



## Effect of dietary fat restriction on vascular function

Baltensperger-Schneider, Heidi Lea

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## **Effect of Dietary Fat Restriction on Vascular Function**

### **Inaugural-Dissertation**

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vorgelegt von

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## **Abstract**

Overweight and obesity are chronic diseases, which are increasingly affecting children and adolescents, and if not treated immediately then fat children from today will become patients from tomorrow.

The objective of this study was to investigate whether dietary fat restriction normalizes body weight, impaired glucose tolerance and endothelium-dependent contractions induced by high dietary fat intake in young rodents. C57BL/6J mice, 4 weeks of age, were divided into Control group, which was fed with standard rodent chow (12% of fat); High-Fat group, which was fed for 30 weeks with the high-fat (HF) diet (41% fat) and Fat Restriction group, which was fed for 15 weeks with the HF diet (41% of fat), followed by standard chow (12% of fat) for 15 weeks. Body weight was monitored and glucose tolerance test was performed. Vascular responses to acetylcholine were investigated in aortic and carotid artery rings *ex vivo* in the absence or presence of nitric oxide and prostanoids.

Body weight was increased in the High-Fat group and was normalized in the Fat Restriction group to similar levels as in the Control group. Impaired glucose tolerance detected in High-Fat group was normalized in the Fat Restriction group. In the High-Fat group, endothelium-dependent contractions were increased in aorta and carotid arteries, and these contractions were attenuated in the Fat Restriction group to the same level as in the Control group. Moreover, the extent and sensitivity of these contractions varied between aorta and carotid arteries in the presence or absence of nitric oxide.

In conclusion, intake of high amounts of fat leads to weight gain, glucose intolerance and enhanced endothelium-dependent contractile responses in aorta and carotid artery of young mice. All these effects were normalized after dietary fat restriction. These findings thus suggest that long term reduction in intake of dietary fat improves the metabolic and vascular function in young mice.

## List of Abbreviations

<b>Ach</b>	Acetylcholine
<b>ATP</b>	Adenosine Triphosphate
<b>BMI</b>	Body Mass Index
<b>CAD</b>	Coronary Artery Disease
<b>cGMP</b>	Cyclic Guanosine Monophosphate
<b>COX</b>	Cyclooxygenase
<b>EDCF</b>	Endothelium-Derived Contracting Factor
<b>EDHF</b>	Endothelium-Derived Hyperpolarisation Factor
<b>ET-1</b>	Endothelin-1
<b>FRS</b>	Framingham Risk Score
<b>HF</b>	High-Fat Diet (41% kcal of fat)
<b>ET-1</b>	Endothelin-1
<b>KCl</b>	Potassium Chloride
<b>L-NAME</b>	N <sup>G</sup> -Nitro-L-Arginine Methylester
<b>Meclo</b>	Meclofenamate
<b>NO</b>	Nitric Oxide
<b>eNOS</b>	Endothelial Nitric Oxide Synthase
<b>PE</b>	Phenylephrine
<b>PKC</b>	Protein kinase C
<b>VSMCs</b>	Vascular Smooth Muscle Cells
<b>WHO</b>	World Health Organisation
<b>WHR</b>	Waist-To-Hip Circumference Ratio

# 1. Introduction

## 1.1. Obesity

The growing prevalence of obesity has become a serious health problem, in both developed and developing countries, regardless of age, sex, ethnicity or socio-economic background.<sup>1</sup>

Obesity is a chronic disease and predisposes to metabolic syndrome, which is a composition of metabolic risk factors that consists of elevated glucose associated with insulin resistance, serum elevations of triglycerides, low levels of high-density lipoprotein, elevated blood pressure, and a prothrombotic and proinflammatory state.<sup>2, 3</sup> A parameter to detect overweight

and obesity is the body mass index (BMI). 
$$\text{BMI} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

A BMI between 20-24.9 indicates normal weight, between 25.0-29.9 is overweight and 30 and above BMI indicates obesity.<sup>4</sup> In general, for each unit of BMI increment, the risk of coronary diseases increases by 8%.<sup>5</sup> However, BMI should not be used as the only criteria for evaluating obesity. There is now substantial evidence suggesting that evaluating the regional distribution of fat is far more important, than simple weight recordings. To assess abdominal fat distribution, the waist-to-hip circumference ratio (WHR) is an important prediction criteria.<sup>6</sup> The deposition of fat in the upper region of the body, or central obesity, is a better predictor of morbidity than excess fat in the lower body.<sup>6</sup> Therefore, recent studies emphasize on the accurate estimations on the distribution of the body fat for better prognosis of pathological changes.<sup>6</sup>

Like in the rest of Europe, the number of individuals being overweight or obese is increasing in Switzerland.<sup>7, 8</sup> A recent national study shows that the prevalence of overweight and obesity is 29.4% and 7.7%, respectively in the Swiss population older than 15 years. This represents a total of 37.1%, which is more than one third of the adult population in Switzerland.<sup>9</sup>

### 1.1.1. Childhood Obesity

Alarming, the prevalence of overweight and obesity is also increasing in children and adolescents. It is estimated that one in 10 children are overweight and under the age of 5 years over 22 million children are overweight worldwide.<sup>1</sup> In Europe, about 30% of children are overweight and approximately one quarter of these are obese.<sup>10, 11</sup> In Switzerland, the prevalence of overweight and obese boys and girls were 17% and 19%, respectively, in 2002.<sup>10</sup> France and Switzerland have data for over 40 years and both countries suggest, that since early 1960s the prevalence of overweight and obesity has increased.<sup>10</sup> The situation in Switzerland is even more dramatic: the incidence of overweight has almost quadrupled from 5 to 22%.<sup>12</sup> It is estimated that the number of overweight children will increase by 17%, and of obese children by over 19% from 2006 to 2010 in the European Union.<sup>12</sup>

The prevalence of overweight and obesity among children is more and more a worrisome clinical problem.<sup>13</sup> Girls with overweight upon entering adulthood have higher chances of developing other complications. When they become pregnant, their risk of developing glucose intolerance and gestational diabetes increases markedly. Consequently, they then produce heavier babies who are themselves prone to become obese in early childhood.<sup>14</sup> A recent review estimated that over 20,000 obese European children presently suffer from type 2 diabetes, most of it is unrecognized and hence untreated, and over 400,000 have impaired glucose tolerance.<sup>12</sup> At least 1 million obese European children are likely to manifest indicators of cardiovascular disease and metabolic syndrome, and almost 1.5 million are likely to suffer from early stages of liver disorder.<sup>12</sup> In children, the relation between systolic blood pressure and elevated visceral fat accumulation, serum insulin, leptin and family history of hypertension was shown by Nishina et al. in 2003.<sup>15</sup> In this study, 109 Japanese obese children aged 6-15 years with a family history of hypertension and 83 Japanese obese children with no family history for high blood pressure were included. The results of the study suggest that the association between systolic blood pressure with visceral fat accumulation,

hyperleptinemia and hyperinsulinemia was independent of a family history of hypertension.<sup>15</sup> Similar results were also reported in studies with adult obesity.<sup>15</sup> In obese children, a lower distensibility of the common carotid artery and a significant lower arterial compliance than in the healthy controls has been reported. These findings suggest that severe obesity is associated with increased endothelium dysfunction and arterial wall stiffness.<sup>16</sup> In another study, overweight children presented an impaired endothelial function and an increased carotid-intima media thickness.<sup>17</sup> The elevation of BMI correlated with the severity of endothelial dysfunction.<sup>17</sup> Thus, childhood obesity may damage or impair vascular function resulting in accelerated development of vascular-related diseases.

## 1.1.2. Diseases Associated with Obesity

### 1.1.2.1. *Atherosclerosis*

Atherosclerosis is a chronic inflammatory systemic disease of the vasculature which is the main cause for most cardiovascular diseases such as coronary artery disease, congestive heart failure, peripheral artery disease, stroke, ischemic bowel disease and diabetic nephropathy.<sup>18-20</sup> In Westernized societies, the reason for 50% of all deaths is atherosclerosis.<sup>21</sup> Stroke accounts for the majority of invalidity. In developing countries the incidence of atherosclerosis is rapidly rising as well.<sup>22</sup> According to the Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study, obesity was associated with coronary atherosclerosis in young men but not in young women.<sup>23</sup> Epidemiological studies have revealed several important environmental and genetic risk factors associated with atherosclerosis. Risk factors include elevated LDL and low HDL cholesterol levels, hypertension, hyperglycemia and diabetes, as well as aging, male gender and estrogen deficiency. Other risk factors are smoking, physical inactivity, genetic variability and chronic inflammatory processes.<sup>18, 21, 24-26</sup>

The development of atherosclerosis, a pathological process, begins already during fetal life.<sup>27</sup> The fetal aorta can already show early lesion of the atherosclerotic plaque, the so-called fatty streak.<sup>27, 28</sup> Maternal hypercholesterolemia may aggravate this formation.<sup>27</sup> Elevated plasma lipids may play an essential role for onset and progression of early fetal atherosclerosis.<sup>28</sup> Juvenile obesity results in unfavorable changes in plasma lipids,<sup>29</sup> and mediates the progression of atherosclerotic lesion formation.<sup>30</sup> Thus, the above studies suggests that atherosclerosis is not only an age-related disease but can also develop in early life.

