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The Effects of a High-Fat Diet on the Brain; A Meta-Analysis of Microglia in the Hypothalamus

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THE EFFECTS OF A HIGH FAT DIET ON THE BRAIN:
A Meta-Analysis of Microglia in the Hypothalamus

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Preface

The words ‘keto,’ ‘vegan’ and ‘paleo’ are currently buzz words, particularly in the health and fitness community. These are diet styles, ‘fads’ some would call them, others would call them ‘lifestyle changes’, that have gained popularity recently. With the pervasiveness of social media, ideas can spread very rapidly; a social media ‘influencer’ could post their opinion of one of these diets, and suddenly thousands of people who follow them may take on that same view, without requiring any scientific evidence that supports it. My aim with this paper is to use scientific evidence to discuss the implications of one of these diet styles (a high fat diet),

specifically implications of how this affects the brain, an aspect which seems to be often overlooked in pursuit of an aesthetic body.

These popular diets largely focus on altering one's macronutrient intake. The term 'macronutrient' or simply 'macros' are referring to are the basic building blocks of the foods we eat; proteins, fats and carbs. Every item of food has a different number of proteins, fats, and carbs, which are broken down differently by the body. They also each provide the body with a different number of kilocalories (we usually shorten this to 'calories'), which are the body's unit of energy. Carbohydrates and protein both give four calories of energy per one gram of carb or protein, while fat equals nine calories per single gram of fat. Examples of foods with a lot of fat are nuts, oils, avocados, and dairy. Fruits, vegetables and grains are almost exclusively made of carbohydrates, and protein is found in beans, tofu, legumes and meats.

One of the most popular diets of recent years is the ketogenic diet, 'keto', which includes eating a high-fat, low-carb diet. People who follow the keto diet eat less than 50 grams of carbs a day (for context, one banana has about 27 grams of carbs). People who follow a keto diet eat mostly fish, meats, butters and a large proportion of oils. Another low-carb diet is the Atkins diet, which does not specify concentration of high-fat foods, but requires its followers consume under 20 grams of carbs per day. On the other end of the food spectrum, some people eat strictly vegan. Vegans do not eat meat, dairy or eggs, which are high-protein and high-fat foods, and thus end up getting a large proportion of their calories from carbohydrates (Rogerson, 2017). All of these programs have strict followers, and there does not seem to be a 'one diet fits all' but I started to wonder if any of these diets have lasting implications on our bodies and brains.

Additionally, beyond just the labelled 'diets' are popular ideas about individual macronutrients. Protein, carbs and fats, all essential to the human diet, experience differing levels

of attention depending on which diet/lifestyle you are following. For example, there is buzz around protein and the amounts we should eat per day. Vegetarians and vegans are often asked the question "where do you get your protein?" by people who are not dieticians but have simply heard that vegetarians/vegans don't get enough protein. Similarly, carbohydrates have gotten a bad reputation; if you type in "are carbs…" into a Google search, some of the top searches are "are carbs bad for you, are carbs fattening, are carbs sugar, are carbs necessary?" (google.com/search). Anecdotally I have heard friends get upset with themselves for eating 'only carbs' and villainizing them. So, I could have chosen either protein or carbohydrates in this paper, because each are equally misunderstood, but I chose to focus on fat, because this seems to be the most polarized and potentially most altering single macronutrient. I also want to note that high fat diets are not just found in recent fad diets, but also are present in the general public; the standard Western diet is generally high in processed fats and sugars it (Varlamov, 2017), so many Americans may consume a high fat diet without realizing it.

Dietary fat has had a dizzying history; from being villainized to praised and back again. In the 1950's heart disease became the most common cause of death, and a few doctors showed preliminary studies that linked heart disease to consuming dietary fat (Gofman et al., 1950; Keys 1968). From there, the American Heart Association published dietary guidelines that included reducing fat intake, and soon many food brands were making 'fat free' versions of all their products (Kritchevsky 1998; Page 1961). This movement has continued in many foods today, and at a grocery store one can often find a regular version of a product and a low fat or 'fat free' version. Unfortunately, many people probably think that that is the healthier version, due to this anti-fat movement in the 60s. However, the problem with these fat free foods is that in order to be fat free, the fat either had to be taken out, which means it was processed unnaturally, or by

adding chemicals to try to make up the taste of the lost fat. Further, not only has the public been confused about fat, there also is still no consensus in the research world about fat as it relates to heart problems. Some studies say that the connection between saturated fatty acids and heart disease is well-established and one of the main things dieticians encourage avoiding (Clifton & Keogh, 2017), while others say that that relationship has been grossly exaggerated (Temple, 2018). This has led to different nutritionists, doctors and dieticians recommending different things to different people, which only confuses the public more. The takeaway here is that there still does not seem to be comprehensive evidence that fat is healthy or harmful; a quick search on PubMed gives an equal number of articles for either argument.

