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Dietary Cholesterol Promotes Adipocyte Hypertrophy and Adipose Tissue Inflammation in Visceral, But Not Subcutaneous, Fat in Monkeys

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Abstract

Objective—Excessive caloric intake is associated with obesity and adipose tissue dysfunction. However, the role of dietary cholesterol in this process is unknown. The aim of this study was to determine whether increasing dietary cholesterol intake alters adipose tissue cholesterol content, adipocyte size, and endocrine function in nonhuman primates.

Approach and Results—Age-matched, male African Green monkeys (n=5 per group) were assigned to one of three diets containing 0.002 (Lo), 0.2 (Med) or 0.4 (Hi) mg cholesterol/Kcal. After 10 weeks of diet feeding, animals were euthanized for adipose tissue, liver, and plasma collection. With increasing dietary cholesterol, free cholesterol (FC) content and adipocyte size increased in a step-wise manner in visceral, but not subcutaneous fat, with a significant association between visceral adipocyte size and FC content ($r^2=0.298$; n=15; p=0.035). In visceral fat, dietary cholesterol intake was associated with: 1) increased pro-inflammatory gene expression and macrophage recruitment, 2) decreased expression of genes involved in cholesterol biosynthesis and lipoprotein uptake, and 3) increased expression of proteins involved in FC efflux.

Conclusions—Increasing dietary cholesterol selectively increases visceral fat adipocyte size, FC and macrophage content, and proinflammatory gene expression in nonhuman primates.

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Disclosures

None.

Visceral fat cells appear to compensate for increased dietary cholesterol by limiting cholesterol uptake/synthesis and increasing FC efflux pathways.

Keywords

adipocyte; cholesterol; dietary cholesterol; white adipose tissue; inflammation; African Green monkey; nonhuman primate

Introduction

Obesity has reached epidemic proportions worldwide. In the US, 67% of adults (>20 yr old) are overweight (≥ 25 kg/m² body mass index (BMI)) or obese (≥ 30 kg/m² BMI), resulting in medical care cost of \$147 billion in 2008 ^{1, 2}. Obesity increases the risk of other chronic diseases, such as type 2 diabetes, coronary heart disease, insulin resistance, and non-alcoholic fatty liver disease ³. Adipocytes are active endocrine cells that secrete adipokines, store excess energy as triglyceride (TG), release fatty acids during fasting states, and control systemic glucose disposal ⁴. During progression of obesity, fat cell size increases due to TG accretion ⁵. Larger adipocytes exhibit decreased responsiveness to insulin, decreased glucose uptake, and increased secretion of proinflammatory adipokines ⁶⁻⁹. Enlarged adipocytes are associated with macrophage infiltration into adipose tissue, resulting in increased production of proinflammatory cytokines and insulin resistance ¹⁰. Diets high in calories, saturated fat, and cholesterol (i.e., Western diets) are sufficient to cause obesity in humans and experimental animals ^{3, 5}. However, the role of individual dietary constituents, particularly dietary cholesterol, in the development of obesity is poorly understood.

Adipose tissue is a major site for cholesterol storage in man ¹¹. In obese states, approximately half of the whole body pool of cholesterol is stored in adipose depots. As adipocyte hypertrophy occurs during development of obesity, cholesterol content also increases ¹². Adipocytes are unique among cells in that nearly all cholesterol (>93%) is stored in the unesterified form (i.e., free cholesterol, FC) and nearly all of the adipocyte FC (~88%) is distributed on the TG droplet surface, with the remainder located in the plasma membrane ^{13, 14}. Dietary cholesterol worsened adipose tissue inflammation in a mouse model of diet-induced obesity, suggesting that high dietary cholesterol can lead to adipocyte dysfunction⁵. Whether dietary cholesterol leads to adipocyte hypertrophy and dysfunction in humans is unknown and is a difficult question to address experimentally.

To address this gap in knowledge, we studied the effect of dietary cholesterol on adipocyte size, adipose tissue cholesterol content, and gene/protein expression in a nonhuman primate atherosclerosis model. We chose this model because of its close phylogenetic relationship to humans, and because lipid and lipoprotein responses to a Western-type atherogenic diet in nonhuman primates mimic human responses better than rodent models ¹⁵. In addition, dietary composition is more easily controlled than in humans, and tissues are readily available for subsequent analyses. Here, we demonstrate that increasing dietary cholesterol alone was sufficient to drive adipocyte hypertrophy specifically in visceral fat, with increased adipose tissue FC and inflammatory gene expression in nonhuman primates.

Results from this study suggest a role for dietary cholesterol in increasing adipocyte dysfunction, a characteristic feature of the pathogenesis of obesity.

Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Dietary cholesterol increases plasma VLDL and LDL cholesterol

To examine the effect of increasing levels of dietary cholesterol on plasma lipids and lipoprotein concentrations in African green monkeys, three isocaloric Western-type diets containing 35% fat with 0.002 (Lo), 0.2 (Med) or 0.4 (Hi) mg cholesterol/kcal were fed to monkeys. During the 10-week diet feeding period, no significant difference was observed in food consumption (data not shown), body weight (Figure 1A) or plasma TG levels (Figure 1B). However, there was a striking increase in plasma total cholesterol concentrations (TC) for the Hi cholesterol group ($P<0.0001$) relative to the Med and Lo groups, which was apparent after 3 weeks (Figure 1C). The increase in plasma TC was due, in part, to a significant increase in VLDL cholesterol (Figure 1D), but was primarily due to large increases in LDL cholesterol (Figure 1E). Plasma HDL cholesterol concentrations were not affected by increasing dietary cholesterol (Figure 1F).

