Associations between variants in the NF-κB1 gene, alone or in combination with saturated fats, and anthropometric traits in young adults.

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Abstract

Animal studies have shown that chronic high consumption of saturated fat (SF) leads to hypothalamic inflammation and ultimately, alters appetite control. This has been shown to be partly due to an increase in the activity of the transcription factor Nuclear Factor-κB (NF-κB), a major regulator of the inflammatory response. The goal of the study was to first confirm the association between SF measurements and anthropometric traits, then to determine the association between single nucleotide polymorphisms (SNPs) in the *NF-κB1* gene and body mass index (BMI) and waist circumference (WC), and finally, to test the interaction between variants in this gene and dietary SF and circulating saturated fatty acids (CSFA) on these anthropometric traits in young adults. A significant positive association was identified between quartiles of CSFA and anthropometric measurements in the total sample (BMI: $p = 0.0003$, WC: $p = 0.0001$) and in South Asians (BMI: $p = 0.004$, WC: $p = 0.01$), but only marginally among Caucasians (BMI: $p = 0.08$, WC: $p = 0.051$) and East Asians (BMI: $p = 0.13$, WC: $p = 0.053$). After correcting for false discovery rate, carriers of the T allele in SNP rs4648022 had higher BMI and WC compared to those with the dominant CC genotype ($p = 0.0003$ and $p = 0.0001$, respectively). Among Caucasians, there was a significant interaction between SNPs in the *NF-κB1* gene and quartiles of CSFA on WC for rs4648095 ($p = 0.002$). Thus, certain SNPs in the *NF-κB1* gene appear to influence BMI and WC and also to modify the association between CSFA and anthropometric traits.

Résumé

Des études chez des animaux ont démontré qu'une consommation élevée en acide gras saturés (AGS) mène à l'inflammation hypothalamique et, ultimement, atténue le contrôle de l'appétit. Ceci a été en partie associé à une hausse de l'activité du facteur de transcription nucléaire-κB (NF-κB), qui est un régulateur important dans la réponse inflammatoire. Le but de cette étude était premièrement de confirmer l'association entre les mesures de gras saturés et les traits anthropométriques, ensuite de déterminer l'association entre les polymorphismes à nucléotide simple (PNS) dans le gène *NF-κB1* et l'indice de masse corporelle (IMC) et la circonférence de taille (CT), et finalement de tester l'interaction entre les variations génétiques de ce gène et les mesures d'AGS sur les traits anthropométriques chez des jeunes adultes. Une association positive a été identifiée entre les gras saturés en circulation et les mesures anthropométriques dans l'échantillon total étudié (IMC: $p = 0.0003$, CT: $p = 0.0001$) et chez les Asiatiques du Sud (IMC: $p = 0.004$, CT: $p = 0.01$), mais seulement marginalement chez les Caucasiens (IMC: $p = 0.08$, CT: $p = 0.051$) et Asiatiques de l'Est (IMC: $p = 0.13$, CT: $p = 0.053$). Après avoir corrigé pour le taux de fausses découverte, les porteurs de l'allèle T dans les PNS rs4648022 ont un IMC et une CT ($p = 0.0003$ et $p = 0.0001$, respectivement) significativement plus élevé comparativement à ceux qui sont porteurs du génotype dominant C/C. Parmi les Caucasiens, une interaction significative a été identifiée entre les PNS dans le gène *NF-κB1* et les acides gras en circulation sur la CT spécifiquement pour rs4648095 ($p = 0.002$). Ainsi, certains PNS dans le gène *NF-κB1* semblent influencer l'IMC et la CT, ainsi que modifier l'association entre les acides gras en circulation et les traits anthropométriques.

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Table of Contents

List of figures

List of tables

List of Abbreviations

AgRP	Agouti-related Peptide
BAFFR	B-Cell Activating Factor Receptor
BCR	B-Cell Receptor
BMI	Body Mass Index
CART	Cocaine-amphetamine-related Transcript
CNS	Central Nervous System
CRP	C-Reactive Protein
CSFA	Circulating Saturated Fatty Acids
DAMP	Danger-Associated Molecular Pattern
FFQ	Food Frequency Questionnaire
HSFD	High Saturated Fat Diet
HWE	Hardy-Weinberg Equilibrium
ICV	Intracerebroventricular
IκB	Inhibitor kappa B
IKK	Inhibitor kappa B Kinase
IL	Interleukin
JAK-STAT	Janus Kinase Signal Transducer and Activator of Transcription
Kcal	Kilocalories
LD	Linkage Disequilibrium
LTBR	Lymphotoxin beta-Receptor
MET	Metabolic Equivalent
MRI	Magnetic Resonance Imaging
NF-κB	Nuclear Factor-kappa B
NIK	Nuclear Factor-kappa B Inducing Kinase
NPY	Neuropeptide-Y
PI3K	Phosphoinositide-3 Kinase
POMC	Pro-opiomelanocortin Hormone
RANK	Receptor Activator of Nuclear Factor-kappa B
SF	Saturated Fat

SNP	Single Nucleotide Polymorphism
TCR	T-Cell Receptor
TLR	Toll-Like Receptor
TNF-α	Tumour Necrosis Factor-alpha
TNFR	Tumour Necrosis Factor Receptor
WC	Waist Circumference

Thesis outline

Chapter 1: Literature Review - Summary of previous research findings leading to the study rationale, hypotheses and research questions.

Chapter 2: Methodology - Detailed explanation of data collection and statistical analyses.

Chapter 3: Results - Presentation and description of results from all three objectives.

Chapter 4: Discussion & Conclusion - In depth interpretation of results and suggestions for future research.

Chapter 1: Literature Review

This chapter details the mechanisms involved in central regulation of energy intake, the impact of high saturated fat diets (HSFD) on specific physiological processes, particularly inflammation, in rodents, the function of the major regulator of the inflammatory response Nuclear Factor-κB (NF-κB) as well as the homeostatic repercussions of hypothalamic inflammation on body composition-related phenotype.

1.1. Gene-environment interactions

In the last several decades, the dietary and lifestyle habits of Canadians have been characterized by a higher dietary intake of lipids without compensating by increasing their level of physical activity, consequently leading to weight gain.[1,2] While the genesis in the accumulation of adiposity is highly understood, where energy intake exceeds energy expenditure, inter-individual differences in controlling body weight encourage further research regarding factors such as genetics as predictors of obesity.[3,4] In fact, the concept of gene-environment interactions has been a predominant field of research in uncovering the etiology of obesity.[4]

Research has suggested that, on a physiological basis, individuals do not respond the same way to nutrients and bioactive food compounds and that one's genotype might be an important predictor of how they will respond or adapt to a specific environment.[4,5] Claude Bouchard and his colleagues conducted a study in 1990 where, after measuring their resting metabolic rate, identical twins were fed six days a week for 100 days a diet that was 1000 kilocalories above their establish baseline energy intake requirements.[6] Interestingly, twins exposed to identical long-term caloric excess demonstrated very similar changes in body composition within pairs, but

a wide range (4.3 to 13.3 kg) in body weight gain was observed between pairs. Indeed, although energy intake and expenditure were stringently controlled, there was a statistically significant difference in changes in body weight, adiposity as well as fat distribution between pairs. Meanwhile, the intrapair similarity in changes in body composition was statistically significant. Thus, genotype may explain, in part, the resemblance between identical twins as well as the variation between twin pairs in changes in anthropometric traits in response to excess nutrient intake.[6,7]

Recognizing that nutrients may possess an ability to interact and modify molecular mechanisms involved in important physiological processes has given rise to a new field in nutrition.[5] With the advance in technology, nutritional genomics is a field of nutritional science that focuses on understanding how the interaction between nutrients and the genome can affect fundamental molecular processes and ultimately health and disease susceptibility.[5,7] Nutritional genomics examines how nutrients affect gene expression (nutrigenomics) and how genetic variations modify the response to diet (nutrigenetics). This thesis will focus on nutrigenetics of inflammatory processes in response to dietary and circulating saturated fat on anthropometric traits.

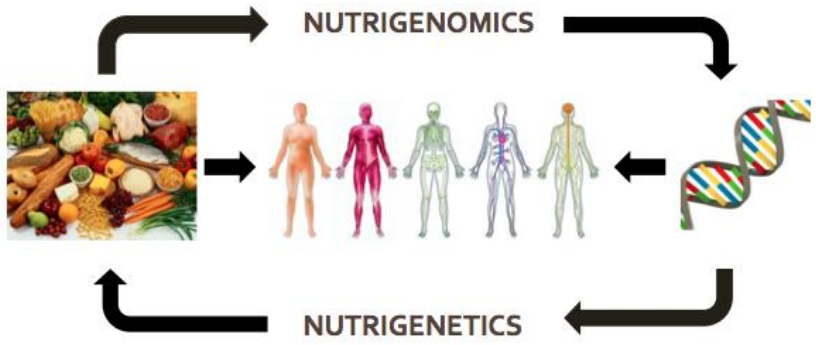

Figure 1. Nutrigenomics vs nutrigenetics.

1.2. The implication of central processes as well as external factors in regulation of energy intake

The hypothalamus is an integral part of the brain that processes many of the body's essential hormones controlling numerous physiological functions of the autonomic nervous system.[8] One of its principal roles is regulating energy homeostasis, which is the biological process involved in balancing energy input and energy expenditure in order to control body energy storage.[9] Energy homeostasis entails the integration of a multitude of hormonal and neuronal signals by a complex network of hypothalamic and extra-hypothalamic neurons, which ultimately regulates energy intake and expenditure through negative-feedback loops in order to maintain body energy stores.[9] Nonetheless, to precisely inform the hypothalamus about the body's homeostatic state, specific signals are required for various types of internal changes. In the instance of carbohydrate ingestion, pancreatic β-cells will secrete insulin in response to a rise in blood glucose concentrations, favouring the uptake of glucose within the cells and triggering glycogenesis.[10] As for lipid homeostasis, white adipose tissue will release leptin in proportion to adiposity.[11] On the other hand, in a fasting state, the hormone ghrelin will inform the hypothalamus that the body's internal energy stores are depleting.[12] In this case, appetite is stimulated and energy expenditure is reduced.[12,13] Although these are only a few examples, it demonstrates how hormone signalling is crucial in maintaining energy balance, and how the hypothalamus plays a major role in integrating these signals and initiating the appropriate homeostatic responses.

The advance of modern science has permitted researchers to uncover the intricate details of the neurocircuitry of the hypothalamus. This central energy regulation is directed from two major groups of neuropeptides, orexigenic and anorexigenic, which are expressed by hypothalamic

neurons in direct proportion to plasma levels of peripheral signals such as insulin and leptin.[14,15] Orexigenic neuropeptides include neuropeptide-Y (NPY) and agouti-related peptide (AgRP), whereas anorexigenic neuropeptides incorporate pro-opiomelanocortin hormone (POMC) and cocaine-amphetamine-related transcript (CART).[14,16] Increased secretion of insulin and leptin triggers the release of anorexigenic neuropeptides, which signals the arcuate nucleus of the hypothalamus to abate hunger and stimulate thermogenesis through catabolic pathways.[11,17–19] On the other hand, a reduction in the release of these hormones, or resistance to them, will promote the opposite as orexigenic neurotransmitters will favour anabolic pathways.[11] Therefore, insulin or leptin resistance due to altered intracellular mechanisms could lead to an important dysregulation of appetite and ultimately, body weight.[20]

The hypothalamus uses negative feedback control to maintain internal conditions.[8,21,22] The mechanism behind this type of regulatory loop is often exemplified using a thermostat. Similarly the hypothalamus uses a preset value, called set point, as a reference to maintain the internal environment within specific conditions.[21] The "receptor" (thermometer) detects changes in the environment and sends the input to a "control centre" (thermostat), which compares the signal to the set point.[21] If the signal falls outside the preset value, the comparator sends the appropriate regulatory command to the "effector" (heater) that will consequently generate the appropriate response to bring variable back to its set point.[21] Negative-feedback control is one of the primary mechanisms that enable the human body to remain within homeostatic conditions and the hypothalamus plays a critical role as a comparator in the central nervous system (CNS).

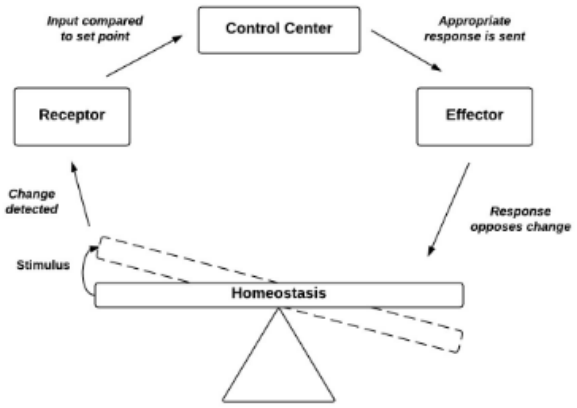

Figure 2. Negative feedback loop diagram.

Despite numerous internal mechanisms involved in controlling appetite, there are external factors which may override our responsiveness to internal signals of satiation and satiety. For instance, palatability, which refers to the pleasure experienced when consuming food, is an important factor influencing our energy intake.[16] It actually stimulates hedonic pathways in the CNS, subsequently triggering a positive emotional response and heightens our desire to eat.[23] Also, the most palatable foods, such as those high in fat, tend to have the least effect on satiety and be the most energy dense, often leading to passive overconsumption.[24] Variety in the food choices available appears to have an effect on appetite as well. A phenomenon known as sensory-specific satiety is believed to reduce the desire to eat foods that have already been tasted and increase the ones that have not been ingested yet.[25] Portion size has also been shown to influence one's intake. Indeed, it was demonstrated that individuals presented with larger portions will consume more food.[26] In addition, a lack of sleep has been suggested to impact appetite hormones leptin and ghrelin. Circulating levels of each hormone is closely linked to the circadian rhythm, where they reach their peak during sleep.[27] However, sleep restriction was demonstrated to reduce leptin and increase ghrelin levels, consequently favoring appetite.[28] Eating in social

situations was also shown to promote greater food intake, partly due to social interactions during the meal distracting from cognitive restraints to eat too much as well as eating in groups most likely leading to longer meal time and thus a greater consumption.[29] This said, there are many external factors that ultimately can override internal mechanisms that prevent us from overeating, and must be taken into account when investigating the complexities of eating behavior.

1.3. Innate immune response in central energy regulator due to nutrient excess

1.3.1. Evidence of innate immune response in the peripheries vs central nervous system

Throughout human evolution, inflammation has played a critical role in the innate immune response, being the first line of defence against infections and microbial pathogens.[30,31] Normally, such a response is triggered by an acute injury and is characterized by an increase in blood flow, capillary dilatation and permeability, which serve to eliminate toxic agents and initiate the repair of damaged tissues.[32] However, research has identified a phenomenon of 'low-grade' (also called 'chronic') inflammatory response which is characterized by an increase in inflammatory biomarkers in multiple organs, such as the liver, pancreas, muscle, adipose tissue and hypothalamus that rises from daily nutritional excess.[18,20,33–39] More precisely, research has specifically demonstrated that the consumption of a HSFD induces inflammation.[17,37,38,40–44] Signs of peripheral inflammation typically develop over weeks to months of being subjected to a HSFD.[33–36,45] However, markers of hypothalamic inflammation can be observed as soon as 24 hours after exposure to HSFD in rodent models.[46,47] Therefore, these findings suggest that first, the overconsumption of saturated fat (SF) triggers an uncharacteristic innate immune response and second, that inflammation in the hypothalamus occurs prior to the peripheries, meaning that it does not originate from peripheral inflammatory processes.

1.3.2. Evidence of multiple pro-inflammatory biomarkers in hypothalamus during HSFD

In normal physiology, the mediobasal hypothalamus coordinates metabolic activities such as feeding behaviour and is an integral part of the central nervous system protecting against pathological expansion of fat mass.[18,48] However, it is now well understood that exposure to a chronic HSFD in animal models raises circulating saturated fatty acid (CSFA) levels, consequently inducing hypothalamic inflammation and dysfunction.[17,18,20,38,39,46] During one of the initial studies exploring the repercussion of HSFD on hypothalamic functioning, researchers subjected experimental male rats to a diet containing 45% of kilocalories (kcal) from lard, which is a high source of SF.[17] They identified a significant increase in the expression of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6 in the hypothalamus compared to the control group fed standard chow containing 10% kcal from fat.[17] In support of these findings, further research found that the expression of pro-inflammatory genes IL-6 in addition to NF- κB both increase by 50% when rodents were exposed to long-term HSFD (60% kcal from lard-based fat) compared to normal chow.[46] Additionally, another study using animal models not only found similar results regarding the upregulation of pro-inflammatory cytokines when subjected to a HSFD, but found no significant differences in anti-inflammatory cytokines such as IL-10 when compared to control rats on a standard chow diet.[37] Therefore, the accumulation of these findings seems to indicate that SF may have pro-inflammatory properties in the hypothalamus when over-consumed.

