Biology

The genetics of obesity: a lab activity

Around 1.5 billion people worldwide are overweight or obese. Are we just eating too much or can we blame our genes? Here's how to investigate the genetics of obesity in the classroom.

By Sarah McLusky, Rosina Malagrida and Lorena Valverde

Obesity is a big problem, in more ways than one. More than 10% of the world's adult population is obese; in some countries this rises to 40%. Heart disease, type 2 diabetes, some cancers, pregnancy complications, joint problems, depression and anxiety have all been linked to obesity[w1].

Readily available calorie-rich foods and a more sedentary lifestyle are usually blamed for the startling rise in obesity, but might our genes be part of the problem? This article introduces some of the current research into the genetics of obesity along with a method developed by the European Xplore Health project for extracting DNA, the first step for researchers looking to identify possible 'fat genes'.

The DNA extraction would be suitable for intermediate (aged 14-16) or advanced (aged 16+) students and could be completed in one lesson. If this method is not appropriate for your students or lab, try one of two alternative methods (based on the same principles, but using more readily available materials), using frozen peas (Madden, 2006) or kiwi fruit[w2].

The topic of obesity also provides a useful way of introducing current genetic research and ethical issues within a context that students are likely to have some personal experi-

An obese, leptin-deficient mouse (left) next to a normal mouse (right)

Image courtesy of US Department of Energy, Oak Ridge National Laboratory

- Biology
- Health education
- Genetics
- Nutrition
- Pharmacogenomics
- Statistics
- Ages 15+

The incidence of obesity is increasing worldwide. Growing awareness of the problem, changing our eating habits and investigating a genetic predisposition could all contribute to its reduction.

This article, which explores recent research into the genetics of obesity and details a practical activity on the topic, could be used for biology lessons on many different topics, including health education, genetics, nutrition, pharmacogenomics, individualised therapy correlated with the presence of specific genes, statistics on obesity in different countries and their link to the diet of the corresponding population.

In addition to providing the starting point for a discussion of obesity, the article could be used for a project on nutrition, starting by recording students' BMI and relating it to their daily diet (e.g. Krotscheck, 2010). In this way, the teacher could propose healthier eating habits, contributing to changing the attitude of students.

Suitable comprehension and extension questions include:

1. Is there a relationship between genetics and obesity? If so, what is it?
2. What are phenotype and genotype? How do these correlate with each other and with the environment?
3. How are epigenetic phenomena associated with phenotype? Are these epigenetic changes inherited?
4. Could the knowledge of our genetic composition help us prevent obesity?
5. Good nutrition is important for health. If your BMI is beyond the normal range, how could you change your eating habits to reduce it?

Panagiotis Stasinakis, 4th Lyceum of Zografou, Greece

REVIEW

ence of and opinions about. After all, how many teenagers don't like fast food and lazing about in front of the TV? Other resources, including a debate pack for exploring the issues further, are available on the Xplore Health website[w3].

What have genes got to do with it?

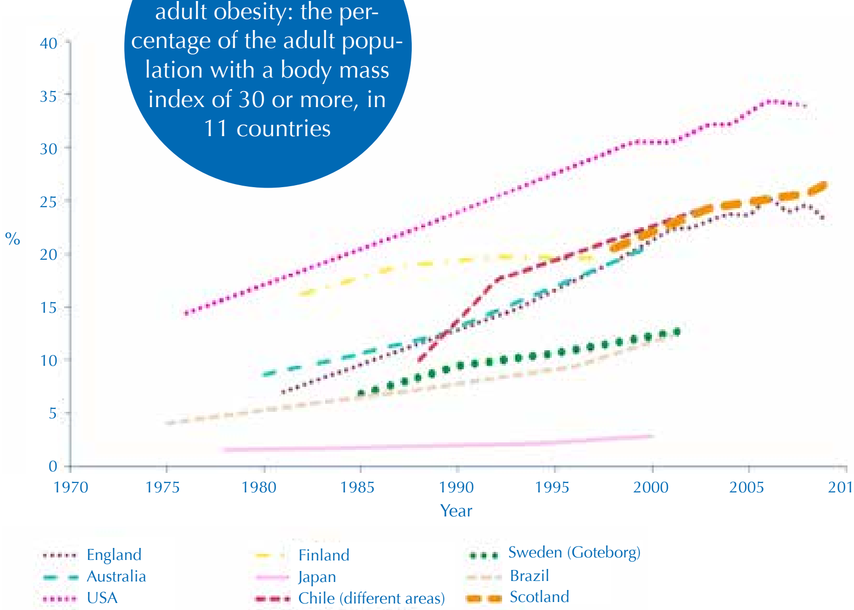
Obesity runs in families. Does that mean obesity is genetic or does it just mean that families share unhealthy eating habits? Perhaps surprisingly, twin studies have shown that obesity could be as much as 70% genetic, as siblings who share the same genes (identical twins) are much more likely to share the same body shape than non-identical twins (O'Rahilly & Farooqi, 2006).

