Tissue-Specificity and Ethnic Diversity in Obesity-Related Risk of Cancer May Be Explained by Variability in Insulin Response and Insulin Signaling Pathways

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Obesity is a predisposing risk factor for several chronic diseases. The link between obesity and cancer appears to be particularly complex. Notably only the risk for development of specific cancers appear to be affected. Moreover, the obesity-related risk of cancer is very different across ethnic groups. African-Americans appear particularly prone, whereas Hispanics appear to be relatively protected. Obesity is associated with increased levels of circulating insulin. These levels of elevated insulin may serve to promote proliferation of fat cells to accommodate the elevated nutrient flux. However, elevated levels of insulin may be a major mediating factor influencing cancer risk. This hypothesis alone cannot explain the complexity of the phenomenon. We suggest here that the different insulin responses to obesity of different ethnic groups may explain their different risk profiles. Moreover, we speculate that tissue-specific variations in the insulin signaling pathways may underlie their differential susceptibility to tumorigenesis in the face of elevated obesity. Elevated cancer risk may be an unwanted side effect of insulin responding to elevated nutrient flux in the obese which it serves to proliferate fat cells that provide a location for storage of ingested fat, which consequently prevents ectopic fat storage. Hence, while Hispanics may be protected from cancer risk in obesity because of their lower insulin response, they have an elevated risk of fatty liver disease. Reduction of insulin levels in obesity as a strategy to reduce cancer risk may pose additional problems unless it is combined also with interventions that aim to limit nutrient influx.

Obesity (2010) **18,** 1071–1078. doi:10.1038/oby.2010.16

BACKGROUND: OBESITY AND ELEVATED DISEASE RISK

Over the past five decades, we have witnessed an enormous increase in levels of body fatness in both the western world, and more recently throughout developing nations (e.g., see refs. 1,2). By the conventional definitions of obesity (BMI >30) and overweight (BMI >25), the population that is obese in the United States has expanded from <5% in 1960 to over 25% in 2004 (3–6). This has been mirrored by an even greater expansion in the numbers of people in the United States that are overweight (increasing from 10 to 35%). However, the largest proportional increase has been in the morbidly obese (BMI >40) (7). The obesity epidemic has also started to affect people at much younger ages than previously, with childhood rates of obesity on the rise globally (8). Although there are some recent indications that the rate of increase is slowing (9), the trends are still upward in most age groups. Obesity is a major health problem because it increases the risk for a number of chronic illnesses. Perhaps the most significant of these is type 2 diabetes (10). Matched with the increased risk of type 2 diabetes is an elevation of insulin resistance and increased risk for cardiovascular disease (11–13), fatty liver disease (14,15), and cancer (16,17).

The precise mechanisms that link obesity to these chronic diseases is an area of intensive investigation, but as yet there is little consensus on mechanisms of causality, or an understanding of why such links might have evolved. An evolutionary framework that has dominated thinking in this area for almost the past 50 years is the "thrifty gene" hypothesis (18). The "thrifty gene" idea is that historically, human populations were exposed to cyclic periods of feast and famine. Under these conditions, it is suggested that selection would favor individuals that had genes allowing them to rapidly deposit body fat during periods of feast, because these fatter individuals

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Received 14 August 2009; accepted 11 January 2010; published online 11 February 2010. doi:10.1038/oby.2010.16

would then have greater reserves to get them through the subsequent periods of famine. Although Neel (18) emphasized increased survival as the primary selective advantage, more recent studies have pointed out that a greater selective benefit may actually be that obese people could retain greater fertility during the famine periods. Neel (18) regarded a mildly diabetic phenotype to be part of this advantageous thrifty genotype helping individuals to deposit fat in times of feast. Obesity and diabetes are then seen as unfortunate consequences of embedding this previously advantageous genotype in a modern environment where food is readily available and easily obtained, allowing individuals to deposit enormous fat reserves in preparation for a famine that may never come (19–26).

