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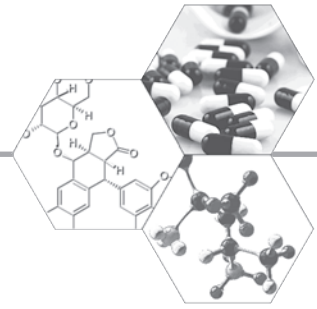

'Energy expenditure genes' or 'energy absorption genes': a new target for the treatment of obesity and Type II diabetes

Several hundred genes associated or linked to obesity have been described in the scientific literature. Whereas many of these genes are potential targets for the treatment of obesity and associated conditions, none of them have permitted the developement of an efficient drug therapy. As proposed by the 'thrifty genotype' theory, obesity genes may have conferred an evolutionary advantage in times of food shortage through efficient energy exploitation, while 'lean' or 'energy expenditure' genes may have become very rare during the same periods. It is therefore a challenge to identify 'energy expenditure genes' or 'energy absorption genes,' whose mutations or single nucleotide polymorphisms do result in reduced energy intake. We submit that such 'energy absorption' or 'energy expenditure' genes (crucial genes) are potential new targets for the treatment of obesity. These genes can be identified in rare genetic diseases that produce a lean, failure-to-thrive, energy malabsorption or starvation phenotype.

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Past & present gene targets in obesity for the treatement of metabolic disorders

Several hundreds of genes have been associated or linked to obesity either as single gene mutation or as loci in a genomic region related to Mendelian syndromes relevant to human obesity [1]. Other studies have estimated the number of genes that are involved in influencing body weight as being several thousands [2]. Directly or indirectly, these so-called 'obesity genes' are all involved in energy management; for example, fatty acid, carbohydrate and protein metabolisms, thermogenesis, appetite, satiety and energy absorption **(Figure 1)**. Among these genes several tens have been proposed as a candidate gene targets for obesity and Type II diabetes treatments and many pharmaceutical companies and biotechnologies are making a huge effort in R&D to identify new molecules for obesity treatment. **Table 1** summarizes past and present gene targets and their stage of development.

These, for instance, include the satiety *OB* gene, the *POMC*, *MC4R*, *NPY* and *CB1* (for review see elsewhere [3–7]), in which all of them are expressed in the CNS where they control food intake (appetite, satiety) among other physiological pathways.

Many of them have been abandoned due to lack of specificity, toxicity and/or side effects. Recently a drug acting on the CB1 receptor (Rimonabant®, Sanofi-Aventis) was retracted from the market and many companies have stopped the development of their molecule against this target [8]. This was essentially due to the side effect of the target, which triggers depression and suicidal thoughts. More recently, the sibutramine (Meridia® or Reductil®), which is involved in serotonin reuptake, a molecule commercialized by Abbott, was also retracted from the European market and is under re-valuation by the US FDA due to a myocardium side effect. The only drug that made it to the market, and is still there, is Orlistat, an inhibitor of the pancreatic lipase. This drug is available today without prescription and is sold over the counter under the name Alli®.

The mitochondrial uncoupling proteins (UCPs) UCP2 and UCP3 (uncoupling the reaction of formation of ATP from ADP) were suggested as good targets for the treatment of obesity by activating UCP, thus enabling the dissipation of energy instead of ATP formation, however, with an associated risk of body temperature elevation [9–11]. Recently the *PRDM16* gene, which can switch myoblastic precursor cells to brown fat cells was suggested as a new opportunity for obesity and Type II diabetes treatment by increasing the metabolic rate and dissipating excess energy (fat) by overexpressing the UCP in brown adipose tissue (BAT) [12,13], here again with a risk of temperature elevation.

Other interesting, new anti-obesity genes that have been proposed as new target for metabolic disorder treatment are:

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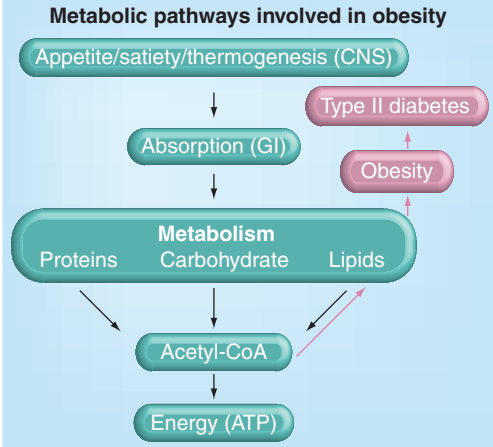

Figure 1. Metabolic pathways involved in obesity.

