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Effects of obesity and weight loss on mitochondrial structure and function and implications for colorectal cancer risk

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Colorectal cancer (CRC) is the third most common cancer globally. CRC risk is increased by obesity, and by its lifestyle determinants notably physical inactivity and poor nutrition. Obesity results in increased inflammation and oxidative stress which cause genomic damage and contribute to mitochondrial dysregulation and CRC risk. The mitochondrial dysfunction associated with obesity includes abnormal mitochondrial size, morphology and reduced autophagy, mitochondrial biogenesis and expression of key mitochondrial regulators. Although there is strong evidence that increased adiposity increases CRC risk, evidence for the effects of intentional weight loss on CRC risk is much more limited. In model systems, energy depletion leads to enhanced mitochondrial integrity, capacity, function and biogenesis but the effects of obesity and weight loss on mitochondria in the human colon are not known. We are using weight loss following bariatric surgery to investigate the effects of altered adiposity on mitochondrial structure and function in human colonocytes. In summary, there is strong and consistent evidence in model systems and more limited evidence in human subjects that over-feeding and/or obesity result in mitochondrial dysfunction and that weight loss might mitigate or reverse some of these effects.

Obesity: Colorectal cancer: Mitochondria: Bariatric surgery

Colorectal cancer prevalence

Colorectal cancer (CRC) is the third most common cancer worldwide with approximately 1·4 million cases diagnosed in 2012[1]. It is predicted that, by 2030, CRC will rise by 60 % and that there will be over 2·2 million new cases[2]. A qualitative analysis of fifty-six observational studies among 7 213 335 individuals and 93 812 CRC cancer cases demonstrated that increased BMI was linked with higher CRC risk[3]. Ning[3] and colleagues also showed that each 5 kg/m^2 unit rise in BMI increased CRC risk by 18 %. This association with BMI was stronger for colon than for rectal cancer and for males than for females[3]. Additionally, obesity is a major risk factor for colorectal adenomas[4], suggesting that higher adiposity is a key player at the early stages of colorectal tumorigenesis[5]. Ma[6] and Keum[7] confirmed a linear dose-dependent relationship between abdominal/visceral

Abbreviations: COX, cyclooxygenase; CRC, colorectal cancer; eNOS, endogenous nitric oxide synthase; mtDNA, mitochondrial DNA; PGC, PPARγ coactivator; ROS, reactive oxygen species; RYGB, Roux-en-Y gastric bypass; TGF, transforming growth factor.

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adiposity and risk of colorectal adenomas suggesting that excess body fatness in and around the visceral organs may explain the positive association observed between increased BMI, increased waist and hip circumference and risk of colorectal adenomas and CRC.

Biology of colorectal cancer development

Most CRC develops sporadically and only 15–30 % are due to inherited causes[8]. CRC result from unrepaired genomic damage to stem cells and their progeny located in the crypts of the colorectal mucosa. Both epigenetic modifications and gene mutations contribute to CRC development by activating oncogenic pathways and by inactivating tumour suppressor genes[9]. This genomic damage includes chromosomal defects, mutations in the nuclear and mitochondrial DNA and epigenetic abnormalities that lead to aberrant gene expression and uncontrolled growth of colonocytes. Through a Darwinian process, damage which provides the nascent tumour cell with a competitive advantage results in the development of cell clones with excessive proliferation and, therefore, neoplastic potential and leads to monocryptal adenomas or aberrant crypt foci. Crypt fission may expand such lesions resulting in the development of non-malignant growths known as adenomatous polyps[10]. With further genetic and epigenetic changes causing hyperplasia, some adenoma develops into malignant adenocarcinoma and some, eventually, metastasise[8]. Inactivating mutations in the tumour suppressor gene *APC* occur early in almost all CRC. Loss of adenomatous polyposis coli function results in aberrant expression of the WNT signalling pathway which contributes to increased cell proliferation and polyp development[11]. In addition, mutations in *KRAS* or *BRAF* occur in 55–60 % of CRC and the proto-oncogene, *KRAS* signals through *BRAF* to activate the mitogen-activated protein kinase pathway[11]. Further mutations in *KRAS* or *TP53*, or in genes regulating key pathways such as the transforming growth factor-β (TGF-β1) signalling pathway, mediate the transformation from polyps to cancer[12–14]. Approximately 30 % of CRC have mutations in the gene encoding the type 2 receptor for TGF-β (*TGFBR2*)[15,16]. Furthermore, other mutated TGF-β signalling pathway members including TSP1, RUNX3, SMAD2 and SMAD4 have been identified in CRC[16–20]. Overall, the most frequently mutated genes in signalling pathways are found in the RAS-RAF-mitogen-activated protein kinase, WNT-APC-CTNNB1, PI3 K and TGFβ1-SMAD pathways[21,22].

