Obesity is linked to increased risk of colon cancer, currently the third most common cancer. Consequently rising levels of obesity worldwide are likely to significantly impact on obesity-related cancers in the decades to come. Understanding the molecular mechanisms whereby obesity increases colon cancer risk is thus a focus for research to inform strategies to prevent the increasing trend in obesity-related cancers. This review will consider research on deregulation of adipokine signalling, a consequence of altered adipokine hormone secretion from excess adipose tissue, with a focus on leptin, which has been studied extensively as a potential mediator of obesity-related colon cancer. Numerous investigations using colon cell lines \textit{in vitro}, \textit{in vivo} studies in rodents and investigations of colon cancer patients illuminate the complexity of the interactions of leptin with colon tissues via leptin receptors expressed by the colon epithelium. Although evidence indicates a role for leptin in proliferation of colon epithelial cells \textit{in vitro}, this has been contradicted by studies in rodent models. However, recent studies have indicated that leptin may influence inflammatory mediators linked with colon cancer and also promote cell growth dependent on genotype and is implicated in growth promotion of colon cancer cells. Studies in human cancer patients indicate that there may be different tumour sub-types with varying levels of leptin receptor expression, indicating the potential for leptin to induce variable responses in the different tumour types. These studies have provided insights into the complex interplay of adipokines with responsive tissues prone to obesity-related colon cancer. Deregulation of adipokine signalling via adipokine receptors located in the colon appears to be a significant factor in obesity-related colon cancer. Molecular profiling of colon tumours will be a useful tool in future strategies to characterise the influence that adipokines may have on tumour development and subsequent therapeutic intervention. Study of the molecular mechanisms linking obesity with cancer also supports recommendations to maintain a normal body weight to reduce the risk of colon cancer.

**Adipose: Colon cancer: Adipokine: Leptin: Cytokine**

**Obesity-related cancer**

Obesity levels have increased dramatically over the past decade and are predicted to continue to increase. By 2007, it was estimated that 30–80% of Europeans were overweight or obese\(^1\). Obesity is now considered to be a global epidemic. It was estimated in 2008 that around 1.5 billion people were overweight worldwide, with 500 million of these individuals being obese, and as many as 10% of children were thought to be obese\(^2\). This has led to intensive research in this area. However, despite this, the trend of rising obesity levels continues with the WHO predicting that about 800 million adults will be obese by 2015\(^3\). This situation is now well recognised as a major public health concern worldwide with a number of co-morbidities associated with being overweight or obese, such as CVD, hypertension, diabetes and liver disease\(^4\). However, there is now considerable epidemiological evidence that obesity

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\(^1\) European Commission, 2006

\(^2\) World Health Organization, 2008

\(^3\) World Health Organization, 2010

\(^4\) World Health Organization, 2011

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**Corresponding author:** Dr Janice E. Drew, fax +44 1224 716629, email j.drew@abdn.ac.uk
has pathophysiological effects that extend beyond these well-known co-morbidities to include a number of organ-specific cancers\(^4\,5\). In particular, the association with increased risk, development and progression of colon cancer is now well established\(^4\,8\).

The epidemiological evidence of links between obesity and cancer was officially endorsed in the last 10-year report from the World Cancer Research Fund and American Institute for Cancer Research\(^9\). The expert panel judged that the strength of evidence causally relating diet and lifestyle factors, such as body and abdominal fat with the risk of colorectal cancer was convincing\(^9\). This was further endorsed more recently by the World Cancer Research Fund and American Institute for Cancer Research Continuous Update Project Report on colon cancer\(^10\). The top three of ten recommendations within the last 10-year report to reduce cancer risk are all linked to obesity\(^9\). These include maintenance of a normal range of body weight, avoiding a sedentary lifestyle and limiting consumption of energy-dense foods and sugary drinks that promote weight gain\(^9\). However, despite establishing unequivocal epidemiological evidence of links between obesity and colon cancer, the mechanisms linking obesity and colon cancer remain elusive. In order to identify these mechanisms it is necessary to understand how obesity interacts at the molecular and cellular level.