- *SIRT1*, a gene that is involved in caloric restriction and was also shown to increase insulin sensitivity [14], was suggested as a new target for obesity treatment. This gene is also involved in extending healthy life span [15,16].
- The Cidea proteins, which are strongly expressed in BAT, are important regulators of energy homeostasis and are linked to the development of metabolic disorders. It was shown that Cidea-deficient mice have a higher metabolic rate and are resistant to diet induce obesity [17].
- Recently, Genome Wide Association Study has identified the fat mass and obesity associated gene (*FTO*; involved in energy balance and energy intake [18,19]), and single nucleotide polymorphisms (SNPs) within a regulatory element of the *MC4R* gene. More than 77,000 European adults participated in this study and three quarters of the genome SNP's were screened [20]. However these genes form a very complex network and in most cases they lack specificity and, consequently, are associated with many side effects when activating or inhibiting one of them. Another problem with centrally acting targets is that they are closely integrated with pathways regulating other key functions. Therefore finding drugs with specific actions is fraught with difficulties.

Indeed in humans, only in rare cases obesity is a consequence of a single gene mutation. In most cases, obesity involves many genes that interact and create a complex network of redundant biochemical pathways to stimulate appetite, satiety and energy management in a very efficient way in order to save energy. The high redundancy of genes involved in energy management makes it unlikely that obesity will ever be controlled by targeting just one of those genes unless such a gene target is associated with a lean phenotype.

Saving energy in the form of fat evolved in our ancestors as a way of surviving long periods of famine as proposed by the **thrifty genome** theory [21,22]. Indeed, when food was scarce, individuals having genotype efficient in energy absorption and metabolism were naturally selected. In the contemporary world, an abundance of food and a sedentary lifestyle contribute with that erstwhile evolutionary advantage result in obesity, Type II diabetes, hypertension and other complex genetic diseases that are becoming so common in our times [21–23].

The evolutionary forces that have favored energy-efficient phenotypes are likely to have suppressed 'lean genes', **energy expenditure genes** or inefficient **energy absorption genes**. An alternative strategy for the treatment of obesity could thus focus on the identification of such genes, which would subsequently be targeted with appropriate drugs. We would like to suggest that future drug-discovery programs centering on any gene target should first establish an association between that gene and a 'lean human phenotype' or 'starvation phenotype'. In other words, instead of looking for new potential obesity gene targets in obese patients, one should seek **cruicial genes** which are not redundant genes that control energy absorption in 'lean' human phenotypes or failure to thrive. To illustrate, if a monogenic slimness disease or a phenotype resulting from a deficiency of energy absorption could be found, the implicated gene would be likely to play a critical role in the phenotype and would be a potential target for new anti-obesity drugs. Furthermore, if this is not compensated by other mechanisms, it would be a more judicious therapeutic target than the foregoing 'obesity genes,' which, as seen earlier, are usually redundant and not specific.

Congenital enteropeptidase deficiency: new target for metabolic disease treatment

Among the genetic pathologies associated with a failure to thrive or a starvation phenotype that have been described in the literature, one attracted our attention: congenital enteropeptidase (EP) deficiency. EP (also termed enterokinase; EC 3.4.21.9) is a serine protease that is present on the brush border of the duodenum and is

Key Terms

Thrifty genome: In contrast with expenditure genes, genes that are efficient in metabolizing energy. In conditions when food is scarce (the usual situation in the evolution of humans), these genes provide a fitness advantage.

Energy expenditure genes: Genes that are inefficient in metabolizing energy. In order to save energy genes that are involved in extracting and metabolizing energy must be efficient in allowing maximum extraction and conversion of food to biological energy (ATP).

Energy absorption genes: Genes that are involved in the digestion and absorption of food from the GI tract.

Crucial gene: A gene that contributes to weight management is major and when mutated is associated to growth retardation, starvation or a lean phenotype.

Table 1. Nonexhaustive list of obesity targets with associated products and their stage of study.