The effects of increasing dietary cholesterol on intestinal cholesterol absorption, cholesterol excretion, and hepatic lipid content were also examined (Table 1). The percentage dietary cholesterol absorption was similar among all three diet groups (average of 52–56%). Fecal cholesterol excretion increased in a stepwise manner with increasing dietary cholesterol. In addition, there was a significant increase of hepatic FC ($p=0.0001$) and CE ($p=0.001$) content with increasing dietary cholesterol, caused primarily by large increases in the Hi cholesterol group. Hepatic TG content trended higher with increasing dietary cholesterol, but there were no significant differences among the diet groups, there was also no visible evidence of hepatic steatosis by histological examination of liver sections (Supplemental figure I). We also observed changes in hepatic gene expression that were compatible with the increase in hepatic cholesterol content (Table 2). Specifically, SREBP2 and HMGCR mRNA expression decreased, whereas ABCG1 expression increased (ABCA1 also trended upward).

Dietary cholesterol increases cholesterol content and adipocyte size in omental, but not subcutaneous, adipose tissue

Several studies suggest that adipose cholesterol accumulation correlates with an increased risk for metabolic syndrome and cardiovascular diseases^{16, 17}. However, it is unclear whether dietary cholesterol directly affects adipose tissue cholesterol accumulation and, if so, whether dietary cholesterol accumulation differs among adipose depots. To address this question, we measured cholesterol levels in omental and subQ adipose tissue. Dietary cholesterol intake up to 0.4 mg cholesterol/Kcal had no significant effect on subQ adipose tissue total, free, or esterified cholesterol content (Figure 2A–C, respectively). By contrast, omental fat total and free cholesterol content was significantly increased in monkeys fed the

Hi vs. Lo cholesterol diet (Figure 2D–E). Omental fat cholesteryl ester (CE) content was low (<10% of total cholesterol) and did not vary among diet groups (Figure 2F). To determine whether dietary cholesterol altered adipocyte hypertrophy in a depot-specific manner, we examined adipocyte size by analyzing digital images of hematoxylin and eosin-stained paraffin sections from omental and subQ adipose tissue. Consistent with cholesterol accumulation, adipocyte size increased with increasing dietary cholesterol in omental fat (Figure 3A; 42.3 ± 2.4 , 49.0 ± 2.7 and 58.9 ± 3.1 μm for Lo, Med, and Hi, respectively), but not in subQ fat (Figure 3B; $p=0.49$). Histograms of adipocyte size distribution also demonstrated a clear shift towards larger size for omental, but not subQ fat. Linear regression analysis showed a significant correlation ($r^2 = 0.298$, $p=0.035$) between omental adipose tissue cholesterol content and adipocyte size, but not subQ adipose tissue ($r^2 = 0.048$, $p=0.44$) (Figure 3C). Collectively, these data demonstrated that increasing dietary cholesterol in monkeys selectively drives increases in adipose tissue cholesterol content and adipocyte size in omental fat. There was also a significant ($P=.0246$, $r^2=.331$) correlation between plasma LDL cholesterol concentrations and omental adipocyte diameter, suggesting that elevations in plasma LDL were a primary determinant of increased adipocyte size (Supplemental Figure II). There was no correlation between VLDL cholesterol concentrations and adipocyte size.

Dietary cholesterol is associated with omental adipose tissue inflammation

To determine whether the increase of omental fat cholesterol content induced by dietary cholesterol altered endocrine function, we examined adipokine gene expression by quantitative real-time PCR. There was a significant increase in expression of the pro-inflammatory genes interleukin (IL)-6 and IL-8 in the Hi cholesterol diet group relative to the other groups (Figure 4A). TNF α gene expression trended upward with increasing dietary cholesterol, but did not reach statistical significance ($p=0.065$). Next, we examined macrophage gene expression in omental adipose tissue. There was a significant upregulation of macrophage recruitment-associated genes, including monocyte chemoattractant protein-1 (MCP-1), C-C chemokine receptor type 2 (CCR2), and the macrophage marker CD68, in Hi vs. Lo cholesterol diet groups (Figure 4B). We observed no increase in M2 type macrophage gene expression (Supplemental Figure III A and B) suggesting increasing macrophage accumulation was primarily M1 type macrophages. Immunofluorescence images of representative omental adipose tissue suggested increased expression of TNF- α and CD68 in the Hi vs. Lo cholesterol group (Supplemental Figure IV), but not subcutaneous adipose tissue (Supplemental Figure V). Cytokine protein expression (TNF- α and IL-6) primarily co-localized with tissue macrophages (Supplemental figure VI). Despite increased inflammation, expression of adiponectin and leptin were unaffected by dietary cholesterol (Figure 4C). In addition, plasma levels of glucose, insulin, or adiponectin were similar among diet groups (Supplemental figure VII) and TNF- α levels were undetectable, indicating that 10 weeks of atherogenic diet feeding did not impair insulin sensitivity or systemic inflammation. These data suggest that increasing dietary cholesterol intake promotes adipose tissue inflammation and recruitment of macrophages into omental fat, but in the absence of body weight differences among diet groups, does not lead to systemic insulin resistance.

Next, we determined whether an increase in omental fat FC content altered expression of genes involved in cholesterol biosynthesis, and cellular cholesterol uptake and efflux (Figure 5). SREBP2, a master regulator of cholesterol biosynthesis, was unaffected by dietary cholesterol (Figure 5A). However, expression of HMG-CoA reductase, the rate-limiting enzyme for cholesterol biosynthesis, was significantly reduced in Med and Hi cholesterol diet groups compared to the Lo cholesterol group (Figure 5A). In addition, VLDLr and LDLr gene expression were significantly lower in the Med and Hi groups compared to the Lo cholesterol group (Figure 5B). Scavenger receptor class B type I (SR-BI) showed a decreasing trend with increasing dietary cholesterol ($p=0.076$). LDLr-related protein (LRP) gene expression level did not differ among diet groups.