In the last decade, there has been another new shift in the fat debate; doctors are now pushing Omega 3 Fatty Acids to aid heart health (Jump, Depner, & Tripathy, 2012). Omega 3 is in some nuts and fish, and some supplements like fish oil tablets, and chances are you have probably heard or read advertisements about consuming omegas. There has also been a craze for other fats like coconut oil (which have claims on Pinterest of being a 'cure-all!'). The problem is, omegas theoretically help heart disease, but they are also still a type of fat (polyunsaturated) versus the saturated fats that potentially cause heart disease, and this can be confusing. Thus, people have deemed omegas and unsaturated fats 'healthy fats' to try to destigmatize dietary fat in general and call saturated and trans fats 'bad fats'. Unfortunately, though, the general public probably only sees the word 'fat' on a nutrition label and either write it off, or they are have heard "fats are actually good for you" and they decide to take it. Again, more muddy waters.

Omegas and fats were also sold to 'enhance brain function'. I recently worked at a fruit/veggie 'smoothie' shop and we sold MCT oil (concentrated fatty acid trains made from coconut oil) and we were supposed to advise customers that it 'helps your brain'. As a

neuroscience major, I wanted to use my knowledge of the brain to describe the benefit of this to customers, but I had no context or science to support the store's marketing push. That same year, my naturopath advised me to fish oil supplements to help my insomnia and 'hyperactive' neurons. This was the first time I had fully contemplated that nutrition affects the brain. I am not alone in this; it was not until recently that researchers began realizing nutrition could alter the brain's structure and function (Bourre, 2004). In the past 15 years, thousands of studies have been done focusing specifically on nutritional neuroscience, and the field is expanding. Over the summer, I came across an article in *Nature* (is this a magazine? Quotes?) that discussed the effects of high fat diets on the brain (Kim et al., 2019) and I realized that this topic is an intersection of my interests, so I decided to make it my thesis topic.

It is fascinating that people seem to be incredibly focused on their body and weight, but that they do not contemplate what a diet can do to their brain, which is arguably the most important organ. If your brain stops working, the rest of your body follows. I specifically set out to find out if high-fat diets are impacting more than the physique; being twenty pounds lighter at the expense of a permanently damaged brain hardly seems worth it; and I wanted to find out if that is something we should warn the public about. I decided to do a meta-analysis, which is a statistics technique where similar studies are run against each other, to determine if there is a consistency between data, an overall 'pattern' that has been shown multiple times. I wanted to know if there is enough evidence that a high fat diet does alter microglial presence in the hypothalamus. I chose this as microglia are inflammation markers, and the hypothalamus is the food center in our brains. Each of these will be described in more detail in the introduction.

List of Figures

I. Introduction

It is important to note right from the start that all the studies I include in my meta-analysis are mouse studies. Rodents are commonly used in neuroscience because their brains are well-mapped and documented. These studies require direct access to the brain in order to calculate microglia count, and this can be done when using rodents. High-fat diets are often what researchers use to induce obesity in rodents due to the high-calorie nature of fats, and because obesity frequency in humans today is in-part due to the high amounts of fat found in modern diets (Article, Heymsfield, Wadden, 2017; Kakizaki et al., 2019;). Due to this, a high-fat diet often greatly increases weight gain in the rodents, one study measured a 36% increase in body weight in the high fat group (Moreas et al., 2009). This means that the rats who undergo a high-fat diet (HFD) in these studies will also likely gain weight which could affect the results (i.e., was the change due to weight gain or HFD alone), but this matches human examples; the two often go hand-in-hand (Steele 2017, Swinburn 2011).

Hypothalamus

The part of the brain that I will focus on in this paper is the hypothalamus. The hypothalamus is where basic drives like hunger, thirst, temperature, sleep and sex come from (Hendelman, 2016). I chose to focus this area because it is the center for hunger and satiety signals, and many studies have looked at the impacts of nutrition here. Different nuclei within the hypothalamus control different aspects of hunger and feeding behaviour. I will be primarily looking at the arcuate nucleus.

The arcuate nucleus (ARC) and the lateral nucleus (LN) of the hypothalamus are both implicated in feeding behaviour. The ARC contains both orexigenic (appetite stimulating) and anorexigenic (appetite supressing) neurons. One prominent orexigenic neuron is Neuropeptide Y (NPY) , which has been shown to be the most powerful central stimulant of appetite (Ross, Caballero, Cousins, Tucker, & Ziegler, 2014). Mice studies have shown that repeated injections of NPY lead to increased feeding and thus weight gain (Bewick et al., 2005). NPY neurons have receptors that are sensitive to changes in hormones that signal that there is food present or absent in the body (Kohno et al., 2007; Willensen, Kristensen & Romer, 1999)

