



**HAL**  
open science

# Is there an ‘obesity paradox’ of cardiac survival in heart disease? A perspective from experimental laboratory studies

Luc Demaison, Evangelia Mourmoura

## ► To cite this version:

Luc Demaison, Evangelia Mourmoura. Is there an ‘obesity paradox’ of cardiac survival in heart disease? A perspective from experimental laboratory studies. 61 p., 2018. hal-01704882

**HAL Id: hal-01704882**

**<https://hal.science/hal-01704882>**

Submitted on 8 Feb 2018

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

**2018, January**

**Is there an ‘obesity paradox’ of cardiac survival in heart disease?**

**A perspective from experimental laboratory studies\***

by Luc Demaison<sup>1</sup> and Evangelia Mourmoura

(1) Human Nutrition Unit, INRA, UMR 1019, Clermont Université,  
Université d’Auvergne, BP 10448, 63000 Clermont-Ferrand, France. Tel.:  
(+33)473608265; Fax: (+33)473608255; email: [luc.demaison@inra.fr](mailto:luc.demaison@inra.fr)

\* Part of this work was performed in the Laboratory of Fundamental and Applied Bioenergetic, INSERM U1055, Université Grenoble Alpes, France.

All our thanks to Pr. Jean-Pierre Iliou of the Servier Research Institute, Cardiovascular Research Unit, 92150 Suresnes, France; Pr. Salvatore Pepe of the Murdoch Children's Research Institute, Department of Cardiology, Royal Children's Hospital, Melbourne, Australia; and Pr. Luc Rochette of the Laboratory of Experimental Cardiovascular Physiopathology and Pharmacology, Medical University, 21033 Dijon, France, for discussing the validity of the scientific arguments presented in the manuscript.

We would also like to thank Salvatore Pepe for skilful improvement of the English version of a large part of this manuscript.

## CONTENTS

<b>ABBREVIATIONS</b>	p. 3
<b>ABSTRACT</b>	p. 5
<b>1 - INTRODUCTION</b>	p. 6
<i>1.1 - Socio-economical context</i>	<i>p. 6</i>
<i>1.2 - Obesity prevention</i>	<i>p. 6</i>
<i>1.3 - Obesity related diseases</i>	<i>p. 8</i>
<b>2 - OBESITY &amp; CARDIOVASCULAR DISEASE</b>	p. 11
<i>2.1 - Obesity effects on morphology and metabolism in a rat</i>	
<i>Dietary model</i>	<i>p. 12</i>
<i>2.2 - Obesity effect on in vivo cardiac function</i>	<i>p. 17</i>
<i>2.3 - Ex vivo cardiac function in Langendorff mode perfusion model</i>	<i>p. 21</i>
2.3.1 - High fat-fed Wistar rats	p. 22
2.3.2 - Lou/C rats compared to Wistar rats	p. 24
2.3.3 - Changes in cardiac function with aging	p. 25
2.3.4 - The Zucker Diabetic Fatty (ZDF) rat	p. 25
<i>2.4 - Discrepancies between in vivo and ex vivo measurements</i>	
<i>of cardiac mechanical function – Limitation of in vitro constant</i>	
<i>pressure perfusion</i>	<i>p. 26</i>
<i>2.5 - Other factors influencing the tissue perfusion and vascular function</i>	<i>p. 32</i>
<i>2.6 - Conclusions</i>	<i>p. 43</i>
<b>3 - OBESITY PARADOX</b>	p. 44
<i>3.1 - Introduction</i>	<i>p. 44</i>
<i>3.2 - Mitochondrial function after 3 months of high fat diet</i>	<i>p. 46</i>
<i>3.3 - Mitochondrial function after 7 months of high fat diet</i>	<i>p. 49</i>
<i>3.4 - Coronary reactivity after the 7-month high fat diet</i>	<i>p. 50</i>
<i>3.5 - Effects of obesity on the severity of ischemia</i>	<i>p. 51</i>
<b>4 - GENERAL CONCLUSIONS</b>	p. 53
<b>5 - REFERENCES</b>	p. 55

## ABBREVIATIONS

Aa: antimycine A  
 Ach: Acetylcholine  
 AFM: Abdominal Fat Mass  
 ArA: Arachidonic Acid  
 BMI: Body Mass Index  
 CaCl<sub>2</sub>: calcium chloride  
 CF: Coronary Flow  
 CO<sub>2</sub>: carbon dioxide  
 dP/dt max and dP/dt min: maximal and minimal derivatives of the left ventricular developed pressure  
 ECVA: Endothelial Cell Vasodilatation Activity  
 EDV: Endothelial-Dependent Vasodilatation  
 EIV: Endothelial-Independent Vasodilatation  
 eNOS: endothelial Nitric Oxide Synthase  
 EPA: EicosaPentaenoic acid  
 GM: Glutamate/Malate  
 GMP: Guanosine MonoPhosphate  
 H<sup>+</sup>: proton  
 H<sub>2</sub>O: water  
 H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide  
 HDL: High-Density Lipoproteins  
 HFD: High-Fat Diet  
 HR: Heart Rate  
 IDF: International Diabetes Federation  
 Indo: Indomethacin  
 INRA: French National Institute for Agronomical Research  
 INSERM: French National Institute of Health and Medical Research  
 Kc: Krebs cycle  
 KCl: potassium chloride  
 KH<sub>2</sub>PO<sub>4</sub>: potassium dihydrogen phosphate  
 LA: Linoleic Acid  
 L-NAME: L-N<sup>G</sup>-NitroArginine Methyl Ester  
 LVDP: Left Ventricular Developed Pressure  
 MA: Middle-Aged  
 MgSO<sub>4</sub>: magnesium sulphate  
 N: Nitroprussiate  
 NaCl: sodium chloride  
 NADP<sup>+</sup>: oxidized Nicotinamide Adenine DiNucleotide Phosphate  
 NADPH: reduced Nicotinamide Adenine DiNucleotide Phosphate  
 NaHCO<sub>3</sub>: sodium hydrogen carbonate  
 ND: Normolipidic Diet  
 NO: Nitric Oxide  
 O: Old  
 O<sub>2</sub>: di oxygen  
 Pearn: Palmitoylcarnitine/malate  
 PG: ProstaGlandin  
 PKC: protein Kinase C  
 Pyr: Pyruvate/malate

RCI: Respiratory Control Index  
Rot: rotenone  
RPP: Rate Pressure Product  
Ser: Serine  
Succ: Succinate/rotenone  
St. 2: State 2 respiration rate  
T2D: Type-2 Diabetes  
TX: ThromboXan  
WHO: World Health Organization  
WHR: Waist to Hip Ratio  
WI: Wistar  
Y: Young  
YA: Young Adult  
ZDF: Zucker Diabetic Fatty  
ZL: Zucker Lean

**ABSTRACT**

Abdominal obesity is associated with the development of cardiovascular disease. However, an ‘obesity paradox’ phenomenon has been identified whereby cardiovascular events that may ultimately lead to heart failure, in a setting of obesity occur with relatively lower severity affording the obese patient a greater chance of survival. The obesity paradox has partly been explained by a higher substrate availability to augmented muscle and fat masses, thus permitting greater resistance to injury and capacity for adaptive remodelling and survival. However, recent research advances show that substrate utilization is probably not the only parameter contributing to the obesity paradox. This book summarizes laboratory data showing that obesity triggers molecular changes at the cardiac level which can partly explain the obesity paradox.

## **1 - INTRODUCTION**

### **1.1 - *Socio-economical context***

Obesity is a major health problem in the industrialized societies as well as emerging countries. According to the World Health Organization (WHO), the yield of obese people has more than doubled since 1980. In 2014, the WHO estimated 1.9 billion adults to be overweight, with 600 million of these to be obese, representing approximately 39% and 13% of the total population respectively. The phenomenon increases with aging, however in the last two decades, the number of children under the age of 5 that are either overweight or obese has been increasing reaching an estimated 41 million in 2014 (1).

Currently the financial cost of obesity is estimated at ~2 trillion dollars per year. However, if the trajectory of obesity continues on its rising trend, the proportion of overweight and obese people will reach half the population in 2030 and the resulting costs will explode according to the McKinsey Global Institute (2). It is thus crucial to reverse the current trend and to slow down the progression of the epidemic, particularly as a large part of obesity may be preventable.

### **1.2 - *Obesity prevention***

The obesity is defined as an excessive fat mass deposition in the body. Body mass index (BMI) which is calculated as the weight in kilograms divided by the square of the size in meter is the index usually used to estimate the build of an individual. Individuals with a BMI ranging from 25 to 30 are considered overweight and those with a BMI higher than 30 are obese. However, fat deposition is not located identically in all individuals and since abdominal adiposity creates a more dangerous health risk, clinicians prefer to utilize the waist-to-hip ratio (WHR) (3). According to the WHO, people displaying a WHR higher than 0.80 for the women and 1.0 for the men are obese (4).

Although some genetic factors have been identified to be involved (5), the obesity epidemic is considered to be mainly related to an unbalanced diet and insufficient physical activity. The Western diet which has progressively expanded to the whole world, due to high abundance of manufactured and refined foods, is excessive in saturated fats and refined sugars. This contributes to fat mass deposition and the phenomenon is further amplified by the lack of physical activity characterizing the modern sedentary life style. The first prescription given by physicians to fight against obesity consists in reducing the calorie intake and increasing the physical activity. Although the ingestion of some specific nutrients have been considered in facilitating the fat loss effort: for example, green tea extract (6-8) and n-3 polyunsaturated fatty acid (PUFA) eicosapentaenoic acid (EPA) (9-15). However, the effects of these nutrients are controversial, at best limited, and cannot totally replace the required effort to limit food intake and increase regular exercise. When obesity is too severe and life threatening, bariatric surgery by fitting a gastric ring or performing a gastric short circuit is efficient in favouring weight loss, though it can trigger vitamin deficiency. Furthermore, it is not known whether all obesity-related disorders are reversed and if bariatric surgery has long-term beneficial effects (16).

Numerous studies show that the build of an individual depends on early events occurring during the perinatal period. For instance, low birth weight (premature) children have a high risk to develop obesity during adulthood (17). To compensate for their low birth weight, these children tend to be overfed in the postnatal period. Similarly, postnatal over-nutrition by reduction of the litter size in rodents translate into obesity during their life span (18). There is thus a critical period surrounding birth during which a metabolic programme sets up and may determine the lifelong dietary behaviour which governs not only the calorie intake but also the level of physical activity during the life span. The central nervous system plays a key role in this governance via the hypothalamic-pituitary axis.