This evolutionary framework for understanding the obesity epidemic and the link of obesity to diabetes has recently been called into question (27–30). The thrifty gene idea has several basic flaws. Firstly, if selection really had been so intense, and obesity and diabetes are so advantageous, then it is difficult to understand why the favorable alleles for obesity and diabetes have not spread through the entire population, making us all overweight or obese. Another problem with the "thrifty gene" interpretation that obesity and diabetes were previously selectively advantageous, is the association between obesity and other disorders. Although there might be some merit in the argument that a prediabetic phenotype might enable "thrift" it seems extremely unlikely that the elevated risk of developing cardiovascular disease and cancer in the obese state has any selective advantage. The links between obesity and chronic disease are consequently more likely to result from metabolic consequences that have never been selectively removed, because historically people never got fat enough to precipitate these diseases. Understanding these associations should therefore focus on trying to identify potential molecular linkages between the respective conditions.

Full details of the links between obesity and cancer are presently emerging. It has become clear that obesity does not uniformly elevate the risk of cancer in all tissues. In particular, the epidemiological links between obesity and cancer are strongest for the following sites: breast cancer, especially postmenopausal breast cancer, endometrial cancer, colon cancer, adenocarcinoma of the esophagus, and renal cell carcinoma (16,17). It is also apparent that in addition to obesity causing differential elevation of risks in different tissues, the risks are also very different between ethnic groups. There are numerous examples of racial differences in cancer risk (31); some examples are shown in **Table 1**.

The mechanism(s) generating these complex tissue-specific and ethnic-specific patterns are currently unclear. One previous hypothesis explaining the link between obesity and cancer relates to elevated insulin levels (32). However, this hypothesis alone is insufficient to explain the diversity in the link between obesity and cancer, that is observed across cancer sites and across different ethnic groups (**Table 1**). Here, we extend this hypothesis beyond the conventional approach of explaining this link through fasting insulin levels or insulin resistance, to other factors involved with insulin, such as postprandial insulin levels throughout the day (i.e., overall exposure of tissues to insulin) as well as downstream factors involved in the insulin signaling process.

EPIDEMIOLOGICAL AND PHYSIOLOGICAL EVIDENCE

Many studies have shown that body fatness is positively associated with circulating fasting insulin levels in both animals and humans (e.g., see ref. 33). Moreover, obesity results in a state of insulin resistance where increasing amounts of insulin need to be secreted to deal with elevated postprandial circulating blood glucose levels. Body tissues of the obese are therefore continuously exposed to elevated background and glucose stimulated levels of insulin. There is also abundant correlational evidence that circulating insulin levels are associated with cancer risk. For example, chronic caloric restriction results in a profound reduction in circulating insulin levels and disruption of the insulin signaling pathways. In rodents a major consequence of chronic caloric restriction is a reduction in the overall risk of cancer development (34–41). This reduction in cancer risk is hypothesized to be one of the major mediating aspects of the link between reduced food intake and extended longevity in rodents.

Moreover, not only is the reduction of insulin during caloric restriction associated with reduced cancer prevalence, but the ethnic differences in the link between obesity and cancer are also correlated with racial differences in circulating insulin levels. It has long been known for example, that African Americans tend to be hyperinsulinemic (at least compared to whites), and this is also evident during childhood (42). This greater hyperinsulinemia in African Americans is even more striking when insulin is examined in response to glucose. In previous studies in children the insulin response to either oral (43), or intravenous (44)

Table 1 Examples of Ethnic Differences in SEER Incidence Rate for Some Forms of Cancer

Disease outcome	White Non-Hispanic	Hispanic	African American
Cancer[a] (all sites)	494.3	361.6	504.1
Male	573.6	426.2	663.7
Female	320.5	320.5	396.9
Breast cancer[a]	140.2	91.2	118.3
Colon cancer[a]	52.0	39.9	62.1
Prostate cancer[a]	166.6	139.7	255.5
Myeloma[a]	5.1	5.7	11.3

Information abbreviated from Goran MI Ethnic-Specific Pathways to Obesity-Related Disease: The Hispanic vs. African American Paradox. Obesity 16: 2561–2565, 2008.
[a]Cancer data based on NCI SEER incidence rate (2000–2004; age-adjusted rate per 100,000 persons) from http://seer.cancer.gov/cgi-bin/csr/1975_2004/search.pl.