#### *1.1.2.2. Coronary Artery Disease*

In obese people, when all risk factors for coronary artery disease coexist, the condition leads to the development of cardiovascular disease (CVD) causing increased morbidity and mortality.<sup>3</sup> Using a cohort of 2005 men and 2521 women, the Framingham Study showed that the 28-year age-adjusted rate (per 100) of coronary heart disease (CHD) in men was 26.3 for a mean BMI of 21.6 kg/m<sup>2</sup> and 42.2 for a mean BMI of 31. Whereas in women the score was 19.5 for a BMI of 20.4 and 28.8 for a BMI of 32.3, respectively.<sup>31</sup> The Framingham risk score (FRS) allows early risk identification for coronary artery disease (CAD).<sup>32</sup> For the calculation of FRS, gender, age, total cholesterol to HDL cholesterol ratio, systolic blood pressure, left ventricular hypertrophy (LVH), type II diabetes and cigarette smoking were used.<sup>32</sup> The relationship between carotid artery intima-media thickness (IMT) and FRS parameters is important in evaluating the development of coronary atherosclerosis.<sup>32</sup> In black and white population, a significant, positive linear relationship between FRS and IMT of the different carotid segments (common, bulb and internal part) were observed.<sup>32</sup> The importance to control multiple risk factors and to prevent early onset of CAD is underscored by these results.<sup>32</sup> Another multivariate analysis, the Gothenberg Study, showed that the waist-hip ratio (WHR) was the strongest predictor of myocardial infarction in 1462 women during a follow up of 12 years.<sup>3, 33</sup> An observation over 20 years by the Honolulu Heart Program

demonstrated that a mean subscapular skinfold thickness of 27.2 mm increased the risk of CHD in Japanese American.<sup>34</sup> All these epidemiological studies indicate that overweight and obesity increases the risk for developing CAD, not only in men but also in women.

#### *1.1.2.3. Diabetes mellitus Type II*

Obesity is strongly associated with type II diabetes mellitus.<sup>35</sup> The manifestation of obesity associated diabetes has become increasingly common and the incidence of diabetes is predicted to double in the next 30 years.<sup>35, 36</sup> Epidemiological studies suggest that most children or adolescents with T2DM (Type II Diabetes mellitus) are obese.<sup>37</sup> Increased accumulation of visceral fat may lead to elevated secretion of free fatty acids and inflammatory cytokines, and a concomitant reduction in anti-inflammatory cytokines<sup>38-40</sup>. This may lead to the development of insulin resistance. The metabolic disorders that characterize diabetes, such as hyperglycemia, increased free fatty acids, and insulin resistance cause vascular dysfunction and mediate cardiovascular complications, including microvascular disease like retinopathy, neuropathy and nephropathy.<sup>41, 42</sup>

#### *1.1.2.4. Other Obesity-Associated Diseases*

Obesity increases the risk of death from all forms of cancers. Obesity elevates the levels of hormones which may influence cancer development. Excess estrogen is linked with cancers of the reproductive system and the adipose tissue is a major site of estrogen synthesis in women.<sup>43</sup>

Other important diseases associated with obesity include abdominal hernias, varicose veins, gout, gall bladder disease, respiratory diseases such as obstructive sleep apnea and obesity hypoventilation syndrome, kidney diseases and liver malfunction.<sup>43</sup>

### 1.1.3. Economic Aspects of Obesity

The cost of obesity-related disorders are estimated in the range of 2-8% of total health care costs, clearly one of the largest expenditure in health care budgets.<sup>10</sup> In addition to the direct costs of obesity as a disease, certain indirect costs are also associated with obesity such as reduced output due to sickness absence and premature death of workers (NAO, National Accounting Office, 2001).<sup>10</sup> In 2002, the UK National Accounting Office (NAO) estimated that total (direct and indirect) cost of treating obesity and its consequences amounted to £3.3-3.7 billion (~ CHF 5.54-6.21 billion).<sup>10</sup> Recently Branca *et al.*, reported that according to WHO the costs of treating obesity-related disorders (such as cardiovascular disease, type II diabetes and certain types of cancer) were 6% of total health care costs in countries of Europe. Similar indirect costs were estimated due to lost productivity.<sup>10</sup> In Switzerland in 2001, the total costs, direct and indirect, were CHF 2648 million. Of these CHF 1374 million were assigned to overweight and CHF 1273 million towards obesity.<sup>9</sup> The costs for overweight and obesity was proportionally equivalent (51.1 vs. 48.9%).<sup>9</sup> Only a small number of studies assess the various potential economic consequences of obesity or high BMI at the individual or household level, the so-called micro-level costs.<sup>10</sup> It is suggested that women, who are overweight, are likely to earn less than women with normal body weight.<sup>44</sup> Obesity is in this way associated with negative effects, like social stigma and decreased labour productivity.<sup>10</sup>

## 1.2. The Vasculature and Basic Structure

### 1.2.1. Endothelial and Smooth Muscle Cells

The vasculature is composed of several layers of tunica. The outermost is known as tunica adventitia, which is composed of external elastic membrane and connective tissue. The middle layer is the tunica media, which is primarily composed of vascular smooth muscle cells and the elastic fibers. The innermost lining of the blood vessel facing the lumen is the

tunica intima, which is composed of the endothelial cells and connective tissues. The endothelial and vascular smooth muscle cells (VSMCs) play a central role in the regulation of cardiovascular tone by providing either contraction or relaxation. Moreover these cells are potentially involved in the maintenance of cardiovascular health and in regulating the progression of disease.<sup>45</sup> Furchgott and Zawadzki described in 1980 that the endothelium is a major regulator of vascular homeostasis.<sup>46,47</sup> The endothelium, built by endothelial cells, is a thin layer of flat cells that lines the interior surface of the entire circulatory system, from the heart to the smallest capillary.<sup>47</sup> It is located between the VSMCs and circulating blood as the inner lining of blood vessels.<sup>47</sup> In a human body with about 70 kg, the endothelium weighs about 1 to 1.5 kg and covers the inner layer of about 700 m<sup>2</sup>.<sup>47</sup> The endothelium releases humoral factors that control relaxation and contraction, thrombogenesis, fibrinolysis and platelet activation.<sup>48</sup> In this way the endothelium plays an important role for the maintenance of blood flow and anti-thrombotic function.<sup>48</sup> Endothelial dysfunction leads to cardiovascular disorders like hypertension, atherosclerosis, heart failure and finally to vascular occlusion and end-organ damage.<sup>47</sup>

The vascular smooth muscle cells surround the endothelial cells.<sup>49</sup> Vascular smooth muscle cells are involved in the development of vascular bed, in vascular related diseases and during repair after mechanical trauma or inflammation.<sup>50-52</sup> The main function of vascular smooth muscle cells (VSMCs) is contraction.<sup>53</sup> These cells show phenotypic variability and flexibility and express VSMCs-specific marker genes like *smooth muscle (SM)  $\alpha$ -actin*, *SM-myosin heavy chain (MHC)*, *caldesmon*, *telokin*, *SM22- $\alpha$* , *h1-calponin* and *smoothelin*.<sup>50</sup> Under normal quiescent condition VSMCs show a very small rate of proliferation and synthetic activity.<sup>50</sup> VSMCs show a high rate of proliferation and migration in early embryonic development.<sup>51, 52</sup> In the embryogenesis, the expression of several gene markers and the production of different proteins such as extracellular matrix protein (ECM) is highly induced.<sup>54, 55</sup> In healthy mature organisms, VSMCs are in differentiated state and regulate

vascular tone, which is their primary function.<sup>53</sup> Vascular diseases or injury can disturb this differentiated state. In this situation a phenotypic modulation or switching of the VSMCs takes place. This process (de-differentiation) is characterized by increased proliferation, migration and decreased expression of VSMC-specific marker genes.<sup>50</sup>

VSMCs are able to perform specialized function, dependent on their anatomical localisation for example arteries versus veins, large versus small arteries, vascular versus gastrointestinal.<sup>51, 52</sup> In the different types of vascular beds, VSMCs mediate different functions like calcium regulation in phasic versus tonic blood vessels and regulating the myogenic tone between large versus small arteries.<sup>56</sup> Important for this different functionality is the diverse embryological origin of the VSMCs.<sup>57</sup> The main population of the VSMCs are derived from the local mesoderm. VSMCs of coronary arteries have their origin from the proepicardial organ and major blood vessels in the head and neck have their origin in the neural crest, that may be important in the morphogenesis of brachial-arch-derived vessels.<sup>58</sup> Circulating stem cells derived from bone marrow can give rise to VSMCs or VSMC-like cells in situation of inflammation, severe vascular injury or tissue/ organ transplantation/ rejection.<sup>58</sup>