The hypothalamic arcuate nucleus possesses neuropeptide Y-secreting neurons which extend to the paraventricular nucleus. Circulating leptin and insulin inhibit the secretion of neuropeptide Y and cause satiety (19). Obese people have high blood concentrations of insulin and leptin because of insulin resistance and high adipose tissue accumulation. However, as obesity triggers leptin- and insulin-resistance of the arcuate nucleus this prevents satiety thus obese individuals continue to eat in unregulated manner. A salvo of studies (20-23) show that postnatal over-nutrition induces disorganization and malprogramming of the arcuate nucleus characterized by leptin- and insulin-resistance as well as augmentation of the number of neuropeptide Y-secreting neurons. This induces inhibition of several neurons of the paraventricular and ventro-medial nuclei by neuropeptide Y and leads to feeding and reduced energy expenditure through expressional changes in the production of orectic and anorectic factors (24).

As a consequence, the body weight of an individual depends greatly on the central metabolic programming occurring in the perinatal period. However, it is also modulated by several factors taking place later during the life: low income-related junk food habits, little exercise due to physical injuries or medication intake, work- and hobby-related inactivity, age-related decrease in capacity to exert physical activity, sarcopenia, etc (25-28). The metabolic program enforces calorie intake, the highest weight gain during these periods. Spontaneous changes in life style factors remains difficult over time, thus bariatric surgery for such obese people offers a more practical direct approach in order to limit and reverse excess fat deposition in the abdominal cavity and the development of numerous pathologies (29, 30).

### ***1.3 - Obesity related diseases***

Obesity increases the risk for disease including hypertension, cardiovascular diseases and certain cancers (31-32). Obesity-related cancers concern

oesophageal carcinoma, pancreatic, colorectal, breast, endometrial, kidney, gallbladder, ovarian, liver, prostate, and blood (leukaemia and lymphoma) cancers. The increased risk for cancer development is due to hormonal malfunctioning. Indeed, adipose tissue is an endocrine system which produces numerous hormones (leptin, adiponectin, resistin, etc.). The increased risk of breast cancers in obese women relates to an excess production of oestrogens by adipose tissue (33).

In addition to cancers other health problem consequences of being overweight and obese are type 2 diabetes and cardiovascular disease. According to the International Diabetes Federation (IDF), the metabolic syndrome is a cluster of the most dangerous heart attack risk factors: diabetes and pre-diabetes, abdominal obesity, high cholesterol and high blood pressure (34). The IDF definition indicates that the metabolic syndrome is diagnosed when an individual displays abdominal obesity with values of waist circumference depending on the ethnic origin plus two of the following factors: raised triglycerides to values higher than 150 mg/dl (or specific treatment for this lipid abnormality); decreased HDL cholesterol to 40 mg/dl in males and 50 mg/dl for females (or specific treatment for this lipid abnormality); increased blood pressure to 130 mmHg for the systolic pressure or 85 mmHg for the diastolic pressure (or treatment of previously diagnosed hypertension); and augmented fasting glucose concentration to 100 mg/dl (or previously diagnosed type 2 diabetes).

The prominent symptom characterizing the metabolic syndrome is insulin resistance which is particularly marked in the skeletal muscles, liver and adipose tissue (35). In adipose tissue, insulin resistance activates lipolysis, contributing to increase plasma non esterified fatty acid concentration (36). When the storage capacities of the adipose tissue are reached, excess circulating lipids invade other tissues usually not dedicated to lipid storage and trigger insulin resistance and or lipotoxicity (37). This is the case for the liver for which insulin resistance

augments gluconeogenesis and glucose delivery into the blood (38). In skeletal muscle glucose uptake capacity decreases with insulin resistance (39). To compensate for insulin resistance, pancreatic  $\beta$ -cells augment insulin secretion, but ultimately become insulin deficient due to excess activity and lipotoxicity (40). Thus, type 2 diabetes (T2D) emerges with hyperglycaemia and hyperlipidaemia.

Obesity strongly increases the risk of T2D which represents 92% of all diagnosed diabetes (41). Excess abdominal adiposity would account for 80-85% of the risk of developing a T2D and obese people are 80 times more likely to develop a T2D than normal-weight individuals. Amputation, nephropathy and heart diseases are the tragic consequences of diabetes. However, 68% of diabetics aged 65 or more die from cardiovascular diseases and 18% from stroke. Adults with diabetes have 2 to 4 times greater risk of death from heart disease. The American Heart Association reports that diabetes is one of the 7 most severe risk factors for cardiovascular diseases (42).

## 2 - OBESITY & CARDIOVASCULAR DISEASE

Obesity- and T2D-related cardiovascular disorders include obstructive and non-obstructive diseases. Atherosclerosis is the predominant obstructive illness with plaque rupture and blood clot formation being responsible for the most severe myocardial infarction and stroke. This vascular pathology is strongly related to obesity, since it is promoted by dyslipidaemia, oxidative stress and inflammation. Atrial fibrillation can also stimulate blood clot formation and stroke. When atria fibrillate, blood dynamics in cardiac cavities are perturbed, blood coagulates and clots are driven to the brain through the blood circulation to trigger stroke. The Framingham cohort study has reported that a BMI increase by 1 unit leads to a 4-5% higher risk of atrial fibrillation (43) indicating that obesity constitutes a strong risk factor for atrial fibrillation.

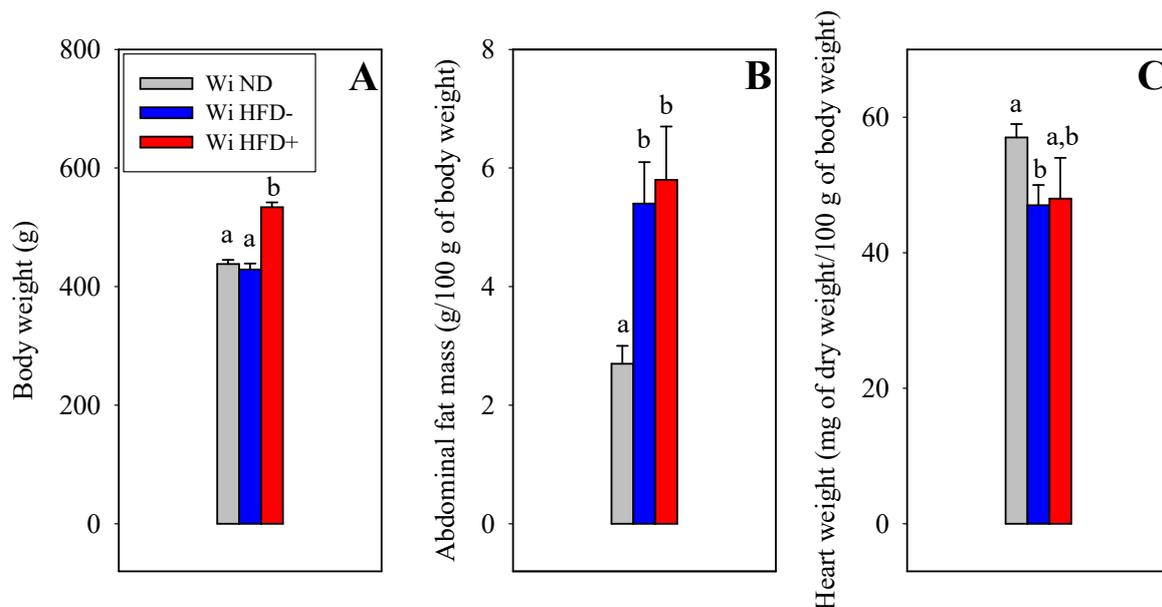
Despite the major progress in cardiovascular medicine leading to fewer patients deaths from obstructive diseases there is a relative increase in cardiac hypertrophic disorder, diabetic cardiomyopathy and heart failure (44, 45). Well demonstrated by Habbout *et al.* (46), obesity also leads to cardiac failure. These researchers showed in the rat that postnatal over-nutrition-induced obesity from the end of weaning to the 9<sup>th</sup> month of life resulted in cardiac failure involving oxidative stress, hypertrophy and fibrosis. Importantly, this cardiac failure was preceded at 3 months of age by a phase when increased cardiac function was stimulated by obesity. Such cardiac stimulation of increased cardiac output may be an adaptive offset for the body weight gain which subsequently leads to the progressive development of the cardiac failure later in the life of the rats, due to excess stimulation and injurious exhaustion and stress of the heart. This hypothesis, although appealing, provides no satisfactory indication of the underlying molecular mechanisms. Thus, as described in the sections that follow, our group sought to determine the biochemical changes occurring with early obesity when cardiac function is stimulated which could explain the progressive

development of the cardiac failure occurring later in the life of the animals. In these studies, rats were fed a high fat diet for 3 months and cardiac function was assessed *in vivo* and *ex vivo*. The functional reserve of the coronary micro-vessels describing the perfusion capacities of the myocardium was also estimated in the absence and presence of a cyclooxygenase inhibitor (indomethacin) in conjunction with the determination of the fatty acid composition of cardiac phospholipids (47-50). Such experiments were repeated in the model of the Zucker Diabetic Fatty (ZDF) rat in which obesity is associated with insulin resistance and hyperglycaemia. The results were supplemented with the measurement of several parameters describing the cardiac oxidative stress (51).

### ***2.1 - Obesity effects on morphology and metabolism in a rat dietary model***

In the first set of experiments, 3-month old male Wistar rats were divided in 2 groups. The first group was fed with a high fat diet (HFD, 54% of lipids in weight) enriched with lard (50% of the diet mass) in order to mimic the Western diet and to induce abdominal obesity; the second group was nourished with a normolipidic diet (ND) with 3.1% of lipids in order to mimic an equilibrated diet. The two diets were consumed *ad libitum* for 12 weeks.