1.3.3. Variations in response according to quantity, source and type of fat ingested in animal models

Following the identification of the negative repercussion of a HSFD, a study conducted by Kelly A. Posey explored whether the level of SF in a rodent's diet may lead to different

physiological responses and weight gain when calories were matched.[18] Accordingly, a first group received *ad libitum* rat chow composed of 10% lard fat (low-fat group), a second group was fed chow *ad libitum* with a 45% lard fat content (high-fat group) and a third group was also given high-fat chow (45%), but calories were matched to the one of the low-fat group (isocaloric high-fat group). Despite having the same energy intake, the isocaloric high-fat group gained significantly more adipose tissue than its paired low-fat group and actually increased their adiposity to levels matching 82% of what the *ad libitum* high-fat group gained. Although this finding is interesting, it does not comply with the laws of thermodynamics, where it would be expected that the same caloric intake would result in the same changes in body weight.[12] A potential explanation for this occurrence may be that exposure to a high source of SF disturbs certain central physiological processes and decreases energy expenditure. In fact, one study examined the repercussion of lesions to the ventromedial hypothalamus in rat models and found a reduction in activity as well as resting metabolic rate compared to nutritionally matched control group.[49] Knowing that HSFD triggers hypothalamic inflammation, the contradicting outcomes from the isocaloric diets with different levels of SF may very well be the result of neural injury and consequently explaining why they led to different patterns of weight gain. Nonetheless, these findings suggest that SF may possess inherent physicochemical and obesogenic properties and consequently, alter the regulatory role of the hypothalamus.

Some studies reveal that the type of fatty acid ingested can lead to different biological effect. For instance, one study demonstrated that the energy intake of rodents given *ad libitum* access to food high in SF was 55% higher than animals fed food high in unsaturated fat and 57% greater than those on a normal chow diet.[50] Actually, numerous other studies have also observed hyperphagic behaviours in rodents exposed to HSFD.[47,51–53] Moreover, another study

demonstrated that intracerebroventricular monounsaturated oleic acid treatment favoured the expression of IL-10, known to have anti-inflammatory properties, and thus, reinforcing the evidence that the type of fat ingested can induce different biological effects as well as the alleged anti-inflammatory property of monounsaturated fat.[37,54] In fact, research suggests that diet high in unsaturated fat might even revert hypothalamic inflammation established by a HSFD.[55] Indeed, rodents who were first submitted to a high lard-source diet for 8 weeks and subsequently introduced to a diet where lard was replaced by flax seed oil or olive oil, both high in unsaturated fat, demonstrated a reduction in food intake, body mass gain as well as a reduction in hypothalamic inflammation, and this independent of oil type.[55] Therefore, cumulating evidence suggests that the type of fat ingested may influence different physiological mechanisms, where SF might promote dysregulation in energy intake and pro-inflammatory processes, while diets high in unsaturated fat seem to favour the opposite.

1.4. Major regulator of the inflammatory response: nuclear factor-kappa B

1.4.1. Nuclear Factor-kappa B activation mechanism

The principal regulator of the inflammatory response is a protein complex called NF-κB, which activates numerous target inflammation-related genes by binding to the κB enhancer.[56] This dimeric molecule can be composed of either RelA (also named p65), RelB, c-Rel, p50 or p52.[57] In unstimulated cells, NF-κB is located in the cytoplasm and is bound to the inhibitor kappa B (IκB), which prevents it from being activated and entering the nuclei.[39,58] Various stimuli binding to different cell surface receptors can activate NF-κB through either the "canonical" pathway or the "non-canonical" pathway (as shown in Figure 3). Receptors involved in the canonical pathway include Toll-like receptors (TLRs), TNF receptor (TNFR), B-cell

receptor (BCR) and T-cell receptor (TCR).[56] Examples of receptors implicated in the non-canonical pathway include B-cell activating factor receptor (BAFFR), CD40, receptor activator of nuclear factor-κB (RANK) and lymphotoxin β-receptor (LTβR).[59] In response to a stimulus activating the canonical pathway, Tak1 activates IκB kinase β (IKKβ) complex, which then phosphorylates IκB, resulting in the release of NF-κB (RelA and P50 subunits).[60] This enables NF-κB to translocate into the nucleus, where gene expression and synthesis of numerous pro-inflammatory cytokines are induced.[57,61] The non-canonical pathway involves NF-κB inducing kinase (NIK) which phosphorylates and activates IKKα.[56,59] Then, p100, which is associated with RelB, is phosphorylated by IKKα, resulting in the ubiquitination of K-48 and the partial degradation of p100 to generate p52.[56,59] RelB-p52 complex can subsequently translocate into the nucleus and binds to specific sequences in the promoter region of target genes.[56,59] This process mediates the transcription of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 as well as many other cytokines.[39] In fact, these cytokines stimulate and are stimulated by NF-κB, leading to a positive regulatory loop resulting in a greater inflammatory response.[62] Nonetheless, during the transcription of pro-inflammatory cytokines, NF-κB also induces the synthesis of IκB, which then attaches to the activated NF-κB and transports it back to the cytoplasm in a negative feedback manner.[62]

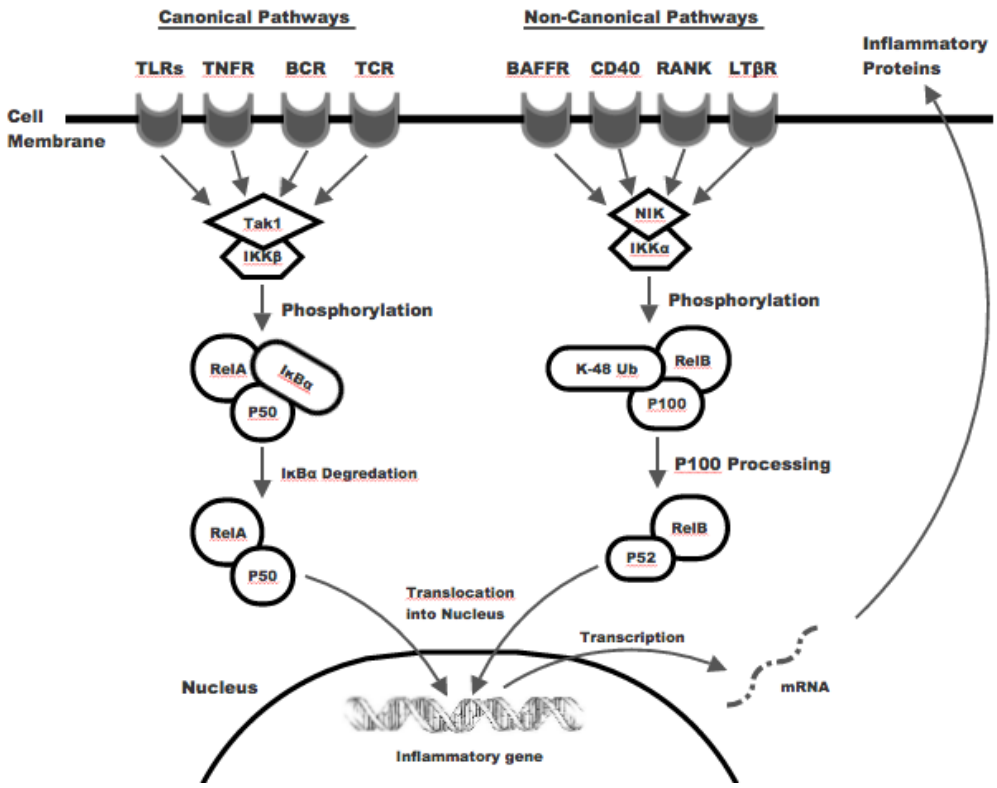

Figure 3. Canonical and non-canonical pathways of NF-κB activation.

1.4.2. Upregulation of NF-κB in the hypothalamus due to HSFD

As previously mentioned, research revealed that nutrient surplus when subjected to a HSFD increases the expression of NF-κB, which directs the inflammatory response. For instance, Zhang *et al.* demonstrated that subjecting mice to a HSFD resulted in an up-regulation in the expression of NF-κB by two folds compared to control group fed a normal chow diet.[39] In addition, they identified during the dissection of the hypothalamus that IKK protein was highly enriched in this organ compared to peripheral organs such as the liver, skeletal muscle, fat tissue and kidneys.[39] They also found higher levels of I*κ*B in the standard chow-fed group compared to the HSFD group, demonstrating why NF-*κ*B was suppressed.[39] Likewise, a study exploring the role of long-chain SF on the hypothalamus in mice also identified heightened activation of NF-*κ*B when fed a

diet where 42% of calories were from milk fat (highly enriched in palmitic acid and stearic acid) compared to standard chow.[44] Moreover, in aim to imitate the effects of high dietary SF intake, another study performed intracerebroventricular injections of palmitic acid directly in the hypothalamus through a third ventricular cannula.[18] Similarly to HSFD, infusion of SF in animal models resulted in an increased phosphorylation of the kinase, which consequently activated NF-κB, as well as decreased its IκB content.[18] Thus, there is evidence in the literature supporting that nutritional oversupply of SF can atypically stimulate NF-*κ*B and its upstream regulator IKK in the primary site of the CNS involved in nutrition sensing and metabolic regulation.

1.5. Repercussion of hypothalamic inflammation from HSFD consumption

1.5.1. Neuron Injury: Evidence in both animal and human models

As previously stated, a classical inflammatory response is usually triggered by an acute injury and is characterized by a localized and rapid response of the immune system to aid and prevent infection.[30] The low-grade inflammation observed during nutrient excess may be an indicator of cellular damage. In fact, studies hypothesized that a rise in intracellular free fatty acid in the hypothalamic neurons from the elevated dietary intake of fat could stress the cells to the point of injury or even death.[63–65] Actually, one of them demonstrated in neural stem cells of the mediobasal hypothalamus of adult male rodents signs of cellular death when exposed to a HSFD through a markedly depleted number of as well as severe impairment in neuronal differentiation.[48] In vitro models of hypothalamic neural stem cells with genetically induced IKK/NF-κB activation, simulating effects of a HSFD, promoted the expression of apoptotic genes and consequently resulting in cell death.[48] Similarly, a group of researchers found that the level of apoptotic cells was significantly higher in animal models subjected to a HSFD and that effects

were predominantly observed in the arcuate and lateral hypothalamic nuclei.[66] Given these findings, elevated intracellular free fatty acids originating from HSFD seem to pose severe stress on the normal cell functioning, activate an inflammatory response through NF-κB and prompt apoptotic mechanisms.

It is suggested that the principal cause of hypothalamic neuron death is not due to caloric excess, but in fact to elevated exposure to SF specifically.[66] For example, in a caloric pair-feeding rodent study, variations in weight between a HSFD group and a standard chow control group were very similar, however hypothalamic pro-apoptotic activity was significantly greater in the HSFD group.[66] Moreover, cleaved caspase-3, an effector molecule involved in apoptosis, was significantly elevated in cultured hypothalamic neurons exposed to palmitic acid compared to control vehicle.[67] As for astrocytes, an essential type of glial cell involved in the regulation of the microenvironment and neuronal synaptic plasticity of the CNS, they responded to elevated CSFA levels by triggering the release of several cytokines, and this at levels that were dose-dependent and significantly higher than exposure to unsaturated fatty acid.[40] Therefore, there is evidence in the literature suggesting that SF can have quite a detrimental impact on the central energy regulator in animal models through the activation of pro-apoptotic mechanisms.

The extensive collection of research findings regarding the repercussion of a HSFD on the hypothalamus of rodents highly suggests that an oversupply of SF can be quite detrimental to functioning of the central energy regulator. Although it is harder to explore its repercussion in humans since most techniques are invasive, a study was able to identify signs of neuron injury in obese human subjects. Joshua P. Thaler and his team hypothesized that individuals that have been subjected to a prolonged hypercaloric diet resulting in excessive weight gain would demonstrate signs of neural injury such as rodents through increased gliosis in the medial basal

hypothalamus.[46] Gliosis, a nonspecific neuroprotective response to injury, is a process often resulting in the production of dense fibrous network of supportive cells called neuroglia and scaring.[68] They can often be used as evidence of neural damage and are identifiable using magnetic resonance imaging (MRI).[68] Thus, Thaler *et al.* performed MRI on the medial basal hypothalamus of 34 subjects ranging from lean to obese (body mass index (BMI): 17.7-44.1 kg/m^2).[46] Across all subjects, signal intensity was positively correlated with BMI, while no associations were discerned with age or by gender. Accordingly, the average signal intensity in the medial basal hypothalamus was significantly higher in obese subjects compared to normal weights subjects.[46] Therefore, nutrient excess seems to also trigger neural damage, and consequently scaring through prominent gliotic activity in the CNS in humans with excess weight, which helps to support that findings uncovered in rodents may be transferable to humans in some cases. It is not clear, however, whether the caloric excess-induced neuroglia and scaring was specifically due to SF as demonstrated in animals.

1.5.2. Insulin resistance

In response to environmental variations, the CNS orchestrates numerous physiological processes that aim to maintain internal parameters within a specific set point in order to preserve an equilibrium.[69] One of the principal signalling hormones involved in energy regulation of food intake is the pancreatic hormone insulin.[70,71] In circulation, it has the ability to penetrate through the blood-brain barrier and reach brain regions such as the hypothalamus.[70,71] There, it binds to its respective insulin receptor on the hypothalamic neurons, subsequently activating a cascade of events that will stimulate satiety and regulate several cellular processes such as glucose homeostasis and lipid synthesis.[71–73] Nonetheless, studies reported that rodents who are chronically or acutely fed a high fat diet demonstrate unresponsiveness to intracerebroventricular

(ICV) administration of insulin, whereas those on a pair-fed low fat diet with comparable body weight and adiposity levels had reduced food intake upon administration.[18,74] In addition, when these pair-fed groups returned to *ad libitum* access to standard chow, low fat diet group were relatively hypophagic and sensitive to insulin, whereas the high fat diet group were hyperphagic, leading to increased body weight and fat mass content.[74] Therefore, these findings suggest that a high fat diet may attenuate the anorexigenic effects of insulin in the hypothalamus and promote uncontrollable eating behaviours.

Some research further investigated the mechanisms behind the inhibition of insulin signalling by elevated CSFA levels. A study conducted by Kelly A. Posey found in rats that hypothalamic insulin resistance caused by HSFD was accompanied with a marked accumulation in the hypothalamus of two saturated acyl-CoA species, palmitoyl- and stearoyl-CoA, which palmitoyl-CoA is known to readily induce insulin resistance in the peripheries.[18] For that reason, they attempted to explore the isolated effect of palmitate on hypothalamic functioning independent of excess caloric intake. Their findings demonstrated that ICV palmitate infusion significantly blunted phosphoinositide-3 kinase (PI3K), an important enzyme in the insulin-signalling pathway, compared to a control vehicle. Inhibition of PI3K and development of insulin resistance was then partly associated with activation of inflammatory signals such as IKK, which appear to diminish hypothalamic insulin signal transduction.[17,18] In support of this, another study reported that genetically engineered mice designed to have a reduction in neuronal IKK/NF-κB signalling and consequently neural inflammation, did not show signs of insulin resistance and thus, the impediments of food intake regulation observed in animal models exposed to HSFD.[39] On the other hand, interventions in animal models where neuronal IKK/NF-κB signalling was enhanced, insulin signalling in AgRP neurons of the hypothalamus was blunted and food intake is markedly promoted.[39,75] Interestingly, ICV administration of monounsaturated fatty acid oleic

acid markedly inhibited food intake attributable to enhanced insulin action, reinforcing that different types of fat have contrastive effect on the hypothalamus.[66,76] Therefore, there is considerable number of findings in the literature indicating that elevated intake of SF particularly palmitate, and not unsaturated fatty acids, may have a negative repercussion on hypothalamic functioning by triggering cellular inflammation, promoting insulin resistance and directly affecting body weight control.

1.5.3. Leptin resistance

Apart from insulin, the adipocyte hormone leptin also plays a crucial role in regulating body weight by informing the hypothalamus about the amount of energy stored in fat tissue.[76,77] Although leptin is involved in lipid metabolism, several studies have revealed that elevated consumption of SF can actually promote leptin resistance, consequently resulting in increased food intake and adiposity. Indeed, dietary SF has been identified to induce hypothalamic inflammation and dysfunction within one week of exposure in rodents.[46,78] Elevated levels of TNF-α, IL-1β and IL-6 have been recognized to stimulate NF-κB inflammatory signalling pathway by phosphorylating and degrading IκB, and ultimately impede leptin signalling.[78] In fact, there is evidence that prolonged activation of NF-κB hinders leptin signalling through Janus kinase signal transducer and activator of transcription (Jak–STAT) signalling cascade.[39] The transcription molecule STAT3 in this signalling pathway is a crucial component in the regulation of leptin gene expression.[79,80] For example, El-Haschimi demonstrated that obese animal models subjected to HSFD for 15 weeks were completely unable to induce hypothalamic STAT3 activation, suggesting the onset of hypothalamic leptin resistance.[80] Another study reported that acute ICV palmitate administration blunted leptin-evoked STAT3-phosphorylation in hypothalamus by 60% compared to pretreatment, indicating that even short-term exposure can

have a negative repercussion on hypothalamic functioning.[81] In fact, transgenic mice with specific deletion of STAT3 from the CNS proved to be hyperphagic and obese, showing the importance of neuronal STAT3 signalling in energy homeostasis.[82] Therefore, additional studies keep supporting that both acute and prolonged exposure to high levels of SF seem to trigger hypothalamic dysfunction and its consequences on weight gain, which in this case is by compromising leptin signalling.