Companies	Target	Product	Function	Stage
Surface Logix	MTP	SLX4090	Fat absorption	Phase II
Alizyme/Takeda	Pancreatic lipase	Cetilistat (ATL-962)	Fat absorption	Phase III
Hoffman-La Roche		Orlistat/Xenical		Approved
GlaxoSmithKline	Sodium/Glucose cotransporter	GSK-189075	Sugar absorption	Phase I
Johnson & Johnson		RWJ-394718		Abandoned
Biovitrum/Amgen	11β HSD-1	AMG221/BVT.83370	Fat metabolism	Phase I
Novartis	DGAT	LCQ908	Fat metabolism	Phase II
Abbott laboratories	Glucocorticoid receptor	A-348441	Fat metabolism	Abandoned
Astra Zeneca	PPAR-α/γ	Galida/AZ242	Fat metabolism	Abandoned
Bristol Myers	PPAR-α/γ	Muraglitazar	Fat metabolism	Abandoned
Eli Lilly/Ligand	PPAR-α/γ	LY818, naveglitazar	Fat metabolism	Abandoned
GlaxoSmithKline	PPAR-α/γ	GSK501516	Fat metabolism	Abandoned
Merck & Co	PPAR-α/γ	KRP 297	Fat metabolism	Abandoned
Novartis Pharma	PPAR-α/γ	DRF415	Fat metabolism	Abandoned
Novo Nordisk	PPAR-α/γ	NN 662/DRF2725	Fat metabolism	Abandoned
Glenmark	SCD1	GRC-9332	Fat metabolism	Preclinical
Xenon Pharmaceuticals/ Novartis	SCD1	GRC-9332	Fat metabolism	Preclinical
Bristol–Myers/AstraZeneca	DPPIV	BMS-477118	Metabolism	Preclinical
GlaxoSmiyhKline	DPPIV	Redona / GSK825964	Metabolism	Abandoned/ Preclinical
Novartis	DPPIV	Galvus	Metabolism	Approved
Chiron	GSK-3	CT98014 / CT98023	Metabolism	Abandoned
Dainippon/Takeda		TAK-677		Abandoned
Eli Lilly		LY-377604		Abandoned
Kyorin/Nisshin Pharma		N-5984		Phase II
Merck & Co		L-796568		Abandoned
CytRx	RIP140	RIP140 inhibitor	Energy burning	Preclinical
RXI Pharma	RIP140	RIP140 inhibitor	Energy burning	Preclinical
Elixir Pharmaceuticals	Sirtuin	SIRT1 agonist /SIRT2 inhibitor	Energy burning	Preclinical/Preclinical
Sirtris Pharmaceuticals	Sirtuin	SRT501	Energy burning	Phase II
Transtech Pharma	AGRP	TTP435	Appetite	Preclinical
Genaera Corp/Magainin	AGRP	Trodusquemine/MSI-1436	Appetite	Phase I
Compellis Pharmaceuticals	Calcium channel	CP404	Appetite	Phase I
Bristol–Myers/Solvay		SLV319		Abandoned
Eli Lilly		LY320135		Abandoned
Sanofi–Synthelabo		Rimonobant/Surinabant		Abandoned
GlaxoSmithkline	CCK-A	GSK181771	Appetite	Abandoned
Sanofi–Synthelabo	CCK-A	SR146131/SSR 125180	Appetite	Abandoned
Elixir Pharmaceuticals	Ghrelin	EX-1314	Appetite	Preclinical
Pfizer/Noxxon	Ghrelin	NOXB11	Appetite	Preclinical
Tranzyme Pharma	Ghrelin	TZP-301	Appetite	Preclinical
Abbott laboratories	H-3 receptor	A-423579	Appetite	Abandoned
Novo Nordisk	H-3 receptor	NNC 0038-0000-1202	Appetite	Abandoned
Abbott laboratories	MCH	A-798	Appetite	Abandoned
GlaxoSmithKline	MCH	GSK803430	Appetite	Abandoned
GlaxoSmithKline	Mu-opioid receptor	GSK-1521498	Appetite	Phase I
Neurogen/Pfizer	NPY	NDG-951	Appetite	Abandoned
Shionogi USA	NPY	S-2367	Appetite	Phase II
7TM Pharma	NPY	TM30338/TM30339	Appetite	Phase II / Phase II
Abbot Laboratories	Serotonin receptor	Meridia/Reductil	Appetite	Approved

Table 1. Nonexhaustive list of obesity targets with associated products and their stage of study (cont.).