The epidemiological evidence clearly indicates common factors linking obesity and colon cancer\(^9,5,11\). Both are associated with consumption of high-energy diets, a sedentary lifestyle, increased age and reduced consumption of fruit, vegetables and fibre\(^9,5,11\). All these factors influence adipose tissue, now firmly established as the body’s largest endocrine organ\(^12\). These factors have the potential to influence the production of adipose-derived hormones and cytokines from the adipose organ\(^13\,16\). Leptin and adiponectin are two of the most abundant and most investigated adipose-derived hormones. It is now clear that in addition to their traditional roles in energy homeostasis\(^17\), they are now implicated as potential mediators of the effects of obesity on colon cancer risk\(^18\,19\). Significantly, adipose-derived hormones and adipocytokines are also linked to inflammatory and immune responses\(^20\,21\). These are processes intimately linked with both obesity and colon cancer\(^22\,24\).

### Adipokine regulation of colon tissue

It has been established that the colon epithelium expresses both isoforms of the adiponectin receptor, ADIPOR1 and ADIPOR2\(^25\) and also leptin receptors\(^25\,26\). This provides support for the potential of adiponectin and leptin to influence regulation of cellular processes within the colon. This epithelial layer is where colon cancer originates\(^27\). This dynamic tissue layer is very tightly regulated to ensure a balance between proliferation, differentiation and apoptosis during the process of constant renewal of the colon epithelium via proliferating stem cells at the base of these crypts\(^27\). Disruption of these processes leads to uncontrolled proliferation, loss of apoptotic regulation and uncontrolled tumour growth\(^27\). Notably insulin receptors are also expressed by the colon epithelium\(^25\) indicating potential cross-talk between metabolic homeostasis, leptin, insulin and adiponectin signalling that are all deregulated in obese individuals.

The role of adipose-derived hormones in regulating the colon epithelium is currently a focus of investigations on links between obesity and colon cancer. Homeostatic regulation of this dynamic tissue layer is implied as well as signalling cross-talk between leptin, adiponectin and insulin signalling pathways. The receptors, expressed throughout normal colon epithelium\(^25\,26\), are, no doubt, responsive to leptin and adiponectin produced as a consequence of normal physiological responses to diet and adipose tissue levels. However, the impact of consuming excess energy and a Western-style diet, and the associated increases in adipose tissue levels have the potential to disrupt regulation of signalling in the colon epithelium as leptin increases\(^17\) and adiponectin levels fall\(^28\) with increased obesity. This has the potential to impact on colon carcinogenesis. The following sections address the implications of altered adipokine regulation of colon tissue associated with obesity and potential molecular mechanisms linked to increased risk, development and progression of colon cancer with a focus on the role of leptin.

### Mechanisms linking leptin to obesity-related colon cancer: in vitro colon epithelial cell line models

Prompted by reports of leptin receptor (both short- and long-form signalling variant) expression by various human epithelial colon cancer cell lines, HT-29, Caco-2, DLD-1, SW480, HCT116, LS174-T and LoVo\(^26\,29\,30\), studies were initiated to investigate molecular mechanisms linking obesity with colon cancer. It was established that leptin stimulation of cultured colon cell lines led to tyrosine phosphorylation of the leptin receptor\(^29\) and activation of major mitogenic signal transduction pathway elements, p42/44 mitogen-activated protein kinase\(^26\,30\), c-Jun N-terminal kinase mitogen-activated protein kinase\(^31\), Src/phosphoinositide 3-kinase/protein kinase B\(^30\,32\) and extracellular-signal-regulated kinase\(^30\,33\). Signal transduction mediated via tyrosine phosphorylation of the long-form signalling leptin receptor\(^29\), leading to the activation of signal transduction pathways\(^26\,32\,33\) possibly drives the observed leptin stimulation of cell proliferation and accompanying DNA synthesis in colon cancer cell lines, such as HT-29 and Caco-2\(^29\). Leptin is also reported to inhibit apoptosis in human colon cancer cells via processes linked to extracellular-signal-regulated kinase, p38 mitogen-activated protein kinase activation and nuclear translocation of NF-κB\(^30\,34\). Anti-apoptotic effects pertinent to colon cancer may also be linked to observed counteraction of Na-butyrate-induced apoptosis\(^35\).