Researchers have discovered a number of gene variants (alleles) that seem to be associated with obesity. Some of these are rare and affect a very small number of people, while others are fairly common and increase the risk of weight gain among large parts of the population (table 1).

The *Ob* gene is a single-variant cause of obesity. It controls appetite by producing a hormone called leptin. People who carry two defective copies of this gene cannot produce leptin and have a constant desire for food. Obese, leptin-deficient people treated with leptin injections have returned to a normal weight. Another gene found to act in a similar way is the *Mc4r* gene, which is part of a signalling pathway that controls eating behaviour (O'Rahilly & Farooqi, 2006).

However, these single-gene mutations are quite rare. Some other,

Changes in the prevalence of adult obesity: the percentage of the adult population with a body mass index of 30 or more, in 11 countries



Data source: International Association for the Study of Obesity (www.iaso.org)

Table 1: Different types of obesity-linked genes identified by researchers

	Rare single gene variants	Multiple common gene variants
Effect on body weight	Account for a lot of extra weight in very few people	Account for a little bit of extra weight in a lot of people
Examples	*Ob* gene, *Mc4r* gene	*Fto* gene, *Tmem18* gene
Association with other clinical conditions	Can be associated with rare diseases, e.g. congenital leptin deficiency, MC4R deficiency	One of many 'normal' varied human characteristics, but can also associate with other common diseases, e.g. type 2 diabetes
How are these found?	Candidate gene studies, animal studies, exome sequencing	Genome-wide association studies
Potential relevance	Prenatal genetic testing and gene therapy	Understanding risk of disease and tailoring disease prevention strategies

much more common gene variants have been identified that, although not a direct cause of obesity, do make carriers more likely to put on weight. Examples include variants of the *Fto* gene and the *Tmem18* gene. Studies have found that people with one copy of the 'at risk' *Fto* variant weigh, on average, 1.2 kg more than people with other variants, whereas those with two copies of the 'at risk' variant weigh, on average, 3 kg more.

So are people who carry these more common 'fat genes' destined to be obese or can they escape their fate? These gene variants simply predispose people to gain weight, but with a healthy diet and lifestyle there is no reason why carriers shouldn't be able to maintain a healthy weight.

Obesity researchers are also exploring epigenetics (the interactions between genes and environment) and how signalling pathways (e.g. hormones and the nervous system) in the body affect metabolism and behaviour. They hope that through a better understanding of the complex nature of appetite, metabolism and fat storage, it will be possible to develop better treatments or strategies for controlling food intake in people who are genetically predisposed to obesity.

BACKGROUND

Defining obesity

Someone is considered obese if they have a body mass index (BMI) of 30 or more. The healthy range is 18.5-25. BMI is calculated by dividing a person's weight in kilograms by the square of their height in metres.

$$\text{BMI} = \frac{\text{weight (kg)}}{(\text{height (m)})^2}$$

Although BMI provides a useful guide, it doesn't allow for variation in build and body composition. For example, athletes who carry a lot of muscle mass would often be considered overweight if judged on BMI alone.

BACKGROUND

Why are there so many different DNA extraction methods?

This is just one of many published methods for extracting DNA from cells (e.g. Madden, 2006, and the Naked Scientists website[w2]). They range from the very simple (using washing-up liquid and table salt) to methods that use chemicals more familiar to molecular biologists. The principles of the extraction are the same in all cases: a detergent is used to break up the cell membranes, an enzyme is added to digest the proteins that keep DNA tightly coiled up, and salt and cold alcohol are then added to create conditions where DNA is insoluble.

The main difference is that the more advanced the method, the purer the resulting DNA will be. For example, in the simplest method, a lot of pectin is mixed in with the DNA. Clearly, obesity researchers and other molecular biologists need their samples to be as pure as possible.

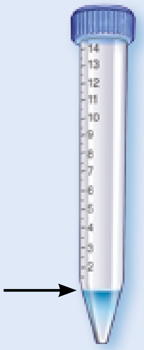
Student activity: Extracting your own DNA

Materials

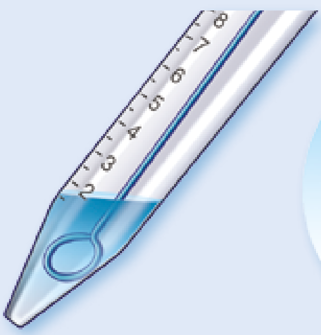
- Micropipettes or graduated transfer pipettes
  If you don't have micropipettes, you can use calibrated / graduated disposable plastic transfer pipettes. On these pipettes the 'stalk' is graduated, allowing volumes of less than 1 ml to be transferred with sufficient accuracy for this experiment.
- Disposable culture loops or buccal swabs
- Small Falcon tube or a test tube with a bung or cap
  Falcon tubes are calibrated test tubes with screw caps. If you don't have them, just use normal test tubes.
- Water bath at 40°C (optional)
- Disinfectant solution
- Lysis solution
- Proteinase K solution
- Sodium acetate solution
- Cold ethanol or isopropyl (rubbing) alcohol (keep in the freezer until required)