By a good teamwork between the endothelium cells and the VSMCs, the functional integrity of the vasculature is ensured.<sup>48</sup>

### **1.3. Vascular Function**

#### **1.3.1. Vasoactive Factors**

The endothelium plays an important role in the control and maintenance of the blood flow, and for different metabolic processes taking place in the vascular wall.<sup>47</sup> The endothelium has the ability to synthesize and release various substances and also controls the tone of smooth muscle cells, platelet aggregation, leukocyte migration, inflammatory processes and responses to circulating substances.<sup>59-61</sup> In obesity, the integrity of the endothelium is disturbed and the

balance between relaxation and contraction is altered towards contraction.<sup>62</sup> The most important vasodilators are acetylcholine<sup>60</sup>, prostacyclin (prostaglandin I<sub>2</sub>)<sup>63</sup>, bradykinin<sup>64, 65</sup> and the endothelium-derived hyperpolarization factor (EDHF).<sup>65</sup> The most important vasoconstrictors include the 21-amino acid peptide endothelin-1 (ET-1),<sup>66</sup> thromboxane A<sub>2</sub><sup>67</sup>, prostanoids, prostaglandin H<sub>2</sub> and components of the renin-angiotensin system such as angiotensin II.<sup>68, 69</sup>

### 1.3.2. Endothelium-Dependent Reactivity

#### 1.3.2.1. *Relaxation*

The endothelium-derived vasodilators are prostacyclin (prostaglandin I<sub>2</sub>),<sup>63</sup> nitric oxide (NO),<sup>70</sup> the endothelium-derived hyperpolarization factor (EDHF).<sup>65</sup> Prostacyclin is primarily produced in the vascular wall by endothelial cells.<sup>48</sup> Prostacyclin increases cyclic 3',5'-adenosine monophosphate (cAMP)-concentrations, which activate ATP-sensitive potassium channels in smooth muscle cells and platelets.<sup>71</sup>

Several isoforms of nitric oxide synthase (NOS) exist in endothelial cells, vascular smooth muscle cells, macrophages, platelets, nerves and the brain.<sup>72</sup> These isoforms are endothelial nitric oxide synthase (eNOS/NOS3), inducible NOS (iNOS/NOS2) and neural NOS (nNOS/NOS1).<sup>72</sup>

Acetylcholine activates endothelium-dependent NO synthesis, which is important for vasodilation.<sup>73</sup> Endothelium-dependent relaxations due to NO involve formation of cyclic 3',5'-guanosine monophosphate (cGMP) via the action of soluble enzyme guanylyl cyclase.<sup>74</sup> L-NAME (N<sup>G</sup>-nitro-L-arginine methylester), an irreversible inhibitor of eNOS, competes with L-arginine at the catalytic site and pharmacologically inhibits NO production.<sup>72</sup> By releasing nitric oxide (NO) the endothelium causes a relaxant response in neighbouring vascular smooth muscle cells.<sup>75</sup> It is remarkable to note, that the vasculature is always in state of vasodilation by a constant basal release of NO.<sup>47</sup> Bradykinin is also involved in the

formation of vasodilating factors such as NO, prostanoids and EDHF. The endothelium-dependent hyperpolarizing factor (EDHF) is another important vasodilator. The molecules mediating EDHF are still unknown. Molecules that probably may be involved are potassium ions, hydrogen peroxide and epoxyeicosatrienoic acids.<sup>76,77</sup>

#### 1.3.2.2. *Contraction*

As already noted the endothelium also mediates contraction. The most important vasoconstrictors (endothelium-derived contracting factors) are the 21-amino acid peptide endothelin-1 (ET-1),<sup>66</sup> vasoconstrictor prostanoids such as thromboxane A<sub>2</sub><sup>67</sup> and prostaglandin H<sub>2</sub> and components of the renin-angiotensin system such as angiotensin II.<sup>68 69</sup> Release of ET-1 is stimulated by thrombin, transforming growth factor-beta, interleukin-1, epinephrine, angiotensin II, arginine vasopressin, calcium ionophore and phorbol ester.<sup>78, 79</sup> To date, two different endothelin receptors are known: ET<sub>A</sub>- and ET<sub>B</sub>-receptors.<sup>47</sup> Both receptors are coupled with a G-protein and linked to phospholipase C and protein kinase C.<sup>47</sup> At low ET-1 concentrations, ET<sub>B</sub>-receptors are involved in dilation via the formation of NO and prostacyclin while at high ET-1 concentrations ET<sub>A</sub>-receptors mediate contraction in smooth muscle cells.<sup>47</sup> Thus, ET-1 can cause vasodilation and contraction depending on the concentration.<sup>78</sup>

Another system, which is also regulated by the endothelium is the renin-angiotensin system. The endothelial cell membrane expresses the angiotensin-converting enzyme (ACE), which converts angiotensin I to angiotensin II. Angiotensin II activates endothelial angiotensin receptors, which stimulates the production of ET-1 and other mediators, leading to vasoconstriction.<sup>80</sup>

Endothelium-derived contracting factors (EDCFs) are generated via the cyclooxygenase pathway. Arachidonic acid, acetylcholine, histamine and serotonin can evoke endothelium-dependent contractions that are mediated by thromboxane A<sub>2</sub> or prostaglandin H<sub>2</sub>.<sup>69</sup>

Thromboxane A<sub>2</sub> and prostaglandin H<sub>2</sub> activate thromboxane receptors in vascular smooth muscle cells and platelets.<sup>69</sup> In both cell types, thromboxane A<sub>2</sub> and prostaglandin H<sub>2</sub> neutralize the vasodilating effect of NO and prostacyclin.<sup>69</sup> The cyclooxygenase pathway mediates contraction by producing superoxide anions, which rapidly inactivates NO to form the potent cytotoxic oxidant peroxynitrite, leading to vasoconstriction.<sup>69</sup> There are two isoforms of cyclooxygenase: COX1 and COX2. A recent study in 2005 showed that endothelium-dependent contraction was absent in the aorta of COX1<sup>-/-</sup> knockout mice and present in that of COX2<sup>-/-</sup> knockout mice. These findings showed that only COX1 is the isoform of cyclooxygenase responsible for the production of EDCF in mice.<sup>81</sup>

## **1.4. Aim of the study**

### 1.4.1. Hypothesis

Obesity is a disease, which has become a worldwide health care problem. The prevalence of overweight and obesity is fast rising in adults and even in children. Restriction of dietary fat or switching from a high-fat to a low-fat diet lowers body weight. Based on this, the hypothesis of the present dissertation was that dietary fat restriction also improves endothelium-mediated vasoreactivity in different vascular beds in a recognized mouse model of obesity, the C57BL/6J mouse, fed with a high-fat diet.

Main topics of interest were:

- Whether dietary fat restriction restores weight gain and blood glucose levels in young animals.
- Whether dietary fat restriction restores endothelium-dependent reactivity.
- Whether vascular function differed between carotid artery and aorta.

## 2. Materials and Methods

### 2.1. Experimental Animals

Healthy male mice (C57BL/6J, Charles River, Sulzfeld Germany), 4 weeks of age underwent different dietary regimes for 30 weeks. The animals were housed at the animal facility of the “Biologisches Zentrallabor” of the University Hospital of Zurich. The animals were exposed on a 12:12-h light- dark cycle, and had free access tap water *ad libitum*. The room temperature was maintained at 22°C. Housing facilities and experimental protocols were approved by the local authorities for animal research (Kommission für Tierversuche des Kantons Zürich) and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996).

### 2.2. Groups and Feeding Protocols

Mice were randomly assigned to one of the following diets (n = 10-12 mice / group): **Control group** was fed with standard rodent chow (12.3% of total kcal from fat, Kliba Nafag 3430, Kaiseraugst, Switzerland) for 30 weeks; **High-Fat group** was fed with a high-fat diet (41% of total kcal from fat, Research Diets D 12079B, New Brunswick, NJ) for 30 weeks and the **Fat Restriction group** was fed with a high-fat diet (41% of total kcal from fat) for 15 weeks, followed by 15 weeks feeding with standard rodent chow (12.3% fat). The macronutrient composition of the diets is listed in **Table 1**. The major source of the lipid fraction of the high-fat diet was composed of anhydrous milk fat, containing approximately 0.3% cholesterol.