1.5.4. Hyperphagia

As previously described, ingesting elevated levels of SF can negatively impact brain mechanisms, such as insulin and leptin signalling, which are important hormones in the regulation of appetite. Accordingly, a number of studies have demonstrated that rodents with *ad libitum* access to a HSFD compared to a low-fat diet induces overeating and contributes to excessive weight gain.[38,50,83,84] For example, in C57BL/6J mice, which are the most known inbred mouse strain and are susceptible to diet-induced obesity, changing their diet from chow to high-lard led to an increase in their mean caloric intake by 75% within 24 hours.[47] Some have attributed the higher energy intake to greater palatability, texture and caloric density of the food in a HSFD[85,86], yet many reports indicate that these properties are not essential for hyperphagic behaviours.[51,52,87,88] Research demonstrated in rats that the consumption of a small "preload-to-test meal" high in SF was associated with an increase in CSFA by 2- to 3- folds and also promoted the expression of orexigenic peptides galanin and orexin.[52] As expected, this group had significantly greater food intake than the low-fat preload group.[52] In support of this, a subsequent study comparing in this case the effect of dietary fatty acid composition on food intake also demonstrated increased expression of orexigenic peptides and hyperphagic behaviours in animal models subjected to a HSFD, but no effect in a group given high levels of unsaturated fat.[50]

Therefore, HSFD may not only induce insulin and leptin resistance, but also seem to favour the expression of orexigenic peptides, which all together lead to increased feeding and excess caloric intake.

1.6. Associations between genetic variations in the *NF-κB1* gene and health conditions

With rapid progress in human genome decoding, it is now possible to identify genetic polymorphisms that are thought to predispose certain individuals to diseases and to uncover the processes involved in disease pathogenesis.[89] Multiple types of genetic variations exist, classified as either single nucleotide polymorphisms (SNPs) or structural variants.[90] The later encompasses insertion–deletions, block substitutions, inversions of DNA sequences and copy number differences.[90] However, SNPs are the most common type of genetic variation and result from a single nucleotide being substituted for another at a given position on the genome, which can lead to amino acid change (coding region), change in gene expression (regulatory region upstream for the gene) or no effect (silent variant).[90]

Multiple SNPs in NF-κB have been associated with increased or decreased risk of some diseases. For instance, NF-κB subunit 1 (*NF-κB1* or p50) rs3774932 was associated with a shorter time to recovery in breast cancer patients.[91] The rs3774968 SNP increased the risk to develop venous thromboembolism in myeloma patients who were given a low dose of aspirin in prophylaxy.[92] It has also been found that the genetic variant *NF-κB1* rs4648022 increases the risk of developing non-Hodgkin lymphoma,[93] whereas, the *NF-κB1* SNP rs4648127 was associated with greater risk of developing lung cancer.[94] Thus, numerous SNPs in *NF-κB1* gene have been linked to several adverse health conditions, particularly several types of cancer, so we can

suppose that they have a functional effect or are in linkage with other causal SNPs, which may result in change in protein synthesis or function.

Although there is evidence of HSFD upregulating NF-κB and triggering an inflammatory response, it is unclear, however, whether SNPs in the *NF-κB1* gene modify the HSFD-inflammation-obesity relationship. Yet, a study using two American populations found an interaction between SF intake and an obesity genetic risk score on modulating BMI, further supporting that it may be worthy to explore the HSFD-*NF-κB1* interactions on anthropometric traits in Canadian populations.[95] No study has so far examined the relationship between SNPs in *NF-κB1* and obesity-related traits. Nonetheless, it is important to add that SNPs individually only explain a fraction of the variance in a predictive model of a health condition. Although there is evidence that genetics may confer a risk for certain adverse health outcomes, it is important to understand that the interaction with the environment may amplify or attenuate genetic predispositions. This said, carriers of a certain SNP in the *NF-kB1* gene may be at risk, but without being exposed to a specific environment, adverse physiological responses will not be triggered and thus, not subjected the individuals to health-related problems.

1.7. Overview, research problem, goals and hypothesis

In summary, the literature suggests that exposure to a HSFD results in elevated CSFA levels, subsequently promoting the entrance of these molecules within cells such as hypothalamic neurons. Governed by NF-κB, an abnormal rise SF in the neuron triggers an innate immune inflammatory response. Activation of inflammatory signals consequently favours pro-apoptotic activity, resulting in neural damage and scaring. In combination with cellular injury, inflammation inhibits important hormone signalling pathways responsible in nutrient sensing and

appetite control. Ultimately, the central energy regulator is unable to regulate food intake, which leads to excessive energy intake and thus, weight gain.

The ensuing step was to investigate the association between genetic variations on anthropometric traits in human subjects, while taking into account the physiological repercussion of a HSFD identified in animals. Although it is known that weight gain results from a greater energy intake than expenditure, the growing field of nutrigenetic research is suggesting that one's genetic make-up, in combination with environmental cues such as diet, may be an important factor influencing one's weight status. At this point, it has been demonstrated in animal models that chronic high consumption of SF leads to the up-regulation of the transcription factor NF-κB, resulting in hypothalamic inflammation and ultimately, dysfunction in the central appetite control centre. In addition, it has been identified that certain SNPs in NF-κB may influence specific health conditions such as cancer. Therefore, knowing that SF activates NF-κB, which controls the expression of a large number of inflammatory genes, the **goal of this study** is to investigate how variants in the NF-*κ*B1 gene, alone or in combination with SF, affect anthropometric traits such as BMI and WC in young adults.

We **hypothesize** that the positive relationship between SF and anthropometric traits will be more pronounced in individuals carrying SNPs which may be associated with increased *NF-κB1* synthesis or activity. Although the molecular mechanisms will not be measured in the proposed project, we anticipate that the greatest hypothalamic inflammatory response will occur in those with high SF intake coupled with risk alleles in the *NF-κB1* gene. This gene-diet interaction would generate dysfunctions in the appetite control centre and lead to increased anthropometric traits.

1.7.1. Specific objectives

Objective 1: To determine whether there is an association between dietary SF intake as well as CSFA levels and anthropometric traits (BMI and waist circumference (WC)).

Objective 2: To determine whether SNPs in the *NF-κB1* gene are associated with differences in anthropometric traits (BMI and WC).

Objective 3: To test the interactions between SNPs in the *NF-κB1* gene and dietary/circulating SF on anthropometric traits (BMI and WC).

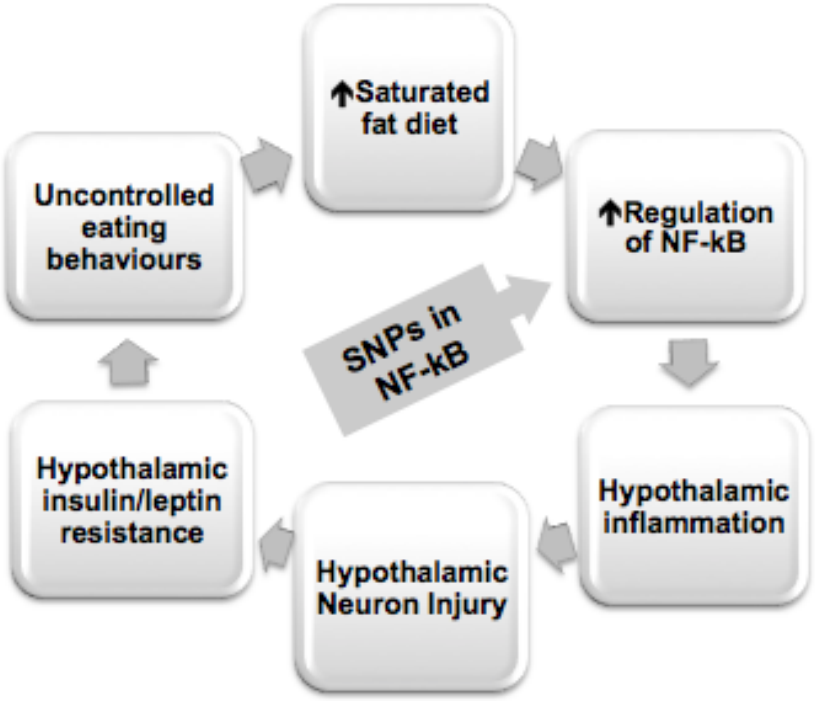

Figure 4. Conceptual framework of the research project.

Chapter 2: Methodology

This chapter describes the study sample as well as explains the procedures involved in data collection. Also, statistical analyses utilized to determine the associations and interactions between our predictors and outcomes of interest are presented.

2.1. Participants

2.1.1. General characteristics

This study is a secondary data analysis from an existing cross-sectional study done on young Canadian adults examining the role of genetics on food intake and food selection as well as gene–diet interactions with regards to biomarkers of chronic diseases. Data was retrieved from the Toronto Nutrigenomics and Health study, which is comprised of a group of free-living young adults (n=1292; 894 women and 398 men) aged of 20-29 years recruited from the University of Toronto campus between October 2004 and December 2010.[96] This age range was chosen because participants are less at risk of being subjected to environmental and lifestyle factors that may alter metabolic functions or lead to disease development over time, which can mask the small effect size that genetic variations, or gene-environment interactions can have on health outcomes.

This study was performed in a highly multi-ethnic city, which was reflected in the diverse origins of the participants. Thus, because of population stratification issues, that is the difference in allele frequencies between subgroups of the population, and due to differences in dietary habits between ethnic groups, participants were regrouped and analyses were stratified into self-reported main ethnic backgrounds. Participants were categorized into three main groups: Caucasians

(n=673), East Asians (n=465) and South Asians (n=154).[96] Caucasians included Europeans, Middle Easterns, or Hispanics. East Asians consisted of Chinese, Japanese, Koreans, Filipinos, Vietnamese, Thais and Cambodians. South Asians comprised Bangladeshis, Indians, Pakistanis and Sri Lankans. Participants were clustered in a group denoted as "others" if they belonged to more than 2 ethnocultural groups, which were not included in the study due to a large within group diversity and variability in the main variables of interest.

Participants were excluded from the study if they were pregnant and breastfeeding due to the numerous hormonal and physiological adaptations happening in many organ systems and the blood[97]; if they had type 1 diabetes, as they are prone to increase inflammatory marker levels[98]; or if they could not provide a venous blood sample. Any participant with a missing value for predictor, outcome or covariate variables was omitted from the study. Moreover, initial exclusion criterion for unlikely energy intake were gathered from previous peer-reviewed articles published in this population; that is, individuals who declared an unlikely energy intake of <800 kcal/day or >3500 kcal/day for women and >4000 kcal/day for men were removed from analyses. However, further analyses to identify under and over-reporters were done using the Goldberg equation (shown below).[99] Energy intake was taken from the FFQ and BMR was measured using Mifflin-St Jeor equation (which included – list of variables), as it has been identified as the most accurate formula.[100] Physical activity level (PAL) was determined using METs. Participants who expended less than 10 METs of modifiable physical activity on a weekly basis, were classified as sedentary/lightly active and given a PAL of 1.4. For those who were between 10 and 15 METs, they were classified as active and PAL was averaged to 1.7. Participants who were very active, expending over 15 METs weekly, were given a PAL score of 1.9. In order to find the *S* value, we utilized the suitable averages for each variable given by

Black who conducted an in depth analysis of the Goldberg equation and the effect of each variable.[99] Accordingly, within-subject coefficient of variation in energy intake (CV_{wEI}) was given a value of 23%, coefficient of variation of repeated BMR measurements (CV_{wB}) was equal to 4% and 15% was used for the coefficient of between-subject variation in physical activity (CV_{tP}). Since each participant was evaluated individually, the number (n) of participants included in the finding the cut-offs was equal to 1. When using a standard deviation of 2, 517 participants were excluded. On the other hand, with a standard deviation of 3, 305 participants were removed. Thus, to avoid losing close to half of our participants and maximizing the power of our analyses, we utilized a standard deviation of 3. As a result, from the 1292 individuals from the original sample group, only 987 were included in the final analyses for dietary SF.

$$\mathrm{EI_{rep}{:}BMR} > \mathrm{PAL} \times \exp\left[\mathrm{s.d._{min}} \times \frac{(S/100)}{\sqrt{n}}\right]$$

$$\mathrm{EI_{rep}{:}BMR} < \mathrm{PAL} \times \exp\left[\mathrm{s.d._{max}} \times \frac{(S/100)}{\sqrt{n}}\right]$$

$$S = \sqrt{\frac{\mathrm{CV^2_{wEI}}}{d} + \mathrm{CV^2_{wB}} + \mathrm{CV^2_{tP}}}$$

2.1.2. Sociodemographic and lifestyle assessment

Participants were asked to fill out a general health and lifestyle questionnaire, which included information on age, sex, ethnicity and smoking status (currently smoke at least one cigarette per day). These specific pieces of information were used for confounder analysis in order to verify their level of influence on the dependent and independent variables tested for. Another questionnaire was developed for this study to assess physical activity level was given to

each participant. Participants were asked to report the types of modifiable physical activities (leisure or occupational activity) that they usually took part of on a weekday and weekend day. For each activity, they specified the intensity and the number of hours spent doing it, which was later converted into metabolic equivalents (METs). A MET is a physiological measurement expressing the energy cost of a physical activity and represents the average energy expenditure for an adult sitting quietly and is approximately equal to 1 kcal per kg of body weight per hour.[101] Accordingly, light activities are equivalent to 2.4 METs, moderate activities represent 3.5 METs and vigorous activities are equal to 7.5 METs.[102] The daily score was then weighted for each weekday and weekend day and then averaged to yield a physical activity score in MET-hours per week.

2.2. Predictor variables

2.2.1. Dietary assessment of saturated fat

In order to assess their habitual food consumption, participants had to complete a modified 196-item Willett semi-quantitative food frequency questionnaire (FFQ) of their dietary intake over the past month. Modifications were made from the original questionnaire in order to improve the dietary assessment of whole grains, fruits, and vegetables.[103,104] For each food item, beverage or dietary supplement, participants were asked to report their frequency of consumption over the past month. Visual aid of portion sizes was shown to participants when instructed on how to complete the questionnaire to help them assess their self-reported food intake. Then, the response to each food item was converted to a daily number of servings. Estimated daily SF intake in grams per day was thus derived from this questionnaire. Although self-report energy intakes are known to have substantial biases and inaccuracies[105,106], analyses using SF from the

FFQ was still used as a means to compare with CSFA measurements as well as other studies.

2.2.2. Assessment of circulating saturated fatty acids

Following a minimum fast of 12 hours, blood samples were collected from the antecubital vein at the LifeLabs medical laboratory services (Toronto, Canada). Blood samples were centrifuged and blood plasma was subsequently frozen at -80°C. After being thawed, gas chromatography was used as previously described[107] in order to quantify all plasma sub-fractions of fatty acids, including CSFA levels. Absolute CSFA measurements were calculated by comparing individual CSFA peaks to the internal standard and were reported as μg of CSFA/mL of plasma. The CSFA analyzed were the following: myristic acid (C14:0), pentadyclic acid (C15:0), palmitic acid (C16:0), stearic acid (C18:0), nonadecyclic acid (C19:0), arachidic acid (C20:0), heneicosylic acid (C21:0), behenic acid (C22:0) and lignoceric acid (C24:0). Absolute measurements were utilized instead of percent composition in order to facilitate comparison between studies. Indeed, the reference value used for relative measurements may vary from one study to another, thus making it unfeasible to compare findings. At the time when these measurements were performed, only 898 samples were available and analyzed. Thus, the study sample differs for dietary SF (n=987) and CSFA analyses (n=898).

2.2.3. Genotyping, SNP selection and linkage disequilibrium

DNA was isolated from white blood cells and multiplex genotyping was performed using the Sequenom MassArray® platform at Princess Margaret Hospital. A tag-SNP approach was chosen to select the SNPs for this study. Tag-SNP selection in the *NF-kB1* gene was performed using HapMap release 27 (https://www.genome.gov/10001688/international-hapmap-project/)

and Haploview 4.2 (https://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) with a minimum minor allele frequency of 5% and r^2 threshold of 0.80 using pairwise tagging. Eighteen tag SNPs in *NF-κB1* gene were identified and genotyped in this cohort. For quality control, 10% of the population was genotyped a second time and a >99% concordance was achieved. Furthermore, since our Tag SNPs were chosen based on a Caucasian population in HapMap, we tested the linkage disequilibrium (LD) in our entire sample and within each ethnic group (Appendix III). LD plots were generated using the *Linkage Format* in Haploview 4.2, with an r^2 threshold of 0.8 (https://www.broadinstitute.org/haploview/chapter-1-using-haploview). LD plots generated for each ethnic group provided information on the frequency at which alleles at different loci are inherited together. The different shades of red for each square represents the degree of association between two alleles, where a darker red means a strong correlation between the two SNPs and white means no correlation. The numbers within some of the boxes represent de correlation coefficient. LD plots will also display "blocks" when SNPs are inherited together and form a block of haplotypes. We used Haploview to determine each tag SNPs within these blocks. Accordingly, within these haplotype blocks, only the SNP tagging for the entire block with the highest minor allele frequency should be analysed (see Appendix III).

2.3. Outcome variables

All anthropometric measurements were taken by trained personnel. Wearing light clothing and without any shoes, body weight was measured to the nearest 0.1 kilogram using an electronic scale.[108] Using a wall-mounted stadiometer, height was measured to the nearest 0.1 centimetre. BMI was calculated using the following equation: weight(kg)/(height(cm))^2. To measure the waist circumference, participants were asked to be upright with their arms to their sides and to

breathe normally. Measurements were carried out using a non-stretchable measuring tape at the mid-point between the lower ribs and the iliac crest. At least two measurements were taken and, if they differed by more than 0.1 cm, a third one was taken. The mean of the waist circumference measurements were utilized for analyses.