Figure 1: *Diagram of the Hypothalamus*

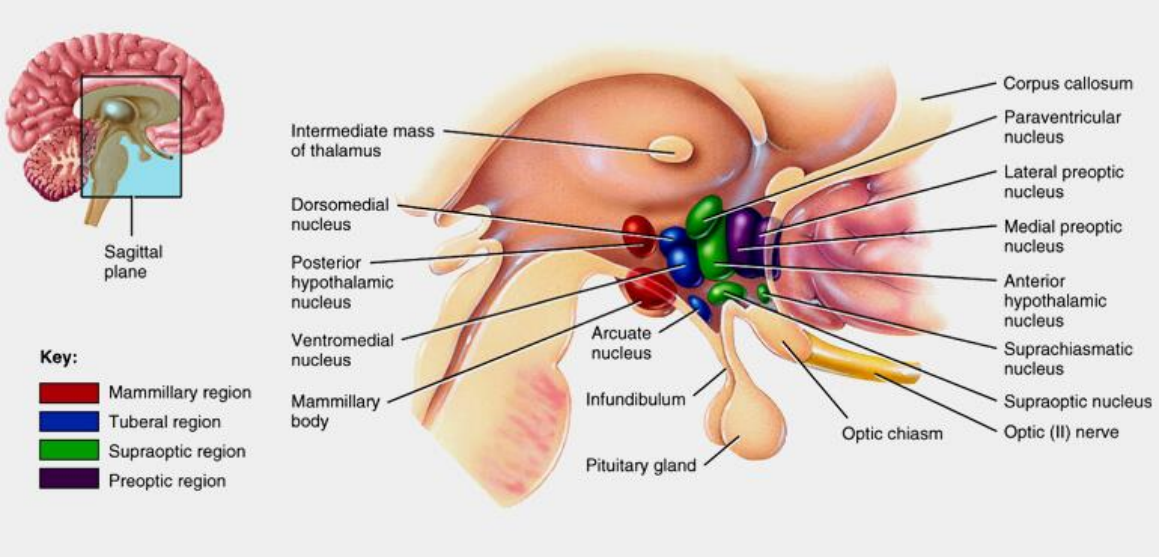


Image retrieved from www.humanbrainfacts.org/hypothalamus.php

One of the most prominent types of anorexigenic cells in the ARC are neurons that express proopiomelanocortin (POMC), the protein precursor for melanocyte-stimulating hormones (MSH) (Millington, 2007 and Ross et al., 2014). MSH neurons are found mostly in the paraventricular nucleus of the hypothalamus, which is dorsal (above) the arcuate nucleus (see Figure 1). Melanocortin receptor 4 (MC4R) is a G-protein coupled receptor that, when MSHs bind, reduce food intake (Schwartz, Woods, Porte, Seeley, & Baskin, 2000 in Ross et al., 2014).

Mice that have had their MC4R and POMC receptor genes removed ('knockout' mice) are usually obese and hyperphagic (Coll, Farooqi, Challis, Yeo, & O'Rahilly, 2004). Further, mutations in the MC4R genes in humans have been shown to result in early-onset obesity (Krude et al., 1998). Lesions in the ARC in general also cause hyperphagia (excessive eating) and weight gain (Clark, Kalra, Crowley, & Kalra, 1984). We can see from these studies that lesions in this area can alter appetite and weight regulation, but there is emerging evidence that this area may be affected by diet alone (Moraes et al., 2009: Thaler et al., 2011). It is also important to note that the arcuate nucleus is one of the few areas of the brain that does not have the blood-brain-barrier, making it a connection point between the body and the brain. In sum, this area can receive and produce signals about food intake and satiety, and stimulation of neurons here can either increase or supress feeding behaviour. A recent body of literature has shown that a high-fat diet can inflame the arcuate nucleus and thus cause a disruption in food signal processing **(**Gao et al., 2009, Valdearcos et al., 2014, Vellloso & Schwartz, 2011).

Microglia

One way that researchers measure inflammation in the brain is by assessing cells called microglia. Microglia are a type of non-neuron brain cells that act as our defense system; they are the immune cells in the brain. They go through our brain and mark and destroy potentially harmful materials like viruses, or foreign or damaged cells. Microglia have projections that are extended and protracted that act in surveillance of these foreign or damaged cells (Hanisch & Kettenmann, 2007; Nimmerjahn, Kirchhoff & Helmchen, 2005), and when the processes are extended the cell is said to be 'ramified'. If a microglia cell detects an object of concern, the

processes retract, the cell is activated and works to phagocytose (engulf) the problem (see figure 2). It is important to note that microglia are always in the brain, which is a good thing, but for my analysis I will only focus on when they are activated, because that means that there is a problem.

There are proteins called pro-inflammatory cytokines that are secreted in response to macrophage activation and increase inflammatory reactions (Zhang & An, 2007). When a microglial cell is activated for inflammation, it is said to be in a state of 'classical' or 'M1' activation (Lively & Schlichter, 2018), which is the state of microglia that my meta-analysis studies will focus on. Researchers can measure microglial presence and activation by looking at proteins that are only present when microglia are activated. Once of which is ionized calcium binding adaptor molecule 1 (iba1-ir), which is upregulated by microglia when they are activated (Gao et al., 2014, Imai, Ibata, Ohsawa & Kohsaka, 1996; Postler, Rimner, Beschorner, Schluesener, & Meyermann, 2000). The studies in the meta-analysis all used iba1 as their indicator of activated microglia.