To determine the effect of dietary cholesterol on cholesterol efflux pathways, we analyzed expression of several genes and proteins involved in adipocyte cholesterol efflux^{18, 19}. Adipose tissue ABCA1 and ABCG1 mRNA expression was similar among diet groups, but apoE expression levels tended to increase with increasing dietary cholesterol ($p=0.12$; Figure 5C). However, Western blot analysis revealed an increase in ABCA1 ($p=0.053$ by ANOVA; $p=0.025$ by t-test, Lo vs. Hi) and apoE ($p=0.016$ by ANOVA) protein expression with increasing dietary cholesterol (Figure 5D), suggesting an upregulation of cellular cholesterol efflux pathways. Finally, we also measured expression of several lipogenic genes and lipoprotein lipase (LPL). Despite increased adipocyte size in omental adipose tissue, expression of lipogenic genes, including transcription SREBP1c, fatty acid synthase (FAS), acetyl coA carboxylase (ACC), and LPL, was similar among diet groups (Supplemental figure IIIC–F). Expression of fatty acid oxidation genes, PPAR α and ACOX, was significantly lower in the Hi vs. Lo cholesterol diet groups (Supplemental figure IIIG–I), suggesting a decrease in adipose tissue fatty acid oxidation with increasing dietary cholesterol.

Discussion

Adipose tissue is the largest free cholesterol storage site in the body, and its cholesterol content affects gene expression and cellular function^{20, 21}. However, it is unclear whether dietary cholesterol has a direct impact on adipocyte size, cholesterol content, and metabolic function. The fundamental question addressed in our study was whether increasing dietary cholesterol drives adipocyte hypertrophy and inflammation and, if so, whether it occurs in a fat depot-specific manner. Increasing dietary cholesterol (from 0.002 to 0.4 mg/kcal) was associated with a selective increase in cholesterol content and adipocyte size in omental adipose tissue; these relationships were not observed in subQ fat (Figures 1–3). Our data also revealed that increased visceral fat cholesterol content induced by Hi cholesterol diet feeding promoted increased expression of genes associated with adipose tissue inflammation and macrophage recruitment (Figure 4), with cytokine expression primarily associated with CD68 positive cells (Supplemental figures IV–VI). These changes occurred in the absence of significant differences in body weight among monkeys consuming the three levels of dietary cholesterol. Collectively, our work shows that increasing dietary cholesterol alone is sufficient to induce adipocyte hypertrophy and adipose tissue inflammation selectively in visceral fat of nonhuman primates.

Whether dietary cholesterol, *per se*, can affect adipocyte hypertrophy and inflammation is unknown. Obesity results from excess caloric consumption that often includes increased dietary cholesterol intake and is associated with adipocyte hypertrophy and increased recruitment of macrophages into adipose tissue¹⁰. Enlarged adipocytes are insulin resistant, secrete more proinflammatory cytokines, and have greater basal and catecholamine-stimulated lipolysis than smaller adipocytes^{6–9, 22}. Fat cell hypertrophy is associated with increased adipocyte cholesterol in humans^{13, 23} and rodents²⁴, but whether this occurs passively with adipocyte TG accumulation during progression of obesity or actively induces adipocyte hypertrophy is unknown.

Past studies have yielded conflicting results regarding the role of dietary cholesterol on adipocyte size and cholesterol content. Angel and Farkas documented an increase in rat adipocyte cholesterol with increasing dietary cholesterol load (0.05% to 5%); however, rats fed a cholesterol-free diet had more cholesterol in adipocytes than those fed diets containing 0.05% or 0.1% cholesterol, suggesting a complex relationship between dietary cholesterol intake and adipocyte sterol homeostasis²⁴. In a recent study, Subramanian et al fed LDLr knockout mice a high fat (60% cal), high carbohydrate (26% cal) diet with (0.15%) or without added cholesterol (0.03% from lard in the diet) and showed that added dietary cholesterol resulted in macrophage infiltration into epididymal fat (i.e., visceral fat depot), but did not increase adipocyte size. On the other hand, inguinal (i.e., subQ fat depot) fat cells were increased in size with added dietary cholesterol, but macrophage infiltration was not observed. Kovanen et al reported that a high cholesterol diet failed to increase adipocyte cholesterol in rats, but did increase plasma and hepatic cholesterol concentrations¹². No study to date has examined the interrelationships between dietary cholesterol intake and adipocyte size, adipose tissue cholesterol content, and inflammatory status.

In this study, we demonstrate that feeding non-human primates diets that were identical except for increasing level of cholesterol was sufficient to increase visceral fat cell size and adipose tissue cholesterol and macrophage content. Furthermore, these increases were not observed in subQ fat and occurred in the absence of body weight differences among diet groups. The difference in our outcomes (increased visceral adipocyte size, cholesterol and macrophage content with increasing dietary cholesterol) from those of Subramanian et al (increased visceral fat macrophage content, but not size) are likely related to the animal model (monkey vs. LDLr knockout mice) and/or diet composition. Our diets are typical of the North American diet in total fat content (35% cal) and fatty acid composition (enriched in saturated and monounsaturated fat). The Hi cholesterol diet is equivalent to consumption of 3–4 eggs/day for a 2000–2500 calorie/day diet, which might be consumed by individuals at risk of developing obesity and coronary heart disease. To our knowledge, this is the first study to show that elevated dietary cholesterol in a typical North American diet can induce adipocyte hypertrophy and inflammation in nonhuman primates. Thus, high dietary cholesterol intake may be one causal factor in development of adipose tissue hypertrophy and inflammation that accompanies obesity, insulin resistance, and type 2 diabetes. Although we did not observe systemic insulin resistance in our study, likely due to similar body weights among our groups of nonhuman primates, a longer feeding period may have resulted in hyperglycemia and/or hyperinsulinemia.