2.4. Statistical analyses

2.4.1. Conceptual models

Based on evidence found in the literature, conceptual models were developed for every objective. These included the independent and dependent variables, confounders, mediator variables and effect modifiers. A confounding variable is an extraneous factor in the statistical model that is associated with both independent and dependent variables and may bias the outcome of the association, consequently reducing the internal validity and increasing the risk of reporting false associations.[109] On the other hand, a mediator is a third theoretical variable that links is caused by the predictor variables of interest and in turn affect the disease or outcome.[110] Effect modifiers are in their case variables that fall outside of the two associative variables, but that influences the magnitude of the outcome and thus, needs to be considered in statistical analyses.[111]

Figure 4 conceptualizes our first objective showing how, according to the literature, consuming SF modifies caloric intake and ultimately, has an impact on anthropometric traits. Ethnicity can influence SF intake since there are differences in dietary habits between cultures. In addition, factors such as age, sex, physical activity and smoking status will need to be taken into account in our analyses since they may change the association between SF intake and anthropometric traits.

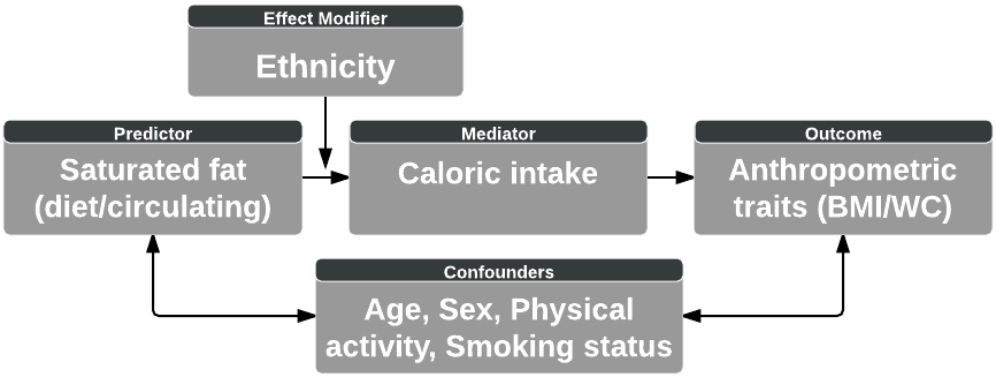

Figure 5. Conceptual model of the association between saturated fat measurement and anthropometric traits.

Figure 5 illustrates our second objective which is the association theorized between SNPs in the *NF-κB1* gene and anthropometric traits. LD between a marker and a causal variant, as well as minor allele frequency, may differ between ethnic groups. Due to the influence of factors such as age, sex, physical activity and smoking status on predictor and outcome variables, these factors needed to be considered during statistical analyses. The literature suggests that inflammation (the outcome from NF-*κB1* gene activation) may cause deregulation in insulin and leptin signalling and thus, mediate energy intake (refer to section 1.5.2. and 1.5.3.).

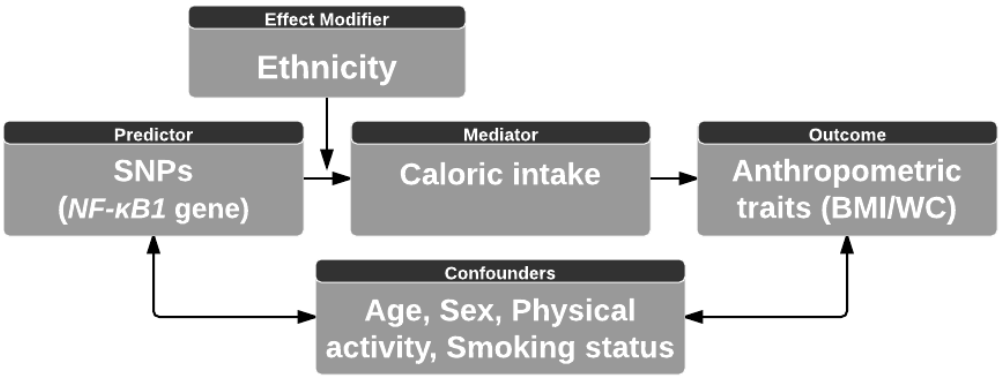

Figure 6. Conceptual model displaying the association between SNPs in the *NF-κB1* gene and anthropometric traits.

Finally, Figure 6 displays the conceptual model of our third objective for the interaction between SNPs in the *NF-κB1* gene and SF measurements on anthropometric traits. As previously stated, ethnicity, age, sex, physical activity and smoking may be associated with one or the other (or both) predictors and the outcomes and modify the interaction and need to be integrated in the statistical model. Moreover, activation of the inflammatory genes has been suggested to affect energy consumption. SF being the highest energy dense macronutrient also affects caloric intake.

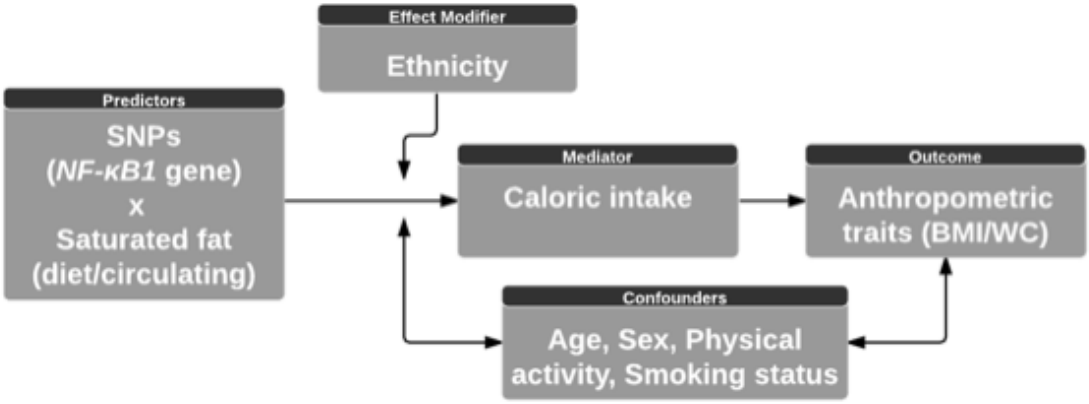

Figure 7. Conceptual model of the interaction between SNPs in the *NF-κB1* gene and saturated fat measurements on anthropometric traits.

2.4.2. Exploratory statistics

The software Statistical Analysis System © (SAS version 9.4) was utilized to perform the statistical analyses for each objective. Exploratory statistics were first performed on the original study sample (n=1639) on all variables for the entire population as well as on each ethnic group (Caucasian n=778; East Asian n=565, South Asian n=175 and others n=119) in order to identify their distribution for categorical variables and the presence of any potential outliers for continuous variables. Accordingly, the ethnic group denoted as "others" was removed due to within group variability in genetic background and dietary habits. Also, the entire sample

distribution for main continuous dependent variables was closer to a normal distribution upon removal of this group. Individuals with missing data for independent (dietary SF n=7; CSFA n=741), dependent (BMI n=3; WC n=3), SNPs of interest (n=140) and other relevant variables (sex n=2; age n=2; ethnicity n=2; physical activity level n=3) were excluded from the analyses. Participants who were classified as being muscular (n=10) according to the research staff were removed from analyses since their anthropometric traits may be misleading and result in false conclusions. Participants who reported a caloric intake outside of the calculated Goldberg cut-off (n=305) were removed from analyses using dietary SF. Women breastfeeding (n=1) were also not included in the study. Finally, scatter plots for independent and dependent variables were used to detect the presence of potential outliers with a cut-off of three standard deviations. However, no data points were found outside of this exclusion criterion.

The distribution of the main continuous independent (dietary/circulating SF measurements) and dependent (BMI, WC) variables was assessed by evaluating skewness, kurtosis and visual inspection of distribution. In order to establish a relationship between two variables and conduct statistical analyses, assumptions such as having a normal sampling distribution are made. Thus, dependent variables were tested for normality using the Anderson-Darling test. These variables failed the stringent parameters of the normality test. Nonetheless, the dependent variables were not modified since, although it is favourable that they are normally distributed in order to make certain inferences, a large sample size helps to overcome deviation from normality and provide results that are reasonably close to what would be expected if this assumption was not violated.[112] Further, upon visual inspection of independent variables, dietary SF appeared to have a normal distribution. However, CSFA was clearly not normally distributed, where two distinct groups could be perceived in its distribution, and no transformation procedures helped in resolving the

lack of normality. Attempts were made to categorize CSFA in either tertiles or quartiles and verify their relationship with the dependent variables. Quartiles of CSFA proved to be the best alternative to separate the clustered distribution evenly, where both clusters were approximately split in half. Further investigation demonstrated a linear relationship with outcome variables. Thus, CSFA was thereafter categorized in quartiles and analyzed as an ordinal variable.

Descriptive statistics, such as mean, standard errors and frequencies, were performed on dependent, independent and other relevant characteristics of both study samples, according to the sample size used with dietary SF (Table 1) and CSFA (Table 2). In addition, we tested the correlation between SF measurements (dietary intake versus circulating levels). This was executed both on the original group as well as after the excluding under- and over-reporters. Confounder analysis was performed by examining the relationship between potential confounders such as age, sex, physical activity, ethnicity, smoking, caloric intake, and c-reactive protein (CRP) measurements (sign of inflammation), and independent (SF and genotypes) as well as dependent variables (BMI and WC). The association between each suspected confounding variable with the main predictors and outcomes of interest was tested independently. If a confounder was associated with both the predictor and the outcome and modified the association (ex. change of 10% in the estimate) when entered in the statistical model, the variable was kept in the final model. CRP was the only potential confounder which analyses were not adjusted for since studies have associated variations in CRP levels with ethnicity[113], diet[114], psychological stress[115], use of oral contraceptives[116] and multiple health conditions not tested for in the present study[117–119]. Thus, knowing how highly sensitive it is to countless internal bodily changes and stressors and, consequently, making the magnitude of its measurements unpredictable, it was removed from the statistical models. Moreover, smoking was used as a covariate for analyses

with SF, but not for CSFA since there was only one smoker in this subgroup.

Interactions between potential confounders and predictor variables on the outcomes of interest were tested. Due to the large number of interactions tested, Bonferroni for multiple testing was applied (new p value threshold = 0.05/number of interactions). No interaction remained significant after multiple testing. Furthermore, in order to detect any potential genotyping errors or population stratification, deviation from Hardy-Weinberg Equilibrium (HWE) using χ-square test with 1 degree of freedom was tested in the entire sample and by ethnic group (Appendix I, Table 1). Several SNPs deviated from HWE but mostly in the total sample size (see Appendix I, Table 1). When stratified by ethnic groups, most of them were in equilibrium, except for 3 SNPs among East-Asians (rs1317745, rs4648090 and rs4648110) and 1 SNP among South Asians (rs4698863). No significant results were observed in our three objectives for these SNPs (except for two; rs3774956 and rsrs4648090; see Results section). Thus we cannot rule out the risk of type II errors for SNPs deviating from HWD for which we did not find a significant association and the risk of type I error for the significant associations found despite departure from HWD. If a genotype represented less than 5% of the total sample, it was grouped with the heterozygous group and a dominant model was used for analyses. Furthermore, multiple SNPs were found to be in LD. Blocks of haplotypes for each ethnic group can be found in Appendix III. It is also worth mentioning that the investigation aiming to identify SNPs in LD was performed after completing the main analyses; therefore findings for objective 2 and 3 were reported for all SNPs. Among the 18 SNPs analyzed, only 1 SNP (rs4648110) could have been omitted from the analyses since it was not a tagSNP in the entire population nor within the three ethnic groups.

2.4.3. Statistical models

Adjusted general linear models were used to assess our three objectives, including gene-diet interactions since our dependant variables are continuous (ex. BMI and WC). An additive model was used to assess the relationships of interest for 7 of the SNPs, whereas a dominant model was used for 11 of them since minor allele frequencies for these genotypes were too low for analyses (≤5%). The distribution of the residuals for each model was also verified, and CSFA again proved to be clustered in two groups, further supporting the need to categorize it. Due to larger number of analyses in objectives 2 and 3 (associations and interactions with all SNPs), we performed corrections for multiple testing for all SNPs, as well as SF and CSF on BMI and WC using the False Discovery Rate. Mean or estimate (ex. slope) comparisons between genotypes within each model were done using the Tukey-Kramer method with SAS 9.4. Significant differences was set at a p value <0.05. Finally, all final models were adjusted for age, sex, physical activity, caloric intake, smoking and ethnicity.

Power calculation was done using Proc Power in SAS version 9.4. Assuming a sample size of 1292 participants, an alpha threshold between 0.01 and 0.001, with 5 to 10 predictors in the model, with an r-square between 0.10 and 0.25 in the full model and if testing for an additive or dominant model; we had at least 80% power to detect an r-square difference of 0.01 to 0.05 between the model with and without the interaction term (i.e. CSFA x *NF-κB1* interaction). However, power was of 70% or more when the alpha=0.001 combined with r-square of 0.15 or lower in the full model.

Chapter 3: Results

This chapter focuses on describing the findings uncovered in each objective. Firstly, the general characteristics of each sample group, respectively categorized by ethnicity and by quartiles of CSFA, are presented. Secondly, the associations between CSFA as well as dietary SF and anthropometric traits are illustrated. Thirdly, the associations between SNPs in the *NF-κB1* gene and anthropometric traits are displayed. Finally, the major findings of the interactions between dietary SF and SNPs in the *NF-κB1* gene on anthropometric traits are presented.

3.1. General characteristics

The majority of the sample (n=1292) consisted of women (n=894) and more than half of the study group were Caucasians (n=398). On average, the participants expended 7.7±0.1 METs of modifiable physical activity per week, classifying them as lightly active. Indeed, the weekly recommendations set by CSEP are 150 minutes of moderate to vigorous physical activity which is the equivalent of 10 to 15 METS.[120] Majority were non-smokers and most fell within the normal-weight and normal-risk categories for BMI and WC, respectively according to Canadian guidelines for body weight classifications in adults (Table 1).[121] General characteristics were then compared between ethnic groups (Table 1), where significant differences were observed for all variables of interest. Consequently, analyses on total sample were adjusted for ethnicity and stratified analyses were also performed within each ethnic group. Since CSFA was not measured in all participants (n=898), general characteristics were also performed in this subgroup as well as between CSFA quartiles (Table 2). Interestingly, there was a significant difference in BMI and WC between quartiles of CSFA, yet no statistical difference was observed in caloric intake. Also, there was no difference in SF intake across CSFA quartiles. Correlation analyses further

demonstrated no relationship between SF measurements (r = -0.0006; p=0.99). This can be explained by the fact that SF can be synthesized de novo[122], thus circulating levels are not a good marker of dietary intake. Finally, physical activity level and ethnic groups differed between quartiles but both were taken into account in subsequent models.

Interactions were then tested between our predictor (SF) and covariates on our outcomes of interest (BMI and WC). We observed significant interactions between quartiles of CSFA and age on WC (p=0.03 for age as a continuous variable and p=0.02 for age categorized in quartiles). An interaction was also found with quartiles of CSFA and sex on WC (p = 0.03). Finally, another interaction was observed between saturated fat intake and smoking on WC (p = 0.047). Analyses were thus stratified accordingly by either age (quartiles), sex and smoking. Then, interactions between SNPs and covariates on the outcome variables were tested for. Significant interactions were observed between SNP rs4648022 and physical activity (p = 0.03), caloric intake (p = 0.004) and ethnicity (p = 0.007) on BMI. Likewise, significant interactions were identified between SNP rs4648022 and calories (p = 0.01) and ethnicity (p = 0.007) on WC (Table 1 and 2, Appendix II).

Table 1. Participants characteristics categorized by ethnicity.

	Total	Caucasian	East Asian	South Asian	*p*
***n* (men/women)**	987 (295/692)	532 (171/361)	350 (81/269)	105 (43/62)	0.006
Age (yrs)	22.74 ± 0.08	23.3 ± 0.1	22.1 ± 0.1	22.1 ± 0.2	<0.0001
Caloric intake (kcal)	2141 ± 18	2228 ± 24	2012 ± 29	2132 ± 52	<0.0001
Dietary SF intake (g)	23.0 ± 0.3	24.4 ± 0.4	21.1 ± 0.4	21.9 ± 0.8	<0.0001
CSFA levels (µg/mL)*	579.2 ± 216.1	598.9 ± 256.2	571.5 ± 176.9	555.7 ± 188.5	0.01
Physical Activity Level (Mets)	7.6 ± 0.1	8.2 ± 0.1	6.8 ± 0.2	7.6 ± 0.3	<0.0001
Smokers *n* (%)	56 (5.7)	43 (8.0)	10 (2.9)	3 (2.9)	0.002
Weight (kg)	63.3 ± 0.4	67.3 ± 0.5	57.5 ± 0.5	63.4 ± 1.1	<0.0001
Height (cm)	166.8 ± 0.3	169.8 ± 0.4	162.9 ± 0.4	164.9 ± 0.8	<0.0001
BMI (kg/m^2)	22.6 ± 0.1	23.3 ± 0.1	21.6 ± 0.1	22.8 ± 0.3	<0.0001
WC (cm)	73.6 ± 0.3	75.6 ± 0.4	70.4 ± 0.4	74.0 ± 0.9	<0.0001

*Values are given as mean ± SE. p-values for differences between ethnicity were calculated using general linear models. Abbreviations: SF = saturated fat; BMI = body mass index; WC = waist circumference.*CSFA was measured in 898 participants. Since this variable was not normally distributed, median±interquartile range are presented. Difference between ethnic groups was tested using a one-way nonparametric test.*

Table 2. Participants characteristics according to quartiles of circulating saturated fatty acid levels.