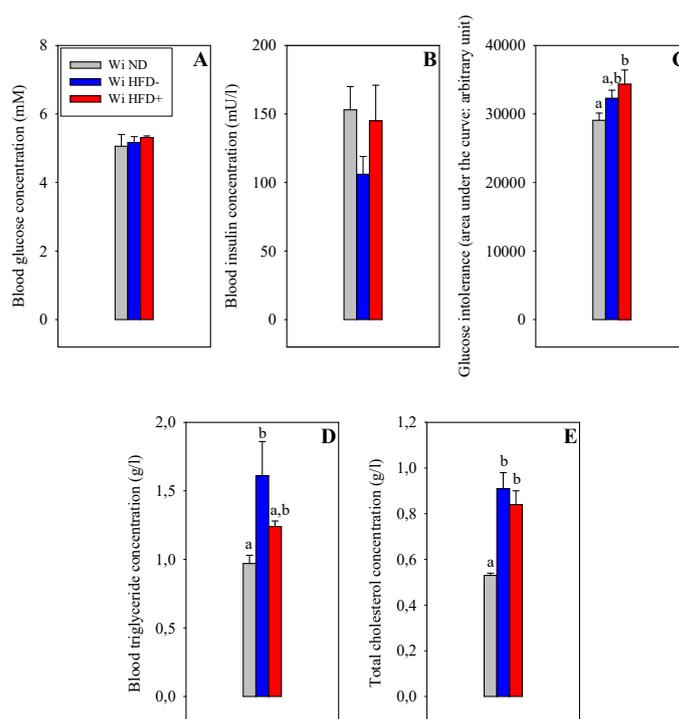
The HFD did not increase the body weight of the animals, but analysis of the data indicated that two subgroups of animals could be distinguished at the end of the feeding period: those which did not gain weight and those which gained weight. Indeed, HFD- fed animals displayed the same body weight as ND-fed rats whereas those of the HFD+ group exhibited a significant 18% increase in their body weight value (Fig. 1). Abdominal fat mass was similarly increased in both HFD-fed subgroups (+100 and +115% in the HFD- and HFD+ subgroups, respectively), but cardiac hypertrophy did not occur as indicated by the significant reduction of the heart weight to body weight ratio (-18 and -16% for the HFD- and HFD+ subgroups).



**Figure 1.** Effects of the high fat diet on the body weight (panel A), abdominal fat mass (panel B) and heart weight (panel C) in Wistar rats. The number of experiments was 10 for the Wi ND group and 5 for Wi HFD- and Wi HFD+ groups. Wi ND: group of Wistar rats fed the normolipidic diet; Wi HFD-: group of Wistar rats fed the high fat diet with a stable body weight; Wi HF+: group of Wistar rats fed the high fat diet with an increased body weight; a,b: means without a common letter are significantly different. Extracted from (47).

At the end of the feeding period, hyperglycaemia did not occur, but glucose intolerance, estimated after oral glucose load by hyperglycemia compared to the control animals, developed particularly in the HFD+ subgroup (Fig. 2) despite unchanged fasting insulin concentrations. Yet, glucose intolerance is considered to be all the more elevated that the lipid storage and lipotoxicity in the skeletal muscles is high. Furthermore, dyslipidaemia was observed mainly in the HFD- subgroup with increased triglycerides and total cholesterol concentrations whereas it partially vanished in the HFD+ subgroup. These results suggest that the lipid storage capacities of the tissues not dedicated to fat storage were low in the HFD- subgroup and high in the HFD+ subgroup which exhibited relatively low circulating lipid concentrations and high glucose intolerance.

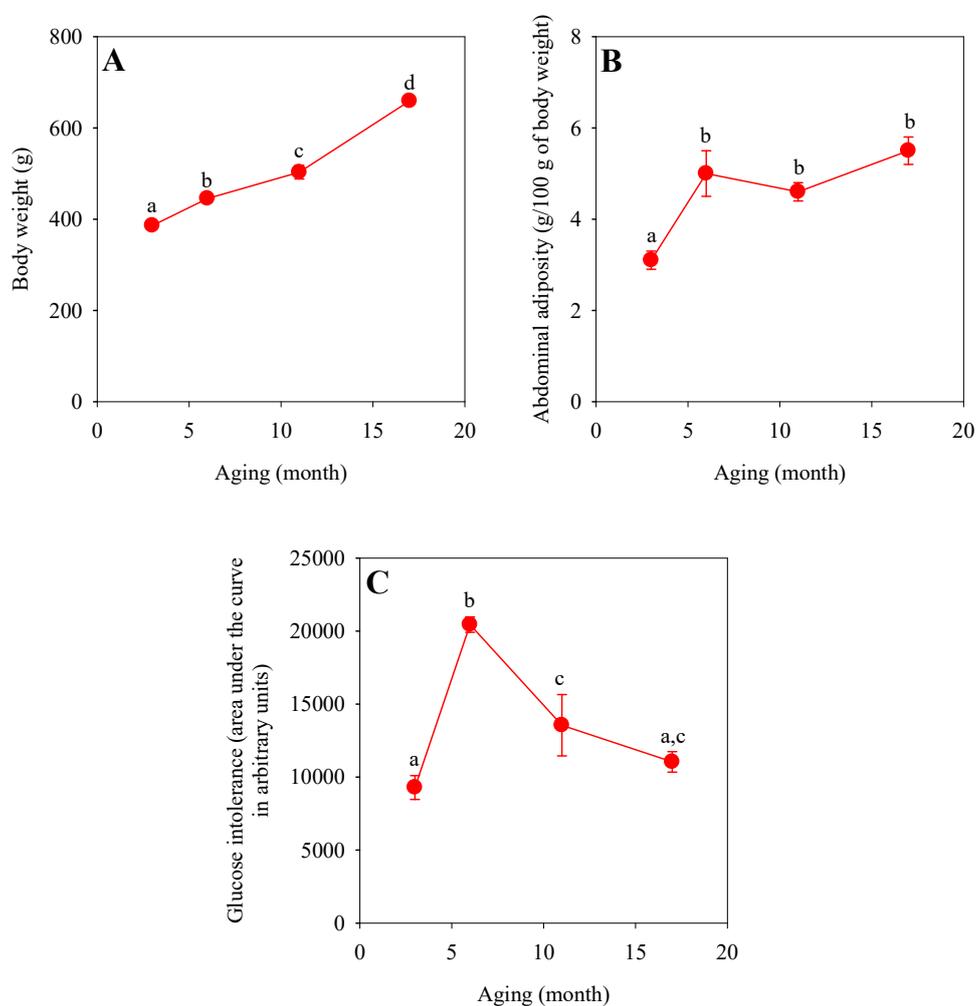
In a second set of experiments, anti-obese Lou/C rats were compared to their Wistar counterparts. In contrast to the Wistar rat, the Lou/C rat does not become fat with aging. Indeed, in our experiments, it weighed only  $188 \pm 11$  vs.  $323 \pm 5$  g for the Wistar phenotype at the age of 3 months. This was partly due to a decreased abdominal adiposity (-43%), but the normalized leg muscle and heart weights were higher (+13 and +22%) suggesting that the Lou/C rat was adapted to physical training. The measurement of the spontaneous physical activity with a spinning wheel revealed that the Lou/C rats ran  $7956 \pm 957$  m/day vs.  $1075 \pm 154$  for their Wistar counterparts. In sedentary animals, fasting glycaemia and insulinemia were not altered by the rat phenotype. When the glucose challenge was performed, the Wistar rats displayed a slight glucose intolerance compared to the Lou/C rat, but the difference was low.



**Figure 2.** Effects of the high fat diet on the glycaemia (panel A), insulinemia (panel B), glucose intolerance (panel C), triglyceridemia (panel D) and cholesterolemia (panel E). The number of experiments was 10 for the Wi ND group and 5 for the Wi HFD- and Wi HFD+ groups. Wi ND: group of Wistar rats fed the normolipidic diet; Wi HFD-: group of Wistar rats fed the high fat diet with a stable body weight; Wi HFD+: group of Wistar rats fed the high fat diet with an increased body weight; a,b: means without a common letter are significantly different. Extracted from (47).

At the end of the feeding period, hyperglycaemia did not occur, but glucose intolerance, estimated after oral glucose load by hyperglycemia compared to the control animals, developed particularly in the HFD+ subgroup (Fig. 2) despite

In a third set of experiments, male Wistar rats were followed during the totality of their life span. The animals were thus divided in four age groups: young (Y), young adult (YA), middle-aged (MA) and old age (O) rats which were killed at the age of 3, 6, 11 and 17 months, respectively. They were fed the commercially available equilibrated diet which contains 3.1% of lipids during all their life span.



**Figure 3.** Influence of aging on body weight (panel A), abdominal adiposity (panel B) and glucose intolerance (panel C) of Wistar rats fed the normolipidic diet. The number of experiments was 10 per group. a,b,c,d: the absence of a common letter between two means indicates a significant difference. Extracted from (48).

Aging modified the body weight of the animals, but also the abdominal adiposity (Fig. 3). A progressive increase of body weight was observed with increasing aging, but abdominal adiposity augmented between 3 and 6 months of age when it was normalized to the body weight. This indicates that energy intake surpassed the needs of the animals during this period. Thereafter, the abdominal adiposity remained stable until the old age. Glucose intolerance was related to abdominal adiposity between 3 and 6 months: it augmented between 3 and 6 months like the abdominal adiposity, suggesting that it occurred when the energy intake exceeded the needs of the animals. However, an adaptation occurred thereafter when the abdominal adiposity was high but stable: glucose intolerance progressively returned to the basal value measured at 3 months of age, implying that the energy intake progressively decreased in order to fit the animal needs. Hypertriglycemia and hypercholesterolemia were observed only at the old age (see reference 48 for more details).

Finally, in a fourth set of experiments, male ZDF rats were compared to their lean counterparts at the age of 11 weeks. Both types of animals were fed with a commercially available diet which was low in lipids (3.1% of the diet mass). However, due to their deficient leptin receptor, ZDF rats were bulimic: they ate almost two fold more diet compared to their lean counterpart. In this case, the body weight of the ZDF animals exceeded that of their lean counterparts at the moment of the sacrifice ( $364 \pm 1$  vs.  $300 \pm 1$  g,  $p < 0.001$ ). Their abdominal fat mass was markedly increased (+265% compared to the lean animals,  $p < 0.001$ ), however no cardiac hypertrophy was evident, according to the heart weight to body weight ratio. These changes were associated with higher blood glucose concentration (+540%,  $p < 0.001$ ), HbA1c level (+130%,  $p < 0.001$ ), as well as plasma insulin (+251%,  $p < 0.001$ ), triglyceride (+97%,  $p < 0.001$ ) and total cholesterol (+81%,  $p < 0.001$ ) concentrations. The ZDF rats displayed type 2 diabetes with hyperglycaemia and insulin resistance, but as evidenced by the high