Risk factors for colorectal cancer and role of obesity

CRC risk increases with age and is modified by lifestyle factors including physical activity, diet, smoking and obesity which influence the acquisition and repair of genomic damage[23,24]. Obesity, inflammation and CRC risk are inter-linked closely[5]. In obesity, a range of pro-inflammatory cytokines and signalling molecules are secreted resulting in systemic low-level inflammation and increased reactive oxygen species (ROS), that accelerate genomic damage[25,26]. With increasing adiposity, leptin concentrations increase[27]. This leads to higher TNF-α, IL-6 and IL-12 production and the accumulation of pro-inflammatory macrophages[27]. Wei and colleagues[28] reported elevated plasma C-reactive protein, TNF-α and IL-6 concentrations in obese individuals, linked with impaired glucose tolerance, insulin resistance, abnormally high concentrations of insulin and insulin-like growth factor 1, and low concentrations of insulin-like growth factor binding proteins, all of which may increase CRC risk. More studies reported that plasma C-reactive protein concentrations are correlated positively with CRC risk[29–31]. Faecal calprotectin concentration (a marker of mucosal inflammation) is positively correlated with obesity and inversely correlated with fibre, fruit and vegetable consumption[32]. This obesity-derived inflammation initiates a mucosal signalling cascade which involves activation of the transcription factor NF-κB and higher expression of both inducible nitric oxide synthase and cyclooxygenase-2 (COX-2)[33]. This altered signalling may play a key role in the suppression of apoptosis, which is a key feature of tumorigenesis[34].

Effects of weight loss on colorectal cancer risk and on biomarkers of colorectal cancer risk following lifestyle-based interventions

A systematic review and meta-analysis investigating the effects of weight change on CRC risk in thirteen studies, found that weight gain was associated with increased CRC risk but that there was no association with weight loss[35]. However, weight loss resulting from lifestyle-based interventions affects biomarkers of CRC risk including expression of inflammatory markers and cell proliferation. In the INTERCEPT Study, 14 % weight loss via an 8-week low-energy liquid diet, in twenty obese adults resulted in reduced Ki-67 expression (a marker of cell proliferation) in the colorectal mucosa and improvements in insulin sensitivity; higher insulin resistance is a potential mechanism underlying the effects of obesity on CRC risk[36]. Nicklas and colleagues[37] reported that weight loss after a low-energy diet reduced plasma concentrations of pro-inflammatory markers including C-reactive protein, TNF-α and IL-6 in obese older people (60+ years). Similarly, a low-energy diet in obese middle-aged women resulted in decreased expression of IL-6 and TNF-α in plasma and in subcutaneous adipose tissue[38]. Although changes in inflammatory markers in plasma and adipose tissue may reflect changes in other tissues, measurements made in colorectal tissue *per se* are more directly relevant. Weight loss (mean 10·1 % of initial body weight) resulting from a very-low-energy diet decreased expression of inflammatory markers including TNF-α, IL-1β, IL-8, monocyte chemotactic protein 1 and of the proto-oncogenes *JUN* and *FOS* in the colorectal mucosa of obese pre-menopausal women[39]. In addition, working with participants in a community-based weight loss programme

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(Slimming World), Kant and colleagues[40] observed lower concentrations of faecal calprotectin (a marker of intestinal inflammation which is increased in colorectal disorders including CRC) only in those participants with a high faecal calprotectin concentration (>50 μg/g) at baseline. Although weight loss in the studies discussed earlier was relatively modest (typically 5–10 %), this was sufficient to lower both systemic and tissue-specific markers of inflammation.

Effects of weight loss following bariatric surgery on colorectal cancer

A systematic review and meta-analysis of studies reporting on 24 321 bariatric surgery patients and 80 866 obese controls found that weight loss induced by bariatric surgery was associated with 27 % reduced CRC risk[41]. In an English cohort study involving more than 1 million obese participants, bariatric surgery did not alter CRC risk but, in this study, the number of participants who underwent bariatric surgery and the number of CRC cases were small (3·9 % of participants underwent bariatric surgery and only 0·1 % of the surgery group developed CRC)[42]. Similarly, investigations of effects of surgically-induced weight loss on biomarkers of CRC risk have yielded conflicting results. In our recent study, at 6 months post-bariatric surgery (mean 29 kg weight loss), markers of systemic and colorectal mucosal inflammation were reduced, glucose homeostasis was improved and crypt cell proliferation was reduced[43]. In contrast, an earlier study found that after bariatric surgery which lowered BMI by 12·6 units, there was increased expression of the pro-inflammatory genes *COX-1* and *COX-2,* decreased apoptosis and increased mitosis in the mucosal crypts[44]. In addition, this increased crypt cell proliferation and greater expression of pro-tumourigenic cytokines persisted until at least 3 years post-surgery in these obese patients who underwent Roux-en-Y gastric bypass (RYGB) one of the most common types of bariatric surgery[45]. Differential effects of weight loss following bariatric surgery on CRC-related biomarkers may be due to subtle differences in the nature of the surgical procedures used[5,43]. For example, we hypothesised that the apparently detrimental effects of the specific type of bariatric surgery reported by the Leeds group[44,45], may be due to greater small bowel malabsorption and, consequently, exposure of the large bowel mucosa to luminal agents such as secondary bile acids that can damage the colorectal mucosa and are associated with increased CRC risk[43]. The most likely reason for lack of evidence for effects of weight loss on CRC risk is the short duration of most relevant studies. In addition, such weight loss studies tend to have a relatively low sample size, the amount of weight loss is modest and weight loss is not usually sustained in the long term. The most convincing evidence is likely to come from long-term follow-up of those who have undergone bariatric surgery because this produces substantial and sustained weight loss.