	Total	Q1	Q2	Q3	Q4	*p*
***n* (men/women)**	898 (260/638)	224 (78/146)	224 (77/147)	224 (50/154)	226 (55/171)	
Age (yrs)	22.7 ± 0.1	22.8 ± 0.2	22.6 ± 0.2	22.4 ± 0.2	22.9 ± 0.2	0.81
Caloric intake (kcal)	1947 ± 21	1950 ± 40	1967 ± 43	1913 ± 43	1957 ± 43	0.87
CSFA levels (µg/mL)	579.2 ± 216.1	435.9 ± 68.1	538.6 ± 42.5	633.7 ± 63.7	923.8 ± 1441	<0.0001
Dietary intake SF (g)	20.8 ± 0.3	20.5 ± 0.5	20.9 ± 0.6	20.8 ± 0.6	21.0 ± 0.6	0.95
Physical activity level (METS)	7.5 ± 0.1	7.5 ± 0.2	7.2 ± 0.2	7.5 ± 0.2	8.0 ± 0.2	0.04
Smokers *n* (%)	1 (<0.01)	0	0	1 (<0.01)	0	0.66
Weight (kg)	63.4 ± 0.4	63.5 ± 0.8	62.4 ± 0.7	62.1 ± 0.9	65.5 ± 0.9	0.01
Height (cm)	166.6 ± 0.3	167.7 ± 0.6	166.9 ± 0.6	165.4 ± 0.6	166.4 ± 0.6	0.06
BMI (kg/m^2)	22.7 ± 0.1	22.5 ± 0.2	22.3 ± 0.2	22.6 ± 0.2	23.6 ± 0.3	0.0003
WC (cm)	73.7 ± 0.3	73.5 ± 0.5	73.2 ± 0.5	72.7 ± 0.6	75.6 ± 0.7	0.0001
Ethnicity *n* (%)	C: 455 (51) EA: 338 (37) SA: 105 (12)	C: 109 (49) EA: 86 (38) SA: 29 (13)	C: 102 (46) EA: 88 (39) SA: 34 (15)	C: 105 (47) EA: 100 (45) SA: 19 (8)	C: 139 (62) EA: 64 (28) SA: 23 (10)	

*Values are given as mean ± SE. p-values for differences between quartiles were calculated using general linear models. Abbreviations: Q = quartile; CSFA = circulating saturated fatty acids; BMI = body mass index; WC = waist circumference; C = Caucasian; EA = East Asian; SA = South Asian.*Since this variable was not normally distributed, median±interquartile range are presented. Difference between ethnic groups was tested using a one-way nonparametric test (Kruskal-Wallis).*

3.2. Associations between saturated fat measurements and anthropometric traits

SF being the most energy-dense and palatable macronutrient, it is often associated with a greater energy intake.[123] Thus, it is assumed that with time, elevated intake of SF would lead to greater anthropometric traits. We examined the relationship between SF measurements and BMI as well as WC in this sample, while adjusting for caloric intake (objective 1). While no

significant association (p = 0.95) was found between dietary SF and anthropometric traits (Table 3), a strong association was found between quartiles of CSFA and both BMI and WC in the entire sample (β±SE: 0.36 ± 0.10; p = 0.0003 and 0.88 ± 0.23; p = 0.0001, respectively) and in South Asians (β±SE: 1.0 ± 0.3; p = 0.004 and 2.2 ± 0.8; p = 0.01, respectively) (Figure 1 and 2).

For analyses on the entire sample, post-hoc analyses confirmed that there was a significant difference between quartiles 1 and 4 as well as 2 and 4 (p = 0.005 and p = 0.001, respectively) for BMI and a marginal difference between quartiles 1 and 4 (p = 0.05) and significant difference between quartiles 2 and 4 (p = 0.01) for WC. No or borderline associations were observed for both BMI and WC in Caucasians and East Asians, while a significant relationship was detected in South Asians (Figure 1 and 2). Post-hoc analyses demonstrated a significant difference between quartiles 1 and 2, and 1 and 4 (p = 0.01 and p = 0.01, respectively) for BMI and quartiles 2 and 4 (p = 0.01) for WC in South Asian.

Table 3. The association between dietary saturated fat (g) and body mass index (kg/m^2) as well as waist circumference (cm) (n=987).

	BMI		WC	
	β±SE	*p*	β±SE	*p*
Total	0.01 ± 0.02	0.46	0.03 ± 0.04	0.43
Caucasian	0.03 ± 0.02	0.24	0.07 ± 0.05	0.16
East Asian	-0.03 ± 0.03	0.32	-0.06 ± 0.06	0.34
South Asian	0.005 ± 0.06	0.93	0.02 ± 0.16	0.91

Values shown are slope±SE. p-values were calculated using general linear model and adjusted for for sex, age, physical activity, calories and smoking status. Total sample was also adjusted for ethnicity.

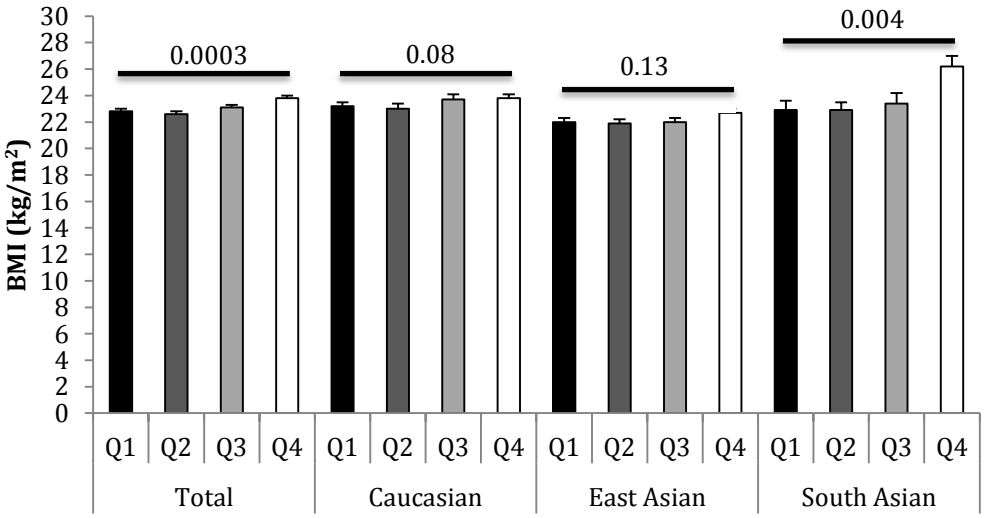

Figure 8. The association between quartiles of circulating saturated fatty acid levels (µg/mL) and body mass index (kg/m^2) for all participants as well as for each ethnic group (n=898). *Values are given as mean ± SE. Abbreviation*: Q = quartiles. *p-values for differences between quartiles were calculated using general linear model and adjusted for sex, age, physical activity and calories. Total sample was also adjusted for ethnicity.*

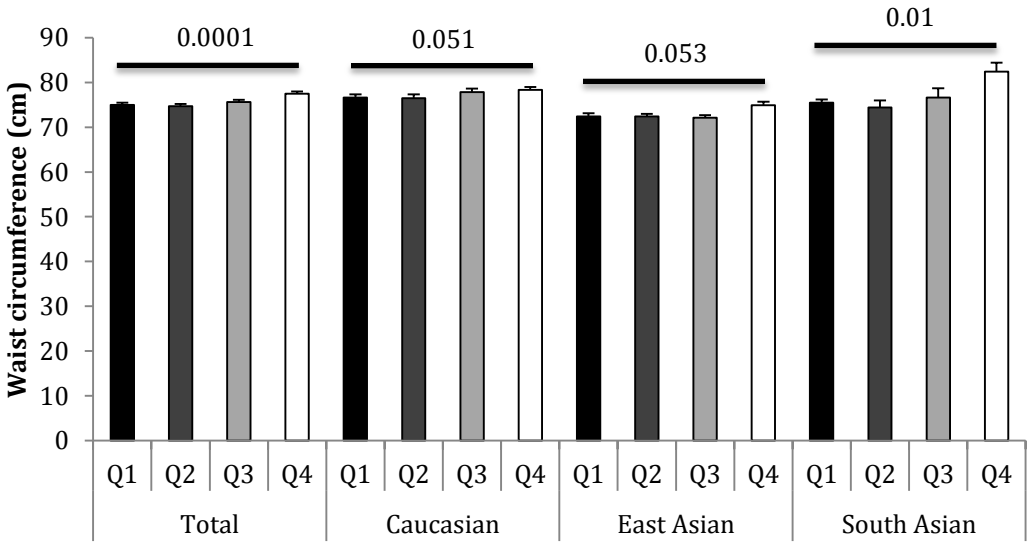

Figure 9. The association between quartiles of circulating saturated fatty acid levels (µg/mL) and waist circumference (cm) for all participants as well as for each ethnic group (n=898).
Abbreviation: Q = Quartiles. p-values for differences between quartiles were calculated using general linear model and adjusted for sex, age, physical activity and calories. Total sample was also adjusted for ethnicity.

3.3. Associations between SNPs in the *NF-κB1* gene and anthropometric traits

We investigated if the genetic variations of interest in the *NF-κB1* gene were associated with anthropometric traits (objective 2), since this gene is known to be activated when exposed to a HSFD and caloric excess which ultimately affect important regulatory processes associated with anthropometric alterations. We found that rs3774956 and rs4648022 were associated with a significant difference in both BMI and WC between genotypes (Table 4). In fact, individuals homozygous for the major allele (wildtype genotype) in these SNPs had significantly lower anthropometric traits than carriers of the minor allele (Table 4). After testing each SNP for deviation from Hardy-Weinberg equilibrium, we found a significant deviation from expected allele frequencies for SNPs rs3774956 in the entire sample and rs4648090 in East Asians (Appendix I, Table 1). Also, upon correcting for multiple comparisons for analyses performed in the entire group, all significant results were lost. Thus, results for these SNPs should be interpreted with great caution.

Other SNPs such as rs1599961, rs3774932, rs3774968 and rs4648006 were only associated with differences between genotypes for WC (Table 4). Post-hoc analysis demonstrated that wildtype genotype in rs1599961 show trends of having lower WC measurements than AG genotype carriers (p = 0.053). SNP rs1599961 was also tested in a dominant model, which improved both its association with BMI (G/G: 23.3 ± 0.2 kg/m^2, carriers of A allele: 23.7 ± 0.2 kg/m^2; p = 0.04) and WC (G/G: 75.9 ± 0.5 kg/m^2, carriers of A allele: 77.0 ± 0.5 kg/m^2; p = 0.02). SNP rs3774932 G/G genotypes demonstrated marginally greater WC than those with the A/A genotypes (p = 0.05). As for SNP rs3774968, individuals homozygous for the major G allele had significantly greater WC than homozygous for the minor A allele (p = 0.04). Finally, the rs4648006 CC genotype was associated with lower WC compared to carriers of the T allele (p =

0.045) and a similar trend was observed with BMI (p = 0.06). However, no significant results remained after performing multiple comparison corrections; therefore results need to be interpreted with caution.

When analyses were stratified by ethnic groups (Table 5), carriers of the minor T allele (n=47) for the SNP rs4648006 had a significantly higher BMI than those with the C/C genotype (n=418) (23.7 ± 0.5 kg/m^2 vs 22.7 ± 0.3 kg/m^2, respectively; p = 0.009) in East Asians. Yet, for the same ethnic group, the association between genotypes in this SNP and WC only showed a marginal association (p = 0.054). Moreover, the two other SNPs rs4648022 and rs4648090 were also associated with differences in BMI and/or WC between genotypes in East Asians (Table 5) and after testing for multiple comparisons, were the only ones to remain significant. However, carriers of the minor allele constituted less than 5% of the entire sample, thus greatly reducing the confidence in the results.

Table 4. Associations between genotypes in the *NF-κB1* gene (n=1292) and body mass index as well as waist circumference (mean±SE).

SNP	Genotype	n	BMI (kg/m^2)	*p*	WC (cm)	*p*
rs11722146	GG	578	23.4 ± 0.2	0.26	76.1 ± 0.5	0.08
	GA	569	23.8 ± 0.2		77.1 ± 0.5	
	AA	145	23.5 ± 0.3		76.8 ± 0.8	
rs1599961	GG	516	23.3 ± 0.2	0.19	75.9 ± 0.5	**0.04**
	AG	584	23.8 ± 0.2		77.0 ± 0.5	
	AA	192	23.5 ± 0.3		76.9 ± 0.7	
rs230511	GG	550	23.4 ± 0.2	0.53	76.2 ± 0.5	0.21
	GA	578	23.7 ± 0.2		76.9 ± 0.5	
	AA	164	23.5 ± 0.3		76.7 ± 0.7	
rs3774932	GG	324	23.6 ± 0.3	0.18	77.1 ± 0.6	**0.02**
	GA	658	23.6 ± 0.2		76.6 ± 0.5	
	AA	310	23.3 ± 0.3		75.7 ± 0.6	

rs3774968	GG	382	23.7 ± 0.2	0.16	77.1 ± 0.6	**0.02**
	AG	642	23.6 ± 0.2		76.6 ± 0.5	
	AA	268	23.3 ± 0.3		75.6 ± 0.6	
rs4698863	CC	575	23.5 ± 0.2	0.98	76.2 ± 0.5	0.32
	CT	554	23.7 ± 0.2		76.9 ± 0.5	
	TT	163	23.3 ± 0.3		76.5 ± 0.8	
rs7674640	TT	311	23.5 ± 0.3	0.89	77.0 ± 0.6	0.28
	TC	661	23.5 ± 0.2		76.4 ± 0.5	
	CC	320	23.6 ± 0.3		76.3 ± 0.6	
rs13117745[1]	CC	990	23.5 ± 0.2	0.80	76.4 ± 0.5	0.37
	CT + TT	302	23.6 ± 0.3		76.8 ± 0.6	
rs1609798[1]	CC	593	23.5 ± 0.2	0.29	76.1 ± 0.5	0.07
	CT + TT	699	23.7 ± 0.2		76.9 ± 0.5	
rs230547[1]	CC	912	23.6 ± 0.2	0.93	76.6 ± 0.5	0.56
	TC + TT	380	23.5 ± 0.3		76.3 ± 0.6	
rs3774934[1]	GG	813	23.6 ± 0.2	0.38	76.8 ± 0.5	0.11
	GA + AA	479	23.5 ± 0.2		76.0 ± 0.6	
rs3774956[1]	CC	466	23.3 ± 0.2	**0.01**	75.8 ± 0.5	**0.009**
	CT + TT	826	23.8 ± 0.2		77.0 ± 0.5	
rs4648006[1]	CC	1174	23.5 ± 0.2	0.06	76.4 ± 0.2	0.049
	TC + TT	118	24.1 ± 0.3		77.9 ± 0.8	
rs4648022[1]	CC	1154	23.4 ± 0.2	**0.02**	76.2 ± 0.5	**0.02**
	CT + TT	138	24.2 ± 0.3		78.0 ± 0.8	
rs4648090[1]	GG	1086	23.5 ± 0.2	0.25	76.3 ± 0.5	0.18
	AG + AA	206	23.8 ± 0.3		77.2 ± 0.7	
rs4648095[1]	TT	1170	23.5 ± 0.2	0.16	76.4 ± 0.5	0.12
	CT + CC	122	24.0 ± 0.4		77.6 ± 0.8	
rs4648110[1]	TT	944	23.6 ± 0.2	0.66	76.5 ± 0.5	0.86
	AT + AA	348	23.5 ± 0.2		76.6 ± 0.6	
rs4648127[1]	CC	1142	23.6 ± 0.2	0.08	76.7 ± 0.48	0.11
	CT + TT	140	23.1 ± 0.3		75.6 ± 0.7	

*[1] Dominant model. Values are given as mean ± SE. p-values for differences between genotypes were calculated using general linear model and adjusted for age, sex, ethnicity, smoking status, physical activity and caloric intake. *All significant results were lost after testing for multiple corrections using FDR. Abbreviations: SNP = single nucleotide polymorphisms; BMI = body mass index; WC = waist circumference.*

Table 5. Associations between genotypes in the *NF-κB1* gene and body mass index and waist circumference (mean±SE) by ethnic groups.

SNP	Caucasian		East Asian		South Asian	
	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)
rs11722146	0.26	0.14	0.40	0.50	0.16	0.22
rs1599961	0.40	0.26	0.06	0.21	0.53	0.73
rs230511	0.47	0.43	0.57	0.58	0.22	0.31
rs3774932	0.39	0.13	0.09	0.17	0.11	0.16
rs3774968	0.60	0.24	0.12	0.12	0.10	0.15
rs4698863	0.26	0.16	0.74	0.74	0.08	0.42
rs7674640	0.68	0.61	0.60	0.56	0.37	0.97
rs13117745[1]	0.63	0.27	0.74	0.92	0.87	0.93
rs1609798[1]	0.28	0.10	0.26	0.32	0.26	0.78
rs230547[1]	0.52	0.26	0.39	0.21	0.86	0.37
rs3774934[1]	0.35	0.24	0.48	0.41	0.90	0.48
rs3774956[1]	0.09	0.07	0.06	0.21	0.96	0.50
rs4648006[1]	0.76	0.32	**0.009**	0.054	0.36	0.44
rs4648022[1]	0.17	0.052	**0.0003**	**0.0001**	0.14	0.55
rs4648090[1]	0.80	0.56	0.08	**0.005**	0.25	0.69
rs4648095[1]	0.68	0.24	0.14	0.38	0.36	0.44
rs4648110[1]	0.72	0.87	0.84	0.66	0.96	0.96
rs4648127[1]	0.56	0.33	0.29	0.35	0.30	0.76

*[1] Dominant model. p-values for differences between genotypes were calculated using general linear model and adjusted for age, sex, smoking status, physical activity and caloric intake. *SNPs rs4648022 and rs4648090 were the only ones to remain significant after testing for multiple comparisons using FDR. Abbreviations: SNP = single nucleotide polymorphisms; BMI = body mass index; WC = waist circumference.*

3.4. Interaction between SNPs in the *NF-κB1* gene and saturated fat measurements on anthropometric traits

The third and last goal of this study was to verify possible interactions between SF (intake and circulating) and SNPs in the *NF-κB1* gene on anthropometric traits (objective 3). These analyses were performed on both the entire sample population and for each ethnic group due to the influence of ethnicity on diet and minor allele frequencies.