glucose administration is two to three times higher in African Americans compared to whites. These observations are independent of any ethnic difference in body composition or fat distribution and demonstrate profoundly higher levels of insulin in the circulation after oral or intravenous glucose administration, and presumably meal ingestion as well. The elevated insulin response is due partly to higher insulin secretion from β-cells and lower insulin clearance by the liver in African Americans (45). Furthermore, African Americans and Hispanics are more insulin resistant than whites, independent of differences in adiposity, and this difference is evident in childhood (46). Interestingly though, the compensatory response to this similar degree of insulin resistance is quite different in African Americans vs. Hispanics (46). In response to the same degree of insulin resistance, African Americans increase circulating insulin levels by both an increase in β-cell secretion (first-phase secretion in response to intravenous glucose) and a reduction in liver insulin clearance. Hispanics on the other hand rely solely on β-cell compensation to increase insulin through a secretory response (especially the second-phase insulin response).

HOW MIGHT TISSUE-SPECIFIC VARIATION IN OBESITY-RELATED CANCER RISK BE LINKED TO OVERALL TISSUE EXPOSURE TO INSULIN?

Correlational evidence summarized above supports both a link between obesity and circulating insulin levels, and between these elevated insulin levels and cancer risk. However, these correlations do not show causality. A key outstanding question concerns the mechanism by which elevated circulating insulin could increase the risk of developing cancer, and do so differentially between different tissues, as highlighted in Table 1. The insulin signaling pathway is notoriously complex and has been subject of several recent reviews (47–50). A summary of some key elements of the pathway as they relate to cell cycle and proliferation is shown in Figure 1. There are two alternative splice variants of the insulin receptor (IR-A and IR-B) (51). Structurally both receptor isoforms have two extracellular domains and two transmembrane domains. The transmembrane domains are associated with a series of insulin receptor substrate proteins (IRS1–IRS4). In addition, insulin may also bind to the insulin-like growth factor-1 (IGF-1) receptor. Binding of insulin to the extracellular domain of the insulin receptors causes a conformational change which results in the autophosphorylation of several tyrosine residues on the transmembrane domain which are recognized by binding sites on the IRS proteins, leading to phosphorylation of tyrosine residues on these substrate proteins. This phosphorylation leads to stimulation of several intracellular signaling pathways (described in more detail below). The efficiency of transduction of the insulin signal and the specific pathways that are stimulated varies with the receptor isoforms and also with the relative abundance of the different IRS proteins (52–54). The two insulin receptor isoforms, the IGF-1 receptor and the IRS proteins are differentially expressed in different tissues (Table 2 and see ref. 55). The basis of our hypothesis is that insulin may stimulate cellular proliferation via these numerous pathways in several different ways, and with different efficiencies, depending on the exact balance of the IR isoforms, IRS proteins, and additional regulatory components of the diverse pathways in distinct tissues. Consequently, tissue-to-tissue variation in the stimulation of these diverse pathways might explain the tissue-dependent variable risks of developing cancer in response to obesity. Moreover, we speculate that the ethnic variation in the risk of developing cancer might also be traced to differences in this signaling cascade.

Perhaps the best characterized pathway downstream from insulin is that involving phosphatidylinositol-3 kinase (PI3K) (Figure 1). PI3K consists of a regulatory