Notably leptin stimulation of proliferation may be confined to colon cancer cells in vitro. Fenton et al.\(^36\) reported that normal colon epithelial cells, YAMC (Apc\(^+\)), isolated from an immortalised murine cell line model that mimics a variety of genetic mutation in cells that can represent stages of carcinogenesis\(^37\), exhibited reduced proliferation in response to leptin, concomitant with induction.
of apoptosis. Conversely, IMCE (Apc\textsuperscript{Min/+}), harbouring a mutation in Apc, a ‘gatekeeper’ gene linked to human colon cancer, exhibit increased proliferation and inhibition of apoptosis in response to leptin. YAMC (Apc\textsuperscript{+/+}) mimics normal colon epithelial cells while the mutation in the Apc tumour suppressor gene in IMCE (Apc\textsuperscript{Min/+}) mimics preneoplastic colon epithelial cells\textsuperscript{(37)}. This provides evidence that leptin stimulates proliferation of colon cells dependent on Apc genotype, to induce auto/paracrine signalling cascades of inflammatory mediators and growth factors\textsuperscript{(38,39)} that could potentially influence colon carcinogenesis \textit{in vivo}. This provokes speculation on the observed differential effects of leptin, inhibiting proliferation of pancreatic cancer cells, but stimulating proliferation of breast, prostate and oesophageal cell lines \textit{in vitro}\textsuperscript{(40)}. Links to leptin regulation of proliferation and apoptosis were further confirmed by studies using colon stem cell clones\textsuperscript{(41)}. This study also revealed that leptin counteracted the cytotoxic effects of the commonly applied colon cancer drug, 5-fluorouracil\textsuperscript{(41)} and provokes speculation on interactions of diet, adipose-derived hormones and cancer therapies.