For dietary SF, a significant interaction for both BMI and WC was found for rs230547 examined in a dominant model and specifically East Asians (p = 0.04 and p = 0.02, respectively) (Table 6). Indeed, individuals with the C/C genotype had both a significantly higher BMI and WC measurements (22.8 ± 0.1 kg/m^2 and 74.0 ± 0.3 cm, respectively) than carriers of the T allele (22.2 ± 0.2 kg/m^2 and 72.5 ± 0.4 cm, respectively). Nonetheless, these interactions were no longer significant after multiple comparison corrections. Results need to be interpreted with caution.

When CSFA were examined (Table 7), significant interactions were observed with SNPs rs1609798, rs3774956 and rs4648095 in Caucasians. Opposite to C/C genotype, carriers of T allele for SNP rs1609798 demonstrated a significant and positive relationship between quartiles of CSFA for both BMI (β±SE; carriers of T allele: 0.58 ± 0.20, C/C genotype: -0.07 ± 0.20; p = 0.02) and WC (β±SE; carriers of T allele: 1.27 ± 0.45, C/C genotype: -0.03 ± 0.44; p = 0.04) (Table 8). Similarly, a significant and positive relationship is observed between quartiles of CSFA for carriers of T allele in rs3774956 for both BMI (β±SE; carriers of T allele: 0.53 ± 0.18, C/C genotype: -0.21 ± 0.23; p = 0.01) and WC (β±SE; carriers of T allele: 1.08 ± 0.40, C/C genotype: -0.18 ± 0.50; p = 0.049), but not for G/G genotypes (Table 8). As for rs4648095, a significant and positive relationship was found between quartiles of CSFA in T/T genotype for both BMI (β±SE; T/T genotype: 0.33 ± 0.15, C/C genotype: -1.33 ± 0.67; p = 0.02) and WC (β±SE; carriers of T allele: 0.81 ± 0.32, C/C genotype: -3.63 ± 1.47; p < 0.01) (Table 8).

Significant interactions were also observed with SNPs rs11722146 (p = 0.03) and rs230511 (p = 0.03) on WC in South Asians (Table 7). Post-hoc analyses for SNP rs11722146 found a significant difference between genotypes A/G and A/A (β±SE; A/G genotype: 1.54 ± 1.63, A/A genotype: -4.28 ± 3.15; p = 0.047), whereas only a marginal difference between G/G and A/A (β±SE; G/G genotype: 3.15 ± 0.98, A/A genotype: -4.28 ± 3.15; p = 0.06) (Table 8). No

significant difference was identified between genotypes in SNP rs230511 when they were analyzed between each other (β±SE; G/G genotype: 3.04 ± 0.98, A/G genotype: 1.54 ± 1.64, A/A genotype: -4.31 ± 3.19; *p* interaction = 0.03). No values are given for SNP rs4648022 for East Asian since all participants are carriers of the wildtype genotype (Table 6 & 7). All of these SNPs were in Hardy-Weinberg equilibrium (Appendix I, Table 1). Yet, after performing false discovery rate analyses, all significant results were lost except for rs4648095 in Caucasians. Thus, other results need to cautiously interpreted.

Table 6. Interaction between each SNP in the *NF-kB1* gene and dietary saturated fat intake on anthropometric traits (n=987).

SNP	All		Caucasian		East Asian		South Asian	
	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)
rs11722146	0.10	0.35	0.40	0.57	0.60	0.63	0.21	0.23
rs1599961	0.27	0.38	0.73	0.70	0.37	0.96	0.41	0.44
rs230511	0.13	0.36	0.50	0.58	0.59	0.59	0.21	0.22
rs3774932	0.40	0.49	0.88	0.72	0.66	0.66	0.27	0.43
rs3774968	0.53	0.98	0.78	0.70	0.65	0.50	0.26	0.41
rs4698863	0.23	0.67	0.84	0.85	0.33	0.92	0.63	0.82
rs7674640	0.62	0.71	0.83	0.66	0.75	0.54	0.34	0.81
rs13117745[1]	0.42	0.58	0.48	0.72	0.68	0.61	0.94	0.83
rs1609798[1]	0.66	0.97	0.72	0.67	0.62	0.71	0.81	0.78
rs230547[1]	0.95	0.84	0.55	0.54	**0.04**	**0.02**	0.78	0.70
rs3774934[1]	0.57	0.83	0.42	0.66	0.38	0.46	0.57	0.91
rs3774956[1]	0.52	0.37	0.76	0.84	0.49	0.99	0.44	0.30
rs4648006[1]	0.73	0.99	0.57	0.86	0.54	0.84	0.99	0.76
rs4648022[1]	0.67	0.78	0.21	0.30	---	---	0.68	0.83
rs4648090[1]	0.13	0.12	0.24	0.14	0.94	0.75	0.96	0.98
rs4648095[1]	0.99	0.68	0.68	0.96	0.52	0.76	0.99	0.76
rs4648110[1]	0.28	0.33	0.29	0.34	0.68	0.60	0.95	0.84
rs4648127[1]	0.24	0.21	0.45	0.11	0.87	0.94	0.30	0.69

*[1] Dominant model. p-values for differences between genotypes were calculated using general linear models and adjusted for age, sex, smoking status, physical activity, caloric intake and ethnicity (only for all). *All significant results were lost after testing for multiple comparisons using FDR. Abbreviations: SNP = single nucleotide polymorphisms; BMI = body mass index; WC = waist circumference.*

Table 7. Interaction between each SNP in the *NF-kB1* gene and circulating saturated fatty acid levels (quartiles) on anthropometric traits (n=898).

SNP	All		Caucasian		East Asian		South Asian	
	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)	BMI (*p*)	WC (*p*)
rs11722146	0.97	0.84	0.10	0.07	0.35	0.70	0.15	**0.03**
rs1599961	0.66	0.98	0.32	0.33	0.72	0.48	0.95	0.48
rs230511	0.81	0.97	0.19	0.12	0.29	0.61	0.17	**0.03**
rs3774932	0.63	0.76	0.42	0.40	0.44	0.42	0.71	0.83
rs3774968	0.63	0.51	0.33	0.33	0.47	0.88	0.71	0.94
rs4698863	0.96	0.99	0.13	0.16	0.24	0.68	0.27	0.13
rs7674640	0.90	0.53	0.23	0.20	0.054	0.34	0.70	0.51
rs13117745[1]	0.90	0.65	0.75	0.77	0.44	0.90	0.48	0.38
rs1609798[1]	0.51	0.42	**0.02**	**0.04**	0.34	0.93	0.34	0.24
rs230547[1]	0.39	0.55	0.78	0.56	0.52	0.99	0.30	0.61
rs3774934[1]	0.75	0.56	0.83	0.57	0.94	0.73	0.84	0.86
rs3774956[1]	0.22	0.52	**0.01**	**0.049**	0.25	0.58	0.97	0.32
rs4648006[1]	0.67	0.59	0.34	0.09	0.23	0.66	0.13	0.24
rs4648022[1]	0.91	0.83	0.50	0.61	---	---	0.49	0.78
rs4648090[1]	0.51	0.87	0.32	0.79	0.73	0.58	0.68	0.78
rs4648095[1]	0.72	0.30	**0.02**	**0.002**	0.37	0.81	0.13	0.24
rs4648110[1]	0.61	0.93	0.30	0.49	0.44	0.90	0.43	0.34
rs4648127[1]	0.99	0.63	0.99	0.70	0.33	0.71	0.70	0.14

*[1] Dominant model. p-values for differences between genotypes were calculated using general linear models and adjusted for age, sex, smoking status, physical activity, caloric intake and ethnicity (only for all). *Only SNP rs4648095 with WC in Caucasians remained significant after testing for multiple comparisons using FDR. Abbreviations: SNP = single nucleotide polymorphisms; BMI = body mass index; WC = waist circumference.*

Table 8. Regression coefficients between quartiles of CSFA and anthropometric traits by *NF-κB1* genotypes for significant interactions among Caucasians and South Asians.

SNP	Ethnicity	Genotype	n	BMI (β±SE)	*p*	WC (β±SE)	*p*
rs1609798[1]	Caucasian	CC	219	-0.07 ± 0.20	0.02	-0.03 ± 0.44	0.04
		CT + TT	236	0.58 ± 0.20		1.27 ± 0.45	
rs3774956[1]	Caucasian	CC	172	-0.21 ± 0.23	0.01	-0.18 ± 0.50	0.049
		CT + TT	283	0.53 ± 0.18		1.08 ± 0.40	
rs4648095[1]	Caucasian	TT	427	0.33 ± 0.15	0.02	0.81 ± 0.32	0.002
		TC + CC	28	-1.33 ± 0.67		-3.63 ± 1.47	
rs11722146	South Asian	GG	61	1.20 ± 0.39	0.15	3.15 ± 0.98	0.03
		AG	35	1.01 ± 0.65		1.54 ± 1.63	
		AA	9	-0.99 ± 1.26		-4.28 ± 3.15	
rs230511	South Asian	GG	62	1.15 ± 0.39	0.17	3.04 ± 0.98	0.03
		AG	35	1.01 ± 0.65		1.54 ± 1.64	
		AA	8	-1.03 ± 1.28		-4.31 ± 3.19	

[1] Dominant model. p-values for differences between genotypes were calculated using general linear models and adjusted for age, sex, smoking status, physical activity and caloric intake. Abbreviations: SNP = single nucleotide polymorphisms; BMI = body mass index; WC = waist circumference.

Chapter 4: Discussion & Conclusion

A considerable amount of research on gene-diet interactions has been conducted in the last decade as there is growing evidence that genetic factors can modify the role of diet in the etiology of numerous health conditions, including obesity.[102,124–130] Although the development of obesity remains a multi-factorial phenomenon, numerous studies on animals, and some in humans, have identified and suggested specific mechanisms and processes which could impair energy regulation (intake and expenditure). Based on studies demonstrating that a HSFD activates the NF-κB pathway and alters central energy homeostasis[38,39,46], we hypothesized that genetic variations in the *NF-κB1* gene modify the relationship between SF (intake and circulating) and anthropometric traits in humans. Accordingly, the objectives of this current study were to determine if SNPs in the *NF-κB1* gene, alone or in combination with levels of SF (intake and circulating), influence BMI and WC measurements in young Canadian adults from a multiethnic cohort. Our findings suggest that genetic variations may in fact predispose individuals to having greater anthropometric traits and, in some ethnic groups such as Caucasians, may also interact with CSFA to affect these traits.

4.1. Association between saturated fat and anthropometric traits

The first aim of the study was to examine the relationship between measurements of SF intake and anthropometric traits, where fat intake has been suggested to play a critical role in weight gain. Similarly to other studies that have shown that intake of fat is significantly correlated with adiposity[123,131], our analyses found a significant and positive association between increments of quartiles of CSFA and BMI/WC when the sample was studied in its entirety. Indeed, fat is known to have the least effect on satiety, while being the most energy-dense

macronutrient.[16] Multiple studies have demonstrated that consuming meals higher in fat often results in passive overconsumption and consequently, greater weight gain over time.[16,85,123,131–133] Participants given high energy density meals (high on fat and low in fiber) consumed on average 52% more calories than when they were given low energy density meals (low on fat and high on fiber).[134] In addition, SF is believed to have greater obesogenic properties than other fatty acids.[16,85,132,133,135] Radioactively labeled saturated and unsaturated fatty acids were given to rodents and unsaturated fat proved to be oxidized at significantly faster rates than SF, where the lowest rates of oxidation observed were palmitic and stearic acid, the two most abundant dietary SF.[136] Since long-chain SF are not as efficiently oxidized, they are more likely to be stored as adipose tissue.[135,136] There is also experimental evidence in animals that diets high in SF can affect energy expenditure, specifically through thermogenesis.[137] Hamsters given a HSFD where energy intake matched their baseline energy requirements demonstrated a reduction in oxygen consumption and consequently, an increase in weight and adiposity over time.[137] Thus, even in the absence of overeating, diets high in SF seem to induce weight gain through a decrease in energy expenditure. These findings taken together further suggest that high intakes of dietary fats, especially SF, may lead over time to excess body fat deposition.[133]

Due to findings from other research, we assumed that SF intake would correlate with CSFA.[138] Intuitively, it makes sense that eating high levels of SF will result in greater circulating levels of saturated fatty acids. Yet, no correlation was observed between these two variables in our study group. This may partially be explained by differences in the metabolic fate in different fatty acids[135,139], inaccurate estimation as well as the likelihood of under- and over-reporters in FFQs[105,106] and differences in CSFA levels depending if the participant is fasted or in a postprandial state as well as inter-individual differences[140]. Also, CSFA measurements in this

study consist of non-esterified fatty acids (NEFA), fatty acids that are not attached to a glycerol and that are free in circulation, meaning that triglycerides and phospholipids were not hydrolyze.[141] However, NEFAs can be created de novo or modified in the liver and are metabolized depending on their level of hydrophobicity.[142] They are mostly destined for energy production (oxidation) and normally increase in function of total lipid oxidation.[142] This said, NEFAs tend to correlate only to a short extent with dietary SF. All these factors may have contributed to the lack of correlation between dietary SF and CSFA.

When stratified between ethnic groups, differences in the association between CSFA and anthropometric traits were also observed. While a marginal association was found between CSFA levels and WC in Caucasians and East Asians, a significant relationship was identified in South Asians. In fact, a study has reported that South Asian females living in the UK have a greater prevalence of developing atherogenic dyslipidaemia compared to white-European adults, which was suggested to be due to diet westernization, change in lifestyle behaviors or genetic predisposition.[143] In support of this, South Asian women immigrating to Canada are upon arrival 10% less likely to be overweight compared to native-born Caucasians, but this gap disappears within 15 years.[144] There is also evidence that South Asians have a greater risk than Caucasians of developing obesity and obesity-related non-communicable diseases such as insulin resistance, metabolic syndrome, type 2 diabetes mellitus and coronary heart disease.[145] A few proposed explanations for this occurrence were differences in body phenotypes (high body fat, high truncal, subcutaneous and intra-abdominal fat, and low muscle mass) and biochemical parameters (hyperinsulinemia, hyperglycemia, dyslipidemia and hyperleptinemia).[145] Although weight gain is clearly multi-factorial, there seems to be an enhance susceptibility to adversely respond to the Western diet, often rich in SF, among South Asians. Interethnic differences between dietary

habits and genetic backgrounds further support the importance of conducting diet-phenotype analyses by ethnic groups.

As for analyses performed on dietary SF intake, no associations were identified. Nonetheless, it is noteworthy to point out that the study sample did not consume, on average, over the recommended intake of SF (approximately 10% of their daily energy intake).[146] In addition, it is important to remind the biases associated with using FFQs to report dietary intake such as likelihood of misreporting (type and amount of food) and social desirability.[105,106] Moreover, any tools used to estimate the dietary intake cannot take into account inter-individual variations in metabolism, SF synthesis and absorption.[141] A study conducted on US women examining the correlation between SF from food intake, and CSFA and erythrocyte SF levels revealed that circulating levels did not coincide with dietary recall measurements (Spearman's partial correlation co-efficient between 0.12 and 20; p not significant).[147] When all of these factors are taken together, it demonstrates that there are many limitations to dietary SF measurements and that CSFA represents a more objective measurement. Actually, it is a more appropriate variable to use to explain the impact of this macronutrient on certain physiological mechanisms taken into account in this study.[141,148] Furthermore, physical activity is also an important variable that needed to be accounted for since, like caloric and fat intake, it can also influence body weight. However, physical activity was also self-declared and estimated by the participant. In addition, the questionnaire had not been validated against another method. Thus, there is a high risk of misreporting and misclassification for this variable, which may have affected the adjustment in our models. The ensuing steps were to investigate if genetic variations may affect anthropometric traits, alone or in combination with SF intake.

4.2. Associations between SNPs in *NF-κB1* gene and anthropometric traits

Although all significant associations in the entire sample were lost after correcting for multiple testing, many of the SNPs identified before correction have actually been associated with other health complications. For example, there is evidence that the G/G genotype for rs3774932 may be a predictor for breast cancer reoccurrence in a shorter period of time after treatment, specifically in African Americans and Hispanics.[91] In our sample, this same G/G genotype was associated with a greater WC measurement compared to the alternative genotypes. In addition, there is evidence that the SNP rs4648022 might not only have an impact on anthropometric traits as shown in our study, but also in the development of cancer. Indeed, carriers of the T allele for rs4648022 in the current sample had significantly higher with BMI and WC than the non-carriers. In comparison, Cerhan and his colleagues demonstrated that the C/T and T/T genotypes are respectively at a 1.7 and 2.1 times greater risk of contracting Non-Hodgkin lymphoma than the G/G genotype.[93] The SNP rs3774968 was also proposed both here and in the literature to be associated with the development of health issues; however, the deleterious alleles reported between studies are inconsistent. Our results demonstrated that the G/G genotype for SNP rs3774968 was associated with significantly higher WC than the A/A and A/G genotypes. On the other hand, Bagratuni reported that there is a higher frequency of carriers of the T/T genotype who develop venous thromboembolism compared to C/C and C/T genotype together.[92] Likewise another study[149] as well PubMed SNP database (https://www.ncbi.nlm.nih.gov/snp) report the alleles to be A and G. Nonetheless, even if other populations have another set of alleles, variations at this given position on the gene suggest that it may have an impact on physiological processes. This said, there is a large body of evidence suggesting that there is an implication of genetic variations in the *NF-kB1* gene in the

development of adverse health conditions. However, our findings were not conducive towards finding a genetic association with anthropometric traits. This may be explained by the fact that the study group consisted mostly of young, healthy and relatively slender individuals, making it difficult to find conclusive differences between genetic variations. Therefore, it would be valuable to repeat these analyses in a larger study group who represent more appropriately the general population.