Mitochondrial structure and function

Mitochondria are eukaryotic organelles residing in the cytosol that are involved in numerous metabolic pathways including intracellular calcium signalling, iron-sulphur cluster biogenesis, apoptosis and maintenance of membrane potential and with their primary function being ATP production via oxidative phosphorylation[46,47].

Every mitochondrion contains multiple copies of a double-stranded closed-circular mitochondrial DNA genome (mtDNA) which are present within the mitochondrial matrix and which are maternally inherited[48]. The mtDNA consists of 16 569 Bp forming an inner light (L; cytosine rich) and an outer heavy (H; guanine-rich) strand encoding a total of thirty-seven genes[48] which include twenty-two tRNA, thirteen proteins of the respiratory chain and two rRNA specific to the mitochondria which are required for mtDNA gene translation[49]. The mtDNA is wrapped together with proteins into mitochondrial nucleoids and every nucleoid comprises one or two mtDNA molecules[48]. This packaging of mtDNA into DNA-protein assemblies (nucleoids) provides an efficient means of ensuring that the mitochondrial genetic material is distributed throughout the mitochondrion and for coordinating mtDNA involvement in cellular metabolism[50]. There are five mitochondrial respiratory chain complexes[51] and, in human subjects, mtDNA encodes the following structural subunits of the mitochondrial respiratory chain: NADH dehydrogenase 1 (MTND1)–MTND6 and MTND4L (complex I), cytochrome b (MTCYB) (complex III), cytochrome c oxidase I (MTCO1) – MTCO3 (complex IV), ATP synthase 6 (MTATP6) and MTATP8 (complex V)[52]. The mitochondrial genome also harbours the non-coding D-loop, which contains the promoters for H and L strand transcription[53]. The majority of mitochondrial polypeptides required for the structure and function of the mitochondria are transcribed from nuclear genes and translated in the cytosol prior to their transport across the mitochondrial membrane[54]. Hence, mitochondrial function depends on both of these genetic systems[54].

Energy metabolism in the mitochondrion and links with mitochondrial DNA damage

Since each cell contains multiple mtDNA copies, mutations can affect either all mtDNA molecules (homoplasmy) or only a proportion (heteroplasmy) of the mtDNA in a given cell[54]. The level of heteroplasmy can vary from 1 % to 99 % between cells in the same organ or tissue, across various organs and tissues in the same individual and between people in the same family[55]. Mitochondrial DNA mutations include single, large-scale deletions (these are rarely inherited and never homoplasmic), point mutations (these are usually maternally inherited) and acquired somatic mutations and are predominantly due to replication errors and ageing[56]. However it has also been shown that they can be generated because of environmental exposures

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such as bacteria and viruses[57] ultraviolet light[58] and tobacco[59,60,47]. A homoplasmic pathogenic mtDNA point mutation usually results in a relatively mild biochemical defect often affecting only one tissue or organ although exceptions have been reported[56]. In contrast, a heteroplasmic mutation may affect multiple organs and the level of heteroplasmy correlates with the extent of organ involvement and degree of severity of the clinical phenotype (with the biochemical defect usually being severe in affected tissues)[56]. The proportion of heteroplasmic mtDNA mutations has to surpass a critical threshold level, typically 60–80 %, before the biochemical defect can be detected[61,62].

Glycolysis and β-oxidation of fatty acids each take place within the cytoplasm but most of the generation of ATP from catabolism of dietary carbohydrates and fats takes place when the common intermediate acetyl CoA enters the mitochondrion and undergoes the citric acid cycle and oxidative phosphorylation. ROS are produced as a by-product of reactions involving the electron transport chain, with complex I and complex III being the major sites of ROS production[63,64]. ROS react with all the macromolecules in the cell including lipids, proteins and nucleic acids and these reactions can lead to reversible or irreversible oxidative modifications of these macromolecules and, subsequently, to cell and organ dysfunction[64]. In addition, ROS production as a result of other dietary and environmental exposures (e.g. alcohol and tobacco use) can cause the development of mtDNA adducts as well as adducts in the nuclear genome via covalent binding of polycyclic aromatic compounds to the DNA. Since DNA repair mechanisms are much less effective in the mitochondrion than in the nuclear genome[65], ROS may have more adverse effects on the mitochondrial genome and may drive disease development[61,66,67].

Mitochondria and colorectal cancer

During malignancy a shift to glycolysis from oxidative metabolism, known as the Warburg Effect, occurs[68]. A recent review argues that the functions of the Warburg Effect for malignancy and tumour cell proliferation remain unknown and evidence on the role of the Warburg Effect in cancer is equivocal[69]. Mouse studies have shown mtDNA mutations in tumour and metastatic tissue. Eukaryotic cells containing the nucleus from one species and the cytoplasm from both the parental species are called cybrids[70]. Cybrids with or without a homoplasmic pathogenic point mutation at nucleotide position 8993 or 9176 in the *MTATP6* gene were transplanted into mice[70]. Mutations in *MTATP6* conferred an advantage during cancer development[70]. A later mouse study also using cybrid technology showed an acquired metastatic potential after mtDNA mutations in the gene encoding NADH were transferred[71]. Somatic mtDNA mutations occur frequently in human CRC and these may contribute to oncogenesis or metastatic spread[72,73]. We observed that older people have higher frequencies of somatic mutations in the mitochondrial genome; however it is unknown whether this increased mitochondrial mutation load may contribute to the age-related CRC risk[74]. When present at high levels, such mutations compromise mtDNA encoded respiratory chain subunits and cytochrome c oxidase activity which causes mitochondrial dysfunction and may be a biomarker of damage[75].