**Mechanisms linking leptin to obesity-related colon cancer: \textit{in vivo} rodent models**

The proposed role of leptin as a growth factor in colon, stimulating proliferation of colon epithelium and inhibiting apoptosis \textit{in vitro} has been conflicted by several studies conducted in rodent models. Leptin failed to promote growth of colon cancer xenografts in nude mice and did not increase intestinal tumorigenesis in Apc\textsuperscript{Min/+} mice\textsuperscript{(29)}. Mutations in Apc predispose the Apc\textsuperscript{Min/+} mouse to development of tumours in the small intestine and colon\textsuperscript{(42)} via loss of regulation of Wnt signalling. Paradoxically, treatment of rats with leptin, following administration of the colon carcinogen, azoxymethane, reduced formation of azoxymethane-induced aberrant crypt foci (markers of pre-cancerous lesions) in rats\textsuperscript{(43)}. Despite an absence of leptin the mutant mouse strain, ob\textsuperscript{ob}, had increased sensitivity to two colon carcinogens, azoxymethane and N-methyl-nitrosourea\textsuperscript{(44)}. However, recent reports that leptin increases colon tumour growth in obesity subsequent to the initiation of colon cancer\textsuperscript{(45)} provide an illuminating insight on the role of leptin in obesity-related cancer. This study reported that obesity, as a consequence of high fat feeding or genetic mutations leading to deficiencies in leptin signalling, led to increased proliferative activity of normal colonic epithelium\textsuperscript{(45)}. The fact that both high fat feeding, previously reported to increase colon cell proliferation in association with the resulting increased plasma leptin\textsuperscript{(46)}, and genetic obesity in mice lacking leptin or functional leptin signalling, implies complex interactions between diet, the adipose tissue and regulation of colon tissue. However, azoxymethane-induced tumours grew more slowly in the leptin-deficient ob\textsuperscript{ob} and leptin-receptor-deficient db\textsuperscript{db} mice\textsuperscript{(45)}. This implies that leptin plays a role in tumour development and progression in addition to other factors associated with obesity. The increased leptin receptor expression in colon tumours of azoxymethane-treated mice is a potential link in the observed differences in tumour growth in mice with functional leptin signalling\textsuperscript{(45)}. Together with the finding that leptin is linked to the activation of Wnt signalling via the leptin receptor signal transducer and activator of transcription 3\textsuperscript{(45)} these results reveal a potential molecular mechanism for obesity-related colon cancer. Uchiyama \textit{et al}\textsuperscript{(47)} presented evidence recently that leptin receptor signalling is enhanced through signal transducer and activator of transcription 3 activation as human colorectal adenoma-tissue progresses to colon cancer. The study by Endo \textit{et al}\textsuperscript{(48)} also supports evidence that leptin stimulation of colon cell proliferation and inhibition of apoptosis is dependent on the Apc genotype\textsuperscript{(38,39)}. Teraoka \textit{et al}\textsuperscript{(48)} also demonstrated that an obese mouse model KK-A(y) that does have an intact leptin and leptin receptor has higher sensitivity to azoxymethane. However, caution must be applied in interpreting sensitivity to azoxymethane, as mouse strains do differ in sensitivity to this chemical dependent on liver metabolism and DNA repair mechanisms\textsuperscript{(49,50)}.

Changes in protein\textsuperscript{(51)} and gene\textsuperscript{(52)} expression profiles indicate a role for leptin in regulating a number of proteins and cellular processes in colon tissues that are associated with pathology and further emphasise links to cellular processes associated with colon cancer. For example, leptin was revealed to induce inflammatory cytokines in colon tissue, IL-6, IL-1β and CXC chemokine ligand 1\textsuperscript{(52)}.

These inflammatory cytokines are all implicated in colon carcinogenesis\textsuperscript{(53–56)}. CXC chemokine ligand 1 is expressed by colon epithelium and is up-regulated in colon tumours\textsuperscript{(56)}. In addition to a role in inflammatory responses, CXC chemokine ligand 1 is notable as an angiogenic cytokine expressed by colon epithelial cells\textsuperscript{(56,57)}. This lends further support to \textit{in vitro} studies demonstrating leptin induction of vascular endothelial growth factor-driven angiogenesis and vascular development in preneoplastic colon epithelial cells\textsuperscript{(58)}. The finding that leptin may also up-regulate its own receptor, specifically the long-form signalling receptor\textsuperscript{(52)}, is also significant in view of the reports that increased leptin receptors expressed by colon tumours lead to increased tumour growth\textsuperscript{(25)}.

**Role of leptin in human colon cancer**

Substantive studies, outlined above, indicating molecular mechanisms linking leptin with increased risk, progression and development of obesity-related colon cancer have prompted further investigation in human populations and colon cancer patients. Leptin receptors are expressed by normal colon epithelial cells in human subjects\textsuperscript{(55,60)}. Examination of leptin receptors reveals altered patterns of expression in human colon tumours\textsuperscript{(47,59–61)}. Furthermore, it has been proposed that leptin receptor expression provides phenotypic information on colon tumour sub-types and overexpression has been associated with better prognosis\textsuperscript{(59,60)}. High expression of the long-form signalling receptor, ObRb, has been associated with increased age, proximally located tumours, high levels of microsatellite instability and lymphocyte infiltration\textsuperscript{(59)}. Association with
lymphocyte infiltration is supported by studies in rodents indicating that ObRb may be an immunological marker and that leptin can activate inflammatory gene targets associated with colon carcinogenesis\(^{52}\) and stimulate inflammatory responses in tumour colonocytes, leading to recruitment of cytotoxic T-cells within the tumour microenvironment\(^{21}\).