Companies	Target	Product	Function	Stage
Orexigen	Unknown/undisclosed	Contrave®/Empatic®	Appetite	Phase III/Phase II
Biovitrum	5 HT6	BVT.5182	Satiety	Abandoned
Arena pharmaceuticals	5HT-2C	APD356	Satiety	Phase III
Biovitrium/GlaxoSmithKline	5HT-2C	BVT.933	Satiety	Abandoned
GlaxoSmithKline	5HT-2C	GW876167	Satiety	Phase II
Vernalis/Roche	5HT-2C	VR1065	Satiety	Abandoned
OSI Pharmaceuticals	MAOI/5-HT1A (SIRUP)	PSN602	Satiety	Phase II
Amylin	Amylin	Symlin/AC137	Satiety	Approved
Regeneron	CNTF	Axokine	Satiety	Abandoned
Shearwater Corp/Regeneron	CNTF	PegAxokine	Satiety	Abandoned
Schering Canada	D1 and D5 receptor	Ecopipam	Satiety	Abandoned
Amylin/Eli Lilly	Exendin-4	Exenatide	Satiety	Approved
Conjuchem	Exendin-4	CJC-1134	Satiety	Phase II
Alizyme	GLP-1	AZM-134	Satiety	Abandoned
Aventis/Zealand Pharma	GLP-1	AVE-100/ZP 10	Satiety	Phase III
GlaxoSmithKline	GLP-1	GSK-716155 Syncria®	Satiety	Phase III
Novo–Nordisk	GLP-1	Liraglutide	Satiety	Phase II
Amgen/Roche	Leptin	OB-1 2nd generation	Satiety	Abandoned
Abbott/Millennium	Melanocortin	MLN4760		Abandoned
Action Pharma	Melanocortin	AP1030		Phase I
IPSEN	Melanocortin	BIM-22493	Satiety	Preclinical
Merck	Melanocortin	MB243		Abandoned
AstraZeneca	PTP-1B	AZD8677	Satiety	Abandoned
Isis Pharmaceiticals	PTP-1B	ISIS-113715	Satiety	Phase II
Amylin/Curis	PYY3-36	AC162352	Satiety	Phase I

involved in the digestion of dietary proteins and lipids. Specifically, EP catalyzes the conversion, in the duodenal lumen, of inactive trypsinogen into active trypsin via the cleavage of the acidic propeptide from trypsinogen. The activation of trypsin initiates a cascade of proteolytic reactions leading to the activation of many pancreatic zymogens, such as chymotrypsinogen, proelastase, procarboxypeptidases A and B and prolipase **(Figure 2)** [24]. EP is highly specific for the sequence $(Asp)_4$-Lys-Ile of the trypsinogen molecule, where it acts to mediate the cleavage of the Lys–Ile bond.

Enteropeptidase is a disulfide-linked heterodimer composed of a heavy chain of 82–140 kDa, and a light chain of 35–62 kDa [25]. Mammalian EPs contain 30–50% carbohydrate, which may contribute to the apparent differences in polypeptide weight. The heavy chain is postulated to mediate association with the intestinal brush border membrane [26], while the light chain contains the catalytic domain localized in the intestine lumen.

Deficiency of intestinal EP results in a failure to thrive, diarrhea, anemia, hypoproteinemia and edema [27–30]. The condition is usually successfully treated by pancreatic enzyme replacement or by administration of dietary protein hydrolysate [28,31–33]. EP deficiency is an extremely rare recessive inherited disorder: so far only 13 cases have been reported in the scientific literature [29]. Affected infants display severe failure to thrive. The analysis of small-intestinal mucosal biopsy specimens suggested that EP deficiency results from the formation of structurally altered enzymes with no EP activity. This was confirmed in a later study that showed that mutations in the EP gene underlie the molecular cause of congenital EP deficiency [34].