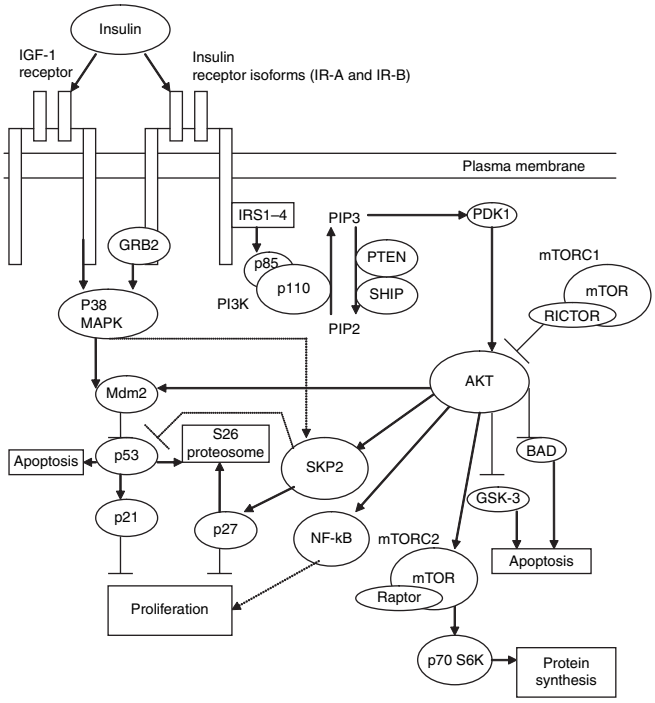

Figure 1 Some key components of the insulin signaling pathway highlighting links to cyclin-dependent kinase inhibitors p27 and p21 that may mediate a link between insulin and cancer susceptibility. BAD, Bcl-2-associated death promoter; GRB2, growth factor receptor-bound protein-2; GSK, glycogen synthase kinase-3; IGF-1, insulin-like growth factor-1; IRS, insulin receptor substrate; MAPK, mitogen-activated protein kinase; Mdm2, mouse double-minute 2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; PIP2, phosphatidylinositol (3,4)-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PTEN, phosphatase and tensin homolog; RICTOR, rapamycin insensitive companion of mTOR; SHIP, Src homology 2 domain-containing inositol phosphatase; SKP2, S-phase kinase-associated protein-2.

Table 2 Relative levels of gene expression for the insulin receptor isoforms IR-A and IR-B and the insulin receptor substrates IRS1–IRS3 in different tissues of the rat (data from Serrano *et al.* 2005)

Tissue	IR-A	IR-B	IRS1	IRS2	IRS3
eWAT	38	62	34	27	39
rWAT	49	51	35	28	37
BAT	41	59	40	32	28
SM – S	100	0	37	37	26
SM –WQ	100	0	66	18	16
H	50	50	42	30	28
L	0	100	40	28	32
K	33	67	36	38	26
P	80	20	60	16	24

Figures represent percentage of total expression for the IR and IRS.
BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; H, heart; K, kidney; L, liver; P, pancreas; rWAT, retroperitoneal white adipose tissue; SM-S, skeletal muscle soleus; SM-WQ, skeletal muscle white quadriceps.

subunit p85 and a catalytic subunit p110. The regulatory subunit contains a number of Src homology 2 domains that recognize the phosphorylation status of the IRS proteins. Hence, phosphorylation of IRS leads to activation of the catalytic subunit of PI3K which phosphorylates its substrate phosphatidylinositol (3,4)-bisphosphate converting it to phosphatidylinositol (3,4,5)-trisphosphate. This reaction can be reversed by several other enzymes including phosphatase and tensin homolog and Src homology 2 domain-containing inositol phosphatase 1. Increased phosphatidylinositol (3,4,5)-trisphosphate results in activation of AKT (v-akt murine thymoma viral oncogene homolog 1, also known as protein kinase B or PKB) by binding it to the membrane where it can be phosphorylated by 3-phosphoinositide-dependent protein kinase-1. AKT is a key signaling protein that can affect several pathways that may be linked to the cell cycle, and hence proliferation and tumorigenesis (56). One recently discovered important link is via the S-phase kinase-associated protein-2 (SKP2). Skp2 is transcriptionally regulated by PI3K/AKT (57) specifically by regulation of E2F1 binding to the SKP2 promotor (58), although a link via glycogen synthase kinase-3 has also been suggested (59). When activated the main function of SKP2 appears to be to ubiquitin tag the protein p27 for degradation by the s26 proteosome (60,61). P27 is a cyclin-dependent kinase inhibitor which represses the cell cycle. Hence activation of SKP2 removes this repression of the cell cycle and results in elevated proliferation. There is some evidence that SKP2 can also be activated via the mitogen-activated protein kinase (p38 mitogen-activated protein kinase) and ERK1/2 signal transduction pathway (57) which are additional signaling routes linking it to the insulin receptor. Moreover, PI3K and AKT may directly interact with other cyclin-dependent kinases regulating the cell cycle like p21 (58). AKT is established to inhibit mouse double-minute 2 which is a negative regulator of p53, which is itself a key regulator of the cyclin-dependent kinase inhibitor 1A (cyclin-dependent kinase inhibitor 1A or p21). AKT activation may also inhibit apoptosis, via inhibition of glycogen synthase kinase-3 which in addition to activating glycogen synthase is a stimulator of apoptosis. AKT can also mediate reductions in apoptosis in other ways, notably by inhibition of the apoptotic stimulator called Bcl-2-associated death promoter, and via its inhibitory effects on p53 which is also proapoptotic (**Figure 1**).