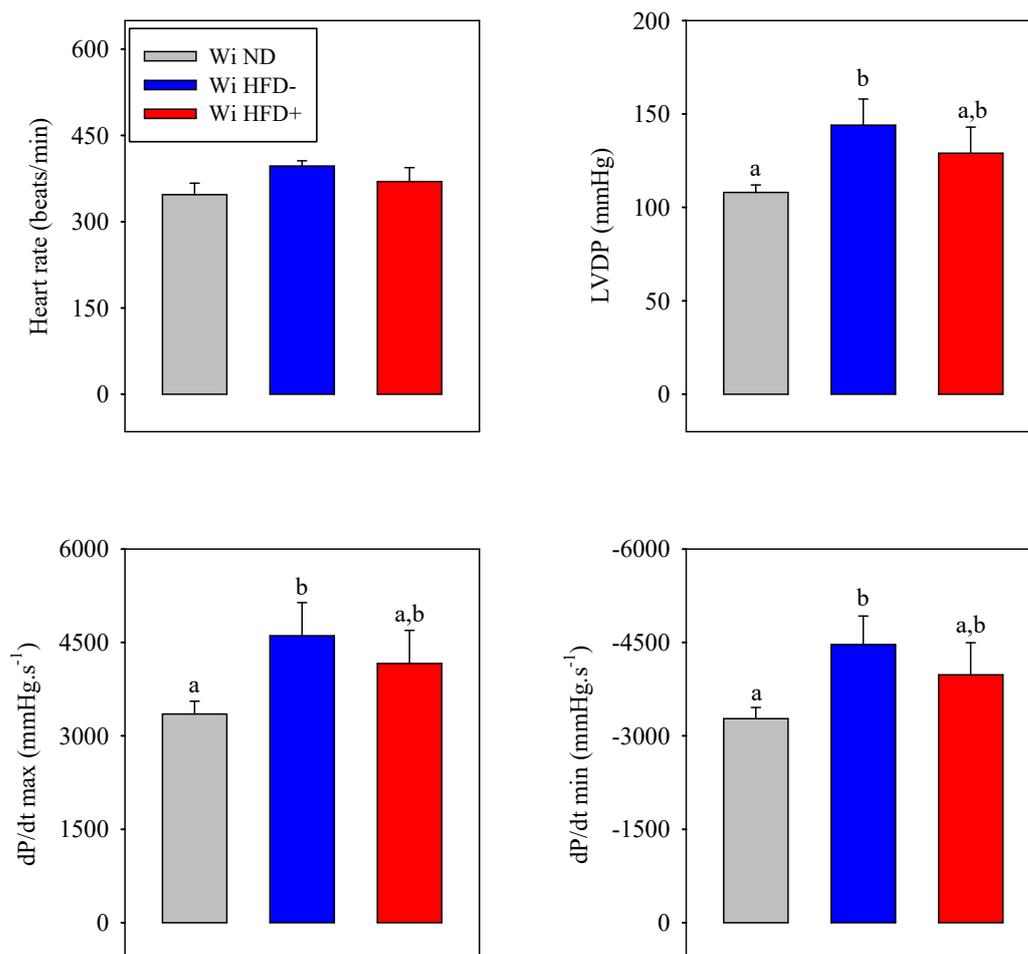
insulin concentration encountered in these animals, the pancreatic  $\beta$  cells were still functional, suggesting that the diabetes progression was in its early development.

## ***2.2 - Obesity effects on *in vivo* cardiac function***

Cardiac mechanical function was determined *in vivo* using a Millar pressure probe introduced in the left ventricle via the right carotid. This probe allowed the determination of the heart rate (HR), left ventricular developed pressure (LVDP), and the positive and negative derivatives of the LVDP with respect to time which reflect contractility and relaxation, respectively.

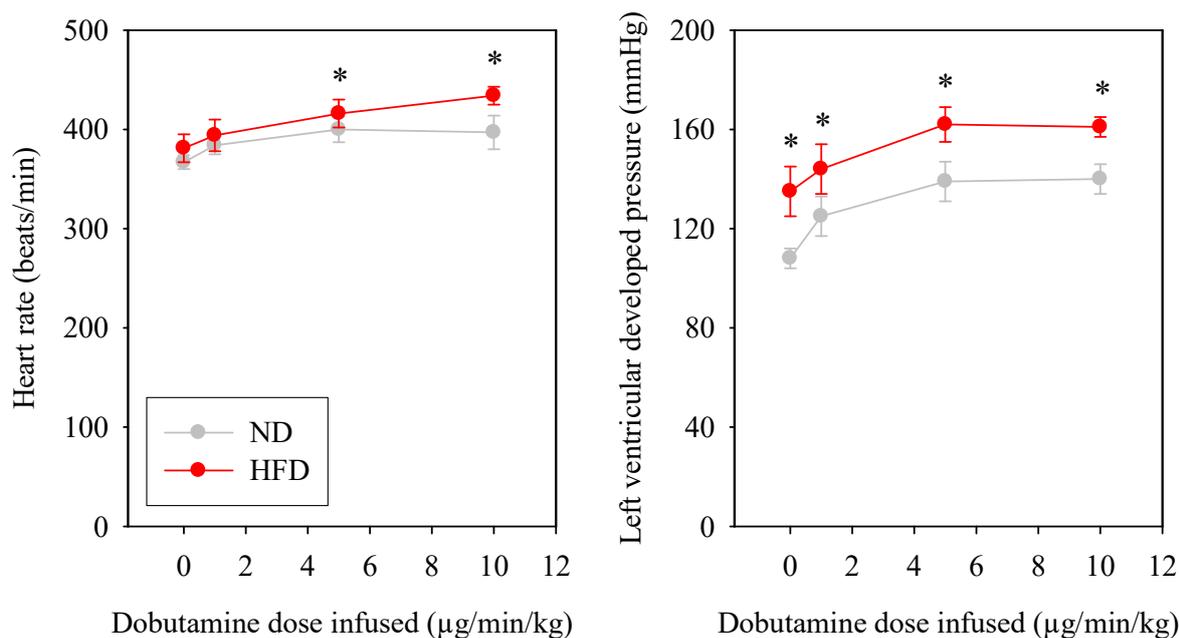
Cardiac function was evaluated in the first set of experiments when Wistar rats were fed with the high fat diet for 3 months. The results, presented in Fig. 4, show that heart rate was not modified by the HFD irrespective of the weight gain of the animals. Conversely, the LVDP was higher in the HFD- group compared to the control one (+33%) and intermediary in the HFD+ group.

A similar pattern was observed for the contractility and relaxation: the HFD- group displayed better contractility and relaxation (+38% and +36%, respectively) than the control group. The HFD+ group exhibited values ranging between these two extremes.



**Figure 4.** Effects of the high fat diet on the *in vivo* cardiac function of Wistar rats. Panel A: heart rate; panel B: left ventricular developed pressure; panel C:  $dP/dt$  max; panel D:  $dP/dt$  min. The number of experiments was 10 for the Wi ND group and 5 for the Wi HFD- and Wi HFD+ groups. Wi ND: group of Wistar rats fed the normolipidic diet; Wi HFD-: group of Wistar rats fed the high fat diet with a stable body weight; Wi HFD+: group of Wistar rats fed the high fat diet with an increased body weight; a,b: means without a common letter are significantly different. Extracted from (47).

A dobutamine test was also performed *in vivo* to stimulate  $\beta$ -adrenergic receptors and a positive inotropic effect. In our study, it was infused via the jugular vein at different doses ranging from 0 to 10  $\mu\text{g}/\text{min}/\text{kg}$ . The cardiac function was monitored by means of the Millar probe. The results are presented in Fig. 5.



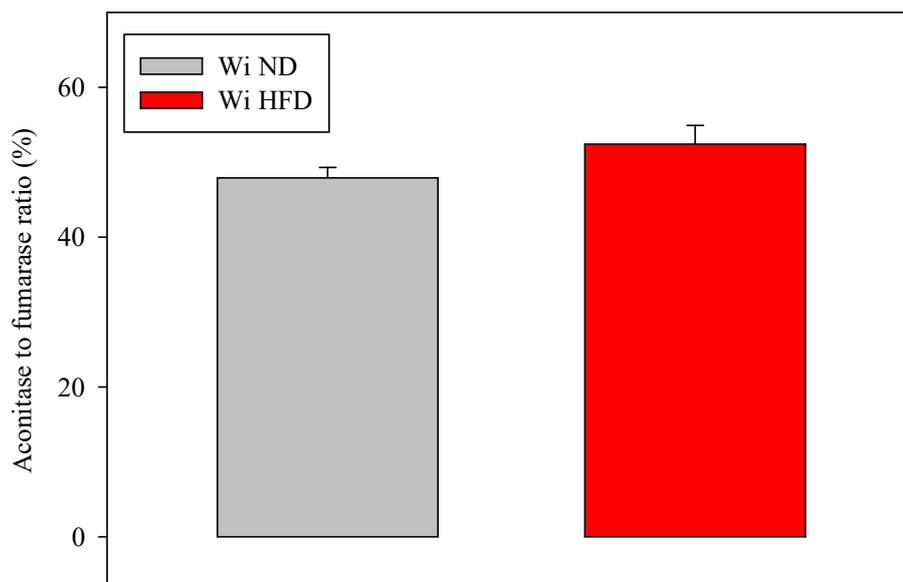
**Figure 5.** Effect of the high fat diet on the *in vivo* cardiac function of Wistar rats during a dobutamine test. The number of experiments was 10 per group. *Wi ND*: group of Wistar rats fed the normolipidic diet; *Wi HFD*: group of Wistar rats fed the high fat diet. \*: significantly different. Unpublished data.

The heart rate and LVDP were augmented by the increasing doses of dobutamine. High fat diet hearts had augmented heart rate when dobutamine was infused at 5 and 10 µg/min/kg. Furthermore, in these high fat diet hearts LVDP was increased regardless of dobutamine dose.

Taken together, these data confirm an inotrope positive effect of abdominal adiposity. This improved cardiac function represents probably a compensatory mechanism in order to maintain the mobility of obese individuals, despite the increased body weight which necessitates higher energy needs to perform a similar degree of activity compared to lean people.

Positive inotropism involves the stimulation of the energy metabolism. Yet, the oxidative stress known to occur with obesity (52, 53) may block the Krebs cycle (Kc) through inhibition of aconitase. This Kc enzyme has been shown to be

inhibited by reactive oxygen species (54) whereas the Kc enzyme fumarase is not. We measured the ratio between the activities of aconitase and fumarase of cardiac homogenates in order to determine the mitochondrial oxidative stress. The results are presented in Fig. 6.