It is well understood that different cultural backgrounds come with diverse genetic make-up.[104] When the analyses were stratified by ethnic groups, our findings only showed significant associations between SNPs in *NF-κB1* gene and anthropometric traits in East Asian participants. After testing for multiple comparisons, WC significantly differed between genotypes for SNPs rs4648090 when analyzed in an additive model, whereas both BMI and WC were significantly different between genotypes for rs4648022 also in a dominant model. Interestingly, these SNPs have been previously associated with other adverse health outcomes as well. As stated earlier, rs4648022 may be a potential determinant for one's overall risk of developing Non-Hodgkin lymphoma.[93] However, this study was also conducted only among Caucasian Americans. It would be interesting to investigate if this gene-cancer association is also observed in East Asians. Moreover, rs4648090 was found to play a significant role in the quality of life of patients after radiation therapy for prostate cancer in a multicultural group.[150] Thus, there is evidence indicating how specific genetic variations play a role in the development of a number of health issues and, in some cases, the associations seem to be more prevalent in certain ethnic backgrounds.

The accumulation of evidence in the literature as well as in this study leads to believe that specific variations within the *NF-κB1* gene may determine the level at which it regulates the transcription of inflammatory genes and consequently, alter normal physiological functioning.

Although specific mechanisms were not analyzed, our study and others suggest that carriers of certain SNPs in this gene may be at greater risk of contracting some adverse health conditions. Interestingly, multiple SNPs were suggested to be associated with both anthropometric traits and cancer. Although inflammation is a critical response and a first line of defense against infection and pathogens, chronic disturbances of metabolic homeostasis can lead to abnormal immune responses.[30] In fact, it is well established that there is a relationship between both cancer[151–153] and obesity[30,154,155], and a deregulation in the inflammatory response. Similarly to obesity, chronic inflammation is perceived as a much greater risk factor for the development of neoplasia than an acute inflammatory response, even though many of the molecular mediators are identical.[63,156,157] In addition, studies demonstrated that obese individuals are characterized with higher levels of pro-inflammatory molecules (TNF and IL6) in circulation compared to non-obese, leading to low-grade and chronic inflammation, and consequently increasing the risk for cancer.[158–160] This said, finding identical SNPs that may both contribute the development of cancer as well as modify anthropometric traits only further support how specific genetic variations play an important role in the development of various health conditions.

Although the implication of inflammation on obesity is a well-studied topic[30,63,157–163], we are the first to our knowledge to identify certain SNPs in the *NF-κB1*, and this exclusively in East Asian, that may influence one's anthropometric traits. The occurrence where SNPs can potentially modify anthropometric traits based on ethnicity may be explained by variances in LD between genetic backgrounds. LD is an evolutionary adaptation involving the non-random associations of alleles at different loci known to be inherited together.[164] Accordingly, specific patterns in gene coding are observed within ethnic groups.[164] In addition, LD promotes variability between gene enhancer elements, which are non-coding segments of DNA that dictate the level

of gene expression.[165] It is believed that differences in LDs between ethnic groups, where the causal variants may fall within enhancers or other coding or non-coding (ex. affecting splicing) regions may contribute to alter the expression of specific gene targets or protein function and, ultimately explain ethnicity differences in the association between SNPs *NF-κB1* gene and anthropometric traits. Overall, our results are suggestive of genetic variations in the *NF-κB1* gene having an impact on BMI and WC in a multicultural group as well as the East Asian group. The ensuing step was to investigate whether diet composition, specifically SF consumption, may modify the association between NF-κB and anthropometric traits.

4.3. Interaction between SNPs in the *NF-κB1* gene and saturated fat intake on BMI and WC

Previous findings in animal studies have shown that SF consumption influences the expression of the *NF- κ B1* gene, energy balance and ultimately body weight and adiposity.[37,38,40,44,83,166,167] We thus hypothesized that the consumption of SF coupled with SNPs in NF-κB have a deleterious effect on inflammation and disrupt energy balance, resulting in changes in anthropometric traits. Our findings showed that in the overall multicultural group, no interactions were identified. Nonetheless, when analyzed by ethnic group, one SNP in Caucasians remained significant after correction for multiple testing. In a dominant model, SNP rs4648095 demonstrated a significant interaction with CSFA on WC. Although rs4648095 has not been reported in previous studies to adversely influence certain health conditions, SNPs rs1609798 and rs11722146, identified before correcting for multiple testing, have both been associated with rectal cancer.[168–170] Indeed, Slattery *et al.* detected a significant interaction between CYP19A1 (an important enzyme involved in the biosynthesis of estrogen) rs700519 and *NF-κB1* rs1609798 on the risk of contracting rectal cancer.[168] Similarly to our results, they identified a greater

probability in carriers of TT genotype for this SNP to develop rectal cancer.[168] Interestingly, another study found gene-diet interactions between SNPs in CYP19A1 and fat intake on total cholesterol levels.[128] Since estrogen concentration has been associated with indices of obesity[171], it may be of interest to further investigate gene-gene interactions between CYP19A1 and *NF-κB1* on anthropometric traits.

There is now evidence suggesting that the level of SF intake may influence the magnitude of the response and consequently, impact energy regulation and body weight. Although many SNPs were identified in the gene-diet interaction of interest, they do not match with the ones associated with anthropometric traits. There are a few potential explanations for this occurrence. First, NF-κB can be activated either by canonical or by non-canonical pathways. Some of the pro-inflammatory signals that triggers the activation of the canonical pathway are described as danger-associated molecular patterns (DAMPs).[172] Interestingly, included in these DAMPs are long-chain SF such as palmitate and ceramide (whereas unsaturated oleate is not).[173] Thus, a HSFD may promote an inflammatory response through the canonical pathway due a rise in CSFA levels. However, it has also been demonstrated in rodents that the expression of non-canonical IKK is dramatically increased (up to 28-fold in adipocytes) in response to a HSFD.[174] Hence, the non-canonical pathway also seems to be activated by nutrient excess. In addition, SNPs may be located within coding, non-coding or intergenic regions of genes. This could affect either transcription factor binding sites and thus gene expression[175], mRNA splicing or other post-translational mechanisms, as well as protein structure or function.[176,177] For example, according to the NF-κB activation pathway that is promoted, binding may be affected by variations in the promoter region due to factors such as conformational differences between RelA-P50 (canonical) and RelB-P52 (non-canonical) complexes. Therefore, binding location and affinity may be

altered not only because of the pathway and the transcription factors activated, but also due to genetic variations within the gene (ex. exons). Such differences could consequently modify the amount of protein produced, the protein structure and function and thus the magnitude of the inflammatory response.

Our findings support our hypothesis that carriers of certain variations in the *NF-κB1* gene may be susceptible to have greater BMI and WC according to their level of SF in circulation. Although the mechanism was not directly investigated, it is believed that greater CSFA levels promote the deregulation of NF-κB, which is known to lead to multiple disease through increased inflammation.[178] NF-κB subsequently activates the SOCS3 gene, which is a critical inhibitor for both insulin and leptin signalling in the hypothalamus.[39,179] The hindrance of these two regulatory hormones results in important energy imbalance.[180] We now suggest that individuals with specific SNPs in the *NF-κB1* gene may be more sensitive to greater CSFA levels. This said, the most prudent approach to solving this problem would be to simply reduce SF intake as much as possible when not knowing one's genotype. An alternative approach could include drugs such as quercetin which has been proven to reduce NF-κB activity in rat models as well as NF-κB transcriptional activity in human adipocyte.[181] This may be an interesting alternative for individuals who are experiencing difficulty losing or managing their weight and who are known to be carriers of specific genetic variations susceptible to greater NF-κB activity. Therefore, as more research is performed to further identify gene-diet interactions in this gene as well as other genes, genetic profiling may one day be an interesting approach in dealing with various health conditions including obesity.

4.4. Limitations

Many limitations in this study need to be taken into account when interpreting the results. First, the database used for these analyses was taken from a cross-sectional study. Measurements taken at one point in time does not allow to investigate the long-term effect of the interaction of interest. Effects of SF intake on NF-κB activity have been observed in animal models as soon as 24 hours after being exposed to a HSFD.[46,47] However, a significant change in anthropometric traits is only discernable over a considerable period of time.[44,50,83,84] This said, associations may be difficult to interpret because obesity is a polygenic and multifactorial condition. Second, the study group was almost entirely composed of young, healthy and slender participants. Indeed, they were mainly university students, who consumed low levels of SF and were relatively active, and very few of them had anthropometric traits classifying them as obese (BMI > 30kg/m^2: n=58; men with WC > 102 cm: n=6; women with WC > 88 cm: n=34). Also, with regards to anthropometric measurements, it would be interesting in future studies to create a variable that includes both BMI and WC, allowing having a measurement that easily distinguishes participants who are slender from those who are overweight or obese. Third, although it was necessary, stratifying our sample group by ethnicity reduced our sample size and consequently, lowered the power of our analyses. Also, self-reported ethnicity is not the most accurate measurement to sub-divide a sample by ethnocultural groups. Using this method to categorize by ethnicity increases the possibility of residual confounding by population stratification. In future studies, ancestry informative marker panels should be used to identify ethnic sub-groups.[182] Finally, upon false discovery rate analyses, all significant results were lost, except for rs4648022. Thus, results must be interpreted with caution.

4.5. Conclusion

Using data retrieved from the Toronto Nutrigenomics and Health study, gene-diet interactions were investigated in a multicultural sample group, as a whole as well as by ethnic groups. Multiple associations, alone or in combination with saturated fat, between the *NF-κB1* gene and anthropometric traits were identified in this study. Interestingly, many of these SNPs have previously been associated with other adverse health conditions, validating that specific genetic variations in this gene may play an important role in the development of adverse health conditions. Although an inflammatory response through NF-κB activation is meant to be a defensive mechanism, there is a lot of evidence in the literature demonstrating how nutrient excess (i.e. HSFD) may contribute to the deregulation of this transcription factor and ultimately, hindering important energy regulation signals. Our analyses now propose that some SNPs in the *NF-κB1* gene may have a greater influence on this process. However, in order to gain confidence in these findings, the associations and interactions of interest need to be further investigated and replicated in other cohorts or ideally in an experimental controlled design where the limitations of the study are offset. For future studies, it would be of interest to explore whether other macronutrients such as carbohydrates would activate NF-κB and trigger the proposed mechanism hindering energy regulation as well. Also, there is evidence that unsaturated fat has anti-inflammatory properties. It would be intriguing to investigate if gene-diet interactions are also observed with this type of fat and determine potential weight control mechanisms using foods high in unsaturated fat. Finally, as previously mentioned, there is evidence in the literature that gene-gene interactions between CYP19A1 and *NF- κ B1* may also have an impact on anthropometric traits. CYP19A1 being an important enzyme in the biosynthesis of estrogens, it would be interesting to analyze differences between sexes in interactions such as this one.

Research in nutrigenetics can help to better understand the physiological processes underlying weight gain in response to diet and provide a unique opportunity for the implementation of preventive strategies using lifestyle modification. With the aim to understand how one's genetic make-up may be an important factor in coordinating specific responses to diet, nutrigenetics may provide insightful information regarding gene-diet interactions for health professionals seeking to optimize nutritional interventions for their patients. Nutrigenetic findings could help create a personalized diet for individuals who are unable or who are unsuccessful at managing their weight. As obesity has become a worldwide crisis and has been identified as a cause for the development of other adverse health conditions, further understanding underlying mechanisms and genetic factors affecting food intake and energy expenditure could help create individualized intervention programs to help overcome this health issue affecting the lives of millions.

My contribution in this study included developing the research problem, hypothesis and objectives of the current secondary analysis of data, performing extensive statistical analyses, including for the dietary, genetics and interactions, interpreting and discussing the results. Trained research personnel collected the data from the Toronto Nutrigenomics and Health study collected all data.

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Appendix I

Table 1. Hardy-Weinberg for each SNP studied.

SNP		Genotype			*p*
		GG	GA	AA	
rs11722146	Total	578	569	145	0.78
	Caucasian	330	284	59	0.85
	East Asian	159	230	76	0.64
	South Asian	89	55	10	0.70
		CC	CT	TT	
rs13117745	Total	990	270	32	**0.01**
	Caucasian	485	166	22	0.10
	East Asian	406	54	5	**0.04**
	South Asian	99	50	5	0.66
		GG	AG	AA	
rs1599961	Total	516	584	192	0.20
	Caucasian	272	298	103	0.16
	East Asian	163	229	73	0.62
	South Asian	81	57	16	0.22
		CC	CT	TT	
rs1609798	Total	593	540	159	**0.04**
	Caucasian	331	277	65	0.53
	East Asian	176	211	78	0.28
	South Asian	86	52	16	0.06
		GG	GA	AA	
rs230511	Total	550	578	164	0.53
	Caucasian	310	289	74	0.59
	East Asian	150	234	81	0.53
	South Asian	90	55	9	0.88
		CC	TC	TT	
rs230547	Total	912	330	50	**<0.01**
	Caucasian	550	117	6	0.94
	East Asian	258	172	35	0.40
	South Asian	104	41	9	0.08
		GG	GA	AA	
rs3774932	Total	324	658	310	0.50
	Caucasian	187	344	142	0.49
	East Asian	101	237	127	0.63
	South Asian	36	77	41	0.99
		GG	AG	AA	
rs3774934	Total	813	387	92	**<0.01**
	Caucasian	549	118	6	0.90
	East Asian	182	213	70	0.55
	South Asian	82	56	16	0.18
		CC	CT	TT	
rs3774956	Total	466	586	240	**0.02**
	Caucasian	255	302	116	0.10
	East Asian	129	230	106	0.86
	South Asian	82	54	18	0.06
		GG	AG	AA	
rs3774968	Total	382	642	268	0.95
	Caucasian	205	341	127	0.48
	East Asian	139	227	99	0.72
	South Asian	38	74	42	0.63

		CC	TC	TT	
rs4648006	Total	1174	118	0	0.09
	Caucasian	614	59	0	0.23
	East Asian	418	47	0	0.25
	South Asian	142	12	0	0.61
		CC	CT	TT	
rs4648022	Total	1154	131	7	0.12
	Caucasian	561	106	6	0.69
	East Asian	464	1	0	0.98
	South Asian	129	24	1	0.92
		GG	AG	AA	
rs4648090	Total	1086	193	13	0.18
	Caucasian	503	159	11	0.69
	East Asian	454	10	1	**<0.01**
	South Asian	129	24	1	0.92
		TT	CT	CC	
rs4648095	Total	1170	122	0	0.07
	Caucasian	622	51	0	0.31
	East Asian	406	59	0	0.14
	South Asian	142	12	0	0.61
		TT	AT	AA	
rs4648110	Total	944	310	38	**0.04**
	Caucasian	438	207	28	0.57
	East Asian	405	55	5	**0.05**
	South Asian	101	48	5	0.81
		CC	CT	TT	
rs4648217	Total	1142	140	10	**0.02**
	Caucasian	603	66	4	0.14
	East Asian	413	49	3	0.25
	South Asian	126	25	3	0.20
		CC	CT	TT	
rs4698863	Total	575	554	163	0.10
	Caucasian	317	286	70	0.64
	East Asian	169	218	78	0.59
	South Asian	89	50	15	**0.05**
		TT	TC	CC	
rs7674640	Total	311	661	320	0.40
	Caucasian	166	355	152	0.15
	East Asian	113	213	121	0.32
	South Asian	32	75	47	0.84

Appendix II

Table 1. Interactions between confounders and SNP rs4648022 (β ± SE) on body mass index (BMI) and waist circumference (WC).

Covariate	Genotypes	BMI	p^1	p^2	WC	p^1	p^2
Physical activity	CC	0.09 ± 0.03	0.007	0.03	0.13 ± 0.09	0.13	0.08
	CT + TT	-0.20 ± 0.10	0.08		-0.35 ± 0.25	0.17	
Caloric intake	CC	0.0012 ± 0.0002	<0.0001	0.004	0.0050 ± 0.0005	<0.0001	0.01
	CT + TT	-0.0005 ± 0.0006	0.40		-0.004 ± 0.001	0.0043	

Values shown were tested using general linear model. p^1 is for the slope and p^2 is for the interaction.

Table 2. Interactions between ethnicity and SNP rs4648022 (means ± SE) on body mass index (BMI) and waist circumference (WC).

	SNP Genotype	Caucasian	p^1	East Asian	p^1	South Asian	p^1	p^2
BMI	CC	23.4 ± 0.1	0.24	21.8 ± 0.1	0.0003	23.6 ± 0.4	0.10	0.007
	CT + TT	23.8 ± 0.3		32.0 ± 2.8		25.2 ± 0.9		
WC	CC	75.7 ± 0.4	0.15	71.1 ± 0.3	0.001	75.8 ± 1.0	0.61	0.007
	CT + TT	77.0 ± 0.8		95.2 ± 7.4		77.1 ± 2.3		

Values shown were tested using general linear model. p^1 is for the difference between genotypes within each ethnic group and p^2 is for the interaction between genotypes and ethnic groups.