The increased visceral fat cholesterol content in monkeys fed the Hi cholesterol diet likely occurred through increased uptake of cholesterol from plasma VLDL and LDL. Supporting this idea, the Hi cholesterol diet significantly elevated plasma VLDL and LDL cholesterol concentrations (Figure 1) and we observed a significant correlation between plasma LDL cholesterol concentrations and omental adipocyte size (Supplemental figure II). Most cells respond to an increase in FC by converting excess FC to cholesteryl ester via acyl CoA:cholesterol acyltransferase (ACAT), decreasing *de novo* cholesterol biosynthesis, decreasing lipoprotein uptake via downregulation of the LDLr, and increasing efflux of cholesterol via ABCA1, ABCG1, and SR-BI²⁵. Adipocytes are unusual in that >95% of cellular cholesterol is FC and most of their FC is located on the surface of the lipid droplet^{12–14}. All of these cellular responses to excess FC are mediated by two master transcriptional factors, SREBP2 (sterol regulatory element binding protein 2)²⁶ and LXR (liver X receptor)²⁷, which prevent cholesterol uptake/synthesis and stimulate FC efflux, respectively. In our study, visceral fat from Hi cholesterol-fed monkeys had 50% more FC compared to monkeys fed the Lo cholesterol diet. Based on visceral fat gene expression (Figure 5 and Supplemental figure IIIG–I), cholesterol biosynthetic (HMGCR), lipoprotein receptor (LDLr and VLDLr), and fatty acid oxidation (PPAR α and ACOX) gene expression were down-regulated, suggesting the visceral fat tissue was attempting to compensate for increased FC content. We¹⁸ and others¹⁹ have shown that ABCA1 expression is critical in the regulation of adipocyte FC content in mice. Although gene expression of LXR-responsive genes involved in FC efflux was unaltered by diet (Figure 5C), ABCA1 and apoE protein expression was significantly higher in Hi vs. Lo cholesterol groups (Figure 5D), suggesting efflux of FC likely plays a critical role in regulating adipocyte sterol homeostasis in nonhuman primates as well. Thus, although increased plasma cholesterol has not been associated with increased adipocyte cholesterol in all studies^{24, 28}, it is the most likely explanation for increased visceral fat cholesterol in this one.

Visceral fat is more metabolically active and susceptible to dysfunction relative to subQ fat²⁹. In our study, subQ fat did not respond to increased dietary cholesterol intake with a change in fat cell size, adipose tissue FC content, or inflammatory cytokine expression. Interestingly, adipocyte size and adipose tissue cholesterol content were 50% greater for subQ vs. visceral fat in monkeys consuming the Lo cholesterol diet (Figures 2 and 3). Feeding monkeys the Hi cholesterol diet increased visceral fat cell size and adipose tissue cholesterol content to levels observed for subQ fat. These results suggest that large fat cell size or adipose tissue cholesterol content, *per se*, does not result in inflammation and dysfunction. The reason for the metabolic differences between visceral vs. subQ fat that lead to divergent responses to increased dietary cholesterol are unknown and will require further investigation.

In conclusion, we provide the first evidence that addition of cholesterol to a typical North American diet selectively induces visceral adipocyte enlargement, adipose tissue cholesterol accumulation, and inflammatory responses in nonhuman primates. Visceral fat cells appear to compensate for increased dietary cholesterol by limiting cholesterol uptake/synthesis and increasing FC efflux pathways. Our results establish new metabolic interrelationships between dietary cholesterol and adipocyte hypertrophy, adipose tissue cholesterol

accumulation, and inflammation that may explain part of the metabolic dysfunction that accompanies diet-induced obesity. Although our study did not specifically address reducing dietary cholesterol as a means of lowering the risk of adipocyte hypertrophy and dysfunction, we speculate that this might be the case. If so, recommending a prudent intake of dietary cholesterol could potentially be a simple strategy to reduce adipocyte hypertrophy and dysfunction in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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SIGNIFICANCE

Obesity has reached epidemic proportions; 67% of US adults are either overweight or obese, increasing the risk of other chronic diseases. During progression of obesity, fat cell size increases, resulting in adipocyte dysfunction and adipose tissue inflammation. Caloric-rich diets containing saturated fat and cholesterol (i.e., Western diets) are sufficient to cause obesity in humans and experimental animals. However, the role of individual dietary constituents, particularly dietary cholesterol, in the development of obesity is poorly understood. In this study, we provide the first evidence that increasing dietary cholesterol alone in a typical North American diet selectively induces visceral adipocyte enlargement, adipose tissue cholesterol accumulation, and inflammatory responses in nonhuman primates in the absence of significant body weight differences among animals. Our results establish new metabolic interrelationships between dietary cholesterol and adipocyte hypertrophy, adipose tissue cholesterol accumulation, and inflammation that may explain part of the metabolic dysfunction that accompanies diet-induced obesity.