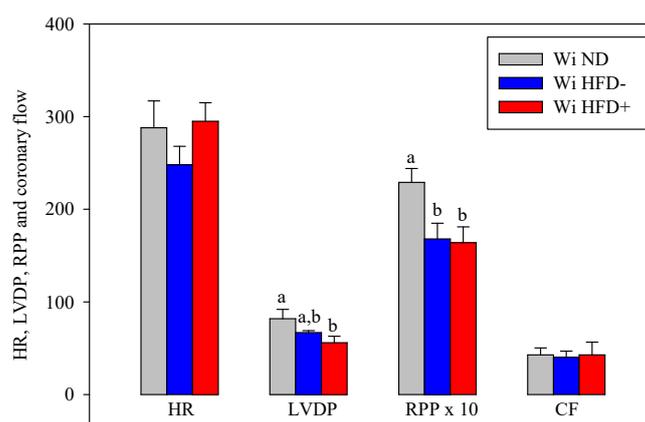


**Figure 6.** Effects of the high fat diet on the aconitase to fumarase ratio of heart collected in the *in vivo* situation. The enzymatic activities were measured at their  $V_{max}$ . The number of experiments was 10 per group. Wi ND: group of Wistar rats fed the normolipidic diet; Wi HFD: group of Wistar rats fed the high fat diet. Extracted from (49).

The ratio tended to be higher in the hearts of the high fat-fed rats, but the difference was not statistically significant. These results suggest that the mitochondrial redox potential was slightly higher when the animals displayed an abdominal obesity. This could result from a better organ perfusion: indeed, the greater the oxygen supply, the greater the production of reduced equivalents available for potential reactive oxygen species scavenging.

### 2.3 - *Ex vivo* cardiac function in Langendorff mode perfusion model

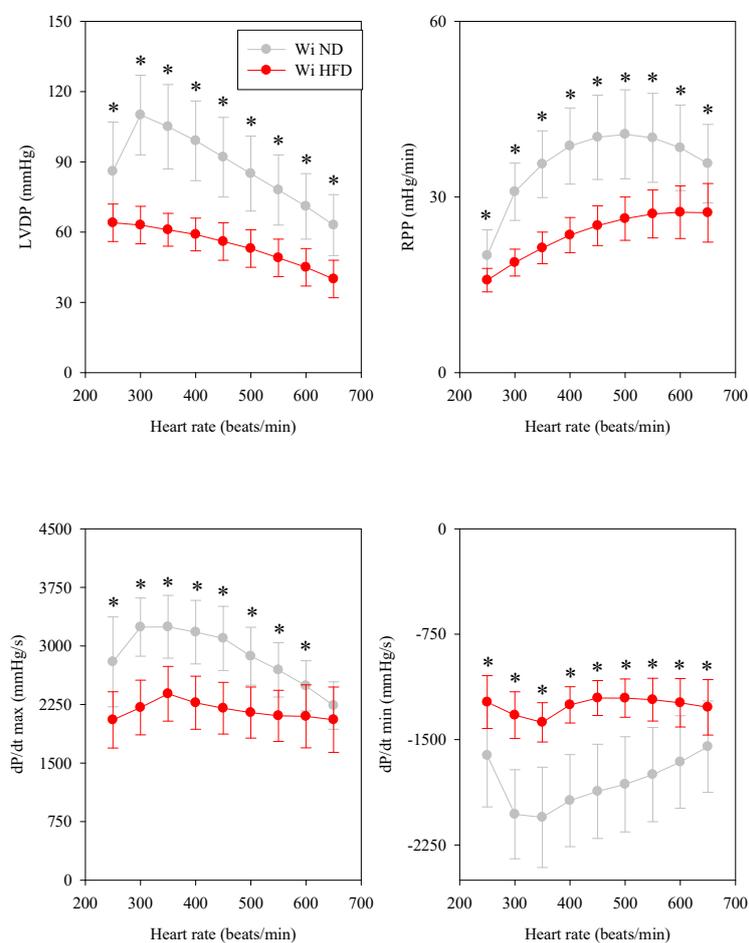
The *ex vivo* cardiac function was assessed using Langendorff-mode isolated heart perfusion. The rat hearts were perfused via the aorta at a constant pressure which was fixed by a column of perfusion fluid via a Mariotte's flask. In this model, the coronary flow depends on the vasodilatation of the coronary micro-vessels: the highest their vasodilatation status, the highest the coronary flow. After rapid collection in the animal, the heart was immediately perfused via the aorta with a perfusion fluid composed of (in mM) NaCl 119, MgSO<sub>4</sub> 1.2, KCl 4.8, NaHCO<sub>3</sub> 25, KH<sub>2</sub>PO<sub>4</sub> 1.2, CaCl<sub>2</sub> 1.2 and glucose 11 mM as sole energy substrate. The perfusion fluid continuously bubbled with carbogen (95% O<sub>2</sub>/5% CO<sub>2</sub>) was maintained at 37°C at the level of the aorta. A latex balloon related to a pressure probe was introduced into the left ventricle in order to record the cardiac cycle and to calculate the left ventricle developed pressure (LVDP), heart rate, rate pressure product (RPP: product between the LVDP and the heart rate), dP/dt max and dP/dt min. The effect of abdominal obesity was then determined in different animal models.



**Figure 7.** Effects of the high fat diet on the *ex vivo* activity of the isolated heart perfused at constant pressure. The number of experiments was 10 for the Wi ND group and 5 for Wi HFD- and Wi HFD+ groups. HR: heart rate; LVDP: left ventricular developed pressure; RPP: rate pressure product; CF: coronary flow; Wi ND: group of Wistar rats fed normolipidic diet; Wi HFD-: group of Wistar rats fed high fat diet with a stable body weight; Wi HFD+: group of Wistar rats fed high fat diet with an increased body weight; a,b: means without a common letter are significantly different. Extracted from (47).

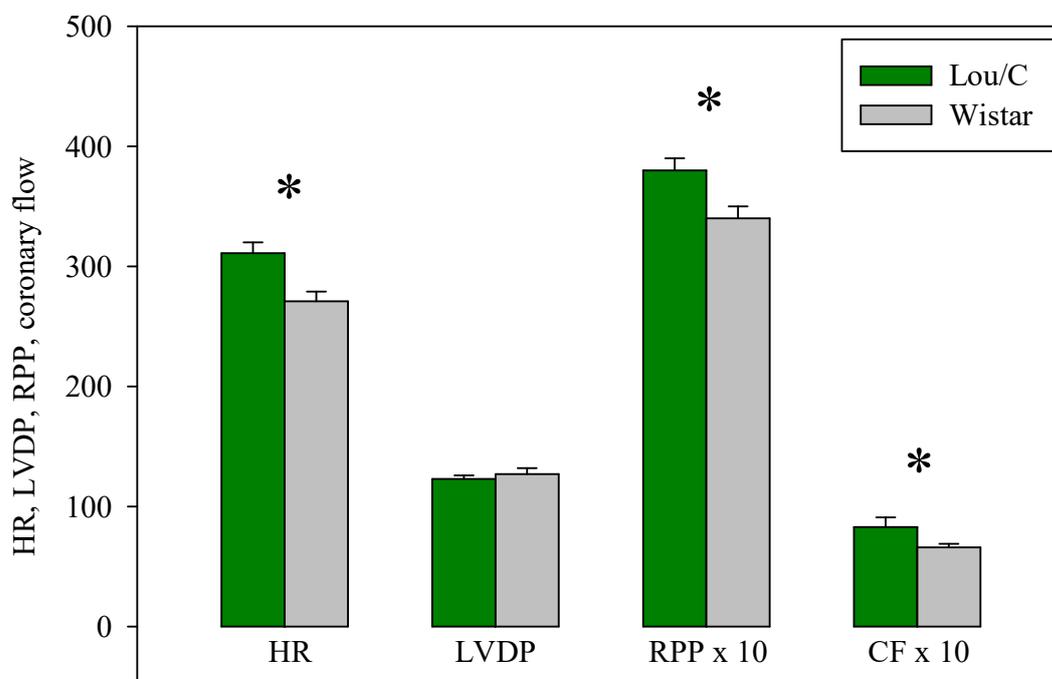
### 2.3.1 - High fat-fed Wistar rats

High fat-fed rats were subdivided in 2 groups, those who did not gained body weight (HFD-) and those who did (HFD+). The coronary flow and heart rate were unaffected by the HFD (Fig. 7), but the rate pressure product (reflecting the cardiac mechanical function) was decreased in the HFD- and HFD+ groups compared to the control group (-27 and -28%). The reduced RPP was due to a decrease in LVDP in the HFD+ group (-32%), but rather to simultaneous tendencies toward reductions of the heart rate and LVDP in the HFD- group.



**Figure 8.** Submaximal cardiovascular effort trial in the ex vivo situation with the influence of the high fat diet. The number of experiments was 10 per group. LVDP: left ventricular developed pressure; RPP: rate pressure product; dP/dt max and min: maximal and minimal time-derivatives of the left ventricular developed pressure; Wi ND: Wistar rats fed the normolipidic diet; Wi HFD: Wistar rats fed the high fat diet. \*: significantly different. Unpublished data.

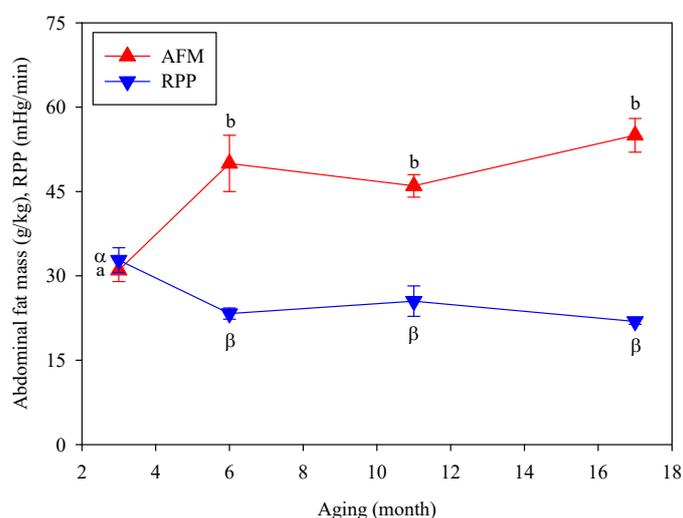
In a second set of experiments, the cardiac function of Wi HFD-fed rats versus Wi ND-fed animals was evaluated by pacing the hearts in a wide range of heart rates. No distinction between the animals gaining or not gaining body weight was performed in this set of experiments. The results are presented in Fig. 8. For each measured parameter (LVDP, RPP, dP/dt max and dP/dt min) and fixed electrical pacing rate, the hearts of Wi HFD-fed animals displayed lower values compared to those of the control group. This finding confirms that abdominal obesity is associated with lower *ex vivo* cardiac mechanical function in this model of perfusion at constant pressure.