Procedure

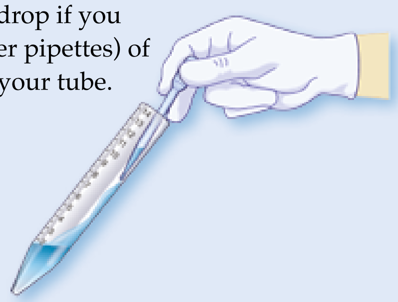
1. Place 1 ml lysis solution in your Falcon tube or test tube.



2. Vigorously scrape a loop or swab around the inside of your cheeks and across your tongue.
3. Place the loop or swab in the lysis buffer and mix it around to dislodge your cells.

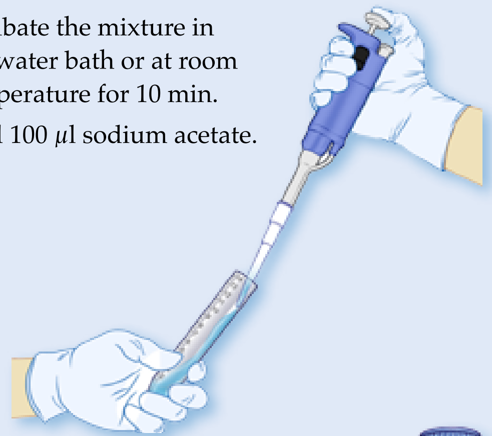


4. Put your loop or swab in the disinfectant.
5. Repeat steps 2-4 twice more to ensure you get plenty of cells. **Use a new loop or swab each time**.
6. Add 20 µl (or 1 drop if you are using transfer pipettes) of proteinase K to your tube.

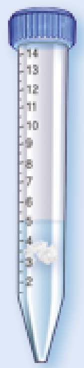


7. Cap the tube and invert it a couple of times to mix.

8. Incubate the mixture in the water bath or at room temperature for 10 min.
9. Add 100 µl sodium acetate.



10. Cap your tube and shake well to mix.
11. Add 3 ml cold ethanol.
12. Cap the tube and invert it very slowly to mix.
13. Your DNA should appear as a whitish stringy precipitate.



Images courtesy of @el taller interactivo, Parc Científic de Barcelona

Safety note:

The solutions can irritate eyes and skin, so wear a lab coat, safety glasses and gloves. Saliva can carry diseases; only handle your own loops or swabs and put used items in the disinfectant.

Disposal: liquids can be poured down the sink with plenty of water. Used loops or swabs can be placed in normal waste after disinfecting for 15 minutes.

Questions for discussion

- What does 'lysis' mean? How does this help extract the DNA?
- The lysis buffer contains a detergent called SDS. Using your knowledge of cell structure, what do you think the detergent does?
- Inside cells, DNA is found tightly coiled up and bound to a variety of proteins. Which step helps to release the DNA from the proteins?
- What does the last step tell you about the solubility of DNA in both salty water and ethanol?
- How could you confirm that the white precipitate really is DNA?

Extension activities

- Compare this method of extracting DNA with the simpler methods using frozen peas (Madden, 2006) or kiwi fruit[w2]. How do they differ? Which one works best? Can you explain why? Can you find out which method is closest to the method that professional geneticists use?
- Simply extracting someone's DNA is not enough to tell if they have a predisposition for obesity. What other tests would have to be done? Find out more about the techniques used in genetic research.
- In many countries, parents who carry serious genetic conditions like cystic fibrosis or haemophilia can opt for pre-implantation genetic diagnostics to avoid having children that carry the disease. Do you think this procedure should be available for parents who have a genetic predisposition for obesity? Do your classmates agree with you?

BACKGROUND

Sourcing and preparing the reagents

Lysis solution (50 ml)

1. Prepare tris-buffered saline (TBS) according to manufacturer's instructions or standard recipe.

   TBS can be purchased as a ready-made solution, in tablet form or made up from scratch[w4].

Safety note: a ready-made sodium dodecyl sulphate (SDS) solution is recommended as powdered SDS is harmful if inhaled. If powdered SDS is used, the teacher should prepare the solution, wearing a mask and using the fume hood.

See also the general *Science in School* safety note on page 57.

2. If using ready-made 10 % SDS solution, add 5 ml SDS to 45 ml TBS.

   If using powdered SDS, dissolve 0.5 g in 50 ml TBS.
3. Store in the fridge until required.