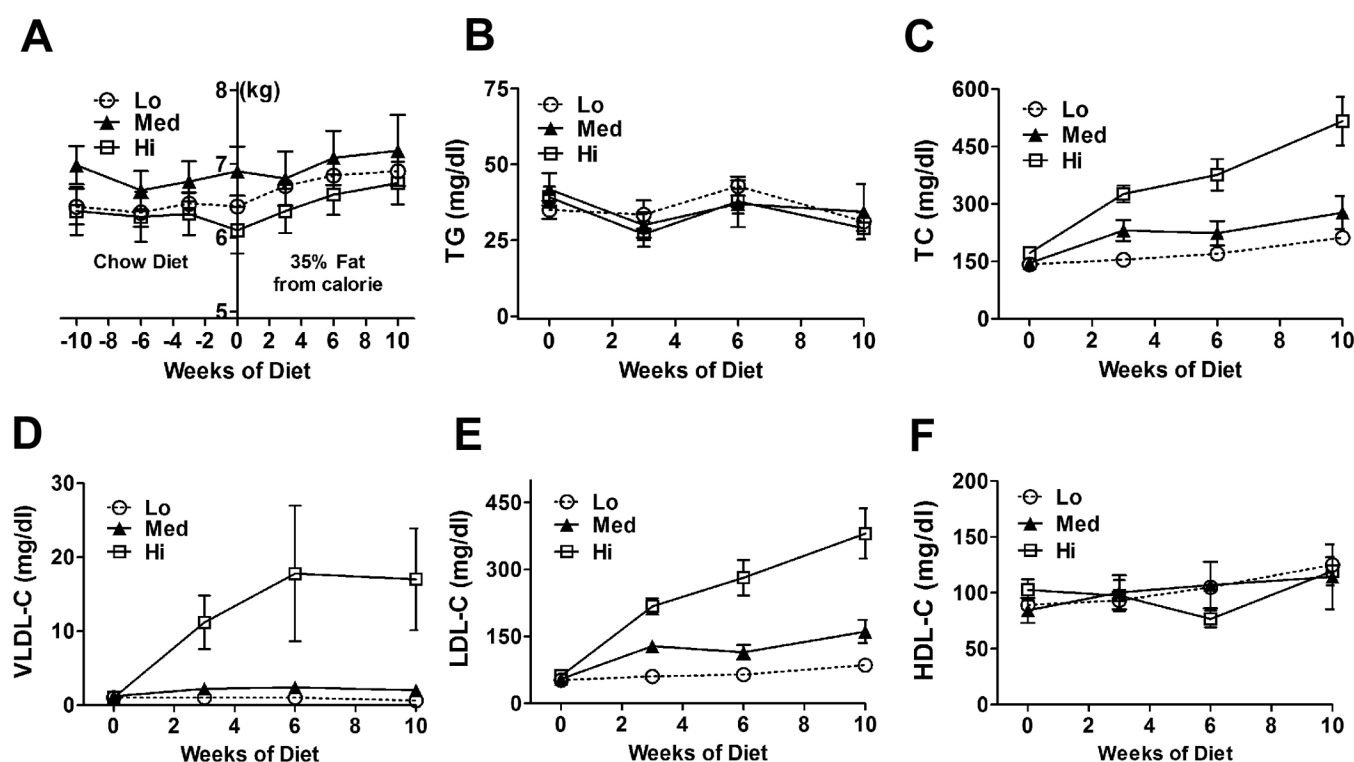


Figure 1.

Supplementation of Hi cholesterol diet increases plasma cholesterol in African green monkeys. African green monkeys were fed atherogenic diets containing 35% calories as fat and 0.002 (Lo, ○), 0.2 (Med, ▲), or 0.4 (Hi, □) mg cholesterol/kcal for 10 wks. Periodic blood samples were taken to measure plasma lipid and lipoprotein cholesterol concentrations, after fractionation of plasma by fast protein liquid chromatography. **A**. Body weight of monkeys before (chow diet) and after diet consumption, **B**. Plasma triglyceride concentrations, **C**. Plasma total cholesterol concentrations; Hi cholesterol group was significantly greater than Med or Lo groups by repeated-measures ANOVA, **D**. Very low density lipoprotein cholesterol concentrations, **E**. Low density lipoprotein cholesterol concentrations; Hi cholesterol group was significantly greater than Med or Lo groups by repeated-measures ANOVA, and **F**. High density lipoprotein cholesterol concentrations. Each data point represents mean $\pm$ SEM; $n=5$ /group.