In light of the foregoing, EP activity may provide a selective and efficient target for the treating metabolic disorders. Furthermore, since EP is a peripheral-acting target that reminiscence the pancreatic lipase target, the only valid target in the market. Partial inhibition of the EP will diminish the efficiency of energy absorption through the gastro-intestinal tract. Reducing the daily absorption of energy derived from proteins and fatty acids by 15–20% should have a significant impact

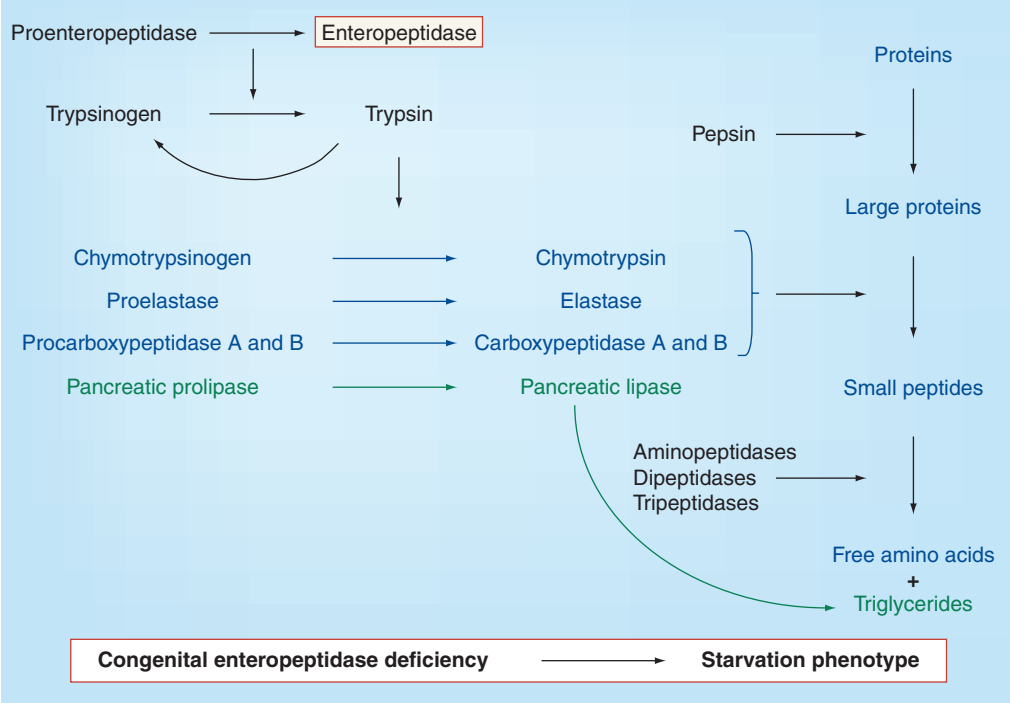

Figure 2. Cascade of biochemical events starting with proenteropeptidase.

on weight management in the long term. Furthermore, such a modest level of inhibition of both fatty acid and protein absorption is likely to trigger few, if any, side effects, such as diarrhea or fluctuation. Indeed, EP knockdown mice present no obvious diarrhea, nor was any diarrhea observed during preliminary *in vivo* evaluation of proprietary EP inhibitors [Braud S, Ciufolini M & Harosh I, Unpublished Data]. It should be noted here that there are, in the literature, several successful popular diets enriched with proteins that work quit well [35,36]. However, here we suggested cutting 15–20% of the total energy absorption.

Future perspective

Extraordinary progress in our understanding of the metabolic disorders has been made during the past two decades. Many genes associated with obesity have been identified and their function elucidated, and many other genes are waiting to be discovered. The number of quantitative trait loci for obesity continues to increase and is estimated to be several hundred. However, none of these genes has been the answer to obesity treatment, despite the numerous pharmaceutical R&D programs conducted worldwide **(Table 1)**. Indeed, such efforts have been plagued by severe side effects due either to the target or the molecule, leading in some cases to withdrawal of approved drugs from the market, and by the meager – if any – therapeutic effect of many experimental drugs.