**Figure 9.** Cardiac function in the anti-obese Lou/C rat. In this experiment, both types of animals were fed the normolipidic diet. The number of experiments was 10 per group. HR: heart rate in beats/min; LVDP: left ventricular developed pressure in mmHg; RPP: rate pressure product in dmHg/min; CF: coronary flow in ml/min/g of dry weight. \*: significantly different. Extracted from (75).

### 2.3.2 - Lou/C rats compared to Wistar rats

Anti-obese Lou/C rats were compared to Wistar rats to evaluate the effect of obesity since Wistar rats become rapidly obese during their life span. In this experiment, the calcium concentration of the perfusion fluid was raised to 2.5 mM in order to stimulate the cardiac mechanical function, as measured by the high rate pressure product shown in Fig. 9. Compared to the anti-obese Lou/C rat, the Wistar rat had a lower rate pressure product (12%). This was due to a lower heart rate (-13%), but the LVDP was unchanged. Interestingly, the coronary flow was significantly reduced by abdominal obesity (-20%). Myocardial oxygen consumption was not significantly modified by the animal phenotype ( $4.9 \pm 0.4$  vs.  $4.1 \pm 0.2$   $\mu\text{moles of O}_2/\text{min/g}$  for the Lou/C and Wistar rats). The metabolic efficiency (RPP to  $\text{O}_2$  consumption ratio) was lower in the Wistar rats compared to its Lou/C counterpart ( $5.4 \pm 0.2$  vs.  $7.3 \pm 0.5$   $\text{mHg}/\mu\text{mole of O}_2$ ).



**Figure 10:** Effect of aging on the ex vivo rate pressure product (RPP) of Wistar rats during a perfusion at constant pressure with the Mariotte's flask. The number of experiments was 10 per group. AFM: abdominal fat mass; RPP: rate pressure product. a,b: significantly different for the abdominal fat mass;  $\alpha,\beta$ : significantly different for the RPP. Extracted from (48).

Notably these results confirm the deleterious effects of abdominal adiposity on the *ex vivo* cardiac function. The high calcium-induced stimulation of the cardiac function evoked the appearance of obesity-related changes in coronary flow and metabolic efficiency.

### ***2.3.3 - Changes in cardiac function with aging***

Fig. 10 shows that the rate pressure product decreased between the 3<sup>rd</sup> and 6<sup>th</sup> month of life when the abdominal fat mass increased. Thereafter, the cardiac mechanical function remained low and stable when the abdominal fat mass was high and stable, from 6 to 17 months of age.

This clearly indicates that the *ex vivo* cardiac mechanical function is inversely related to the abdominal fat mass.

### ***2.3.4 - The Zucker Diabetic Fatty (ZDF) rat***

The homozygous fa/fa Zucker rat exhibits hyperphagia caused by a non-functioning leptin receptor which promotes onset of obesity and diabetes (55). In our study, 11 week-old animals displayed hyperglycaemia (~5 g/l), hyperinsulinemia (~4.5 ng/ml), hypertriglyceridemia (~1.8 g/l) and hypercholesterolemia (~1.4 g/l) compared to their lean counterpart (~1 g/l, 1.2 ng/ml, 0.9 g/l and 0.75 g/l for glycaemia, insulinemia, triglyceridemia and cholesterolemia). Their abdominal fat mass was increased by 365%.

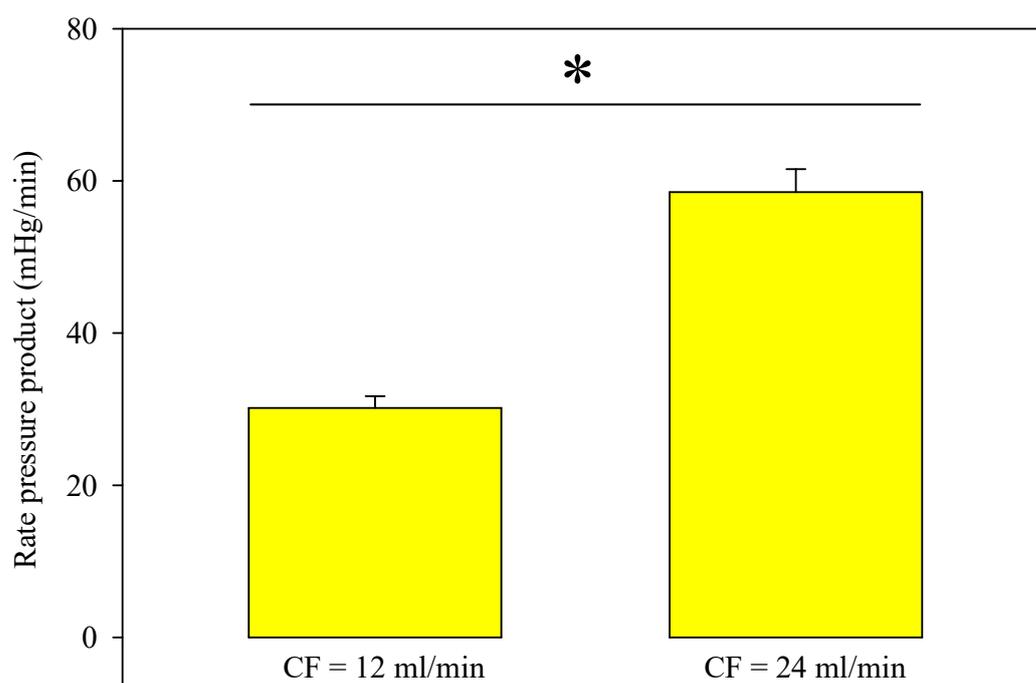
The rate pressure product of these obese animals was decreased by 35% compared to their control and this was due to a reduction of the heart rate (-42%), since the LVDP was augmented (+18%). The reduction of cardiac mechanical function observed in the ZDF rats was associated with a decrease in the coronary flow (-25%).

#### ***2.4 - Discrepancies between in vivo and ex vivo measurements of cardiac mechanical function - Limitation of in vitro constant pressure perfusion***

Our data show that abdominal obesity increased *in vivo* cardiac mechanical function. Meanwhile, the utilization of the isolated heart perfused at constant pressure indicates the inverse relationship. Why such a discrepancy? Of course, the valid method to evaluate the cardiac mechanical function is *in vivo*. So what are the limitations of *ex vivo* perfusion?

In the whole animal, the cardiac function depends on the contractile activity of the cardiomyocytes, but also on several parameters fixed by other organs. For instance, the peripheral circulation regulates the blood pressure at the level of the left atria and thus the volume of blood fulfilling the cardiac cavities during diastole. This volume is of crucial importance in determining the strength of the cardiac contraction. Indeed, according to the Starling's law, this parameter is all the more elevated that the myofibril stretching is important: a high systolic pressure is thus synonymous of a strong contraction. Moreover, the *in vivo* cardiac function depends on the neurohumoral status of the organism. This might have a high importance during obesity when the inflated adipose tissue produces more leptin and less adiponectin (56). Another difference concerns the substrate availability. The isolated heart received only glucose as a substrate whereas all the possible substrates (glucose, lactate, fatty acids, certain amino acids, ketone bodies, etc.) are available to the *in vivo* heart. These differences between the *in vivo* and *ex vivo* situations could partly explain the discrepancies observed in our studies, but some defaults of our *ex vivo* perfusion model can also be involved. When isolated hearts are perfused at constant pressure, the coronary flow is high at the beginning of the perfusion procedure (higher than 22 ml/min). However, when the duration of the perfusion increases, the coronary flow gradually decreases to progressively reach values around 10-12 ml/min: this reflects

incremental vasoconstriction of the resistance arteries of the coronary circulation. Yet, the oxygen and substrate supplies to the cardiomyocytes which are mainly determined by the rate of coronary flow have a strong effect on the contractile function. Similar to a car engine: the highest the air (oxygen) and fuel (substrate) supplies, the highest the pulling force (contraction) of the engine. Figure 11 illustrates this relationship. Isolated hearts were perfused at 2 different coronary flows thanks to a peristaltic pump: 12 and 24 ml/min. The cardiac mechanical activity was then determined using a latex balloon introduced in the left ventricle and related to a pressure probe. Doubling the coronary flow almost multiplied the rate pressure product by a factor 2. Irrigation of the coronary bed thus plays a fundamental role on cardiac contractile activity.



**Figure 11.** Influence of the coronary flow rate on the cardiac mechanical activity of Wistar rats. The number of experiments was 10 per group. CF: coronary flow. \*: significantly different. Unpublished data.