**Table 1** Major macronutrient constituents of diets given in percent of kcal

<b>Diet</b>	<b>Control</b>	<b>High-Fat</b>
Protein	22.4	17
Carbohydrate	65.4	43
Fat	12.3	41

### **2.3. Glucose Tolerance Test**

In the week of the experiment, mice were fasted overnight for 14 h and body weight was measured. Venous blood was obtained from the tail vein (0 min) for baseline glucose measurements. Mice were subsequently injected (i.p.) with 2mg/g body weight D-glucose and blood was collected at 5, 10, 15, 30, 45, 60, 90 and 120 min. Blood glucose was determined with an Accu Chek Advantage glucose meter (Roche Diagnostics, Switzerland).

### **2.4. Tissue Harvesting**

At the end of the feeding protocol, mice were anesthetized with xylazine (100 mg/kg body weight (BW)); ketamine (23 mg/kg BW) and acepromazine (3.0 mg/kg BW), all intraperitoneal. When pain reflexes (in response to strong pain triggers on the toes) were absent, abdomen and chest were opened by laparotomy and medial sternotomy. Then the mice were subsequently exsanguinated via cardiac puncture. Blood vessels were identified and carefully excised. The thoracic aorta and carotid artery were isolated and placed in cold (4°C) Krebs Ringer bicarbonate solution of pH 7.4. The composition (in mmol/l) of Krebs buffer is as follows: NaCl 118.6, KCl 4.7; CaCl<sub>2</sub> 2.5; MgSO<sub>4</sub> 1.2 ; NaHCO<sub>3</sub> 25.1; KH<sub>2</sub>PO<sub>4</sub> 1.2; EDTA Na<sub>2</sub>Ca, 0.026; glucose 10.1). Under a microscope (Olympus SZX9, Volketswil, Switzerland) adherent connective, fat and muscle tissue was removed carefully from the arteries with microsurgical instruments. Special care was taken not to damage the endothelium or the

integrity of the smooth muscle cells during this procedure. Vessels were cut into 3.0 mm rings for thoracic aorta and 2.0-2.5 mm rings for carotid artery.

## **2.5. Vascular Function Experiments**

### 2.5.1. Organ Chambers

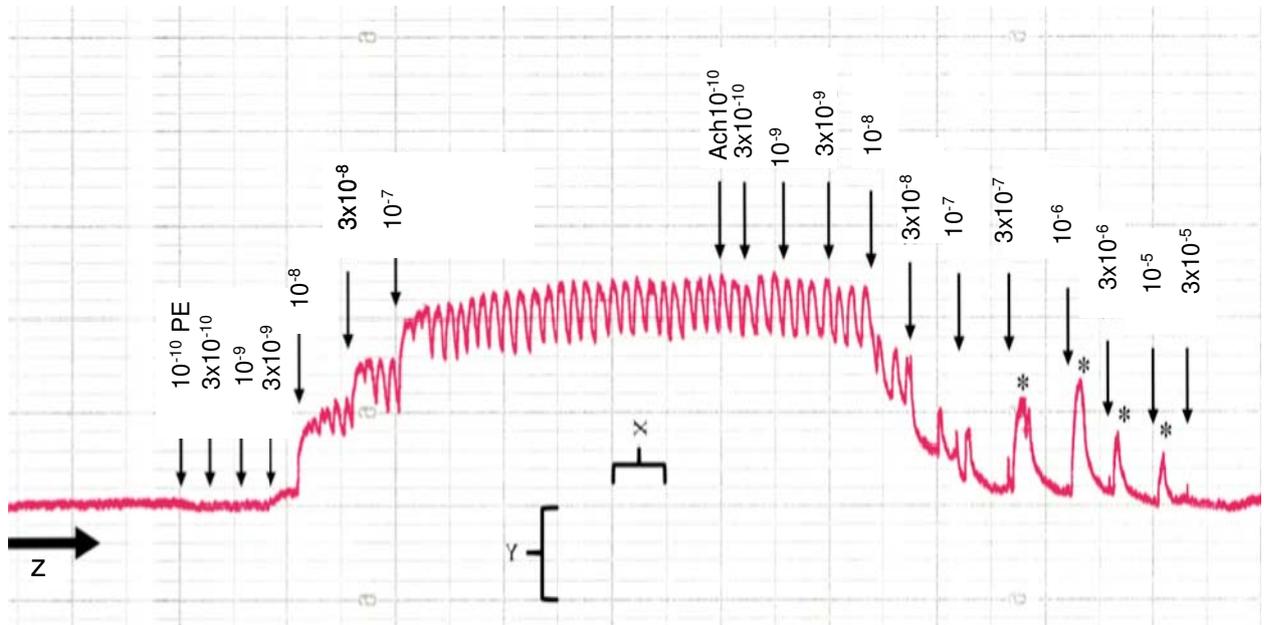
Vascular rings were mounted onto two tungsten wires (100  $\mu\text{m}$  diameter) under the microscope. The aortic and carotid artery rings were transferred into water-jacketed prewarmed organ baths containing gassed (95%  $\text{O}_2$ , 5%  $\text{CO}_2$ ) Krebs solutions at 37  $^\circ\text{C}$  with pH 7.4. Chamber volume was 10 ml. The rings were connected to a force transducers (Hugo Sachs Elektronik, Mach-Hugstetten, Germany), that allowed recording of isometric tension via an anchor. Before stretching the vessels, an equilibration time of 30 minutes was provided. To obtain an optimal level of passive tension, vessels were stretched step by step. Optimal stretching weights for aorta and carotid rings were 2.5g and 1.75g, respectively. These optimal weights were standardized based on earlier experiments in the laboratory. After another equilibration period of 20 minutes, the integrity of the vascular smooth muscle cell layer was verified by repeated exposure to potassium chloride (KCl, 100 mmol/L) until a stable response were achieved. The recordings were transferred to an X/Y-plotter (Rikadenki Electronics, Tokyo, Japan) and printed.

### 2.5.2. Experimental Protocols

#### 2.5.2.1. *Acetylcholine*

To measure responses to acetylcholine (Ach), vessels were either left untreated, or incubated with the non-selective cyclooxygenase inhibitor meclofenamate (Meclo, 1  $\mu\text{mol/L}$ ) or the NO- synthase inhibitor  $\text{N}^{\text{G}}$ -nitro-L-arginine methylester (L-NAME, 300  $\mu\text{mol/L}$ ). After 30 minutes of incubation, vessels were precontracted to 50% KCl using cumulative

concentrations of phenylephrine (PE; 0.1-100 nmol/L). Endothelium-dependent responses to Ach were then examined by adding Ach (0.1 nmol/L-300  $\mu$ mol/L) in a concentration-dependent manner as shown in **Fig. 1**.



**Fig. 1:** Representation of a typical contraction and relaxation curve of a carotid artery. X correlates to 5 minutes, Y correlates to 0.25g. Z indicates the direction of the protocol, starting with phenylephrine 0.1 nmol/L. \* indicates endothelium-dependent contractions to acetylcholine.

## 2.6. Drugs

Acetopromazine (Fatro, Ozzano Emilia, Italy), xylazine (Bayer, Zurich, Switzerland) and ketamine (Chassot AG, Bern Switzerland) were used for anesthesia. Acetylcholine hydrochloride, phenylephrine hydrochloride and meclofenamate were obtained from Sigma-Aldrich (Buchs, Switzerland), and L-NAME was obtained from ALEXIS Biochemicals (Lausen, Switzerland). Drugs were dissolved in purified water (Millipore®, Volketswil Switzerland) and diluted with fresh cold Krebs solution to attain the concentration needed. Concentrations are expressed as final molar concentration in the organ chambers.

## 2.7. Calculations and Statistical Analysis

The printed charts were manually digitized. The peak of the vascular response to the second exposure of potassium chloride (KCl, 100 mmol/L) was taken as reference value for contractions. Relaxations were expressed as percentage of precontraction in response to PE. For statistical analysis Microsoft Excel® for Windows® version 2003 and Statview® for Windows® version 5.0.1 were used. A *P* value smaller than 0.05 was considered significant and “n” is the number of animals used for the experiments. Two-tailed paired or unpaired Student’s *t*-test, ANOVA for repeated measurements followed by Bonferroni’s corrections were used when appropriate for the dataset. Values were represented in bar-graphs, line-graphs or tables. To emphasize the difference between relaxation and contraction, percentage for relaxations were shown as negative values, whereas percentages for contractions were displayed as positive values.

### 3. Results

#### 3.1. Normalization of Weight Gain after Dietary Fat Restriction

The changes in body weight were monitored throughout the experimental feeding period of 30 weeks. In **Table 1**, the gain in weight in the Control, High-Fat and Fat Restriction groups are listed. The gain in weight was calculated by subtracting the initial weight at 4 weeks of age, measured before starting the diet, from the final weight at 34 weeks of age. Feeding of high-fat diet for 30 weeks increased mouse body weight versus the Control group. Interestingly, animals subjected to fat restricted diet resulted in weight-loss and at the end of feeding protocol the weights were similar to control mice.