Figure 2: *Microglia States*

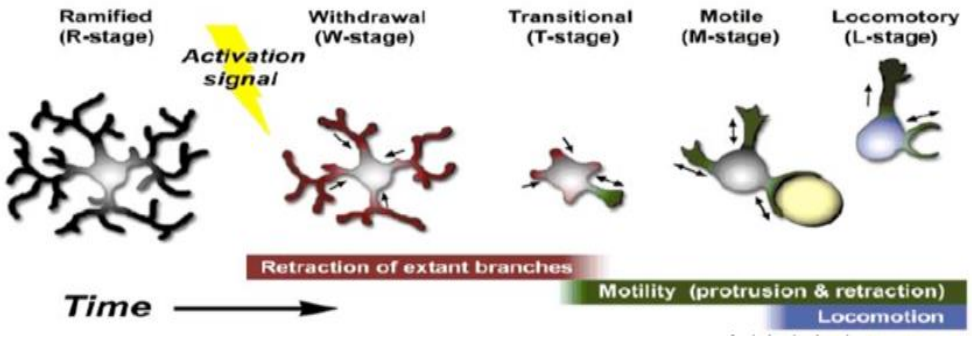


image retrieved from https://www.researchgate.net/figure/Microglial-activation-sequence-This-image-explains-the-morphological-differences-and_fig2_202653882

There are certain patterns of proteins found on problematic material that label them as potentially hazardous material to the microglia, called pathogen-associated molecular patterns (PAMPs) that microglia respond to (Kigerl, de Rivero Vaccari, Dietrich, Popovich, & Keane, 2014). Excess amounts of dietary fat have been shown to activate these PAMPs (Mendes, Kim, Velloso, & Araújo, 2018) and thus induce microglia presence (Baufeld, Osterloh, Prokop, Miller, & Heppner, 2016). Hypothalamic microglia have been shown to change morphology in as little as eight hours after a liposaccharide (fat) injection (Buttini, Limonta, & Boddeke, 1996). Thaler et al. (2012) found that within just 24 hours of HFD exposure, there was evidence of increased inflammation markers in the hypothalamus

Fats, Leptin and Obesity

Why might inflammation in the hypothalamus be detrimental? Studies have indicated that inflammation can disrupt the signals our body sends about food intake and satiety (Huang et al., 2019). Inflammation is thought to change sensitivity to these signals, making the brain less responsive to them over time. The primary signals for satiety are leptin and insulin and their mechanisms are described below.

Leptin is a hormone that lives in fat cells and gets released by those fat cells to signal satiety. Receptors for leptin are found primarily in the arcuate nucleus. A phenomenon called leptin resistance happens when there is a high level of serum leptin, yet the receptors fail to receive the signal (Campfield, Smith, Guisez, Devos, & Burn, 1995). Leptin resistance is common in obesity (Oh et al., 2019), and a genetic lack of leptin causes severe, early-onset

obesity (Haslam & James, 2005). There is some debate about which causes which: obesity or leptin resistance (Myers, Leibel, Seeley, & Schwartz, 2010), but either way we can assume that obese rodents will likely have leptin resistance. Leptin and orexins signal to each other in the ARC to regulate appetite and metabolism, so altered leptin signalling will also alter orexin signalling (Funato et al., 2009).

Insulin is a hormone that works similarly to leptin: when you eat food, cells in your pancreas signal a release of insulin that tells the body to start breaking the food down. Insulin resistance happens when the body no longer responds to insulin signals, and therefore cannot use blood glucose for energy, causing an increase in blood sugar (webmd.com). Insulin resistance is one of the factors that underlies type 2 diabetes (Kahn, Hull & Utzschneider, 2006). A four-week study showed that a high fat (specifically saturated fat) diet decreased insulin sensitivity in obese and overweight adult humans (von Frankenberg et al., 2017). Furthermore, a review published in *Nature* listed extensive evidence that Type 2 Diabetes is an inflammatory disease (Donath & Shoelson, 2011), which brings us back to the topic of inflammation.

Inflammation

We know inflammation in the body to be bad, but the mechanisms of what it could do to the hypothalamus are a bit more nuanced, and not yet completely understood. Studies looking at peripheral organs have shown that a high-fat diet can lead to obesity which leads to low-level inflammation and the macrophages at work can lead to insulin resistance (Baufield 2016; Hotamisligil, 2006; Weisberg, 2003).

Recent studies have found similar results when looking at inflammation in the brain: one study found insulin resistance as a result of a high-fat-diet in the hypothalamus directly (De

Souza et al., 2005). Others yet have suggested that microglial activation in the ARC caused by a high-fat-diet altered the metabolic regulation in the hypothalamus (Gao et al., 2011; Thaler et al., 2012). The reason behind this was that the hyperactivated state of the microglia produced too many pro-inflammatory cytokines, which can be toxic to cells in excess, which damaged POMC (anorexigenic neurons in the ARC) thereby decreasing the metabolic function of that region (Gao et al., 2011: Smith, Das, Ray & Banik, 2012). There are still more studies being done, but there seems to be evidence that inflammation caused by a high-fat diet can injure areas in the brain essential to weight control, and thus contribute to problems like obesity and metabolic syndrome (Thaler et al., 2011).

To understand whether the inflammatory effects of HFD on the hypothalamus are consistent in this burgeoning literature, I conducted a statistical meta-analysis, which focused specifically on microglia activation in the hypothalamus, as measured by proteins (iba1) only present in active M1 microglia, after a high fat diet. The meta-analysis will share an overall average effect size of a high fat diet on microglia count from various studies that measure this and share similar methods. The effect size will show how far apart, on average, mean numbers of microglia are when there is a high-fat diet versus a control low-fat diet. These numbers will indicate whether there is an overall trend in these studies of how much consuming a high fat diet changes average microglial count.

II. Methods

Study Search and Collection.