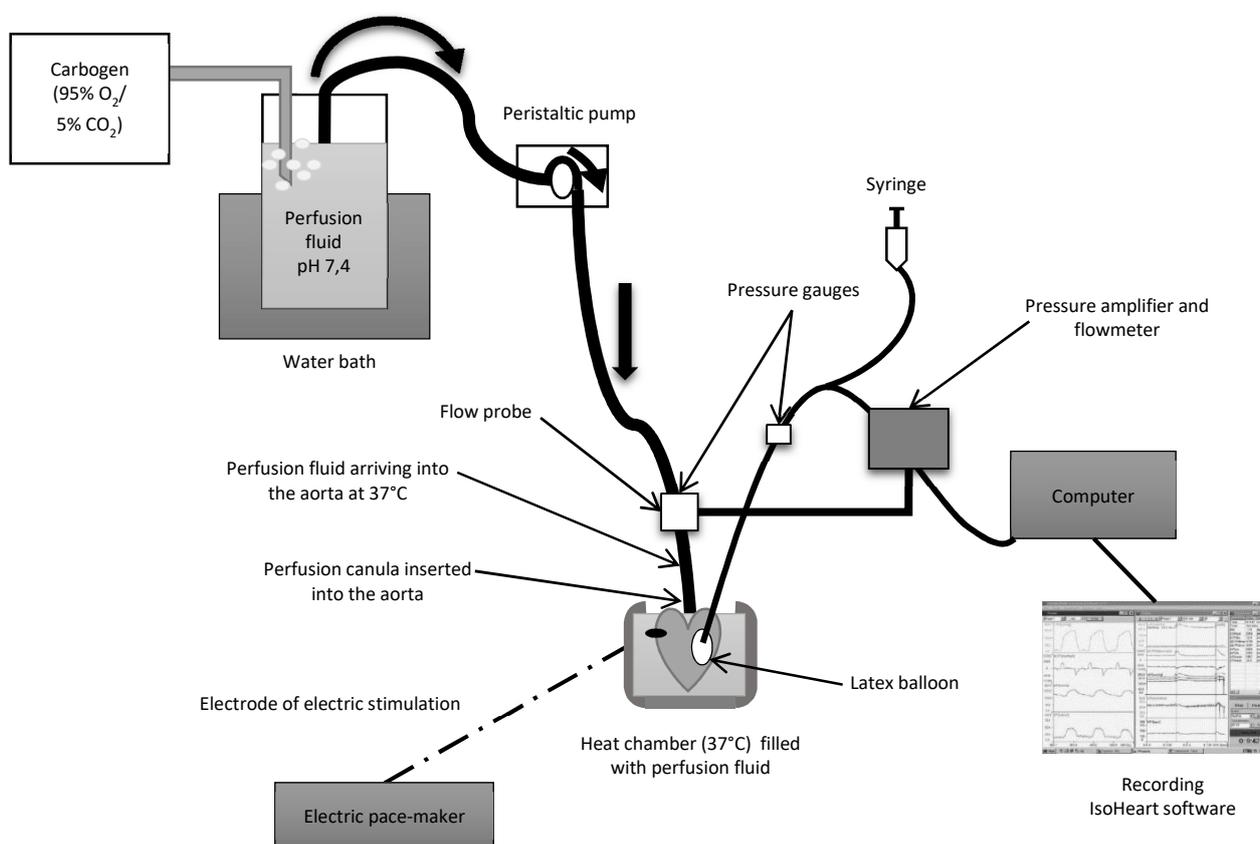
A metabolic index describing the extent of coronary bed irrigation and the cardiomyocyte oxygenation is the mitochondrial aconitase to fumarase ratio. Aconitase and fumarase are two Krebs cycle enzymes with differing redox potential regulation. Aconitase is strongly inhibited by the oxidative stress whereas the fumarase is not (54). The ratio between the activities of aconitase and fumarase informs on the mitochondrial redox potential and thus the degree of mitochondrial oxygenation, thus the greater the aconitase to fumarase ratio, the index suggests a greater extent of mitochondrial oxygenation. We thus determined the aconitase to fumarase ratios of the homogenates of hearts collected directly from the *in vivo* or *ex vivo* perfused hearts. The results are presented in Table 1. In the *ex vivo* situation, the hearts from lean animals (ZL and Wi ND groups) displayed a high aconitase to fumarase ratio. Conversely, ZDF and high fat diet-fed Wistar rat hearts had a low ratio compared to their lean counterparts. This contrasted with the *in vivo* situation where the lean and obese animals displayed ratios of similar magnitude. Thus, the *ex vivo* perfusion is inadequate to the study of cardiac function since an abnormal hypo-perfusion seems to occur principally for the hearts of rats with abdominal obesity. This hypo-perfusion does not occur *in vivo*.

**Table 1. Effects of abdominal obesity on the aconitase to fumarase ratio measured in the *in vivo* and *ex vivo* situations**

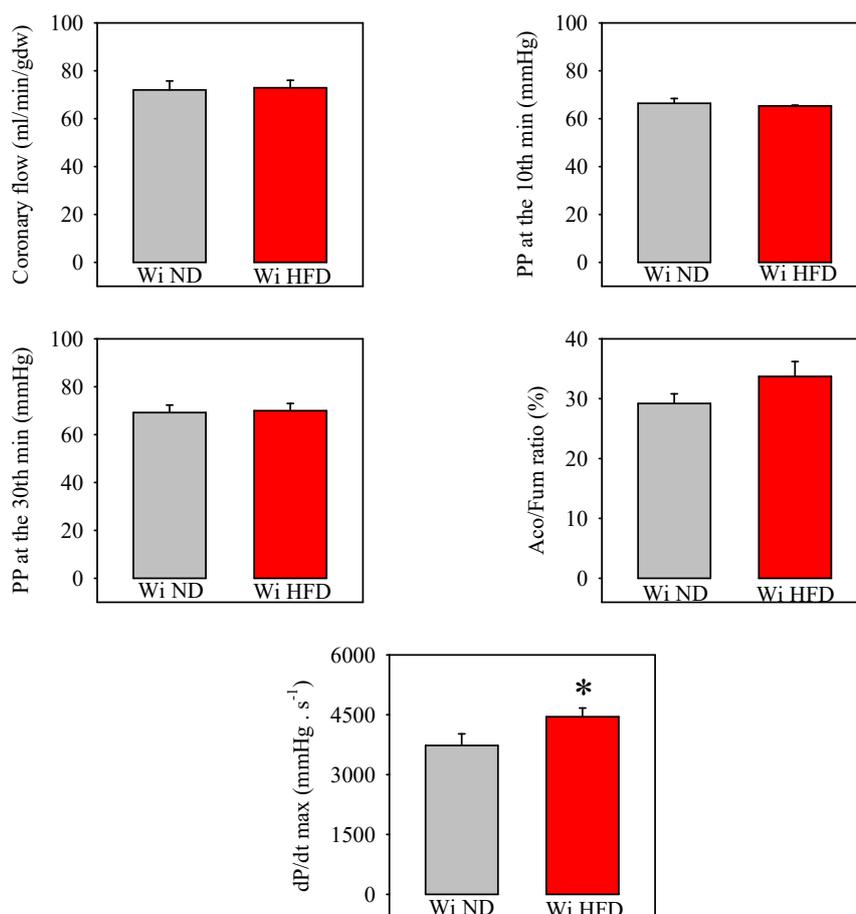
Group	Aconitase to fumarase ratio
ZL <i>ex vivo</i>	21.5 ± 2.9
ZDF <i>ex vivo</i>	12.8 ± 2.9 *
Wi ND <i>ex vivo</i>	26.2 ± 1.9
Wi HFD <i>ex vivo</i>	15.7 ± 1.3 *
Wi ND <i>in vivo</i>	19.0 ± 1.6
Wi HFD <i>in vivo</i>	22.4 ± 1.7

*In the ex vivo situation, the hearts were perfused at a constant pressure fixed by a Mariotte's flask. The aconitase and fumarase activities were measured at their Vmax. The number of experiments was 10 per group. ZDF: Zucker Diabetic Fatty rats; ZL: lean counterparts of ZDF rats; Wi ND: Wistar rats fed the normolipidic diet containing 3% of lipids; Wi HFD: Wistar rats fed a diet containing 54% of lipids; \*: significantly different. Extracted from (49) and (51).*

We decided thus to improve our perfusion method by perfusing the hearts at a constant coronary flow fixed by a peristaltic pump. The perfusion pressure was set at 65 mmHg during the first 10 min of perfusion in order to avoid oxygenation differences due to the size of the heart. The coronary flow measured at the 10<sup>th</sup> min of perfusion was then maintained constant during all the perfusion procedure by means of a peristaltic pump. The hearts were paced electrically at a rate of 370 beats per min. A representation of the perfusion model is presented in scheme 1.



**Scheme 1.** Perfusion at constant coronary flow by means of a peristaltic pump.



**Figure 12.** Effects of the high fat diet on the characteristics of ex vivo cardiac perfusion. The enzymatic aconitase and fumarase activities were measured at their  $V_{max}$ . The number of experiments was 10 per group. PP: perfusion pressure; Aco/Fum ratio: ratio between the activities of aconitase and fumarase;  $dP/dt$  max: maximal time-derivative of the left ventricular developed pressure; Wi ND: group of Wistar rats fed the normolipidic diet; Wi HFD: group of Wistar rats fed the high fat diet. \*: significantly different. Extracted from (47).

The measurements of the mechanical function were performed at the 30<sup>th</sup> min of perfusion. In these standardized conditions, the positive derivative of the left ventricular pressure with respect to time ( $dP/dt$  max) is an excellent index of the cardiomyocyte contractility. We evaluated the influence of the high fat diet-related abdominal adiposity on the coronary flow, perfusion pressure at the 10<sup>th</sup> and 30<sup>th</sup> min of perfusion, aconitase to fumarase ratio and cardiac mechanical function (Fig. 12). The perfusion pressure at the 10<sup>th</sup> min of perfusion was very similar in the HFD and ND groups: no difference in the coronary flows resulted

from the fixed perfusion pressures. Interestingly, the perfusion pressures at the 30<sup>th</sup> min of perfusion were only slightly increased compared to those fixed at the 10<sup>th</sup> min of perfusion, suggesting that no major vasoconstriction of the coronary resistance arteries occurred, even in the HFD group.

In addition, the aconitase to fumarase ratios of both groups were high and unaffected by abdominal adiposity. Interestingly, the dP/dt max was increased in the HFD group (+19%) compared to the control group. The results of the *in vivo* situation were thus recovered in this model of perfusion at fixed coronary flow: the 3-month HFD increases the contractility of the cardiomyocytes provided that the oxygenation of the cardiac tissue is maintained.

Thus, differences between the *in vitro* perfusion models impacted modifications of the physiological status of the cardiac cells, with the constant pressure perfusion model (with progressive decrease in coronary flow) being inferior to the latter constant coronary flow, constant pressure and paced heart rate model. An important second messenger involved in coronary vasodilatation is nitric oxide (NO) resulting from the transformation of L-arginine to citrulline by the endothelial nitric oxide synthase (eNOS) according to the following reaction:



After L-arginine utilization by the eNOS, restoration of its cellular pool depends on 2 pathways: the first one is based on the uptake of circulating L-arginine through the system y<sup>+</sup> transporter (57). Plasma L-arginine concentration in the rat surrounds 80 μM (58); the second one is a rescue pathway which regenerates L-arginine from citrulline through an energy-dependent process (59). Moreover, the eNOS can be uncoupled by the oxidative stress (60) and this results to lower NO production and vasoconstriction. In our *ex vivo* perfusion models, L-arginine is not added to the perfusion fluid and L-arginine availability depends only on the energy-dependent rescue pathway. When the perfusion at fixed coronary flow is