#### Weight Gain

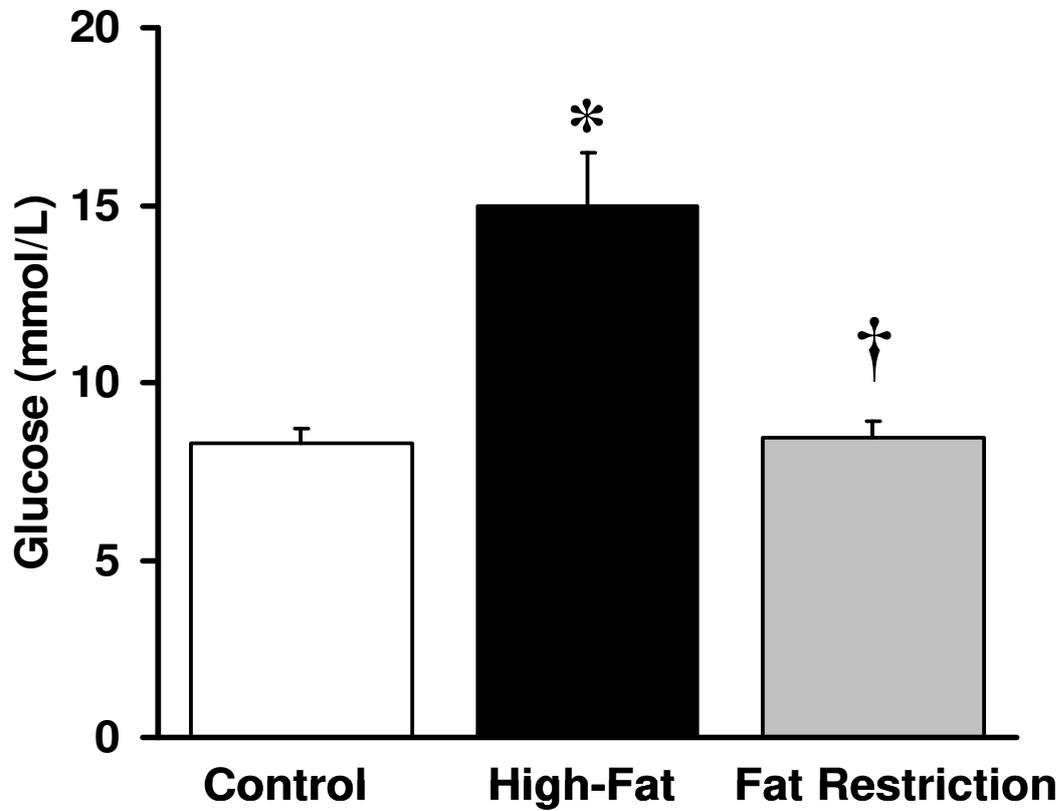
Group	Control	High-Fat	Fat Restriction
Weight gain (g)	15.1± 0.7	19.6 ± 0.6*	15.1 ± 1.6†

**Table 1.** Weight gain of mice in grams in Control, High-Fat and Fat Restriction Groups after 30 weeks of feeding (n= 5-13/ group). \* $P < 0.05$  vs. Control; † $P < 0.05$  vs. High-Fat. Values represent means ± standard error.

#### 3.2. Effect of High-Fat Diet and Dietary Fat Restriction on Glucose Levels

Basal glucose levels (at 0 min) in starved animals were similar across all groups (Control: 5.43±0.5 mmol/l; High-Fat: 6.15±0.2 mmol/L; Fat Restriction: 6.47±0.4 mmol/L). At 90 min after intraperitoneal injection of D-glucose (2mg/g of body weight), the blood glucose levels in all groups were higher compared to the 0 min (**Fig. 2**). The glucose level in High-Fat group at 90 min increased markedly in comparison to the Control group (High-Fat: 14.96 ±1.6 mmol/L vs. Control: 8.31± 0.44 mmol/L;  $P < 0.05$ ; **Fig. 2**). In the Fat Restriction group at 90

min, the blood glucose level became similar to the Control group ( $8.46 \pm 0.36$  mmol/L; Fig. 2).

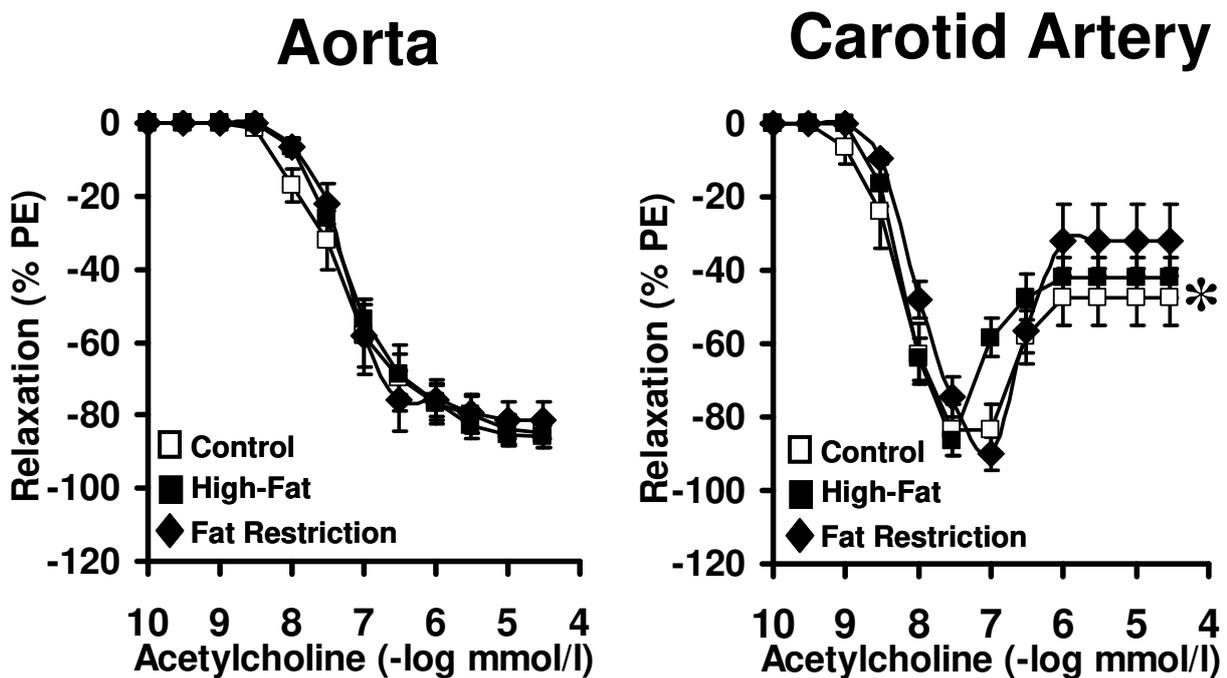


**Fig. 2:** Blood glucose levels at 90 minutes after injection of D-glucose (2mg/g body weight) in Control, High-Fat and Fat Restriction groups (n=10-11/group). Values represent means  $\pm$  stand error. (\*  $P < 0.05$  vs. Control; †  $P < 0.05$  vs. High-Fat group).

### 3.3. Effect of High-Fat Diet and Dietary Fat Restriction on Vascular Function

#### 3.3.1. Acetylcholine-Dependent Relaxation

Endothelium-dependent relaxation to acetylcholine was analyzed in the aorta and carotid rings in Control, High-Fat and Fat Restriction groups. Vascular relaxation was investigated in presence of prostanoids and nitric oxide as shown in Fig. 3.