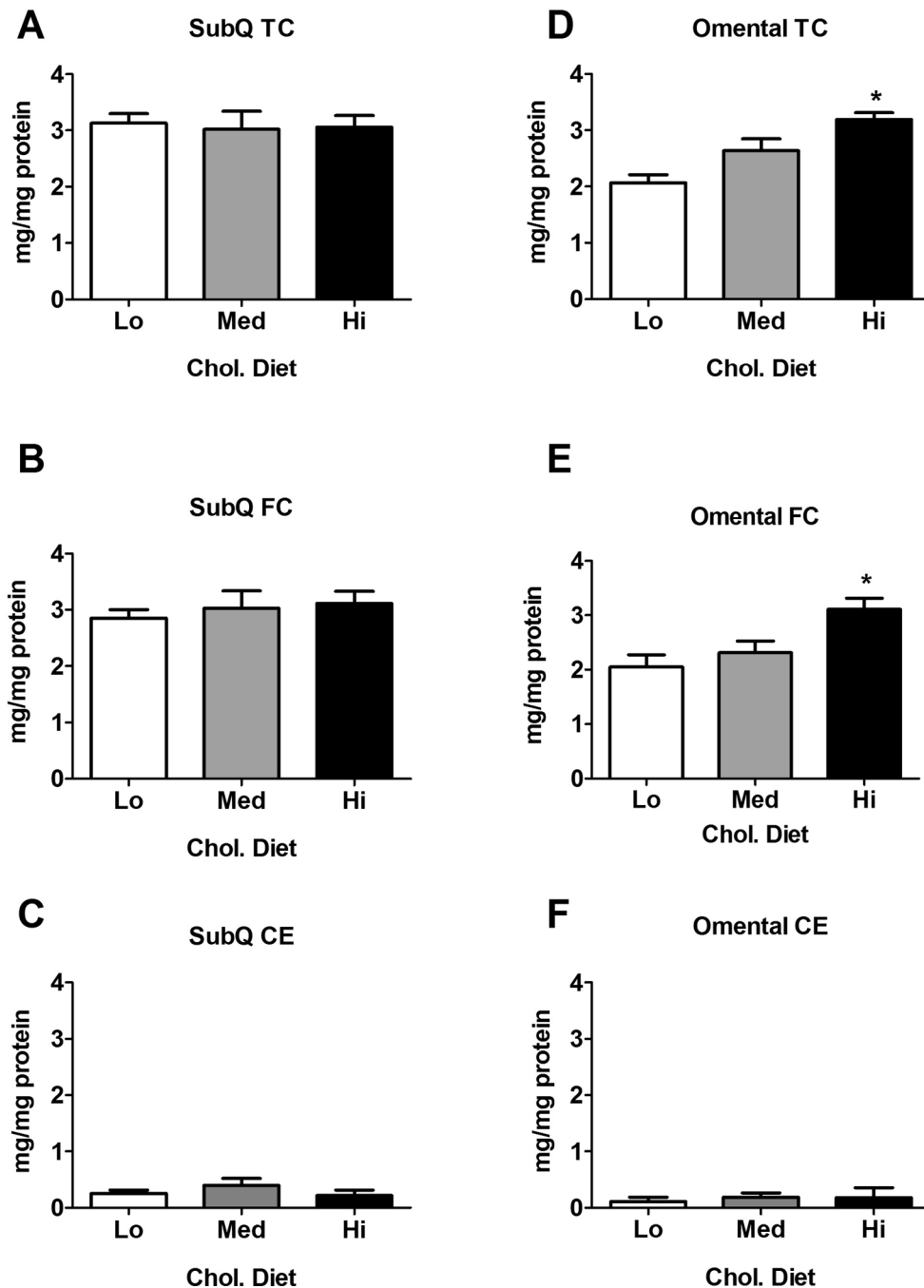


Figure 2.

Dietary cholesterol consumption selectively increases cholesterol content in visceral, but not subQ, adipose tissue. After 10 wks of diet consumption, monkeys were euthanized, adipose tissue was collected and lipid extracted, and cholesterol content was quantified by gas-liquid chromatography and normalized to protein content. **A–C**, Subcutaneous (subQ) total cholesterol (TC), free cholesterol (FC), and cholesteryl ester (CE). **D–F**, Omental TC, FC, and CE. Mean $\pm$ SEM; $n=5$ /diet group. * $p<0.05$ by one-way ANOVA.

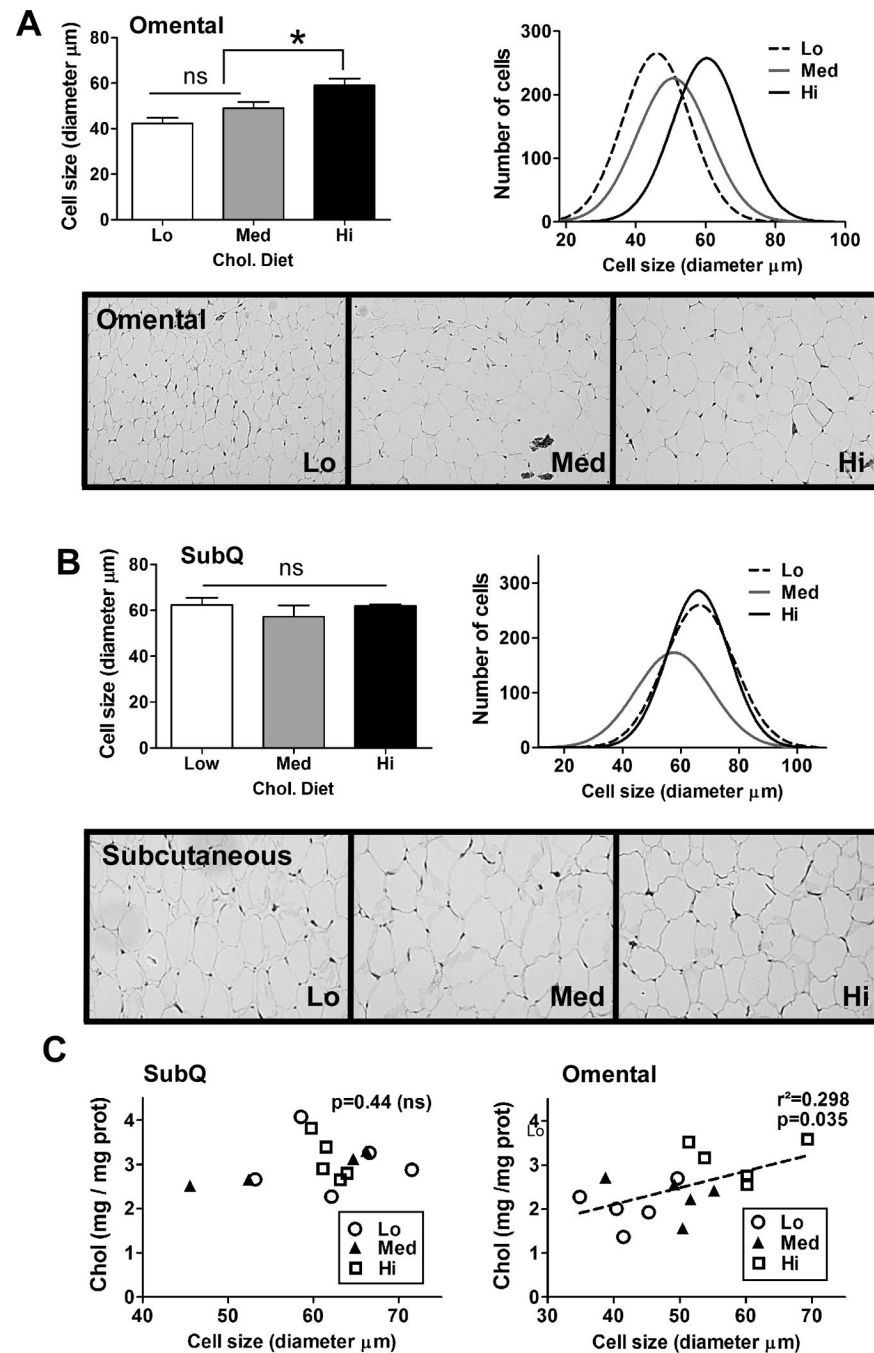
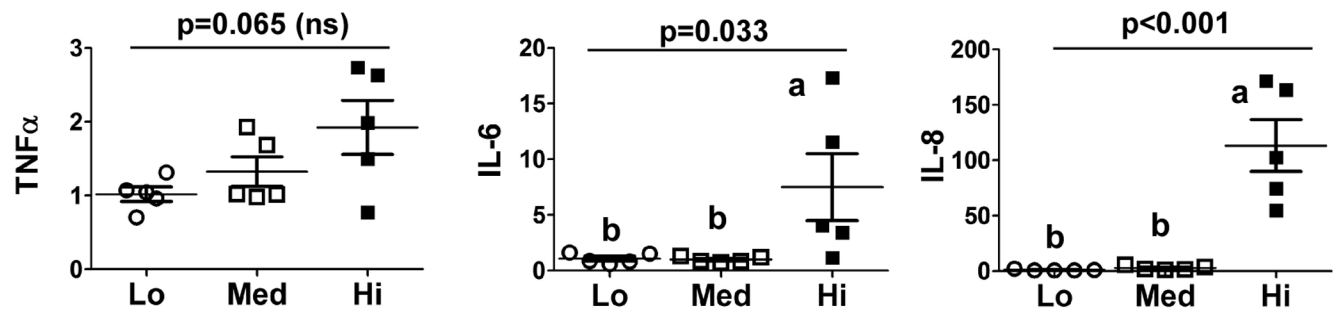