All studies for the analysis were found on Pubmed and limited to ‘Free full text’. These studies were also all published in peer-reviewed journals. The MeSH function within PubMed was

used to find all related words for keyword. The total search keywords were: (((((("diet, high-fat"[MeSH Terms] OR ("diet"[All Fields] AND "high-fat"[All Fields])) OR "high-fat diet"[All Fields]) OR (("high"[All Fields] AND "fat"[All Fields]) AND "diet"[All Fields])) OR "high-fat diet"[All Fields]) AND ((("microglia"[MeSH Terms] OR "microglia"[All Fields]) OR "microglia's"[All Fields]) OR "microglias"[All Fields])) AND ("hypothalamus"[MeSH Terms] OR "hypothalamus"[All Fields] AND ("rodentia"[MeSH Terms] OR rodents[Text Word] OR "rats"[MeSH Terms] OR rats[Text Word] OR "mice"[MeSH Terms] OR Mice[Text Word]) and this search yielded 42 studies (as of January 9, 2020). From this pool, studies were eliminated if they: did not directly measure microglia, did not have a control non-high-fat-diet group, if all of the animals were genetically modified, or if they did not report their n values. These criteria excluded 29 studies, leaving 13 to be analyzed. These 13 different studies had a total of 23 data sets to run.

Data Collection and Analysis

Only one study (Gao et al. 2017) reported means and standard error of their microglia count in numerical form in their results, the others only gave graphs of their results. The Java program “DataThief” was then used to extract means and standard error from each of the graphs. Axes were set using crosshairs and each point was placed in the topmost part of the line for the most consistent results. These data were entered into an online calculator to find the effect size of each study (http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php). From each study, specific details of each study were also documented.

The R programming language (R Core Team, 2019) in conjunction with the metafor package (Viechtbauer, 2010) was used to conduct this analysis. The data for this meta-analysis are publicly available at https://rstudio.cloud/project/1058952. The standardized mean difference, Cohen's d, was used as the measure of effect size in this meta-analysis. The initial meta-analysis examined the average effect across all 23 studies from the 13 experiments. Five additional moderator analyses were conducted examining if they would change the overall effect size. These moderators were: sex of mice, brain region measured, percent high-fat-diet, or length of the high-fat diet, and lab idiosyncrasies. The complete data file containing study identifiers, standardized mean difference, and all moderators are presented in Appendix A.

III. Results

Overall Meta-Analysis

A random-effects meta-analysis indicated that the average effect size for this data set was large, as mice on an HFD presented microglia count in the hypothalamus that were over 2 standard deviations greater than the microglia count for mice in control diets; Cohen's d = 2.7258, [1.7921, 3.6595], $z = 5.7219$, $p < .0001$. To show the strength of these results, one only needs to look at the lower level of the 95% confidence interval estimate for Cohen's d. The study with the smallest effect size showed that HFD increased microglial count by 1.79 standard deviations, an effect that is more than double the standard definition of Cohen's standard of a large effect as a d = .8. Figure 3 presents a forest plot highlighting the results of this meta-

analysis, 19 of the 23 datasets found that HFD increased activated microglia. Furthermore, the forest plot highlights how a meta analyses works. Specifically, one can see how the estimates of error for each study [the tails] are much more dispersed than the estimates of error for the meta-analysis [the tails on the aggregate terms]. The meta-analysis pinpoints the areas of overlap between each effect and uses these areas of overlap to estimate not only the true effect size but also the variance around that true effect size. Overall, it is clear, HFD increases activated microglia by somewhere in the range of 1.79 standard deviations to 3.66 standard deviations. This is a very large effect.

Moderators

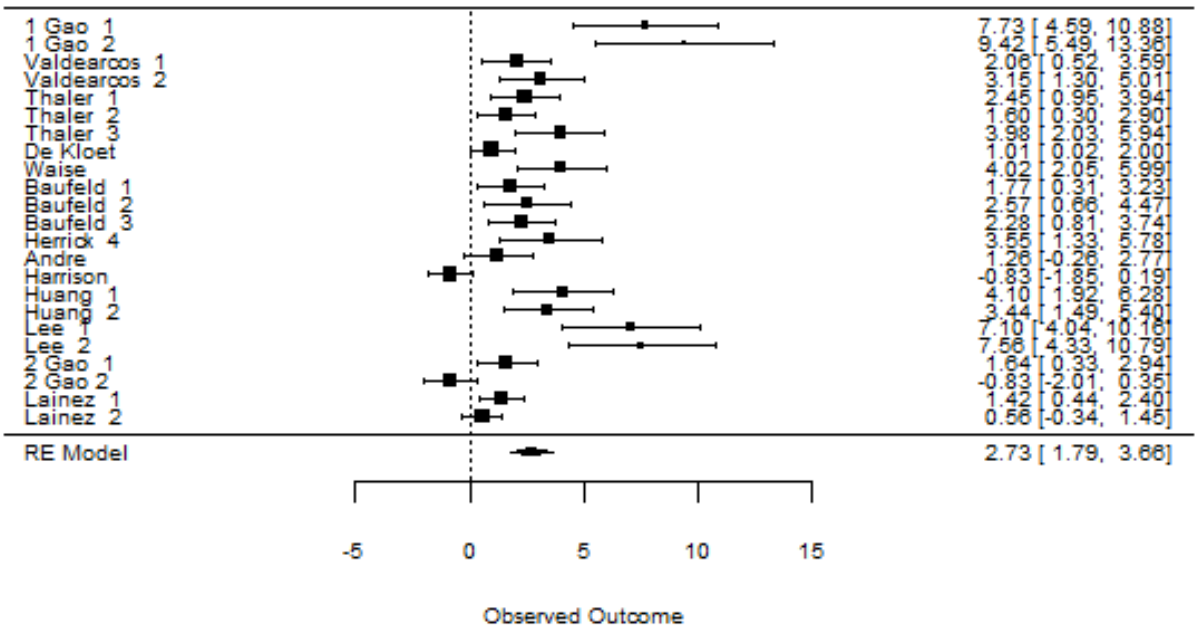
While this is a large effect overall, results indicate substantial heterogeneity. Heterogeneity means genuine differences in effect sizes across the studies that are not due to chance but to differences in the very essence of the studies. The I^2 statistic is used to quantify the total variation among the effect sizes in this meta-analysis that is due to heterogeneity. The I^2 for this study indicates that 88.08% of the variation among effect sizes in this meta-analysis are due to fundamental differences in the studies while 21.92% of variation between these effect sizes is due to chance: $I^2 =$ 88.08%, Q(df = 22) = 129.3166, p < .0001. An I^2 score of 25% is considered to be minimal heterogeneity, 50% is considered moderate and over 75% is considered to mean high heterogeneity. When there is substantial heterogeneity found, it suggests that at least one moderator variable is accounting for the variation among these effect sizes.