There are at least two other pathways via which AKT may affect the cell cycle. First, AKT is a known regulator of the transcription factor nuclear factor κB, which promotes transcription of several genes that stimulate cellular proliferation (62,63). Second, AKT activates the complex of mammalian target of rapamycin (mTOR) and the regulatory-associated protein of mTOR (mTOR complex 2: mTORC2). This activation may be direct and/or via phosphorylation of TSC2 which is a negative regulator of mTORC2. The mTORC2 complex plays a key role in cellular proliferation (64) because it regulates protein synthesis (via p70 S6kinase) (65). The interactions between mTOR and AKT, however, are multifaceted because mTOR in complex with the rapamycin insensitive companion of mTOR (RICTOR) (mTOR complex 1: mTORC1) negatively regulates AKT.

In addition to the signaling cascade via PI3K and AKT there are other signaling cascades via which insulin may affect cellular proliferation, in particular the p38 mitogen-activated protein kinase (p38 mitogen-activated protein kinase) pathway. This pathway is stimulated directly from the insulin receptor (particularly IR-A), independent of the IRS proteins, via the protein growth factor receptor-bound protein 2 (66) and also can also be stimulated from the IGF-1R. p38 mitogen-activated protein kinase has many downstream effects but these include stimulation of mouse double-minute 2 (hence p53 and p21 inhibition) and also a direct effect on SKP2, hence p27 degradation.

There is considerable evidence from genetic disruption of these pathways that they are associated with cancer risk. For example, global knockout (KO) of IRS1 leads to a long lived mouse with reduced cancer risk, in spite of elevated insulin levels (67), consistent with insulin being the primary mediator of cancer risk, and blunted insulin signaling interfering with this association. Conversely, KO of IRS2 produces a mouse that is profoundly diabetic and dies long before altered susceptibility to cancer could be detected (68), clearly illustrating the different signaling roles played by the different IRS proteins (see also refs. 69,70). The effects of tissue-specific KO of IRS substrates has complex and disputed effects (71,72). Global KO of the IR is lethal. However, mice have been produced with tissue-specific KO of the IR—the FIRKO, MIRKO,

and SIRKO mice with the IR knocked out respectively in fat, muscle, and skin cells. FIRKO mice have much reduced fat tissue and an extended lifespan (73), but data on cancer is lacking. SIRKO mice, however, have reduced rates of melanoma (74), and MIRKO mice have reduced rates of colon cancer (75).

Phosphatase and tensin homolog which reverses the conversion of phosphatidylinositol (3,4)-bisphosphate to phosphatidylinositol (3,4,5)-trisphosphate catalyzed by PI3K is recognized as a tumor suppressor gene (76). Mutations and deletions in the *PTEN* gene or the downregulation of phosphatase and tensin homolog have been reported in various malignant tumors (77) and in leukemia (78). Conversely mutations in PI3K are also often linked to cancer but in this case the mutations causing cancer increase the activity of the PI3K. Pharmacological inhibition of PI3K by wortmanin or LY294002 both result in inhibition of proliferation in cell culture (79). Cancers that are resistant to the inhibitory effects of caloric restriction tend to have mutations in the insulin signaling pathway—particularly in PI3K (80), suggesting the anticancer effects of caloric restriction are contingent on lowered insulin levels and an intact signaling pathway to transduce these lowered levels into modulated levels of apoptosis, and perhaps other effects such as altered proliferation (80). Studies of isoforms of PI3K subunit p110 suggest a link to cancer development (81,82) and there is evidence that the risk of development of cancer appears to hinge on the balance of the insulin receptor isoforms, with IR-A being especially overexpressed in some cancers (83–85).