Figure 3.

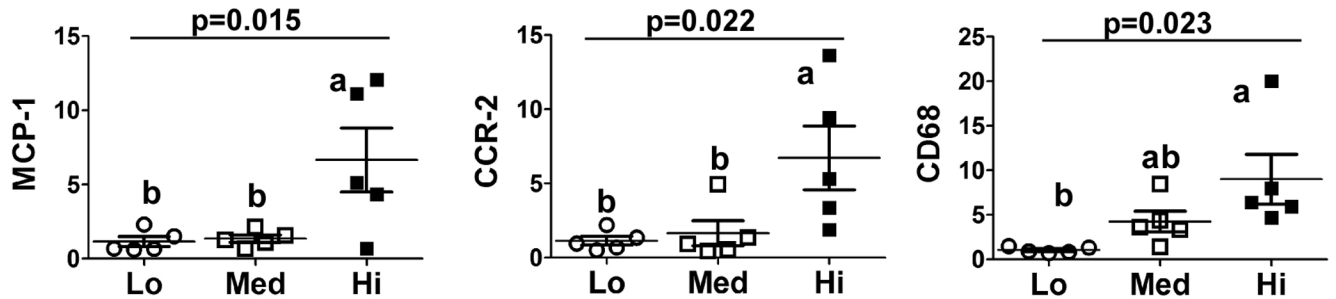
Dietary cholesterol is associated with increased omental adipose tissue cholesterol content and size. Adipocyte cell size ($n=200/\text{animal}$) was determined from digital images of paraffin-embedded, hematoxylin and eosin-stained omental (**A**) and subQ (**B**) adipose tissue sections using Image Pro[®] software. Mean $\pm$ SEM, $n=5/\text{diet group}$. Adipocyte cell size histograms for all animals within each diet group are shown in the right panel. Representative images from omental and subQ fat for each diet are shown below the plots in

panels A and B. **C.** Correlation of subQ and visceral adipocyte size with cholesterol content using linear regression analysis. The line of best fit is shown for each plot.

A Inflammation



B Macrophage recruitment



C Adipokines

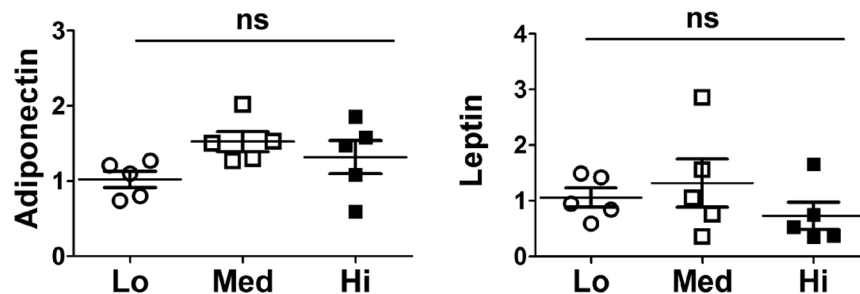
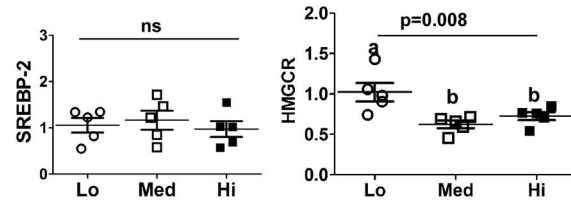
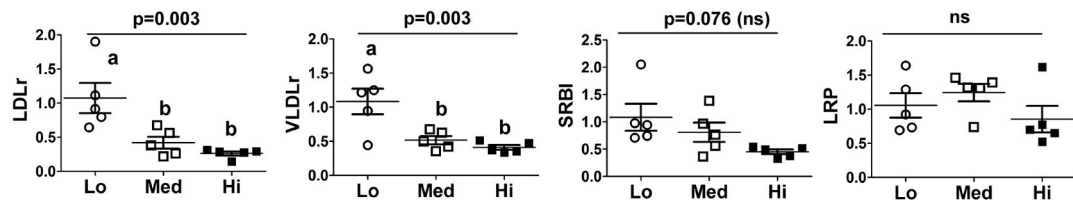
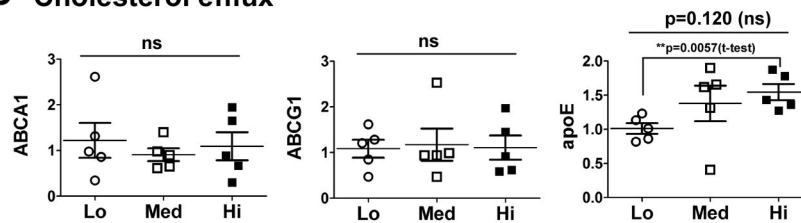
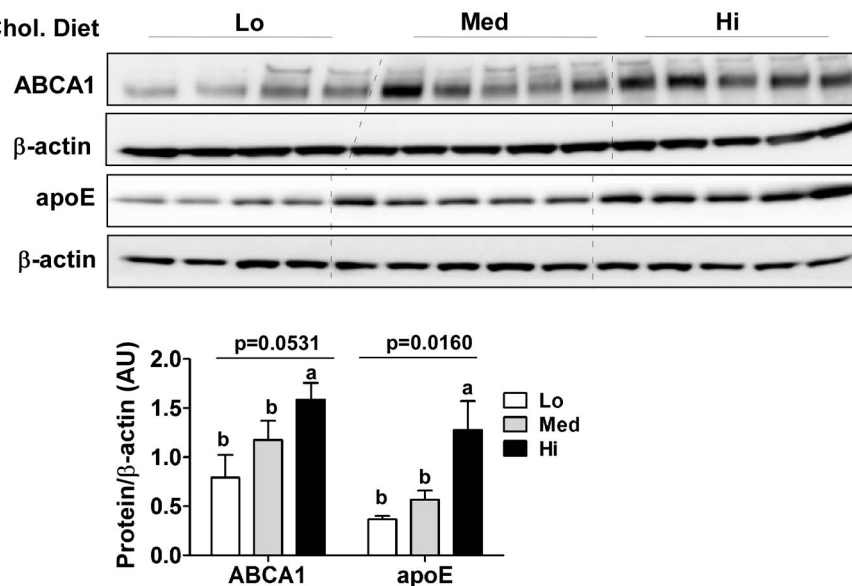