Five moderator analyses were conducted using the meta-regression function of the metafor package. The first moderator was percent high-fat diet; and this moderator did not significantly change the effect size; Beta = -.0806, standard error = .0693, *z* = -1.1632, [.2447, -

.2164], p = .0552 nor reduce heterogeneity by much: I^2 = 87.68%, Q(df = 21) = 115.1601, p = <.0001. The second moderator was length of exposure to high fat diet (ranging from three days to 22 weeks) and this also did not have an impact on average effect size: Beta = .0456, standard error = .0801, z = .5697, [-.1113, .2026], p = .5689. This also did not change the heterogeneity; I^2 = 88.40%, Q(df = 21) = 128.0101, p = <.0001. The third moderator was sex of animal; though it should be noted there were only two cohorts of female rats, versus 21 groups of male rats, and this did not impact effect size (Beta = -.9205, standard error = 1.7001, z = -.5414, [-4.2526, 2.4116], p = .5882) nor heterogeneity (I^2 = 88.02%, Q(df = 21) = 126.5194, p = <.0001). The fourth moderator analyzed was area where microglia were counted, either the arcuate nucleus specifically or the entire hypothalamus. This also did not change the effect size with a beta of -.1365, standard error = 1.5826, z = -.1064, [-2.6504, 2.3775], p = .9153. These results also did not change heterogeneity: I^2 = 88.87%, Q(df = 21) = 124.6204, p = <.0001. The last moderator analyzed was idiosyncrasies of different laboratories, comparing different labs to one another, (ie comparing the Gao lab with the Lee lab), which still did not change heterogeneity much: I^2 = 86.72%, Q(df = 21) = 110.5231, p = <.0001 or the overall effect size: beta = -.185, standard error = .116, p = .112. Clearly, there is a moderator that was unable to be found.

Figure 3

Forest Plot of Confidence Intervals



Note. Confidence intervals shown for each study, and the average confidence interval is given below.

IV. Discussion

This meta-analysis showed that a high fat diet has a significant impact on activated microglial count in the hypothalamus in mice studies. This follows the raw data from the studies— nearly all found a significantly higher amount of microglia in the high-fat group (there were four datasets with no significant results). There was also a large amount of heterogeneity between the studies, indicating the studies varied greatly from one another. Thus, one must proceed with caution when comparing them to each other, as they might be too different to properly compare. Moderators, parts of the studies that varied slightly between studies, were extracted to determine if they were the reason for the heterogeneity. However, out of the five moderators identified (percent high-fat diet, length of high-fat diet, sex of animal, region tested,

and different labs), not one changed the heterogeneity score nor the overall effect size. This is curious as is indicates, for example, that there is no difference between microglial count from a diet that lasted one week versus twelve weeks versus 22 weeks. It also would suggest that there is no microglial difference between when there was a 40 percent-fat diet versus a 72 percent-fat diet. It is possible that it this is correct; that any amount of fat higher than normal (for any length of time) causes microglial activation, or these results could be due to flaws in the analysis.

It is important to reiterate that not every single study done on this topic was used in this analysis. The studies were found exclusively on PubMed and with the restriction 'free full text' which means that any studies that were not published or not peer-reviewed would not be included. Further, the results were backcalculated from graphs for 21 of the data sets because raw result numbers weren't given. This undoubtedly means that some of the means and standard errors extracted were not exact, which would lead to a different effect size for that study, which would also alter the overall effect size. A full meta-analysis should attempt to use every study published in that field (provided it meets the criteria) and raw numbers should be used to ensure accurate results, so there is certainly room for improvement for this study. Additionally, these studies were exclusively mice studies; it is unfair to make a directly transfer these results to humans, since humans and mice are anatomically very different. Not to mention that a prescribed diet of exactly 60 percent fat every single day is unlikely to be followed by humans. Even people who do eat a high fat diet won't have it be the exact same amount for multiple weeks in a row.