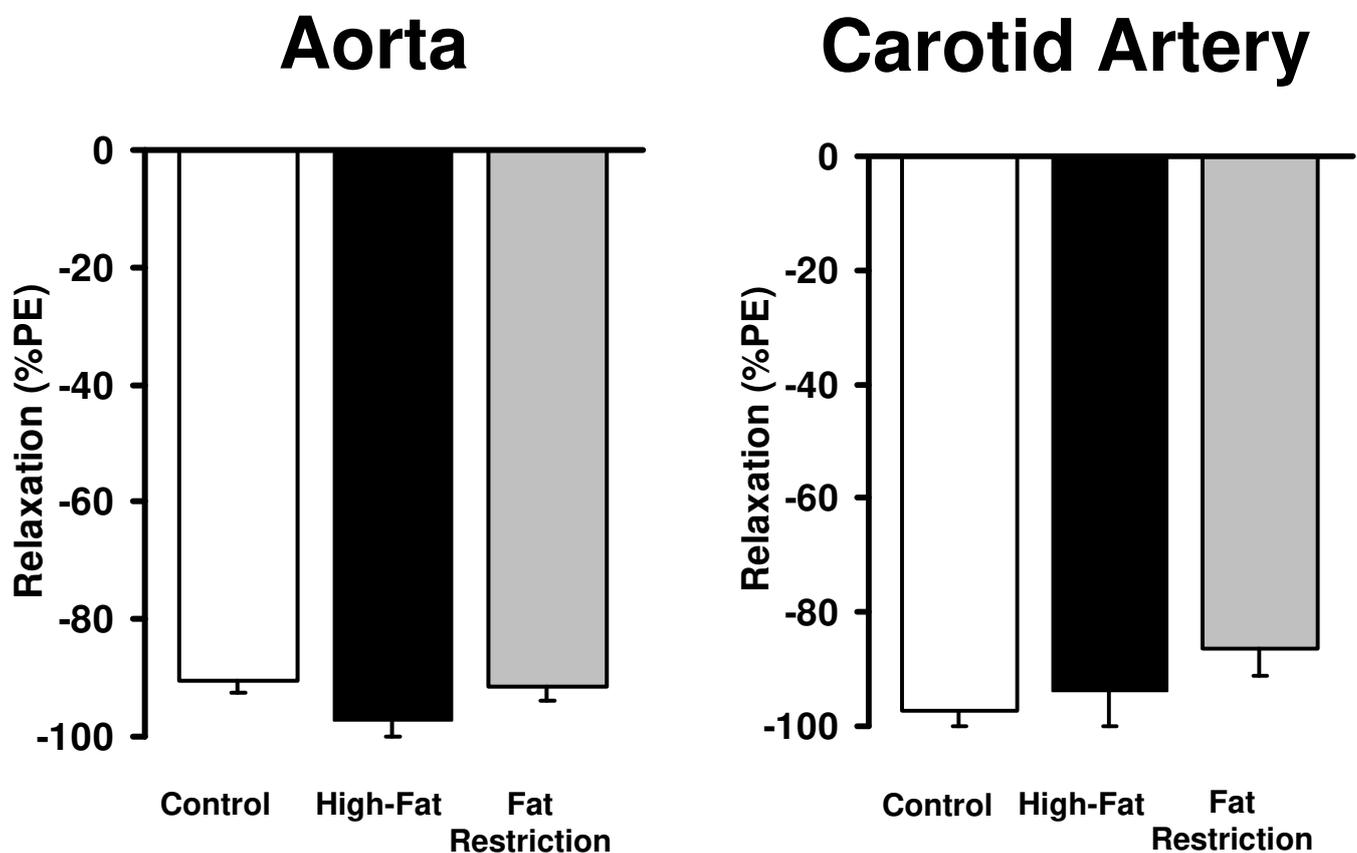
**Fig. 3:** Endothelium-dependent relaxation to acetylcholine in aorta and carotid artery in Control, High-Fat and Fat Restriction groups. Vascular rings were precontracted with phenylephrine before adding acetylcholine (0.1 nmol/L- 30  $\mu$ mol/L) in a concentration-dependent manner. Data are expressed as a percentage of precontraction with phenylephrine. Aorta: n=8-13/group; Carotid artery: n=5-8/group. \*P<0.05 for carotid artery vs. aorta.

In the carotid artery, acetylcholine evoked a biphasic response, which was not seen in the aorta. In response to low concentrations of acetylcholine ( $\leq 100$  nmol/L), carotid artery rings showed a relaxation (maximal relaxation as percentage of concentration to PE at 30 nmol/L: Control:  $-83.42 \pm 7.05$ ; High-Fat:  $-86.33 \pm 4.12$ ; Fat Restriction:  $-74.62 \pm 5.52$ ), whereas above this concentration a contractile response was observed (final relaxation percentage at 30

$\mu\text{mol/l}$ : Control:  $-47.33\pm 7.86$ ; High-Fat:  $-41.9\pm 5.65$ ; Fat Restriction:  $-31.77\pm 9.71$ ). In aortic rings, the contractile response was absent (maximal relaxation as percentage of contraction to PE: Control:  $-85.09\pm 3.64$ ; High-Fat:  $-86.01\pm 3.02$ ; Fat Restriction:  $-81.33\pm 5.08$ ).

### 3.3.2. Acetylcholine-Dependent Relaxation in the Absence of Prostanoids

To examine whether prostanoids are involved in the acetylcholine-dependent response, vessels were pretreated with meclofenamate ( $1 \mu\text{mol/L}$ ), a non-selective COX-inhibitor, (Fig. 4).

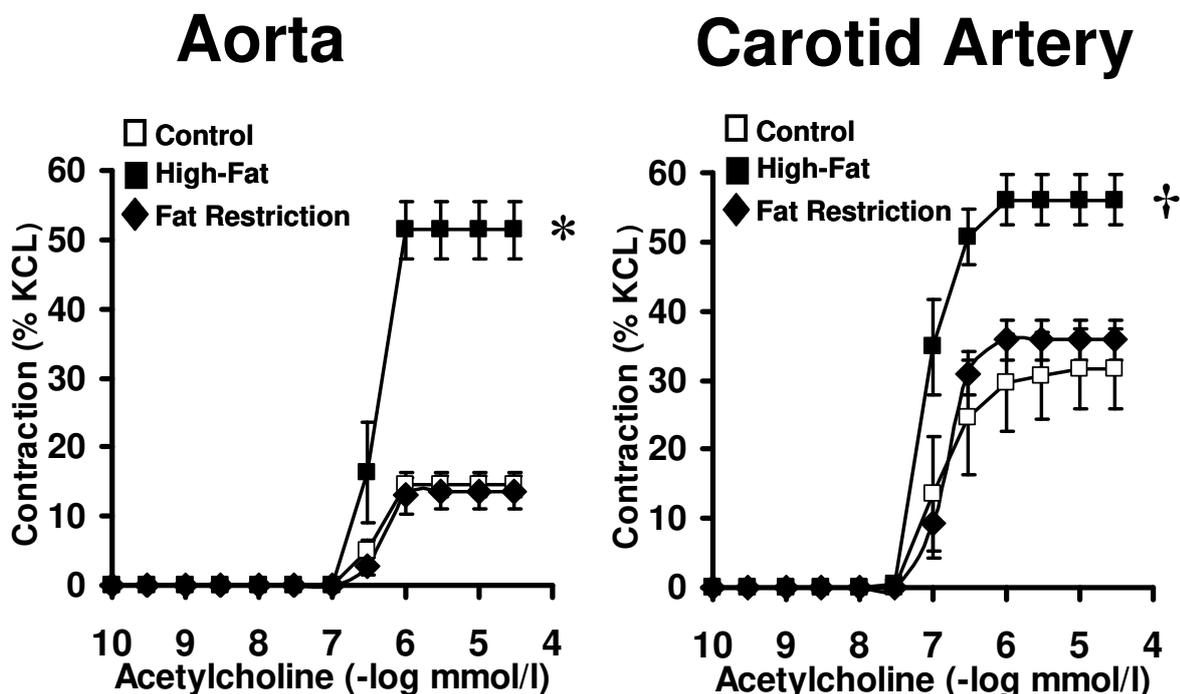


**Fig. 4:** Endothelium-dependent relaxation to acetylcholine in aorta and carotid artery in Control, High-Fat and Fat Restriction groups. Phenylephrine precontracted vessels were pretreated with meclofenamate ( $1 \mu\text{mol/L}$ ) for 30 min followed by acetylcholine treatment ( $10 \mu\text{mol/L}$ ). Data are expressed as a percentage of precontraction with phenylephrine. Aorta:  $n= 8/\text{group}$ ; Carotid artery:  $n= 4/\text{group}$ ).

In the carotid artery, pretreatment with meclofenamate completely abolished the contractile response at high concentrations of acetylcholine and the rings dilated in all the three diet groups (**Fig. 4**). The final relaxation percentage in the carotid artery was: Control:  $-97.22 \pm 2.77$ ; High-Fat:  $-93.75 \pm 6.2$ ; Fat Restriction:  $-86.30 \pm 4.8$ . The final relaxation percentage in aorta was: Control:  $-90 \pm 2.21$ ; High-Fat:  $-97.16 \pm 2.71$ ; Fat Restriction:  $-91.46 \pm 2.3$  (**Fig. 4**)

### 3.3.3. Acetylcholine-Dependent Vasoreactivity in the Absence of Nitric Oxide

To examine the response to acetylcholine in NO-depleted conditions, L-NAME (300  $\mu\text{mol/L}$ ) was used. The relaxant response after acetylcholine addition was blocked after pretreatment with L-NAME (**Fig. 5**), and only contractions were observed.



**Fig. 5:** Endothelium-dependent contractions to acetylcholine in aorta and carotid artery of mice from Control, High-Fat and Fat Restriction groups. Phenylephrine precontracted vessels were pretreated with L-NAME (300  $\mu\text{mol/l}$ ) for 30 min followed by acetylcholine (0.1 nmol/L - 30  $\mu\text{mol/L}$ ) treatment. Data are expressed as a percentage to KCl. Aorta:  $n=5-9/\text{group}$  and Carotid artery:  $n=5-8/\text{group}$ . \* $P < 0.05$  vs. Control and Fat Restriction, †  $P < 0.05$  vs. Control and Fat Restriction.