Effects of obesity on mitochondrial function

There is evidence from model systems as well as from direct experimentation in human subjects that obesity, or over-feeding, leads to mitochondrial dysfunction.

In vitro *studies*

A few studies have investigated the effects of overfeeding and/or obesity on mitochondrial structure and function *in vitro*. For example, treating differentiated 3T3-L1 adipocytes for 48 h with high glucose, high NEFA, or high glucose plus high NEFA resulted in abnormal mitochondrial size, morphology and biogenesis[64]. These treatments led to the loss of mitochondrial membrane potential, reduced intra-mitochondrial calcium concentration, lower concentrations of mitofusion protein Mfn1 and increased mitofission protein Drp1[64]. In addition, the high glucose and high glucose plus high NEFA treatments downregulated expression of *NRF1, PGC-1α* and *mtTFA* at the mRNA level and reduced PPARγ coactivator (PGC)-1β concentration, all of which are important factors in mitochondrial biogenesis[64]. Such treatments attempt to mimic the causes (or consequences) of obesity and demonstrate reduced mitochondrial size, morphology and biogenesis.

Studies in animal models

To date, the effects of over-feeding and/or obesity on mitochondrial structure and function appear to have been little studied in non-mammalian animal models. However, feeding a high sucrose diet to Drosophila induced obesity and caused mitochondrial dysfunction in the ovary[76]. This was observed as increased ovarian mtDNA copy number and reduced expression of key mitochondrial regulators including *cytochrome c oxidase I*, *mtTFB1*, *Parkin* and Drosophila homologs of *PGC-1α* and *NRF-2α*[76].

A high-fat diet (21 d) led to reduced expression of *PGC-1α* and *PCG-1β* mRNA, reduced PGC-1α and cytochrome c protein concentrations and downregulation of genes encoding oxidative phosphorylation proteins including complex I–IV in mouse muscle[77]. Later studies of high-fat feeding also observed mitochondrial dysfunction in skeletal muscle[78,79] and impaired expression of genes encoding for mitochondrial biogenesis in the rat liver[80]. Diet-induced obesity led to reduced mitochondrial mass and function, increased mitochondrial fission rates in rat liver and skeletal muscle, as well as decreased expression of the *OPA1* gene and decreased *Mfn2* expression which may contribute to mitochondrial dysfunction during obesity[81]. Significantly lower endogenous nitric oxide synthase (eNOS) mRNA and protein concentrations were found in white adipose tissue

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of obese mice, obese Zucker rats and high-fat diet-induced mice, and in brown adipose tissue of obese mice and obese zucker rats when compared with controls[82]. This downregulation of eNOS was accompanied by lower mtDNA content, and reduced mitochondrial proteins involved in cell respiration including COX IV and cytochrome c, and regulators of mitochondrial biogenesis, including PGC-1α, nuclear respiratory factor-1 (NRF1) and mitochondrial transcription factor A (Tfam) in white and brown adipose tissue of obese rodents[82]. TNF-α downregulates eNOS and it was suggested that this affects mitochondrial biogenesis[82]. A high-fat diet also led to reduced complex IV, cytochrome c, HSP60, CORE I, PGC-1α and mtDNA copy number in adipose tissue mitochondria in male rats[83]. There is strong evidence that feeding a high-fat diet results in reduced mitochondrial function and biogenesis in multiple organs and tissues in rodents evidenced by reduced mtDNA content, PGC-1α concentrations, and expression of cell respiration proteins namely cytochrome c and complex IV.

Obesity causes metabolic disturbances such as insulin resistance and subcellular low-grade inflammation and results in increased oxidative stress and mitochondrial dysfunction in mice suffering from cardiomyopathy[84,85]. In a comparison of the expression of mitochondrial proteins in the liver, muscle and adipocytes of normal, obese and diabetic mice, only diabetic mice revealed low concentrations of ATP synthase α and β, complex II and complex III in adipocytes. Additionally, abnormal mitochondrial morphology, reduced mtDNA content, β-oxidation and respiration rates were seen in obese and diabetic mouse adipocytes suggesting an important mitochondrial dysfunction[86]. Skeletal muscle of obese mice showed impaired mitochondrial dynamic behaviour via increased fission (increased Fis1 and Drp1 protein concentrations) and reduced fusion (reduced Mfn1 and Mfn2 protein concentrations), reduced mitochondrial respiratory capacity and low ATP content[87].

Effects of over-feeding and of obesity on mitochondrial structure and function in human subjects

In addition to evidence from *in vitro* and animal model studies, human studies have demonstrated mitochondrial defects in obesity or when feeding a high-fat diet. These studies are summarised in Table 1 and are discussed in more detail below.