By contrast, the EP gene promises to be an extraordinary target for the treatment of obesity and associated metabolic disorders. The gene has a strong association with a starvation phenotype, it is tissue specific (expressed exclusively in the brush border of the duodenum), it is not redundant and peripheral target with all its advantages compared to central acting targets. Growth retardation effects are of no concern in adult subjects, and in any event they may even be treated in newborns and infants by the dietary administration of amino acids or, alternatively, of pancreatic juice containing the necessary activated enzymes [28,31–33]. We have shown that EP knock-out mice display a growth retardation phenotype analogous to the human new born [Braud S, Ciufolini M & Harosh I, Unpublished Data], and that *in vivo* inhibition of EP has a direct effect on weight management due to the inhibition of absorption of fatty acids and proteins [101,102].

Because thousands of genes and multiple environmental factors are involved in metabolic disorders, we believe that a successful strategy for the treatment of obesity must target nonredundant genes associated with a 'lean human phenotype.' There are several

interesting lean phenotypes associated with a single gene that are waiting to be studied (e.g., Anderson's disease). Other genes can be discovered and validated by using different SNPs databases and correlating the polymorphism with a lean phenotype, either by generating knock-out mice or associating the lean phenotypes with lean families or cohorts.

We are confident that the next generation of targets for the treatment of metabolic disorders will have a strong genotype–lean phenotype association, thus reducing the likelihood of a side effect inherent to the gene. Nature did it – we should be able to identify those lean genes and duplicate the lean phenotype with drug therapy.

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Executive summary

- Many obesity-related genes have been proposed as potential targets for the treatment of obesity and Type II diabetes.
- None of these genes have yet provided an efficient drug therapy.
- We therefore pose the following questions:
  - Is there any genetic disease or phenotype associated with 'lean human phenotype'?
  - Is the phenotype associated with one gene (monogenic)?
  - Is the gene target tissue-specific?
  - Is there any redundancy of the gene target?
- Congenital enteropeptidase deficiency is an extremely rare pathology that fulfils all these criteria, suggesting it may be an excellent target for metabolic disorders treatment.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Rankinen T, Zuberi A, Chagnon YC *et al.* The human obesity gene map: the 2005 update. *Obesity (Silver Spring)* 14(4), 529–644 (2006).

2 Reed DR, Lawler MP, Tordoff MG. Reduced body weight is a common effect of gene knockout in mice. *BMC Genet.* 9, 4 (2008).

3 Tartaglia LA, Dembski M, Weng X *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83(7), 1263–1271 (1995).

4 Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372(6505), 425–432 (1994).

5 Friedman JM. Obesity: causes and control of excess body fat. *Nature* 459(7245), 340–342 (2009).

▪ **Good question and answer article on obesity.**

6 O'Rahilly S. Human genetics illuminates the paths to metabolic disease. *Nature* 462(7271), 307–314 (2009).

▪ **Excellent review article.**

7 Horvath Tl. Endocannabinoids and the regulation of body fat: the smoke is clearing. *J. Clin. Invest.* 112(3), 323–326 (2003).

8 Jones D. End of the line for cannabinoid receptor 1 as an anti-obesity target? *Nat. Rev. Drug Discov.* 7(12), 961–962 (2008).

9 Boss O, Samec S, Paoloni-Giacobino A *et al.* Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.* 408(1), 39–42 (1997).

10 Bouchard C, Perusse L, Chagnon YC, Warden C, Ricquier D. Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. *Hum. Mol. Genet.* 6(11), 1887–1889 (1997).

11 Fleury C, Neverova M, Collins S *et al.* Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* 15(3), 269–272 (1997).

12 Kajimura S, Seale P, Kubota K *et al.* Initiation of myoblast to brown fat switch by a PRDM16-C/EBP-β transcriptional complex. *Nature* 460(7259), 1154–1158 (2009).

13 Seale P, Bjork B, Yang W *et al.* Prdm16 controls a brown fat/skeletal muscle switch. *Nature* 454(7207), 961–967 (2008).

14 Sun C, Zhang F, Ge X *et al.* SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing ptp1b. *Cell Metab.* 6(4), 307–319 (2007).

15 Frye RA. Phylogenetic classification of prokaryotic and eukaryotic SIR2-like proteins. *Biochem. Biophys. Res. Commun.* 273(2), 793–798 (2000).

16 Lin SJ, Defossez PA, Guarente L. Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae. Science* 289(5487), 2126–2128 (2000).