Assessment of leptin signalling in tumour tissue needs further investigation to determine the proposed impact on prognosis. The complexity of this is confounded by various aspects associated with leptin production and signalling in human colon cancer patients. Leptin expression is reported to be elevated as tumorgenesis progresses\(^ {24,62}\). Leptin expression in colon tissue may be positively correlated with tumour features that are associated with improved survival of colorectal cancer patients\(^ {62}\). However, a study by Stachowicz et al.\(^{61}\) failed to detect the mRNA encoding the leptin protein in samples collected from human colon cancer patients. The impact of plasma leptin is confounded by inconsistent and conflicting data reported from studies on colon cancer patients\(^ {47,63-67}\). Some studies report reduced levels of serum leptin in colon cancer patients\(^ {65,66}\), while others indicate that increased serum leptin is associated with incidence of colon cancer in men, but not in women\(^ {63,64}\). Salageanu et al.\(^ {67}\) also assert that serum leptin is low in colon cancer patients compared to controls and that levels decreased with tumour progression and aggressiveness\(^ {67}\). A more recent report failed to determine significant differences in serum leptin in colon cancer cases and controls\(^ {47}\). Conflicting reports may be due, in part, to cachexia and anorexia that are common in colorectal cancer patients\(^ {68}\). However, Arpaci et al.\(^ {69}\) measured low-serum leptin in colon cancer patients without cachexia or anorexia in a small patient population (thirty-six cases and controls). The observation that colon cancer patients have increased omental fat deposits may also lead to increased localised levels of leptin\(^ {70}\).

Summary and conclusions

It is becoming clear that the promotion of colon cancer by diet and adipose interactions is potentially very important. The recognition of positive and negative leptin receptor tumour sub-types\(^ {59,60}\) indicates the potential for diet and obesity levels to impact on colon carcinogenesis and has implications for prognosis and treatment of individual tumours. Deciphering implications for obesity-related colon cancer and colon cancer patients will require further characterisation of genetic variants attributed to SNP in the Ob gene and ObRb or altered regulation of these genes, either as a consequence of mutation or methylation changes to promoter regions, both of which are features of tumours, may also be important. Additionally, serum leptin is commonly assessed in obesity-related cancer studies, but luminal levels of leptin may be equally significant considering the assertion that leptin induces autocrine/paracrine signalling cascades in colon tissue\(^ {39}\). Secretion of leptin from omental fat\(^ {70}\) and colon epithelium\(^ {24,62}\) has the potential to impact on leptin receptors in normal and colon tumour tissues. The identification of leptin-regulated genes and cellular processes linked to inflammation\(^ {52}\) and Wnt signalling\(^ {38,39}\) present us with particularly interesting targets for follow-up studies to elucidate links between obesity, leptin and cancer.

Current knowledge lends support to recommendations that cancer survivors maintain a normal body weight and avoid weight gain during treatment for colon cancer\(^ {38,18,71}\). In summary, it will be important to dissect the mechanisms linking obesity to cancer to determine an individual’s risk of developing obesity-related cancers and strategies required to reduce this risk and prevent chemoresistance and recurrence. In order to achieve this it will be important to assess multiple interacting pathways conducted both in vitro and in vivo to elucidate the complex interplay between diet and adipose tissues and responsive tissues prone to obesity-related cancers.

Acknowledgements

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. The author is grateful for support from the Scottish Government RESAS, TENOVS, The Rank Prize, Tayside Tissue Bank (Ninewells, Dundee, Scotland) and Biomathematics and Statistics Scotland for contributions and support of some of the research referred to in this paper.

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