obese men. Moreover, increased activity of citrate synthase was observed in skeletal muscle, which is a commonly used marker for mitochondrial functioning. In a follow-up of this study, Konings et al. (2014) described decreased mean adipocyte size in the obese subjects treated with resveratrol. Collectively, these findings do support the contention that resveratrol acts via SIRT1 to induce increased mitochondrial functioning.

The contradicting results observed in human studies with resveratrol supplementation might be due to methodological differences. The age of the individuals and extent of obesity were not the same and it is currently unknown to what degree these differences affect the function of resveratrol (Malhotra et al., 2014). In addition, the dose and route of resveratrol administration differed between studies. Yoshino et al. (2012) administered resveratrol together with food, which might result in a lower degree of absorption (La Porte et al., 2010). Interestingly, not all studies controlled for resveratrol metabolites in plasma during the administration period, although interindividual variation in bioavailability of resveratrol is indicated (Tomé-Carneiro et al., 2013).
Discussion

The growing number of people suffering obesity and related metabolic diseases are reaching epidemic proportions in several countries (Timmers et al., 2013). Lifestyle changing strategies, such as CR and exercise, turned out to function inadequate to handle the progressively growing obesity problem (Timmer et al., 2011). Therefore, other strategies such as the therapeutic resveratrol are of increased interest. The non-flavonoid polyphenol resveratrol might induce metabolic benefits due to its function to activate SIRT1 (Howitz, 2003). SIRT1 activity plays a major role in the regulation of energy homeostasis, particular via its effect on mitochondrial functioning (Yu & Auwerx, 2009). This thesis discussed the potential molecular mechanisms by which resveratrol induces anti-obesity effects. In particular, the focus was on the modulation of mitochondrial functioning by resveratrol via its effect on SIRT1 activity.

Sirtuin1 activation by resveratrol

There is debate about the targets of resveratrol. Based on in vitro and animal studies, the activation of SIRT1 by resveratrol is most likely. However, other indirect and SIRT1 independent pathways are described in literature (Zhang, 2006; Park et al., 2012). Consequently, to what extent SIRT1 is responsible for the metabolic benefits induced by resveratrol remains questionable (Zhang, 2006). Novel insights into the complex role of activated SIRT1 by resveratrol might be provided by tissue specific deletion of the SIRT1 gene (Chaudhary & Pfluger, 2009). In addition, more studies with SIRT1 knockout mice are needed to exclude or confirm SIRT1 independent pathways of resveratrol. Another suggested pathway is indirect stimulation of SIRT1 via AMPK (Park et al., 2012). Consequently, alternative molecules which activate AMPK hold considerable potential to be more effective compared to resveratrol. Other AMPK or SIRT1 activators can also circumvent the limitation of low bioavailability of resveratrol (Malhotra et al., 2014).

In brief, there is abundant room for further research to investigate the mechanism of SIRT1 or AMPK stimulation by resveratrol or other molecules that contribute to its metabolic benefits.

Results of animal studies translated to humans

One of the major limitations of animal studies is the unclear value of the results for humans. Metabolism and absorption of resveratrol might differ between animals and humans, which probably leads to consequences for the proposed mechanism of action in animal models. Further research in humans is required to confirm whether the proposed effects of resveratrol in animal studies also apply to humans. In addition, to enhance the value of the potential effects on BAT in rodents, the physiology of human BAT should be first determined.

Dose dependent effects of resveratrol

To achieve desired plasma concentrations of resveratrol metabolites, the metabolism and absorption pathways need to be more clearly understood. Published studies with animal models utilized resveratrol doses with a range from 0.1 to 1000mg/kg per day (Malhota et al., 2014). In human studies given doses range from 10 to 2000 mg per day. However, the conclusions were not proportional to the
used resveratrol doses. Some studies with high resveratrol doses showed enhanced effects, while others with high doses did not observe any effect at all. The same contradictory results occurred in studies with low doses of resveratrol (Yoshino et al., 2012). In addition to different doses, duration and way of administration, such as combination with particular dietary constituents, differed between studies. Interestingly, interindividual variance in bioavailability is not taken into account in these studies. These limitations and methodological differences might explain some of the contradictory results found in human studies. In further research concentrations of resveratrol metabolites should be measured in plasma of the subjects during the administration period. In this respect, proteomics comprising of liquid chromatography with tandem mass is indicated as an appropriate technique therefore (La Porte et al., 2010). In this way, dose response relationship of metabolites in plasma can be determined. In addition, the effect of different resveratrol metabolites should be investigated. As a consequence, other formulations of resveratrol might enhance the effectiveness or duration of action.

Considering the used doses of resveratrol in the discussed animal studies, it is unlikely for humans to achieve the desired amount of resveratrol by dietary intake. For example, a dose of 75mg is equivalent to the amount resveratrol of 8 liters of red wine (Burns et al., 2002). However, the best administration method and absorption pathway are not yet clearly understood. Moreover, cumulative effects of long-term administration in humans are not addressed, leaving the possibility that frequently consuming red wine may play a part in the prevention of obesity and related metabolic diseases.

Taken these findings together, further research is required to determine dose-dependent effects of plasma resveratrol metabolites on efficacy of short- and long-term resveratrol supplementation in humans.

Safety of desired doses of resveratrol

It is important to note that as a result of the pleiotropic effects of resveratrol, it may alter undesired pathways and processes in several tissues. In literature, adverse effects of resveratrol on whole body condition are not mentioned frequently (Meydani & Hasan, 2010). However, toxicity to very high doses of resveratrol has been observed in rats and resulted in liver dysfunction, anemia and nephrotoxicity (Crowell et al., 2004). From the discussed human studies in this thesis, Porte et al. (2010) was the only study which reported an adverse effect. Six of the eight participants got diarrhea after resveratrol supplementation.

Before therapeutic application of resveratrol in humans is possible, it is necessary to establish the safety of the desired doses of resveratrol on short- and long-term usage.

Limitation of this thesis

The major limitation of this thesis is the narrow focus on affected metabolic pathways by resveratrol. Besides mitochondria function, resveratrol influences body composition, insulin sensitivity and liver fat accumulation (Aguirre et al., 2014). In addition, SIRT1 activation regulates several fundamental cellular processes in the whole body (Chaudhary & Pfluger, 2009). A full discussion of the effects of resveratrol on metabolic pathways lies beyond the scope of this thesis. However, all these effects of resveratrol may play an additive role in the anti-obesity effects of resveratrol and are important to take into account in further research.
Conclusion

In this thesis, the underlying mechanisms of the anti-obesity effect of resveratrol on mitochondrial functioning have been discussed. With the focus on SIRT1, the conclusion is that the effects on fatty acid oxidation, thermogenesis and mitochondrial biogenesis all seem to contribute partly to the metabolic health benefits of resveratrol. Based on animal and in vitro studies, resveratrol acts most likely via direct or indirect activation of SIRT1, which subsequently leads to deacetylated PGC-1α. This process results in promotion of the switch from glucose to fatty acid metabolism via stimulation of the enzymes CPT1, MCAD and LCAD, which are critical in the process of fatty acid oxidation. In addition, resveratrol improves thermogenesis via increased energy dissipation via UCPs. Finally, the induced mitochondrial biogenesis influences mitochondrial functioning in a positive way.

Although this thesis focused on limited pathways of resveratrol, the findings in animal studies to date already suggest a promising role of resveratrol in the treatment or prevention of obesity and related metabolic diseases. However, confirmation of these metabolic health benefits in humans remains limited and further research should concentrate on this topic. The safety and other mechanisms of action of resveratrol need to be examined before therapeutic application of resveratrol is possible.

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References