17 Zhou Z, Yon Toh S, Chen Z *et al.* Cidea-deficient mice have lean phenotype and are resistant to obesity. *Nat. Genet.* 35(1), 49–56 (2003).

18 Fischer J, Koch L, Emmerling C *et al.* Inactivation of the FTO gene protects from obesity. *Nature* 458(7240), 894–898 (2009).

19 Olszewski Pk, Fredriksson R, Olszewska AM *et al.* Hypothalamic FTO is associated with the regulation of energy intake not feeding reward. *BMC Neurosci.* 10, 129 (2009).

20 Bogardus C. Missing heritability and GWAS utility. *Obesity (Silver Spring)* 17(2), 209–210 (2009).

21 Coleman Dl. Obesity genes: beneficial effects in heterozygous mice. *Science* 203(4381), 663–665 (1979).

22 Neel JV. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? *Am. J. Hum. Genet.* 14, 353–362 (1962).

23 Sharma AM. The thrifty-genotype hypothesis and its implications for the study of complex genetic disorders in man. *J. Mol. Med.* 76(8), 568–571 (1998).

▪▪ **Very interesting article on the evolution of metabolic diseases.**

24 Kitamoto Y, Yuan X, Wu Q, Mccourt DW, Sadler JE. Enterokinase, the initiator of intestinal digestion, is a mosaic protease composed of a distinctive assortment of domains. *Proc. Natl Acad. Sci. USA* 91(16), 7588–7592 (1994).

▪▪ **Role of enteropeptidase in the digestive system.**

25 Mann NS, Mann SK. Enterokinase. *Proc. Soc. Exp. Biol. Med.* 206(2), 114–118 (1994).

26 Fonseca P, Light A. The purification and characterization of bovine enterokinase from membrane fragments in the duodenal mucosal fluid. *J. Biol Chem.* 258(23), 14516–14520 (1983).

27 Follett GF, Macdonald TH. Intestinal enterokinase deficiency. *Acta Paediatr. Scand.* 65(5), 653–655 (1976).

28 Ghishan FK, Lee PC, Lebenthal E, Johnson P, Bradley CA, Greene HL. Isolated congenital enterokinase deficiency. recent findings and review of the literature. *Gastroenterology* 85(3), 727–731 (1983).

29 Hadorn B, Tarlow MJ, Lloyd JK, Wolff OH. Intestinal enterokinase deficiency. *Lancet* 1(7599), 812–813 (1969).

▪▪ **Discuss the importance of enteropeptidase.**

30 Haworth JC, Gourley B, Hadorn B, Sumida C. Malabsorption and growth failure due to intestinal enterokinase deficiency. *J. Pediatr.* 78(3), 481–490 (1971).

▪▪ **Discuss the importance of enteropeptidase.**

31 Amat L, Martin E, Mateu L, Rubio C, Ballesta A. Malnutrition in congenital deficiency of enterokynase (author's transl). *An. Esp. Pediatr.* 11(3), 219–226 (1978).

32 Hadorn B, Haworth JC, Gourley B, Prasad A, Troesch V. Intestinal enterokinase deficiency. Occurrence in two sibs and age dependency of clinical expression. *Arch. Dis. Child.* 50(4), 277–282 (1975).

33 Polonovski C, Laplane R, Alison F, Navarro J. Trypsinogen pseudo-deficiency caused by congenital enterokinase deficiency. Clinical study. *Arch. Fr. Pediatr.* 27(7), 677–688 (1970).

▪▪ **Disucss how congenital enteropeptidase deficiency can be treated.**

34 Holzinger A, Maier EM, Buck C *et al.* Mutations in the proenteropeptidase gene are the molecular cause of congenital enteropeptidase deficiency. *Am. J. Hum. Genet.* 70(1), 20–25 (2002).

35 Astrup A. The satiating power of protein – a key to obesity prevention? *Am. J. Clin. Nutr.* 82(1), 1–2 (2005).

36 Westerterp-Plantenga MS, Nieuwenhuizen A, Tome D, Soenen S, Westerterp KR. Dietary protein, weight loss, and weight maintenance. *Annu. Rev. Nutr.* 29, 21–41 (2009).

■ Patents

101 OBE Therapy Biotechnology. WO/2009/071601 (2008).

102 OBE Therapy Biotechnology. WO/2006/050999 (2006).