Short-term (3-d) over-feeding with a high-fat diet in healthy men resulted in reduced expression of *PGC-1α* and *PCG-1β* mRNA, reduced PGC-1α and cytochrome c protein concentrations and downregulation of genes encoding oxidative phosphorylation proteins including complex I-IV in vastus lateralis and gastrocnemius muscle[77]. Given the short duration of the intervention, it is not possible to determine whether the observed effects on biomarkers of mitochondrial function are due to changes in adiposity, as distinct from changes in macronutrient intake. Other studies showed that excess nutrient intake may lead to reduced mitochondrial size and number and to reduced oxidative phosphorylation in ectopic brown adipose tissue[93].

Obese individuals showed reduced expression of genes encoding oxidative phosphorylation proteins and reduced oxygen consumption, indicative of a reduction in mitochondrial function[93]. Yin and colleagues[89] observed reduced mitochondrial oxidative activity in adipocytes of obese individuals, which may be due to overall adiposity instead of adipocyte hypertrophy. Another study revealed that subcutaneous adipose tissue of obese twins had lower mtDNA content, that ninety-six out of 130 CpG sites of mitochondria-related transcripts and upstream regulators were hypermethylated and reduced mtDNA-encoded transcripts (12S rRNA, 16S rRNA, COX1, ND5, CYTB) and OXPHOS subunit proteins (complex III-V)[90]. More recently, Kras[92] found that obesity resulted in seventy-three and forty-one differentially expressed proteins in subsarcolemmal and intermyofibrillar mitochondria respectively in skeletal muscle of seventeen obese individuals. Kras[92] observed that proteins making the TCA cycle and complex II were increased, whereas proteins forming ATP synthase and complex I and III were decreased in intermyofibrillar mitochondria of the obese. In obese compared with lean people, mitochondrial network, shape and number differed in adipose-derived stromal stem cells[91]. In addition, *TBX15* (a negative regulator of mitochondrial mass) was hypomethylated and TBX15 protein concentration was higher in cells from the obese individuals[91].

In summary, there is strong and consistent evidence that over-feeding and/or obesity result in mitochondrial dysfunction in model systems as well as in human subjects. Downregulated PGC-1α has been reported consistently *in vitro*, animal model and human studies. Reduced mitochondrial content and reduced expression of complex IV and cytochrome c are prominent in animal and human studies, whereas effects on other outcomes such as mitochondrial protein and enzyme concentrations, and on β-oxidation are less consistent.

The potential mechanisms underlying the effects of obesity on mitochondrial dysfunction

Evidence for potential mechanisms underlying the effects of obesity on mitochondrial dysfunction comes largely from *in vitro* and animal studies with much more limited data from human studies. In addition, this mechanistic work has been undertaken in several different cell types and tissues, with relatively little research undertaken in colonocytes.

A recent review summarised evidence showing that obesity leads to reduced β-oxidation and to mitochondrial dysfunction through excess ROS, oxidative stress and an obesity-induced inflammatory response in tissues such as muscle, liver and adipose tissue[94,95]. Rogge[94] found that, during these processes, impaired mitochondria may initiate a vicious cycle of reduced mtDNA content, mitochondrial biogenesis and β-oxidation. Impaired β-oxidation results in increased TAG synthesis and ectopic deposits of lipids, which can lead to impaired

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Table 1. Effects of obesity on mitochondrial structure and function in human subjects

Study	Tissue	Investigation	Key findings
[88]	Adipocytes	Obesity	Reduced PGC-1α concentration
[77]	Male vastus lateralis and gastrocnemius muscle	High-fat diet	No changes in mtDNA content, Tfam, or NRF1 Reduced concentrations of PGC-1α mRNA, lower activity of cytochrome C oxidase and Citrate synthase
[89]	Adipocytes	Obesity	Reduced mtDNA content, oxygen consumption and citrate synthase activity
[90]	Subcutaneous adipocytes	Obesity	Reduced mtDNA content, ninety-six out of 130 CpG sites of mitochondria-related transcripts and upstream regulators were hypermethylated, reduced mtDNA-encoded transcripts (12S rRNA, 16S rRNA, COX1, ND5, CYTB) and OXPHOS subunit proteins (complex III-IV)
[91]	Adipose-derived stromal stem cells	Obesity	Altered DNA methylation: *TBX15* was one of the most differentially hypomethylated genes
[92]	Skeletal muscle	Obesity	Increased expression of proteins of the TCA cycle and complex II and, decreased expression of proteins forming ATP synthase and complexes I and III

PGC, PPARγ coactivator; Tfam, mitochondrial transcription factor A; NRF, nuclear respiratory factor; ND, NADH:ubiquinone oxidoreductase core subunit 5; CYTB, cytochrome b; mtDNA, mitochondrial DNA.

cellular functions and oxidative stress via increased ceramide formation, increased lipid peroxidation by-products, increased nitric oxide synthase concentrations, greater inflammatory cytokine production and excess ROS[94]. Excess ROS including superoxide anions, perioxynitrite, hydroxyl radicals and hydrogen peroxide can damage lipids in membranes, proteins (especially OXPHOS enzymes) and nuclear and mitochondrial nucleic acids leading to further cellular damage[94]. When fatty acids accumulate in the cytosol, β- and ω-oxidation are activated in peroxisomes and microsomes, respectively[94]. Ω-oxidation can damage mitochondria through uncoupling oxidative phosphorylation and disrupting the mitochondrial membrane proton gradient leading to loss of ATP production[94]. Furthermore, in obesity, the mitochondria are overloaded with glucose and fatty acids, which increases acetyl-CoA production and, in turn, results in high NADH concentrations produced by the Krebs cycle[95]. As a consequence, this increases electron availability to the mitochondrial respiratory chain complexes and increases ROS production which activates transcription factors e.g. NFκB that regulate the inflammatory response[95]. The earlier evidence demonstrated that obesity reduces mitochondrial number, biogenesis and respiratory capacity which results in mitochondrial dysfunction[95].