Appendix III

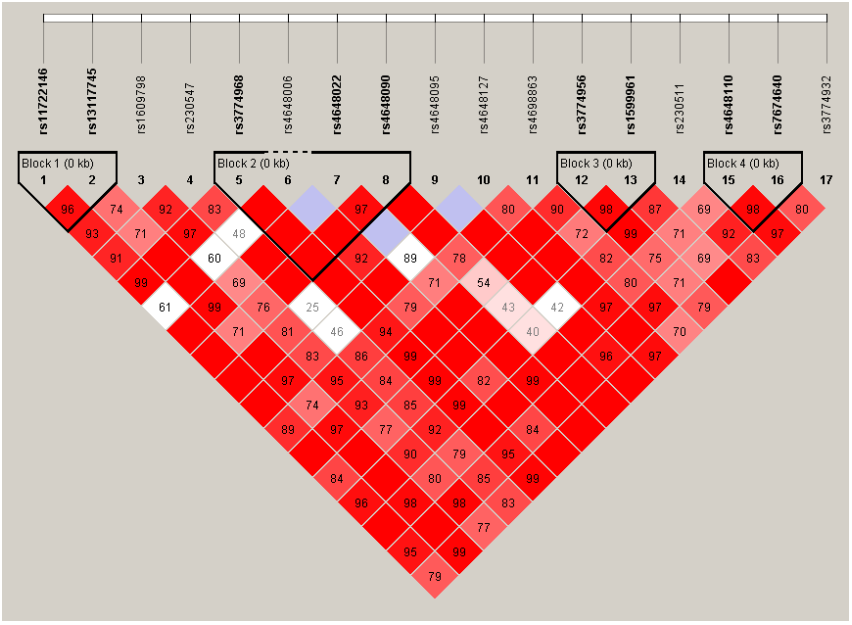

Figure 1. LD plot for all participants.
Tag SNPs in the entire sample were: rs11722146, rs230547, rs3774932, rs3774956, rs4648090, rs4648127, rs4698863 and rs7674640.

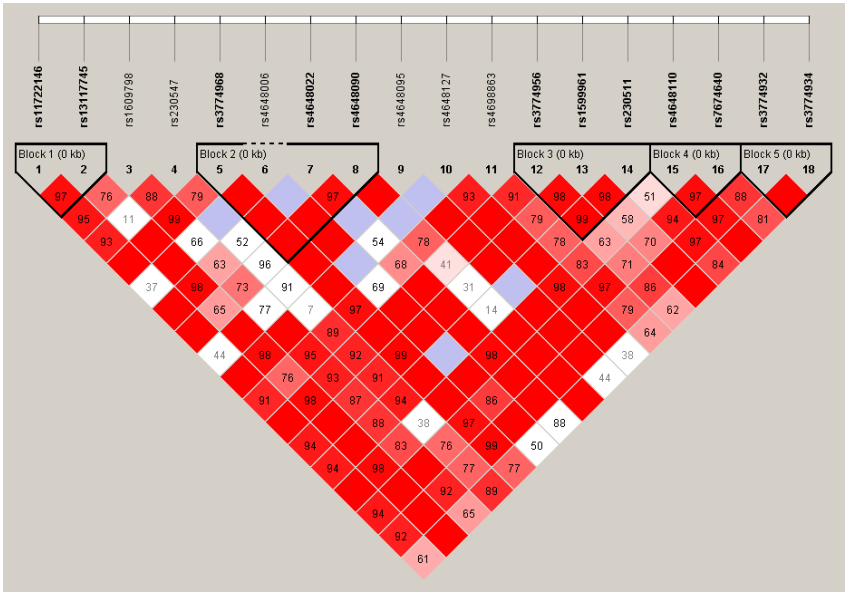

Figure 2. LD plot for Caucasian group.
Tag SNPs in among Caucasians were: rs13117745, rs1609798, rs230547, rs3774932, rs3774956, rs4648090, rs4648127 and rs7674640.

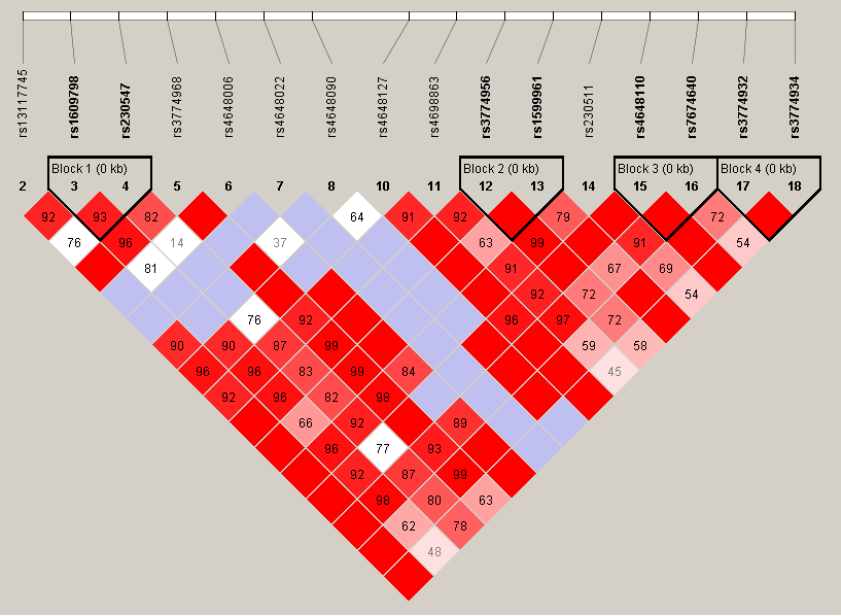

Figure 3. LD plot for East Asian group.
Tag SNPs among East Asians were: rs11722146, rs1599961, rs230511, rs230547, rs3774932, rs3774968, rs4648006, rs4648022, rs4648090, rs4648095, rs4648127, rs4698863 and rs7674640.

Figure 4. LD plot for South Asian group.
Tag SNPs among South Asians were: rs11722146, rs13117745, rs3774934, rs3774956, rs3774968, rs4648022, rs4648095, rs4698863 and rs7674640.

General Health and Lifestyle Questionnaire

1. Gender
   - ❑ Male
   - ❑ Female

   Today's Date_____
   Day Month Year

2. Age _____

3. Date of Birth _____ Country of Birth _____
   Day Month Year

4. Highest level of education:
   - ❑ Elementary
   - ❑ High school
   - ❑ Some college or undergraduate training
   - ❑ College or Undergraduate degree received
   - ❑ Graduate degree received

5. Please describe your ethnicity (write as many as necessary).

   _____

6. Please indicate your blood type:
   - ❑ A
   - ❑ B
   - ❑ AB
   - ❑ O
   - ❑ don't know

   and Rh factor:
   - ❑ positive
   - ❑ negative
   - ❑ don't know

7. Do you currently, or have you ever, experienced headache attacks (including migraines, cluster headaches, etc.)?
   ❑ Yes ❑ No

   At what age did these attacks start? _____yrs old

   How long ago was your last headache attack? _____

   How many headache attacks have you had in the last year? _____

   How long do the attacks usually last? _____

8. Within the past **two weeks**, have you had

	Yes	**No**
a piercing, tattoo, acupuncture?		
medical or dental procedure?		
a vaccination or immunization?		

a flu?		
an infection?		
a fever?		

9. Please indicate if you have ever been diagnosed with any of the following conditions.

Condition	**Tick if YES**	**Age or Date of Diagnosis**
High cholesterol		
High blood pressure		
Depression		
Anxiety disorder		
Chest pain or shortness of breath		
Kidney problems		
Food allergies, specify_____		
Other allergies, specify_____		
Asthma		
Diabetes		
Cancer, specify_____		
Crohns		
Ulcerative Colitis		
Ulcer		
Thyroid conditions		
Celiac disease		
Diverticulitis		
Arthritis		
Osteoporosis		
Other condition, specify_____		

10. Has any family member ever been diagnosed with any of the above conditions?

Your....	**Health conditions**
Mother	
Father	
Brothers	
Sisters	

11. What medications have you taken within the **last month**?

Medication name	Reason	Amount	Duration

12. For **Females** only (Males skip to #13 on the next page)

Are you pregnant or nursing? ❑ Yes ❑ No

Are you using hormonal contraception (e.g., birth control pill or patch)?

❑ Yes ❑ No

If yes, what type? _____

how long? _____

Please indicate next to each of the following PMS (premenstrual syndrome) symptoms, the degree to which you experience them *within the 5 days before the onset of your period and ending by the 4th day of your period*:

Symptoms	None	Mild	Moderate	Severe
Acne, skin blemish				
Bloating, swelling, breast tenderness				
Cramping				
Mood swings, crying easily, irritability, angry outbursts				
Increased appetite, food cravings				
Fatigue				
Headaches				
Anxiety, tension, nervousness				
Clumsiness				
Confusion, difficulty concentrating, forgetfulness				
Sexual desire/activity change				
Insomnia				
Nausea				
Depression				
Desire to be alone				
Other (specify)_____				

13. What vitamins, minerals, herbal supplements or other nutritional supplements have you taken within the **last month** (i.e., multivitamin, protein powder, gingko)?

Name	Reason	Amount	Duration

14. Please list any food restrictions (e.g., salt, fat, carbohydrate, etc.) or special diets you are have been on in the **last month** (e.g., Atkins, South Beach, vegan) and the reason (e.g., health, religious or other reasons).

Food Restrictions/ Special Diet	Reason	How long ?

15. Do you **currently** smoke (at least 1 cigarette per day for 1 month or longer)?

❑ Yes ❑ No

If yes, how many years have you been smoking? _____

how many cigarettes do you smoke per day? **_____**

Are you a **past** smoker (have previously smoked at least 1 cigarette per day for 1 month or longer but have not smoked at least 1 cigarette per day in the last month)?

❑ Yes ❑ No

If yes, when did you quit (approximate date)? _____

how many years did you smoke? _____

how many cigarettes did you smoke per day? _____

16. Do you currently, or have you ever, consumed caffeine-containing beverages (e.g., coffee, tea, cola) *regularly?*

- ❑ Yes, I currently consume them *regularly*
- ❑ Yes, I used to consume them *regularly* but do not anymore
- ❑ No, I have never *regularly* consumed them (GO TO Q17)

If **yes**, please indicate next to each of the following **withdrawal** symptoms the degree to which you experience(d) them **up to 48 hours** after ceasing to consume caffeine-containing beverages.

SYMPTOM	**Don't know**	**None**	**Mild**	**Moderate**	**Severe**
Headache					
Tiredness/ Fatigue					
Decreased energy/activeness					
Decreased alertness/attentiveness					
Drowsiness/ Sleepiness					
Decreased contentedness/well-being					
Depressed mood					
Difficulty concentrating					
Irritability					
Foggy/ Not clearheaded					
"Flu-like" symptoms					
Nausea/ Vomiting/ Upset stomach					
Muscle pain/ Stiffness					
Anxiety/ Nervousness					
Other, please specify _____					

17. Do you experience any of the following effects **up to 12 hours** after consuming **one** caffeine-containing beverage (e.g., coffee, tea, cola)?

EFFECT	**Don't know**	**None**	**Mild**	**Moderate**	**Severe**
Headache					
Increased energy/activeness					
Increased alertness/attentiveness					
Elevated mood					
Increased heart rate					
Anxiety/ Nervousness					
Panic attacks					
Restlessness					
Agitation					
Tremors/ Jitters/ Shakiness					
Dizziness					
Insomnia/ Impaired sleep					
Upset stomach					
Laxative effect					
Other, please specify_____					

18. Do you try to avoid or limit your consumption of caffeine?

- ❑ No (GO TO Q19)
- ❑ Yes

If **yes**, please indicate the reason(s) below

REASON	**YES**	**NO**
Fear of dependence/addiction		
Fear of experiencing withdrawal symptoms		
Increased heart rate		
Increased blood pressure		
Anxiety/ Nervousness		
Panic attacks		
Restlessness		
Agitation		
Tremors/ Jitters/ Shakiness		
Dizziness		
Insomnia/ Impaired sleep		
Upset stomach		
Laxative effect		
General health		
Dislike taste of caffeine-containing beverages		
Other, please specify _____		

19. What other food(s), beverage(s) or ingredient(s) do you **avoid/dislike**?

Food, beverage, ingredient	**Reason you avoid/dislike**

20. On a usual weekday and weekend day in the **last month**, how much time did you spend on each of the following activities? *Total for each type of day should add up to 24 hours.*

Activity	**Effects**	Usual **week** day (hours/day)	Usual **weekend** day (hours/day)
Sleeping			
Sitting or reclining activity (studying, eating, reading, desk work, computer activity, watching TV, etc.)	• Minimal movement • Minimal exertion		
Light activity (office work, driving a car, strolling, standing, etc.)	• Some movement • Weak exertion		
Moderate activity (housework, light sports, regular walking, golf, yard work, lawn mowing, ballroom dancing, cycling, etc.)	• ↑ heart rate • Moderate exertion		
Vigorous activity (strenuous sports, jogging, aerobic dancing, swimming, brisk walking, rollerblading, cycling, etc.)	• ↑↑↑ heart rate • Strong exertion • Get out of breath • Sweating		
		= 24 hours	= 24 hours

Contact Information

Full Name: _____

Address _____

_____

_____

Phone number _(_____)_____

e-mail address (please print **clearly**)

_____

Would you be interested in being contacted for a follow-up study?

❑ Yes ❑ No

Thank You!

UNIVERSITY OF TORONTO

OFFICE OF THE VICE-PRESIDENT, RESEARCH AND INNOVATION

PROTOCOL REFERENCE # 24100

July 3, 2014

Dr. Ahmed El-Sohemy
DEPT OF NUTRITIONAL SCIENCES
FACULTY OF MEDICINE

Dear Dr. El-Sohemy,

Re: Your research protocol entitled, "Nutrigenomics and biomarkers of chronic disease"

ETHICS APPROVAL	**Original Approval Date: June 3, 2009** **Expiry Date: June 2, 2015** **Continuing Review Level: 1** **Renewal: Data Analysis Only**

We are writing to advise you that you have been granted annual renewal of ethics approval to the above-referenced research protocol through the Research Ethics Board (REB) delegated process. Please note that all protocols involving ongoing data collection or interaction with human participants are subject to re-evaluation after 5 years. Ongoing research under this protocol must be renewed prior to the expiry date.

Please ensure that you submit an Annual Renewal Form or a Study Completion Report 15 to 30 days prior to the expiry date of your protocol. Note that annual renewals for protocols cannot be accepted more than 30 days prior to the date of expiry as per our guidelines.

Any changes to the approved protocol or consent materials must be reviewed and approved through the amendment process prior to its implementation. Any adverse or unanticipated events should be reported to the Office of Research Ethics as soon as possible. If your research is funded by a third party, please contact the assigned Research Funding Officer in Research Services to ensure that your funds are released.

Best wishes for the successful completion of your research.

Yours sincerely,

Elizabeth Peter, Ph.D.
REB Chair

Daniel Gyewu
REB Manager

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Université d'Ottawa University of Ottawa
Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

Certificate of Ethics Approval

Health Sciences and Science REB

Principal Investigator / Supervisor / Co-investigator(s) / Student(s)

First Name	Last Name	Affiliation	Role
Bénédicte	Fontaine-Bisson	Health Sciences / Nutrition	Supervisor
Jeremy	Bauman-Fortin	Health Sciences / Human Kinetics	Student Researcher

File Number: H03-15-20

Type of Project: Master's Thesis (Secondary use of data)

Title: The effect of single nucleotide polymorphisms in the NF-kB gene on the association between saturated fat intake and indices of obesity in young adults

Approval Date (mm/dd/yyyy)	Expiry Date (mm/dd/yyyy)	Approval Type
04/14/2015	04/13/2016	Ia

(Ia: Approval, Ib: Approval for initial stage only)

Special Conditions / Comments:
N/A

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Bureau d'éthique et d'intégrité de la recherche Office of Research Ethics and Integrity

This is to confirm that the University of Ottawa Research Ethics Board identified above, which operates in accordance with the Tri-Council Policy Statement and other applicable laws and regulations in Ontario, has examined and approved the application for ethical approval for the above named research project as of the Ethics Approval Date indicated for the period above and subject to the conditions listed the section above entitled "Special Conditions / Comments".

During the course of the study the protocol may not be modified without prior written approval from the REB except when necessary to remove subjects from immediate endangerment or when the modification(s) pertain to only administrative or logistical components of the study (e.g. change of telephone number). Investigators must also promptly alert the REB of any changes which increase the risk to participant(s), any changes which considerably affect the conduct of the project, all unanticipated and harmful events that occur, and new information that may negatively affect the conduct of the project and safety of the participant(s). Modifications to the project, information/consent documentation, and/or recruitment documentation, should be submitted to this office for approval using the "Modification to research project" form available at: http://research.uottawa.ca/ethics/submissions-and-reviews.

Please submit an annual status report to the Protocol Officer 4 weeks before the above-referenced expiry date to either close the file or request a renewal of ethics approval. This document can be found at: http://research.uottawa.ca/ethics/submissions-and-reviews.

If you have any questions, please do not hesitate to contact the Ethics Office at extension 5387 or by e-mail at: ethics@uOttawa.ca.

Germain Zongo
Protocol Officer for Ethics in Research
For Daniel Lagarec, Chair of the Sciences and Health Sciences REB

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