3 M Sodium acetate solution (for 50 ml)

1. Dissolve 12.3 g anhydrous sodium acetate in 50 ml distilled water.
2. Add dilute HCl to adjust to pH 5.2.
3. Store in the fridge until required.

Proteinase K (100 µg / ml)

1. Dissolve 1 mg proteinase K in 10 ml tris-buffered saline.
2. Only a very small amount of the enzyme is required, so you might want to make up a smaller volume if you have a sufficiently accurate balance. Simply adjust the quantities accordingly.
3. Store in the freezer until required.

Disinfectant solution

Suitable disinfectants include 0.015 M sodium hypochlorite solution, 1 % Virkon® solution or 5 % domestic bleach. After soaking for at least 15 min, the loops can be transferred to a plastic bag (wear gloves) and disposed of with normal waste.

To help you find the necessary reagents, a list of product numbers for Sigma-Aldrich is given in table 2. However, you will also be able to source them from other suppliers.

Reagent	Sigma-Aldrich product number
Tris buffered saline (TBS)	Tablets: TS030 or 94158
Sodium dodecyl sulphate (SDS)	Powdered: L3771 10% solution: 71736
Sodium acetate	S2889
Proteinase K	P6556

Table 2: Sigma-Aldrich product numbers for the reagents used in this protocol

Image courtesy of colros / Flickr

Heart disease, type 2 diabetes, some cancers, pregnancy complications, joint problems, depression and anxiety have all been linked to obesity.

References

Krotscheck F (2010) Using cutting-edge science within the curriculum: balancing body weight. *Science in School* **16**: 19-26. www.scienceinschool.org/2010/issue16/obesity

Madden D (2006) Discovering DNA. *Science in School* **1**: 34-36. www.scienceinschool.org/2006/issue1/discoveringdna

O'Rahilly S, Farooqi IS (2006) Genetics of obesity. *Philosophical Transactions of the Royal Society of London Biological Science* **361(1471)**: 1095–1105. doi: 10.1098/rstb.2006.1850

Web references

w1 – The World Health Organization's factsheet number 311 *Obesity and Overweight* is available on the WHO website (www.who.int) or via the direct link: http://tinyurl.com/62hyt96

w2 – A simple DNA extraction method that uses kiwi fruit and does not require any specialist chemicals or equipment can be found on the Naked Scientists website (www.thenakedscientists.com) or via the direct link: http://tinyurl.com/8aqhkhj

w3 – You can download the DNA extraction method detailed in this article, as well as lesson ideas, games and videos related to obesity and other health science topics on the Xplore Health website (www.xplorehealth.eu). The website and most resources are available in English, Spanish, French, Polish and Catalan. Xplore Health is a European education project by scientists, educators, science centres and museums. It offers young people innovative multimedia and hands-on experiences about cutting-edge health science, to bridge the gap between research and education.

To learn more about obesity, its causes, consequences and treatment, as well as the ethical, legal and social aspects associated with obesity, download the background information for educators, *A Crisis of Fat?* (available in English, French, Polish and Spanish), from the Xplore Health website (www.xplorehealth.eu) or use the direct link: http://tinyurl.com/cpq7esd

w4 – A recipe for making tris-buffered saline from scratch is available on the Protocols Online website (www.protocolsonline.com) or via the direct link: http://tinyurl.com/br4or3t

Resources

The experiment was developed for the Xplore Health project with the help of scientists from the Institute for Research in Biomedicine in Barcelona, Spain. To learn more about the research, see the web page of the molecular pathology and therapy in heterogenic and polygenic diseases research group (www.irbbarcelona.org or use the direct link: http://tinyurl.com/bt93a7o) or read one of the original research articles:

Argyropoulos G, et al. (1998) Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. *Journal of Clinical Investigation* **102(7)**: 1345-1351. doi: 10.1172/JCI4115

An animation of the DNA extraction process is available on the University of Utah's Learn Genetics website (http://learn.genetics.utah.edu) or via the direct link: http://tinyurl.com/cuf8rzf

The *Genetics and Obesity* factsheet can be downloaded from the website of the UK's National Genetics Education and Development Centre (www.geneticseducation.nhs.uk) or via the direct link: http://tinyurl.com/cjezjxb

If you found this article useful, why not browse all biology-related articles in *Science in School*? See: www.scienceinschool.org/biology

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Image courtesy of Tobyotter / Flickr

More than 10% of the world's adult population is obese; in some countries this rises to 40%.

Lorena Valverde holds a degree on biology from the University of Barcelona, Spain and a master's in immunology from the University of Barcelona and the Autonomous University of Barcelona. Lorena is currently doing a PhD in biomedicine and working as a teacher of the University of Barcelona. She has collaborated with Xplore Health, offering experimental workshops for students and the general public at the Barcelona Science Park.

To learn how to use this code, see page 57.