Effects of weight loss on mitochondrial structure and function

There is evidence from animal models as well as from direct investigations in human subjects that weight loss and/or nutrient and energy restriction leads to enhanced mitochondrial integrity, capacity, function and biogenesis.

Studies in animal models

Effects of dietary energy restriction. Animal studies have investigated the effects of dietary energy (caloric) restriction on mitochondrial function. Zid and colleagues[96] revealed that energy restriction in Drosophila potentiated mitochondrial activity by increasing ribosomal loading of genes encoding complex I and IV of the respiratory transport chain. Energy restriction resulted in increased mitochondrial biogenesis, fusion, increased ATP production and increased expression of mRNA of *NRF1, TFAM, COX4, Cyt C, MFN1, MFN2, eNOS* and *PGC-1α* in various tissues of male mice[97]. Raffaello and Rizzuto[98] demonstrated that many signalling pathways are involved in the expression of genes involved in the stress response; for example genes which reduce mitochondrial ROS production and promote mitochondrial activity and function. Energy restriction downregulated the insulin-like growth factor-1 signalling pathway and induced transcription of the mitochondrial antioxidant gene *SOD2*[98]. Energy restriction can activate the SIRT1 and/or AMPK signalling pathway(s) which consequently increase PGC-1α concentrations[98]. Other studies also found that energy restriction in aged animals activated PCG-1α which, subsequently, activated AMP-activated protein kinase and sirtuins; this improved mitochondrial integrity, biogenesis and reduced mitochondrial-derived ROS and damage[99–101]. Overall, there is consistent evidence that dietary energy restriction improves mitochondrial function via reduced oxidative stress and that this results in the activation of PGC-1α and AMP kinase. Evidence suggests that metabolic inputs tightly regulate mitochondrial fusion and fission rates which can improve mitochondrial function[102]. Nutrient starvation leads to reduced fission rates, by triggering protein Kinase A mediated phosphorylation of Drp1 (a mediator of mitochondrial fission) in mouse embryonic fibroblasts[103]. Additionally, nutrient depletion leads to interconnection and elongation of mitochondria through downregulation of Drp1.[103]. This increased mitochondrial network, as a result of nutrient depletion, protects against autophagosomal degradation[103]. Lee and colleagues[102,104] found that glucose restriction in mouse skeletal muscle deacetylates and activates Mfn1, a mitofusin implicated in the regulation of

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mitochondrial morphology and, subsequently leads to mitochondrial fusion which serves as a protection against oxidative stress. McKiernan[105] found no differences in mitochondrial electron transport enzyme abnormalities in skeletal muscle between energy restricted and control rhesus monkeys, but reported large mtDNA deletions (which removed a large proportion of the genome of at least one of the three mitochondrial encoded COX subunits) in fibres with abnormal mitochondrial enzyme activities. In animal models, there is consistent evidence that nutrient and energy depletion improves mitochondrial function through reduced fission and increased mitochondrial fusion rates as a result of downregulated Drp1 and upregulated Mfn1, respectively. However, there is a lack of evidence on the effects of nutrient and energy depletion on mtDNA content, β-oxidation and expression of mitochondrial proteins and enzymes (encoded by the nuclear or mitochondrial genome).

Effects of weight loss on mitochondrial structure and function in human subjects

In addition to evidence from animal model studies, human studies have demonstrated improved mitochondrial structure and function after energy depletion and weight loss. These studies are summarised in Table 2 and discussed in more detail later.

The effects of weight loss via diet and/or exercise. In thirty-six young overweight subjects, induction of negative energy balance by 25 % (through dietary energy restriction, or dietary restriction plus increase in energy expenditure through exercise) resulted in increased expression of genes involved in mitochondrial function (*PPARGC1A, TFAM*, *eNOS, SIRT1* and *PARL*), and increased mtDNA content but had no effect on mitochondrial enzyme activity (citrate synthase (for TCA cycle), β-hydroxyacyl-CoA dehydrogenase (for β-oxidation) and cytochrome c oxidase II (for the electron transport chain)) in muscle[107] Improvement in aerobic capacity, mitochondrial content and reduced mitochondrial size in skeletal muscle were observed in a diet plus exercise intervention (mean 8·5 kg weight loss achieved) but not in a diet alone weight loss intervention (mean 10·6 kg weight loss achieved)[108]. The larger effects of the combination of diet and exercise on mitochondria might not be due to weight loss *per se* but because exercise has independent, and synergistic, effects on mitochondria to those of dietary energy reduction alone[108].