In the aorta, the contractile response occurred only at high concentration of acetylcholine  $\geq 100$  nmol/L. The contraction was enhanced by 3.4-fold in the High-Fat group in comparison with the Control group in the aortic rings (Control:  $14.58 \pm 1.78\%$  vs. High-Fat:  $51.37 \pm 1.78\%$ ;  $P < 0.05$ ). Interestingly in the Fat Restriction group, the contraction was attenuated to a level similar to the Control group (Fat Restriction:  $13.48 \pm 2.32\%$  vs. Control:  $14.58 \pm 1.78\%$ ). The carotid artery rings were more sensitive to acetylcholine and showed a contractile response starting at  $\geq 30$  nmol/L of acetylcholine. In the carotid artery, the contractile response was enhanced in the Control group in comparison to the aorta. Maximal contraction was observed in the High-Fat group in carotid artery (Control:  $31.64 \pm 5.76\%$  vs. High-Fat:  $56.04 \pm 3.69$ ;  $P < 0.05$ ). The contraction in the Fat Restriction group was attenuated similar to the Control group in the carotid artery (Fat Restriction:  $35.8 \pm 2.96\%$  vs. Control:  $31.64 \pm 5.76\%$ ).

## **4. Discussion**

### **4.1. Summary**

In the present study, high dietary fat feeding increased body weight and impaired glucose tolerance in young mice, and these effects were restored to a similar level as in control animals after dietary fat restriction. Feeding of high dietary fat did not alter endothelium-dependent relaxation in aortae and carotid arteries in the presence or absence of prostanoids. Dietary fat restriction also had no effect on relaxation. However, in the absence of nitric oxide (NO), endothelium-dependent contractions were induced in aortae and carotid arteries and these were enhanced after high dietary fat intake. Interestingly, these contractions were attenuated after dietary fat restriction to similar levels as in control animals. In the carotid artery, acetylcholine evoked a biphasic response with an initial relaxation, followed by a contractile response in all the treatment groups, whereas in the aorta this contractile response was absent and only relaxation occurred. After depletion of prostanoids with meclofenamate, the contractions present in carotid artery were absent and only relaxation was obtained. In the absence of endogenous NO, acetylcholine-mediated relaxations were abolished and only endothelium-derived contractions were observed in both carotid artery and aorta. In the carotid artery, acetylcholine mediated strong contractions in all the three groups in comparison to the aorta. These data suggest that endothelium-dependent reactivity differs between aorta and carotid artery. Moreover, endothelium-dependent contractile responses are further enhanced after high-fat diet. Switching of high-fat to low-fat diet attenuates these contractions to a similar level observed in control animals. This suggests that long-term reduction in dietary fat intake has beneficial effects on metabolism, and also on vascular function in children.

## 4.2. Weight Gain and Glucose Tolerance

In the present study, mice fed with high-fat diet were significantly heavier than the control animals fed with standard chow. Additionally, the glucose tolerance was markedly impaired after high-fat diet in young animals. Body weight and glucose tolerance improved significantly after mice were fed with a fat restriction diet and the values were similar to those of the control animals. This suggests that reduction in intake of high-fat diet normalizes body weight and glucose tolerance in young animals.

The suitability of the C57BL/6J mice fed with a high-fat diet as a useful rodent model for metabolic changes and vascular research has been shown in different studies.<sup>82, 83</sup> The metabolic changes in C57BL/6J mice after high-fat are comparable to the metabolic changes in humans, which are mainly characterized by insulin resistance due to obesity.<sup>83</sup> In the present study, C57BL/6J mice were analyzed at the age of 34 weeks, which is comparable with early adolescence in human beings and thus is an useful model for understanding metabolic and vascular changes in childhood.<sup>84</sup>

Obesity is associated with an excessive intake of a high caloric diet and decrease in physical activity.<sup>85</sup> To promote overweight, which consists of an increase in fat tissue in visceral regions, the typical “western diet” is used.<sup>82</sup> The classical western diet is rich in carbohydrates, especially oligo- and disaccharides, and lipids, mainly consisting of saturated fatty acids.<sup>82, 86</sup> These macronutrient pattern leads to metabolic changes, like insulin resistance, hyperinsulinemia, dyslipidemia, elevated blood pressure and vascular dysfunction.<sup>87</sup>

In a previous dietary fat restriction study, the effects of switching from high-fat to a low-fat diet in Sprague-Dawley rats (SD) were investigated.<sup>88</sup> Fat restriction was performed by feeding 3 week-old male SD rats with a high-fat diet (HFD) for 23 weeks followed by low-fat diet (LFD) for 20 weeks. The body weight of rats exposed to dietary fat restriction reduced in comparison to rats fed only a HFD for 43 weeks, however the body weights were higher than

control mice fed only a LFD. This data does not corroborate to our findings since in our study the body weights in mice after dietary fat restriction were similar to control animals. The reasons may be: Firstly, we fed the high-fat diet only for 15 weeks, whereas the duration of their high-fat feeding was 23 weeks. Secondly, their rodent model was rat, while we used C57BL/6J mice, which may contribute to non-comparable metabolic effects. In these rats even though there was no difference between serum insulin, glucose, total cholesterol, triglyceride levels between the groups but the liver showed steatosis even after dietary fat restriction. The authors suggest that a long-term high-fat diet can induce hepatic steatosis, which can be an irreversible effect and cannot be improved even after switching to a LFD.<sup>88</sup>

Excessive calorie intake and subsequent obesity increases not only the risk of developing chronic diseases, it also decreases life expectancy.<sup>89</sup> Already in 1935 Mc Cay et al., published the first scientific paper to report that calorie restriction in rats, when carried out after puberty, extended median and maximum life span and prevented or attenuated the severity of chronic disease.<sup>89</sup> Data from different studies using in laboratory rodents showed that, in part, calorie restriction increases longevity by preventing or delaying chronic diseases, like diabetes, atherosclerosis, cardiomyopathy, autoimmune diseases, and cancer.<sup>90-94</sup> The mechanisms responsible for calorie restriction-mediated effects on aging include decreased production of reactive oxygen species and modulation of the endogenous antioxidant system, which decrease oxidative stress and free radical-induced tissue damage.<sup>95</sup> Another calorie-restriction-mediated effect is a decrease of sympathetic nervous system activity,<sup>96</sup> which leads to a decrease in body temperature and whole-body resting energy expenditure from baseline.<sup>97, 98</sup> Furthermore, other studies showed a reduction in systemic inflammation by a decrease in plasma concentrations of inflammatory cytokines and modest increase in levels of circulating cortisol.<sup>99-102</sup> Two ongoing studies in rhesus monkeys show similar beneficial effects of calorie restrictions such as lower body weight,<sup>103</sup> lower core temperature and resting energy expenditure,<sup>97, 98</sup> reduced T<sub>3</sub> (Triiodothyronine, one of the hormones, produced by thyroid)

concentration<sup>104</sup> and improvement in risk factors for cardiovascular diseases like blood pressure, serum lipid profile, serum glucose and insulin concentration and insulin sensitivity,<sup>105-107</sup> decreased inflammatory markers and increased oxidative stress.<sup>108</sup> Similar positive effects of calorie-restriction have been seen in humans including low percentage of body fat, low systolic and diastolic blood pressures, improved lipid profile, increased insulin sensitivity, low plasma concentrations of inflammatory markers, low serum concentration of T<sub>3</sub> and low levels of circulating growth factors.<sup>109-111</sup> In addition, left ventricular diastolic function with better viscoelasticity and less stiffness was suggested as another beneficial effect of calorie restriction.<sup>111</sup> The left ventricular heart function was even comparable to those who were 16 years younger.<sup>111</sup> It is evident that obesity is associated with impaired function of most organ systems and premature mortality.<sup>112-114</sup> Weight loss, due to reduced energy intake, improves metabolic risk factors for cardiovascular disease and other medical abnormalities due to obesity.<sup>115</sup> Interestingly, bariatric surgery-induced calorie restriction leads to long-term weight loss, improves obesity-related complications and can decrease mortality rate in extremely obese human.<sup>116, 117</sup> But liposuction, by removing large amount of body fats, does not improve insulin sensitivity or other metabolic risk factors for cardiovascular disease.<sup>118</sup> Thus dietary fat restriction, which is a form of calorie restriction, is one of the corner stones to reduce the raising prevalence of overweight and obesity.<sup>89</sup>

### **4.3. Effect of High-Fat Diet and Dietary Fat Restriction on Vascular Tone**

In the present study, endothelium-dependent vascular relaxations to acetylcholine were examined in the presence of prostanoids and nitric oxide. Vascular rings were precontracted with phenylephrine before adding acetylcholine in a concentration-dependent manner. Interestingly, the responses between the carotid artery and the aorta were different. In the aorta only relaxation occurred whereas the carotid artery showed a biphasic response,

relaxation followed by contraction. In carotid artery, the relaxations occurred at lower concentrations of acetylcholine and maximal vascular relaxation was similar in carotid artery and aorta, and was unaffected by high-fat diet or dietary fat restriction. At higher concentrations of acetylcholine, a contractile response was observed in carotid artery in the three diet groups and this effect was absent in aorta. Reasons for this different reactivity patterns between the two vascular beds may be: 1. the anatomy of the vascular ring, particularly the proportionality of smooth muscle cells; 2. differences in the expression of acetylcholine-specific receptors; and 3. differences in the expression or activity of the intracellular signaling molecules.