It is also important to note that the studies included in this analysis did not restrict carbohydrate or sugar consumption, so most of them were a combination of both (as fitting with the Western diet). One study even found that it was the combination of high fat and sugar, not fat alone, that caused the microglial increase (Gao et al., 2017). Not enough of the studies discussed

their levels of carbohydrates to allow this to be a moderator, but it also could contribute to the differences of results. And since none of them mentioned carbohydrate restriction, we can assume that there was no restriction, and thus not representative of the ketogenic diet. A keto diet study would have a limited amount of carbohydrates, putting the body into a completely different metabolic process called ketosis. The keto diet has been found to improve multiple neurological disorders (McDonald & Cervenka, 2018; Wheless, 2008) which is quite the opposite from the high-fat-high-sugar diet discussed in this paper.

It is unquestionable that further studies should be done, especially in animals like primates which are more like humans, but that does not mean that these results should be dismissed. Mice and rats are the most common animals used in neuroscience research, and their studies can still inform us. The results of this analysis are not sufficient to show that high fat is inherently bad or can permanently alter a human's brain, but it means we should, at the very least, be cautious and understand the possible implications.

There are two types of microglia: pro-inflammatory and anti-inflammatory. The pro-inflammatory was the M1 style that these studies looked at, so named because when they are activated, they release the cytokines that induce inflammation (Smith et al., 2012). This mechanism is meant to be beneficial in the short-term; the distress signal of the microglia indicates that excess lipids need to be removed from an area, however chronic activation is detrimental (Lee et al., 2019). Chronic activation of these microglia, which can be induced by a high fat diet as we have seen in these studies, can lead to chronic inflammation. Chronic inflammation in the arcuate nucleus has been implicated in damaging the blood vessels (angiopathy) which can lead to a leaky blood-brain-barrier (Lee et al., 2019; Thaler et al., 2012). This could lead to a reduction in the receiving of satiety signals from the blood like leptin and

ghrelin (Cai and Liu, 2011). This could mean the body doesn't know if it is full or not and might continue to eat. This excess eating leads to more fat cells which leads to more leptin release, but no receptors to hear it, so it becomes this positive feedback loop in which more food leads to less signalling and more eating. Overeating can of course lead to obesity and the health implications that come with it. Indeed, chronic inflammation of the arcuate has been shown in preliminary studies to directly lead to leptin resistance, systematic insulin resistance and glucose intolerance (Arruda et al., 2011; Lee et al., 2019; Velloso, Araújo, & de Souza 2008).

Further, other studies have even found a connection between a high-fat diet and risk of Alzheimer's Disease, as Alzheimer's is thought to be connected with insulin resistance and other metabolic disorders (Biessels & Kappelle, 2005; Kothari et al., 2017; Luchsinger, Tang Shea, Mayeux, 2002). Further, some rat studies showed that a high fat diet and related insulin resistance caused changes in cognitive ability and spatial working memory (Arnold et al., 2014, Kothari et al., 2017).

While this study focused specifically on the hypothalamus, I want to briefly touch on studies that have been done on the effects of a high-fat diet on other parts of the brain. Some concerns about the effects of a high-fat-diet on the brain include increase in impulsivity (Steele et al., 2017), and other altering of the dopamine mesolimbic reward system that could even lead to psychiatric disorders and amphetamine sensitivity (Naneix et al., 2017). There was also a primate study that showed that if a mother ate a high-fat diet when she was pregnant, her offspring 67% more likely to show adverse behavior (aggression in males, anxiety in females) due to disturbances in the serotonergic systems from the prenatal high-fat (Sullivan et al., 2010). Multiple studies have also showed that a prenatal high-fat diet can impact learning and memory, by altering gene expression in the hippocampus (Cordner, & Tamashiro, 2015; Cortés-Álvarez et

al., 2020; Page, Jones & Anday, 2014). These studies are all preliminary and in animals, but still have concerning implications. More studies need to be done, though, because the initial evidence suggests that an excessive and chronic high-fat diet can alter almost every aspect of our brains and behaviors.

Summarized in one sentence: the implications of a diet with too much fat are pointing to possibilities of metabolic disease as well as neurological diseases. However, this does not mean we should stop eating fat, all macronutrients (proteins, carbohydrates, fats) are important for bodily function, but we should be mindful about how much of our diets are coming from fats (especially saturated fats).

One last disclaimer is that I am not a doctor nor am I prescribing nutritional or medical advice, simply taking information from the scientific community and compiling it into a research project. Please consult a physician before changing your diet

V. Conclusion

This thesis process started with my personal interest in fad diets and the fitness industry, but over the months the thesis evolved and the patterns that emerged were far larger and more impactful than my own personal interest. I initially thought high fat diets were crash diets, only undertaken people desperate to lose a pound, but the more I read the more I learned that it goes far beyond that. I could no longer ignore that this could be applicable to all Americans, and that this was connected to the obesity epidemic. Foods high in fat (and sugar) have become the marker for ‘classic American’ food— cheeseburgers, fries, bacon and milkshakes come to mind.