The effects of weight loss following bariatric surgery. Expression of Mfn2, which is an essential mitochondrial fusion protein and contributes to mitochondrial network integrity, was reduced in skeletal muscle of obese individuals. However, 2 years after bilio-pancreatic diversion surgery which caused a 25 kg/m^2 unit fall in BMI (resulting in mean 31 kg/m^2 BMI). Mfn2 expression was increased significantly suggesting that Mfn2 expression is inversely proportional to body weight[106]. A study involving 101 RYGB patients allocated either to an exercise or health education control intervention, found at 6 months follow-up that a mean 23·6 kg weight loss by RYGB in addition to the exercise intervention enhanced mitochondrial respiration in vastus lateralis muscle tissue. Although the RYGB plus health education achieved similar weight loss (mean 22·1 kg) to the RYGB plus exercise intervention it did not alter mitochondrial respiration. Neither intervention arm showed a change in OXPHOS content and all patients remained obese (mean 30·4 kg/m^2 BMI) at follow-up[109]; it is unclear if the effects were due to weight loss *per se*. Fernstrom[112] reported that 6 months after RYGB, mean weight loss in eleven obese females was 25·5 kg and resulted in increased coupled respiration in vastus lateralis muscle. However, there were no effects on respiratory control index (a quality measure of isolated mitochondria) and uncoupled respiration (oxygen consumption without ADP phosphorylation), and although patients achieved significant weight loss they remained overweight post-operatively with a mean BMI of 29·6 kg/m^2. These studies show that sustained and significant weight loss following bariatric surgery results in increased mitochondrial fusion protein Mfn2 and enhanced mitochondrial (coupled) respiration in muscle tissue[106–112].

Jahansouz[110] investigated the short-term (7·5 d) effect of RYGB (*n* 8) and adjustable gastric banding (*n* 8). Although at this stage weight loss was small and non-significant (mean 0·9 kg/m^2 unit fall in BMI), expression of *PGC-1* α, *NRF1, Cyt C, Tfam* and *eNOS* were increased. Expression of these genes is associated with mitochondrial biogenesis, and protein carbonylation, a marker of oxidative stress, which was lower in the adipose tissue. These effects were evident after RYGB but adjustable gastric banding had no effect. Following bariatric surgery a rapid improvement in glycemic control occurs, prior to weight reduction, suggesting that the observed changes in gene expression might be due to metabolic changes linked to bariatric surgery, rather than to weight loss *per se*. Obese women (*n* 18) were allocated to a normoglycemic group and to an insulin resistant group before they underwent bariatric surgery. Subsequent investigations in subcutaneous adipose tissue 13 months post-operatively revealed that the normoglycemic group (14·2 kg/m^2 unit fall in BMI) showed decreases in mitofilin and PGC-1α concentrations, whereas the insulin resistant group (17·5 kg/m^2 unit fall in BMI) had changes in the opposite direction for mitofilin and PGC-1α concentrations[111]. This suggests that the effects of surgically induced weight loss on mitochondrial function may depend on initial metabolic status[111]. In nineteen obese patients, individuals who achieved mean 33 % weight loss at 1-year post-RYGB had smaller adipocytes which were richer in mitochondria[113]. Significant and sustained weight loss following bariatric surgery results in an increased number of mitochondria, upregulated gene expression (coding for mitochondrial biogenesis, function and dynamic) and reduced oxidative stress[110–114].

To date, the studies investigating the effect of weight loss by bariatric surgery have focused on effects in muscle and adipose tissue only and more studies in other tissues are warranted. These studies varied in duration of follow-up (7·5 d to 13 months), type of bariatric surgery procedure and weight and BMI loss achieved

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Table 2. Effects of weight loss on mitochondrial structure and function in obese human subjects

Study	Tissue	Weight-loss intervention	Key findings
[106]	Skeletal muscle	Bilio-pancreatic diversion	Increased Mfn2 expression
[107]	Muscle	Dietary energy restriction with/without increased physical activity	Increased expression of *PARGC1A, TFAM, eNOS, SIRT1* and *PARL* and increased mtDNA content
[108]	Skeletal muscle	Dietary energy restriction with/without increased physical activity	No change in diet-only group Increased mtDNA and NADH-oxidase activity, improvement in aerobic capacity and mitochondrial content in the diet plus exercise group
[109]	Vastus lateralis muscle	RYGB with exercise intervention or health education	Increased OXPHOS proteins, NADH oxidase, citrate synthase, creatine kinase and cardiolipin in the RYGB with exercise intervention
[110]	Subcutaneous adipose tissue	RYGB	Improved mitochondrial biogenesis via increased concentrations of *PGC-1α, NRF1, Cyt C, Tfam* and *eNOS;* reduced protein carbonylation
[111]	Subcutaneous adipose tissue	Bariatric surgery	No effect in normoglycemic women, increased *PGC-1α* and reduced mitofilin in initially insulin-resistant women
[112]	Vastus lateralis muscle	RYGB	Increased coupled and uncoupled respiration, oxidative phosphorylation ratio and citrate synthase activity
[113]	Muscle and adipose tissue	RYGB	Adipocytes became smaller and richer in mitochondria

(11·6 kg–25·5 kg and BMI 0·9–25 kg/m^2, respectively) and these differences in study design may explain the lack of consistent results on mitochondrial structure and function. One important limitation of all studies discussed earlier is that the patients remained overweight and/or obese post-surgery and there is a lack of evidence of effects of weight loss leading to a normal weight on these mitochondrial outcomes. Finally, physical activity/exercise seems to have additional benefits on mitochondrial outcomes beyond those of weight loss *per se*, but this is beyond the scope of the present paper and will not be discussed here.