As might be expected, knocking out SKP2 stops p27 being tagged for degradation. This p27 then acts as a cell proliferation repressor and these mice have much reduced adipocyte cell numbers (61,86) and reduced β-cell numbers (86,87) but also reduced susceptibility to cancer. These effects on proliferation of adipose tissue can be eliminated by simultaneous KO of p27, showing that the effect of SKP2 on adipogenesis is mediated exclusively via p27 (61). In contrast knocking out p27 alone results in tissue proliferation. SKP2 is frequently overexpressed and/or p27 repressed in a variety of human cancers (88) including prostate cancer (89–91), cervical cancer (92), thyroid cancer (93), colorectal cancer (94), breast cancer (95,96), lung cancer (97), and leukemia (98). Compounds inhibiting SKP2 are currently under exploration as novel antitumor agents (87,99). While the link to SKP2 is often associated with lowered p27 levels, it has been suggested that SKP2 may also promote tumorigenesis by inhibiting p53-mediated apoptosis (100,101).

An additional factor that may potentially be important in this signaling pathway in obesity is IGF-1 which binds to both the IGF-1R and IRs (**Figure 1**). Although there is a stimulatory effect of insulin on IGF-1 production by adipocytes, which probably has an autocrine/paracrine role in stimulating growth of adipose tissue under conditions of overnutrition, circulating IGF-1 levels are decreased in obese subjects (102–105). This reduction in IGF-1 would be expected to have a protective effect against cancers (106). However, circulating IGF-1 is carried by a number of binding proteins (IGF-binding proteins (IGFBPs)), some of which enhance its activity (e.g., IGFBP-3) whereas others inhibit it (e.g., IGFBP-1). Production of the inhibitory IGFBP-1 in the liver is strongly reduced by insulin (107). Hence, the balance of the effects of obesity on bioactivity are unclear. It has been recently suggested that the overall reduction in IGF-1 levels in the obese is offset completely by the reduction in IGFBP-1 (108) leading to no overall effect of obesity on bioactive levels of IGF-1. Moreover, there is no evidence that the associations between both IGF-1 and the IGFBPs and obesity vary among ethnic groups.

To summarize, the evidence strongly implicates several elements of the insulin signaling pathway as primary mediators of susceptibility to cancer. Elevated insulin and increased insulin resistance in obesity may then stimulate this pathway causing the increased cancer risk. The complexity of the intracellular pathways that may mediate these links combined with tissue specific and potentially ethnic variations in the expression of the different receptor isoforms, substrate proteins, and other regulatory components of the pathways, provides a potential explanation of why different tissues and different ethnic groups are differentially susceptible to the effects of globally elevated circulating insulin levels resulting from obesity.

SUMMARY: WHY DO THESE LINKAGES EXIST AND WHAT ARE THE IMPLICATIONS?

We suggest that the link between obesity and cancer that is mediated via tissue insulin exposure, may be an unwanted side effect of insulin responding to elevated nutrient flux. Elevated insulin may stimulate cellular proliferation in fat cells and pancreatic β-cells (87). This mechanism ensures additional insulin production and also provides additional storage for the increased nutrient load (57,61). This may have substantial advantages because it provides cells that can hold on to ingested fat and prevent its ectopic distribution elsewhere in the body. It is notable that African Americans that have elevated insulin in obesity and increased cancer risk have reduced risk of developing fatty liver disease even at an early age (15), while hispanics are particularly prone to fatty liver disease. Supporting our hypothesis some of the ethnic variation in susceptibility to fatty liver disease has been traced to polymorphic variation in the adiponutrin gene *PNPLA3* (Patatin-like phospholipase domain-containing protein 3) (109) and this gene has been linked to insulin secretion (110). This is important because it suggests that reducing levels of insulin in obesity as a strategy to prevent obesity-related cancers may have the unwanted side effect of reducing fat cell proliferation and promotion of fatty liver disease, and other ectopic fat deposition, unless it is combined with additional interventions to limit nutrient influx. However, the effects of insulin on cellular proliferation depends on specific tissue distributions of the various regulatory components of insulin signaling. Consequently, tissue-to-tissue variation in the stimulation of

these diverse pathways might explain the tissue-dependent variable risks of developing cancer in response to obesity (**Table 1**). Moreover, we speculate that the ethnic variation in the risk of developing cancer might also be traced to differences in this signaling cascade.

DISCLOSURE

The authors declared no conflict of interest.

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