In the present study, treatment with meclofenamate, a non-selective cyclooxygenase inhibitor, abolished these endothelium-dependent contractions in the carotid artery. Thus, cyclooxygenase-derived endoperoxides contribute to the endothelium-mediated contractile responses and depletion of prostanoids mediated relaxation in the three diet groups. Treatment with L-NAME led to a nitric oxide-depleted condition and blocked the relaxation response after acetylcholine treatment and only contractions were observed. In this condition, the carotid artery was more sensitive to acetylcholine showing a contractile response already at lower concentrations of acetylcholine compared to aorta. The contractile response was enhanced after high-fat diet. Interestingly, the contraction in the Fat Restriction group was attenuated similar to the Control group. In the aortic rings, the contractile response occurred only at high concentrations of acetylcholine and was also enhanced after high-fat diet. Dietary fat restriction attenuated the contraction to level similar to the Control group. These findings suggest that the induction of endothelium-dependent contraction depends on the vascular bed. Decreased availability of nitric oxide induces endothelium-dependent contractions, which are further amplified after a high-fat diet. Thus a reduction of the bioavailability of nitric oxide (NO) is linked to the development of vascular stress<sup>47, 119</sup>, which can be related to the increased endothelium-dependent contractions.

In a recent study using B6D2F1 male mice, carotid artery dilation was impaired in older mice, fed standard rodent chow *ad libitum* in comparison to young mice fed standard rodent chow *ad libitum*.<sup>120</sup> With calorie restriction, the impaired dilation in old mice was improved and was similar to the younger mice, fed *ad libitum* or a calorie restriction diet. These findings show that short-term calorie restriction restores vascular endothelial function in old mice and improves endothelium-dependent dilation.<sup>120</sup> In the present study, we did not observe any alteration in endothelium-dependent relaxation between the groups. In another study, a 12-week low-carbohydrate diet has been shown to improve postprandial vascular function in human brachial arteries.<sup>121</sup> The authors showed a positive effect of dietary carbohydrate restriction (CRD) in comparison to low-fat diet (LFD) on flow-mediated dilation.<sup>121</sup> After 12 weeks, peak flow-mediated dilation at 3 hours increased from 5.1% to 6.5% in the CRD group and decreased from 7.9% to 5.2% in the LFD group.<sup>121</sup> Several other reports indicate that 6 months of weight reduction and exercise improve macrovascular endothelial function and reduce selective markers of endothelial activation and coagulation in obese subjects with metabolic syndrome regardless of the degree of glucose tolerance.<sup>122, 123</sup> A recent study showed that weight reduction with very low-calorie diet improved flow-mediated vasodilation in obese individuals and the improvement was related to the reduction in plasma glucose concentration.<sup>124</sup> 1 year of a multidisciplinary weight reduction program caused a reduction in body weight by 10% along with a reduction in cytokine and adhesion concentrations and an improvement of vascular response to L-arginine in 56 healthy premenopausal obese women with a mean body mass index 37.2 kg/m<sup>2</sup>.<sup>125, 126</sup> After 2-week of low calorie diet (800 kcal/d), a significant improvement in flow-mediated dilatation was observed in obese hypertensive patients.<sup>127</sup> In children, an improvement of endothelial function has been observed when both diet and exercise were associated.<sup>128</sup> Thus, in line with our findings these studies also suggest a beneficial role of dietary fat restriction on endothelial function.

#### **4.4. Limitations of the study**

In the present study, following were the limiting factors: **1.** The number of animals is in the range of 4 to 13 per group, causing a limitation in statistical significance. **2.** In the present study, animals were fed only for 15 weeks of standard chow after high-fat diet feeding for 15 weeks. Therefore the possible effects associated with decreased duration of treatment with standard chow after high-fat feeding was not studied. **3.** Whether similar effects also occur in older animals were not examined. **4.** Only elastic-type of arteries were examined.

#### **4.5. Conclusion and Clinical Implication**

The present study shows that increased endothelium-mediated contractions occur in elastic arteries of obese adolescent mice due to high-fat diet intake. These effects were accompanied by weight gain and impaired glucose tolerance. These changes can be improved, and even normalized, by restricting the intake of fat or by switching from classical western type diet to a normal diet, low in fats. In healthy vessels, acetylcholine causes normally vasodilation, but by enhanced fat intake the homeostasis of vascular tone is disturbed and thus the vascular bed shows a greater tendency to vasoconstriction. Therapeutic strategies that reduce vascular oxidative stress, increase NO bioavailability and reverse impairment in endothelium-dependent reactivity may have important clinical implication for the prevention of cardiovascular disease in humans.<sup>120</sup>

The main topic of this study was to obtain more knowledge of vascular changes induced by fat restriction compared to high-fat feeding. Whether, the effects mediated by short-term dietary fat treatment were irreversible even after reduction in dietary fat content was not clear. In this study, we show that reduction in dietary fat content rescued endothelium-dependent contractions. It is important to realize that metabolic and vascular changes initiate already in early life. But even more important and more difficult is to counteract these early changes. And in this study, we show that significant reduction in dietary fat content for a long period

normalized effects contributed by high-fat diet. Since the present study deals with young animals, we are not sure whether similar results will be observed once adulthood is reached. Moreover, aging is an additional cardiovascular risk factor.

In the last few decades, our lifestyle has changed dramatically. In earlier years, human beings had to work more physically to earn money for their existence. The daily routine was more dependent on body-work. Today, with the modernity, everything has become more easy, surely more specialized and professional, but often associated with less body-activity, that means less body-work. With the growing culture of computer, internet and other virtual activities, including games and chats, our children and adolescents are more attracted in spending their free-time in front of a computer or playing other indoor games, without much physical activity, than playing outdoor activities. This modernization and acceleration of our daily life and life content is an important co-factor of the increasing obesity incidence and childhood obesity, in particular.

Another important reason is the socio-economic aspect. In spite of more knowledge and growth, the socio-economic problems has not become smaller, in contrary, the gap between the different social leagues have become even greater. That's why today, even in a normal under or middleclass 4-or 5-head family, a one person's income is mostly not enough and both parent's need to work to run the family's needs. This means, that children have to create their day often alone, especially they have to eat alone, which does not lead to a behavioral nutrition. Tim Lobstein describes in his work in 2008 a so-called "obesogenic economy", that is characterized by two main points. The first point is, that actually food companies influence what the consumer wants. Data from 2003 in UK show that food companies spend a high amount of money on marketing their products, mostly high in fats, sugar and or salt, and low in fresh fruit or vegetables. Only a small budget was spent by government for the promotion of healthy diets.<sup>129</sup> The second point is that, food market is considerably supported by government incentives to produce high levels of meat, milk butter and cheese, sugar and oil

and only a small budget is used for the production of fruit and vegetables and fish.<sup>129, 130</sup> This “obesogenic economy” is surely responsible to the growing prevalence of overweight and obesity with all its consequences.

In this study, we investigated the importance of reduction in fat intake to avoid obesity and to reach and keep an “optimal health”. The “optimal health” for each individual person is difficult to define, but to sum it up it can be declared as the state in which there is the highest possible attainment of mental, physical, and social well-being and the lowest risk of developing diseases in future.<sup>89</sup> Suggested clinical criteria to provide optimal health are lower than previous cut-off values; for systolic and diastolic blood pressure (< 115/75 mmHg vs. a previous threshold of 140/80 mmHg),<sup>131, 132</sup> concentration of plasma low-density lipoprotein cholesterol (1.3-1.8 mmol/L vs. a previous threshold of < 2.6 mmol/L),<sup>133</sup> and lower concentrations of fasting plasma glucose (4.2 mmol/L vs. a previous threshold of < 5.6 mmol/L)<sup>134, 135</sup>. The precise amount of the optimal calorie intake or body mass index for each individual associated with “optimal health” is not known till now, but the WHO and other groups have proposed that a BMI between 18.5 to 24.9 is optimal.<sup>89</sup>

It is not yet clear whether these suggested clinical criteria are realizable and do not lead to a “medical over-treating” of our society. But fact is, we have to take care of our fat intake and body weight, beginning already in early age to prevent the onset of overweight and obesity with all its clinical and economical consequences.

## 5. References

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## **6. Acknowledgments**

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