There is strong and consistent evidence that weight loss by dietary intervention or bariatric surgery leads to an increase in fusion proteins and PGC-1α concentrations, and a reduction in oxidative stress in both animal and human studies. Expression of genes such as *Tfam* and *eNos* were increased after a diet and exercise intervention and RYGB in human subjects indicating improved mitochondrial capacity. Weight loss increased gene expression of proteins encoding the respiratory transport chain in animals and human subjects but evidence of effects on enzyme activity is lacking for both animal and human studies. More studies in females have investigated the effects of bariatric surgery and found increased mitochondrial respiration and differential mitochondrial gene expression leading to improved mitochondrial function. We are not aware of studies that have investigated the effect of weight loss on existing mitochondrial genomic damage. The evidence on increased mtDNA content after weight loss is limited in both animal and human. Overall, there is some evidence that weight loss results in improvements of mitochondrial structure and function but more studies are needed to confirm the limited findings to date.

The potential mechanisms underlying the effects of weight loss on mitochondrial function

Energy and nutrient restriction, either via fasting, dietary energy restriction or increased physical activity, increases cAMP concentration and AMP:ATP ratio which triggers the PKA/CREB, SIRT1 and AMPK signalling pathways and, in turn, activates PGC-1α[115,116]. PGC-1α is the key regulator of mitochondrial biogenesis which activates downstream targets including Tfam, Nrf1 and Nrf2, resulting in upregulation of mitochondrial activity and biogenesis[116].

Effect of weight loss on mitochondrial structure and function in the human colon

Animal and human studies provide evidence of causal links between increased adiposity and mitochondrial dysfunction. In addition, weight loss in those who are overweight or obese results in enhanced mitochondrial structure and function in various tissues, particularly skeletal muscle and adipose tissue. However, few studies have investigated the effects of obesity on mitochondrial function in the colon; all of these have been *in vitro* or in animal models and we are unaware of any published evidence on the effects of obesity or of weight loss on mitochondrial structure and function in the human colon.

For example, in a rat model of diet-induced obesity, two groups, rats from the lowest and highest quartile of obese body weight, were selected. Principal component analysis showed that increased adiposity in the group with the highest body weight was associated with twenty-seven out of the sixty-nine colon mitochondrial-associated proteins in colon tissue. Over half of these proteins were downregulated suggesting reduced ATP production, protein transport and folding and, increased oxidative stress during obesity; however, these changes in mitochondrial-associated proteins were not correlated with their corresponding gene expression in the colon in response to increased adiposity[117]. To verify if obesity contributes to increased CRC risk by causing mitochondrial dysfunction and reducing OXPHOS gene expression, Nimri[118] exposed MC38 and CT26 mouse colon cancer cells to conditioned media obtained from adipose tissue of mice that were fed a high-fat diet. Nimri[118]

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found a reduced oxygen consumption rate and a downregulation of mitochondrial gene expression mediated by the JNK/STAT-3-signalling pathway. In human HCT116 colon cancer cells, those exposed to media from cultured human visceral adipose tissue fragments of obese individuals had reduced expression of mitochondrial respiratory chain complexes e.g. *COX1, COX2, COX4* and *SDHs* compared with those exposed to media from non-obese individuals. This supports the notion that media from obese individuals may induce mitochondrial dysfunction (reduced mitochondrial respiration and function) in HCT116 cells[119]. These findings suggest that mitochondria may play a role in obesity-induced colorectal tumorigenesis but more evidence is needed to confirm this hypothesis.

We are investigating the effect of obesity and of weight loss following bariatric surgery on mitochondrial function in the human colorectal mucosa. Using immunofluorescence, we have quantified expression of oxidative phosphorylation proteins, namely complex I and IV, in colonocytes from obese individuals before and 6 months after bariatric surgery when they had lost 27 kg body mass in comparison with matched non-obese controls[43].

Conclusion

CRC risk is increased by obesity and by its lifestyle determinants including physical inactivity, sedentary behaviour and poor diet. Mutations accumulate during ageing leading to mitochondrial dysfunction; however it is unknown whether these are more prevalent in obese individuals. There is limited evidence suggesting that weight loss may reduce CRC risk and enhance mitochondrial activity, integrity and biogenesis. Furthermore, most of the evidence is derived from animal studies and from other tissues such as adipose tissue or skeletal muscle. In conclusion, the role of obesity and weight loss (including surgically-induced weight loss) on mitochondrial structure and function in the human colon is currently unknown and warrants further investigation.

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Conflict of Interest

None.

Authorship

The concept for this manuscript was developed by S. P. B. and J. C. M., S. P. B. drafted the manuscript, J. C. M. edited the manuscript and the final version was agreed by all authors.

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