Not only have these types of dense foods become more popular in the US in our meals but snacking on less nutritious food has also increased in the American population in the last 50 years (Piernas & Popkin, 2010). Increases in both calorie-dense food type and frequency of consumption of such foods have led to the rates of obesity in recent times (Drewnowski, & Specter, 2004). The fact that there is new evidence that these types of eating behaviors could not only be affecting our bodies in such ways, but that it could also be (perhaps permanently) altering our brain is something we need to take seriously. This research could impact our entire culture, but there are some populations that could be affected by this worse than others.

I end with one last Jesuit/Honors point: this research is even more imperative for underprivileged populations. Foods that are high in fat and sugar are often the cheapest and sometimes the only type of food that underprivileged communities have access to (Drewnowski, & Specter, 2004; Pechey & Monsivais, 2015). Pechey and Monsivais (2015) did a study that showed that SES was directly related to food quality; those in higher-income households purchased more produce and healthier foods than those in lower-income households. This was due to multiple factors like certain neighborhoods having lower quality supermarkets, which was related to ethnicity and wealth (Baker, Schootman, Barnidge, & Kelly, 2006; Molaodi, Leyland, Ellaway, Kearns, & Harding, 2012), as well as being able to go grocery shopping less frequently, (ie. due to longer hours at work or multiple children) (Pechey & Monsivais, 2015). It is no surprise, then, that obesity and diabetes are more common in these populations (Baker, et al., 2012; Baltrus, Lynch, Everson-Rose, Raghunathan, & Kaplan, 2005).

This is already a public health concern of its owns, but what about their brains? Eating a high-fat high-sugar seems to trigger a feedback loop that makes that person crave more high-fat high-sugar foods, and since those are the foods that are easiest available, they keep eating them

and the cycle continues. This can then lead to the leptin and insulin problems, which can cause long-lasting detrimental effects on their brains and bodies… So not only do they not get access to nutritious food, affecting their bodies, but their brains are also comprised, specifically to crave more of the food that could be detrimental. All because of their socioeconomic position. There is a very real problem of food inequality and food injustice in our country.

So, 'so what who cares?'— we should, because this research could be affecting our whole country, and particularly the underprivileged communities who already have systematic injustices working against them. The field of nutritional neuroscience is relatively new but needs to keep expanding so we can have not only bodily health, but brain health for all.

To conclude, please be aware that diet can affect more than physique. The body and brain are inextricably connected in ways we have yet to complete understand, but the research continues to grow and every day and the best thing we can do is to continue to educate ourselves. We only have one body and one brain in this lifetime, so it is imperative that we treat them with care and compassion.

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Appendix A

Studies Included in the Meta-Analysis

Study	Percent HFD	Sex of Mice	Length of HFD	Area Measured	Cohen's D	Variance
Gao et al.. 2014 (1)	58	male	6 weeks	arcuate	7.73463	2.582
Gao et al.. 2014 (2)	58	male	6 weeks	arcuate	9.4209	4.0314
Valdearcuateos et al.. 2014 (1)	42	male	4 weeks	arcuate	2.0559	0.611327
Valdearcuateos et al.. 2014 (2)	42	male	16 weeks	arcuate	3.1531	0.897097
Thaler et al.. 2012 (1)	60	male	3 days	arcuate	2.446	0.582628
Thaler et al.. 2012 (2)	60	male	1 week	arcuate	1.5985	0.4398
Thaler et al.. 2012 (3)	60	male	2 weeks	arcuate	3.9843	0.994766
De Kloet et al.. 2014	60	male	8 weeks	arcuate	1.01	0.25337
Waiseet al.. 2014	60	male	12 weeks	hypothalamus	4.022	1.007353
Baufeld et al.. 2016 (1)	60	male	3 days	hypothalamus	1.7678	0.556256
Baufeld et al.. 2016 (2)	60	male	4 weeks	hypothalamus	2.5666	0.945042
Baufeld et al.. 2016 (3)	60	male	8 weeks	hypothalamus	2.2788	0.559225
Herrick et al.. 2018	60	female	12 weeks	arcuate	3.5544	1.289617
Andre et al.. 2017	60	male	3 weeks	arcuate	1.2574	0.5988
Harrison et al.. 2019	58	male	22 weeks	arcuate	-0.8299	0.271523
Huang et al.. 2019 (1)	61.6	male	3 months	arcuate	4.0974	1.239425
Huang et al.. 2019 (2)	61.6	male	4 months	arcuate	3.4433	0.992803
Lee et al.. 2018 (1)	58	male	4 weeks	arcuate	7.1018	2.43483
Lee et al.. 2018 (2)	58	male	20 weeks	arcuate	7.5597	2.714518
Gao et al.. 2017 (1)	58	male	4 weeks	arcuate	1.6368	0.44497
Gao et al.. 2017 (2)	78.7	male	4 weeks	arcuate	-0.8326	0.362215
Lainez et al.. 2018 (1)	60	male	4 weeks	arcuate	1.4229	0.250617
Lainez et al.. 2018 (2)	60	female	4 weeks	arcuate	0.5573	0.207765

Note. HFD=High fat diet. The parentheses after the year of the study indicate the different trials within each study