Figure 4.

An increase in dietary fat is associated with adipose tissue inflammation. Quantitative real-time PCR analysis of omental fat gene expression in monkeys fed Lo, Med, or Hi dietary cholesterol. **(A)** Expression of the proinflammatory genes TNF α , IL-6, and IL-8; **(B)** expression of the macrophage recruitment genes MCP-1, CCR2, and CD68 and **(C)** expression of the adipokine genes adiponectin and leptin. Data from individual animals are shown with mean $\pm$ SEM denoted by the horizontal lines, n=5/diet group. P values were obtained by one-way ANOVA with Tukey's multiple comparison test. Diet groups with different letters are statistically different, p<0.05.

A Cholesterol synthesis**B Lipoprotein uptake****C Cholesterol efflux****D Chol. Diet****Figure 5.**

An increase in dietary cholesterol is inversely associated with adipose tissue lipoprotein receptor gene expression. Omental fat from monkeys fed Lo, Med, or Hi cholesterol diets (n=5 per group) was analyzed for gene expression by quantitative real-time PCR. Expression of genes involved in cholesterol biosynthesis (**A**), lipoprotein/cholesterol uptake (**B**), and cholesterol efflux (**C**). Western blot analysis of ABCA1, apoE, and β-actin from omental adipose tissue (n=4–5). Relative band intensity quantified by ImageJ software (**D**). Results

are mean $\pm$ SEM. P value was obtained by one-way ANOVA with Tukey's multiple comparison test. Diet groups with different letters are statistically different, $p < 0.05$.

Table 1

Cholesterol absorption, fecal cholesterol excretion, and hepatic lipid content in African green monkeys fed different amounts of dietary cholesterol

	Lo-cho	Med-cho	Hi-cho	p value
Cholesterol content (mg/Kcal diet)	0.002	0.2	0.4	-
Cholesterol absorption (%)	56.0 ± 5.3	52.0 ± 5.8	52.4 ± 2.2	0.81
Fecal excretion (mg/day/kg BW)	5.5 ± 0.4 ^a	11.40 ± 1.9 ^{ab}	15.62 ± 2.1 ^b	0.003
Hepatic FC (mg/g protein)	14.33 ± 0.3 ^a	17.4 ± 0.7 ^a	31.2 ± 1.8 ^b	0.0001
Hepatic CE (mg/g protein)	11.36 ± 1.6 ^a	25.0 ± 5.1 ^a	158 ± 40.9 ^b	0.001
Hepatic TG (mg/g protein)	39.92 ± 10.3 ^a	57.0 ± 12.6 ^a	67.6 ± 9.4 ^a	0.22

Mean ± SEM, n=5 per diet group. Values with different superscript letter are statistically different (p<0.05) by one-way ANOVA with Tukey's multiple comparison test.

BW = body weight; CE = cholesterol ester; FC = fecal cholesterol; TG = triglycerides.

Table 2

Hepatic gene expression in African green monkeys fed different amounts of dietary cholesterol

Gene	Lo-cho	Hi-cho	p value
SREBP2	1.03 ± 0.13	0.51 ± 0.13	0.02
HMGCR	1.07 ± 0.20	0.48 ± 0.15	0.05
apoE	1.06 ± 0.17	1.22 ± 0.24	n.s
ABCA1	1.13 ± 0.31	1.76 ± 0.55	n.s.
ABCG1	1.17 ± 0.31	3.93 ± 0.84	0.01
SRB1	1.18 ± 0.29	0.72 ± 0.22	n.s.
LDLr	1.18 ± 0.29	3.38 ± 1.90	n.s.
FAS	1.15 ± 0.26	3.72 ± 1.81	n.s
CD36	1.22 ± 0.23	1.47 ± 0.25	n.s

Gene expression was determined by quantitative real time PCR and results were normalized to GAPDH. Results were then normalized to the mean value for each gene in the Lo-cho group, which was set to a value of 1. Mean ± SEM, n=5 per diet group.