

Diabetes research in Dundee

# Black Tea Mimicry

Type 2 diabetes caused by insulin resistance is one of the most widespread diseases in the western world. Amy Cameron *et al.* of Dundee have found that components in black tea have insulin-mimetic properties and may contribute in the search for a therapeutic molecule that can substitute for insulin.

Amy Cameron, a second year PhD student at the Ninewells Hospital and Medical School of the University of Dundee and first author of a recent paper in *Aging Cell* (vol. 7(1):69-77), explains: "Type 2 Diabetes is considered as epidemic in America and in Europe as well. It is a serious disease, which causes people to go blind or lose limbs. Perhaps what is more striking is it considerably increases your risk of heart disease which is still the leading killer of men and women. Historically people over 40 years of age have developed it; initially they are able to control it to some degree by changes in their diet and with exercise. Only later on would these patients need to take medication. The even more worrying aspect of this disease is that younger adults and even children are developing this type of diabetes. This earlier onset is being driven by increased obesity which is becoming widespread in our culture."

In a healthy body the blood sugar level is regulated by hormones released from the pancreas which then act in the liver. Insulin promotes the storage of glucose in the liver and muscle tissue if there is an excess of available sugar, e.g. after a meal. Insulin simultaneously down-regulates a number of genes that are involved in the generation of glucose (gluconeogenesis).

In diabetes this homeostasis is impaired and abnormally high blood sugar levels are observed (hyperglycaemia). In Type 1 Diabetes this is due to insufficient levels of the hormone insulin that is produced within the  $\beta$ -cells of the islets of Langerhans in the pancreas. The situation is different in type 2 diabetes which results from insulin resist-

ance (IR). In IR the target tissue of insulin is (at least partly) resistant to its effects and the blood glucose levels stay higher. In turn the  $\beta$ -cells produce more insulin but ultimately they are unable to meet demand. The direct cause of insulin resistance is unknown but it is suspected that it is related to an energy-rich "western diet". Recommended initial treatments for insulin resistance are weight loss and exercise.

The insulin signaling pathway begins with binding of insulin to the insulin receptor (INR), a tyrosine kinase transmembrane receptor. The insulin receptor substrate (IRS) becomes phosphorylated and in turn activates Phosphoinositide 3-kinases (PI 3-kinases or PI3Ks). As a result, FOXO1a becomes phosphorylated at several sites. FOXO1a belongs to the forkhead transcription factor family O (FOXO). Its members, and in particular FOXO1, have important roles in the regulation of body energy metabolism. After being phosphorylated, FOXO1a binds to 14-3-3 proteins which dissociate FOXO from the DNA and enable it to be excluded from the nucleus. FOXO1a is now inhibited as it can no longer transcribe its target genes such as phosphoenolpyruvate carboxykinase (PEPCK), which results in reduced gluconeogenesis.

## Flavins with exciting properties

Dr Graham Rena, the lead author of the paper, began studying the forkhead (FOX) transcription factors during his time as a post-doc in the MRC Protein Phosphorylation Unit in Dundee. He was especially interested in the phosphorylation events that occur on FOXO1a in response to different kinases. After establishing his own group at the Neuroscience Institute at Ninewells Hospital a few years back, the group was able to show that Epigallocatechin gallate

(EGCG), a polyphenol in green tea, can mimic insulin action through phosphorylation of the transcription factor FOXO1a (*Cellular Signalling*, 2007, vol. 19, p.378). EGCG has been shown to be effective in decreasing PEPCK expression in rodent models (*J Nutr*, vol. 136, p.2512).

## Somewhere up the cascade

The majority of polyphenols in green tea are flavonols, also known as catechins. When black tea is made, in a process called fermentation, the monomeric flavanols undergo oxidative polymerisation leading to the formation of oligomers like theaflavins and thearubigins. In collaboration with the Scottish Crop Research Institute (SCRI) in Dundee (funded in part by the Scottish Government and the European Union) Rena's group was able to show that these dimerisation products of the previously studied catechins also phosphorylate FOXO1a (*Aging Cell*, 2008, vol. 7, p.69).

In a screen using HEK293 cells, the authors looked for induction of phosphorylation of FOXO1a at different phosphorylation sites by dietary compounds. Black tea extract was identified as being able to phosphorylate FOXO1a in a manner similar to IGF-1. The extract was analysed and consisted mainly of theaflavins. Amy says "Theaflavins have insulin-like properties but they are not as powerful as insulin. Theaflavins need a higher concentration to achieve the same effect." Using different kinase inhibitors, the responsible kinase was narrowed down to PI3K. "Theaflavins work somewhere upstream of the PI3K cascade but we don't know exactly where yet." In the hepatoma cell line HL1c, a good model system to study the effect of insulin related repression of gluconeogenesis, theaflavins were also able to repress PEPCK activity.

"It takes EGCG approximately two hours to initiate FOXO1a phosphorylation while the theaflavins are able to phosphorylate



"Tea expert" Amy Cameron



FOXO1a in a much shorter time; phosphorylation is detectable after 5-10 minutes. We suspect that the protracted delay in the EGCG effect occurs because EGCG is required to undergo oxidative dimerisation before it can act on the pathway.”

### Are they bioavailable?

Further evidence for this hypothesis comes from the observation that phosphorylation of FOXO1a is prevented if the formation of catechin dimers is blocked. This can be achieved either by modification of the dimerising phenol-ring or by using superoxide dismutase (SOD). As theaflavins have already dimerised, their induction of FOXO1a phosphorylation is not blocked by SOD and is more rapid.

It remains to be seen if black tea polyphenols and especially theaflavins are sufficiently bioavailable to produce similar effects *in vivo*. Amy says, “Studies in humans have not been able to show clear results yet. Plasma levels of catechins go up after drinking a cup of tea, however, only for a short period of time. These compounds are not very stable and there is even less data on theaflavin bioavailability. Theaflavins are also known to oligomerise further to thearubigins, which were also active in the assay. Unfortunately however, no mass spectroscopy data are available for them as they are structurally too complex and we don't know exactly what they look like.”

By screening more food compounds in the future, the researchers hope to identify more insulin-like substances. Amy says “We are looking for the point of action and the mode of action of these insulin-mi-

metic molecules. We want to identify the target(s) that all these compounds have in common and then find a small molecule with the same properties.” Conducting a high throughput screen, costing around £20,000, would be a big challenge for the small team, currently consisting of one Ph.D. student and some honours and summer students. “The important thing about designing a small molecule is that it should ideally not use the same binding site as insulin does. It would ideally have a separate binding site so that there would be no interference with any residual action of insulin that might still be present in the patient's body.”

### Overwhelming media reaction

A press release in February about the research sparked considerable interest in the media (*BBC*, 2<sup>nd</sup> March 2008: “Tea could help combat diabetes”). Amy says, “We hoped for a little interest and were overwhelmed by the reaction from the media! There were even reports in India and Australia about our studies. The public can easily relate to tea as it is something they experience on a daily basis. Though, of course, the experiments are still in the early stages, so people with diabetes should continue to follow their medication as directed by their general practitioner rather than drinking masses of tea. I would recommend that anyone who is interested in our results has a look at the summary that is available on the Diabetes UK website.” [[http://www.diabetes.org.uk/en/About\\_us/News\\_Landing\\_Page/Can-black-tea-help-diabetes-control/](http://www.diabetes.org.uk/en/About_us/News_Landing_Page/Can-black-tea-help-diabetes-control/)] ANDREA HERB

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