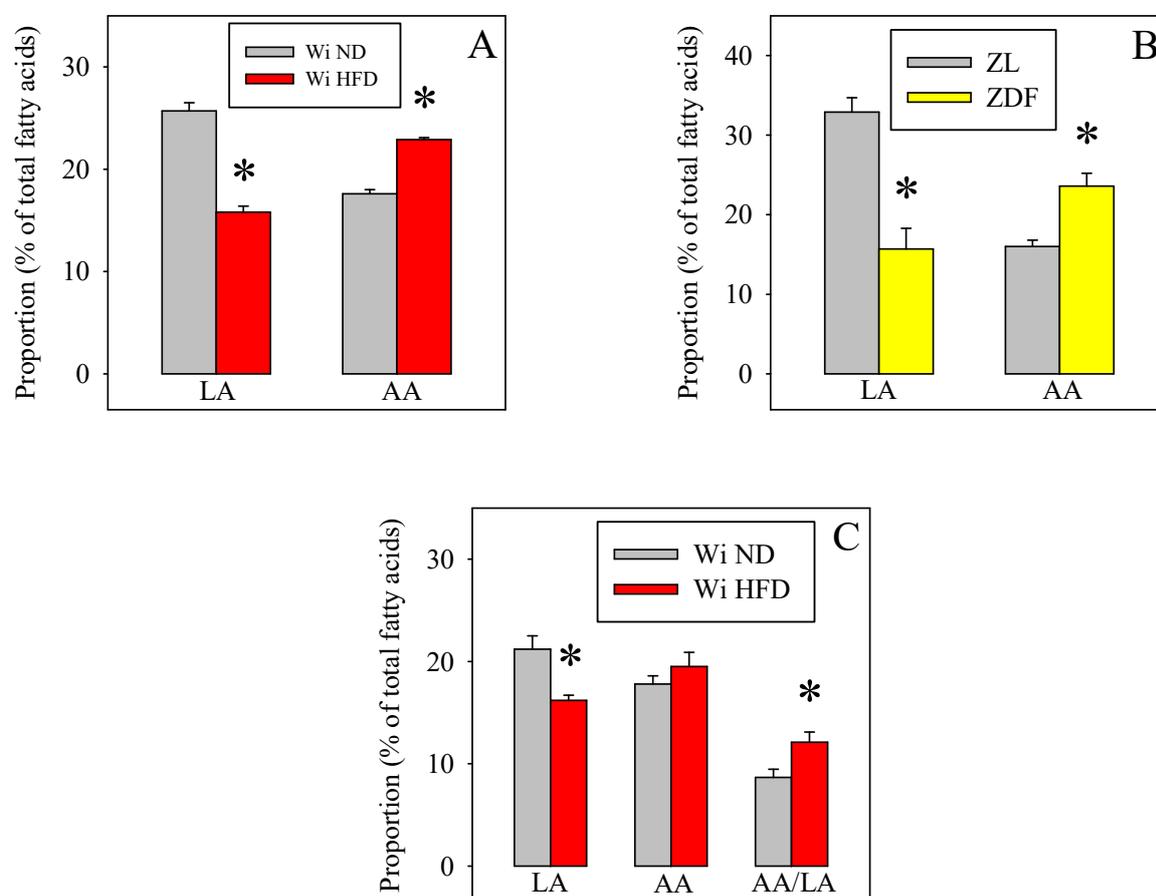
performed, the upholding of the coronary flow by means of the peristaltic pump allowed the approximate maintenance of the coronary pressure, which suggests that the energy metabolism was still high enough to insure the upholding of the L-arginine pool. However, with the constant pressure model, the progressive decrease in the coronary flow reduced the energy synthesis capacities and/or augmented the oxidative stress. This may have resulted in a vicious cycle during which the L-arginine pool did not replenish, resulting in further augmented decrease in the coronary flow. The impact of this was particularly strong in hearts from rats with abdominal adiposity, suggesting a pathological status induces important disturbances of the coronary vessels in their energy metabolism and/or oxidative stress. The true reasons for these alterations (alterations of energy metabolism or biopterin depletion) cannot be directly discovered in the whole heart and complementary studies in cultured cells appear necessary.

### ***2.5 - Other factors influencing the tissue perfusion and vascular function***

Amongst the mediators susceptible to produce a coronary vasodilatation, we can mention prostaglandins (PG) which are produced from polyunsaturated fatty acids of membrane phospholipids, namely arachidonic acid (C20:4 n-6 or ArA) and eicosapentaenoic acid (C22:5 n-3 or EPA). A cascade of enzymes leads to the formation of these prostaglandins: the initial step of this cascade is catalysed by the cyclooxygenase enzyme which allows the synthesis of prostaglandin H (PGH<sub>2</sub> and PGH<sub>3</sub> from ArA and EPA). Thereafter, the PGH molecules are transformed in different prostaglandins by several PG synthases: they include PGI<sub>2</sub>, PGD<sub>2</sub>, PGE<sub>2</sub> and PGF<sub>2</sub>α for the 2 series prostaglandins produced from ArA and PGI<sub>3</sub>, PGD<sub>3</sub>, PGE<sub>3</sub> and PGF<sub>3</sub>α for the 3-series prostaglandins synthesized from EPA. Thromboxane (TX) A<sub>2</sub> and A<sub>3</sub> are formed by a thromboxane synthase. Each prostaglandin has a specific activity: for instance, PGI<sub>2</sub> also named prostacyclin triggers vasodilatation and inhibits platelet

aggregation; PGD2 and PGE2 have similar effects. Conversely, TX A2 and PGF2 $\alpha$  have opposite effects, provoking vasoconstriction and platelet aggregation. The series-3 prostaglandins have in general either no effect or inverse effects as compared to the series-2 prostaglandins.

One of the most important discovery that we have done in our laboratory about abdominal obesity is that 3-month of a high fat diet in the rat strongly modify the profile of n-6 polyunsaturated fatty acids of cardiac phospholipids (Fig. 13A).

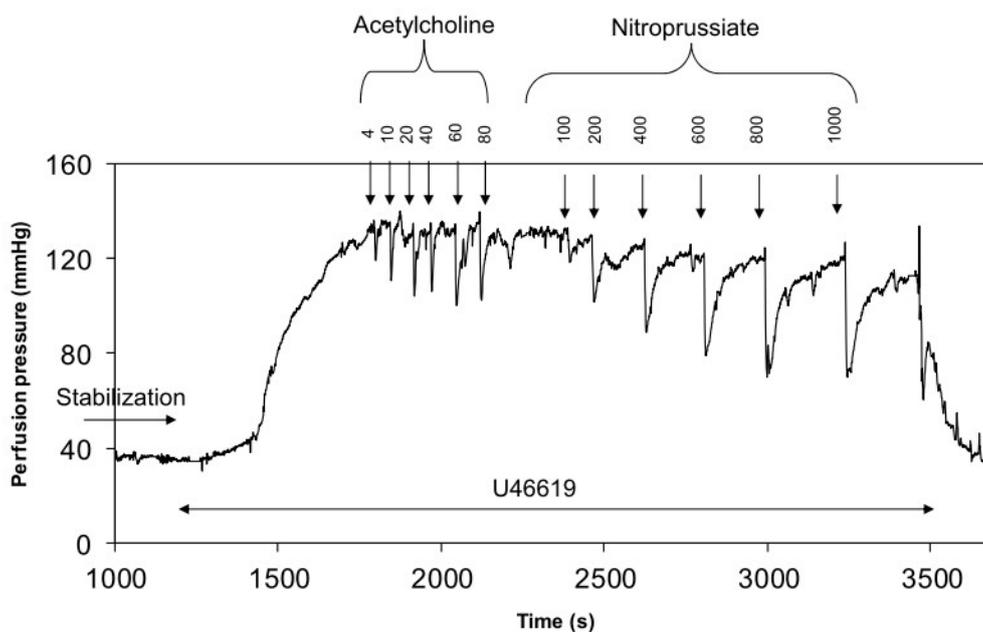


**Figure 13.** Fatty acid composition of cardiac phospholipids of high fat-fed Wistar rats (panel A), Zucker Diabetic Fatty rats (panel B) and of total plasma lipids of high fat-fed Wistar rats (panel C). The number of experiments was 5 per group. LA: linoleic acid (C18:2 n-6); AA: arachidonic acid (C20:4 n-6); Wi ND: group of Wistar rats fed the normolipidic diet; Wi HFD: group of Wistar rats fed the high fat diet; ZL: Zucker lean animals fed the normolipidic diet; ZDF: Zucker Diabetic Fatty rats fed the normolipidic diet. \*: significantly different. Extracted from (49) and (51).

Indeed, the proportion of linoleic acid (C18:2 n-6 or LA) is decreased (-39%) and that of ArA is increased (+30%). Advised reviewers could suggest that this difference was due to the change in the fatty acid composition of the diet: indeed, the high fat diet is composed of the low fat diet supplemented with lard. Yet, lard has a different fatty acid composition than the basal diet: it is richer in saturated and monounsaturated fatty acids and poorer in n-6 and n-3 polyunsaturated fatty acids. However, the ZDF rats ate the same basal diet than that consumed by their lean littermates. The unique difference was the amount of diet eaten by each phenotype: it was almost double for the ZDF animals compared to the ZL rats. The analysis of the fatty acid composition of cardiac phospholipids (Fig. 13B) indicates that the same excessive conversion of LA to ArA occurred in the ZDF rats compared to their lean counterpart. The differences were even ampler (-52 and +48% for LA and ArA, respectively) compared to the HFD-fed rats. Thus, the accumulation of ArA was not due to the fatty acid composition of the diet, but to the excessive energy intake. The reasons for these changes are not known. What we know is that the changes were already seen in the plasma after the 3-month high fat diet (Fig. 13C): LA was decreased by 24% and ArA was augmented by 10%. The increase in ArA was not significant, but circulating ArA was probably captured avidly by the organs, which tended to decrease its proportion in the plasma. In any case, the ArA to LA ratio of total plasma lipids was increased significantly by the HFD (+40%). Our data suggest thus that the conversion of LA to ArA did not occur in the heart, but in an upstream organ closer to the digestive tract. It could be the liver or even the microbiota.

ArA released from phospholipids by the phospholipase A2 in cultured cardiomyocytes acts as a cellular messenger. It increases both the amplitude of calcium transients and contraction of electrically stimulated cells (61). The target of ArA would be the PKC and ionic channels. The enrichment of cardiac membranes with ArA can thus explain the positive inotropic effect of the high fat

diet observed in our studies *in vivo* and *ex vivo* when the hearts were perfused at fixed coronary flow. However, positive inotropism must be associated with adequate adaptation of the coronary circulation. Otherwise, the system is not in equilibrium, energy needs surpass energy production and ischemia installs. We expected thus a vasodilatation effect induced by abdominal obesity.



**Figure 14.** Typical plot of the perfusion pressure obtained during the measurement of the coronary reactivity. The doses of acetylcholine and nitroprussiate injected are expressed in pmoles. U46619: analogue of thromboxane A2 with vasoconstriction properties.

In this purpose, we evaluated the coronary reactivity to acetylcholine (endothelial-dependent vasodilatation or EDV) and nitroprussiate (endothelial-independent vasodilatation or EIV). Acetylcholine (Ach) acts directly on endothelial cells: when it interacts with its membrane receptor, it favours the entry of calcium in these cells. Increased calcium stimulates the eNOS enzyme which produces more NO. NO triggers relaxation of smooth muscle cells via a cyclic GMP-dependent mechanism and dilates the coronary vessels. Ach action reflects thus the simultaneous activities of endothelial and smooth muscle cells. When it is

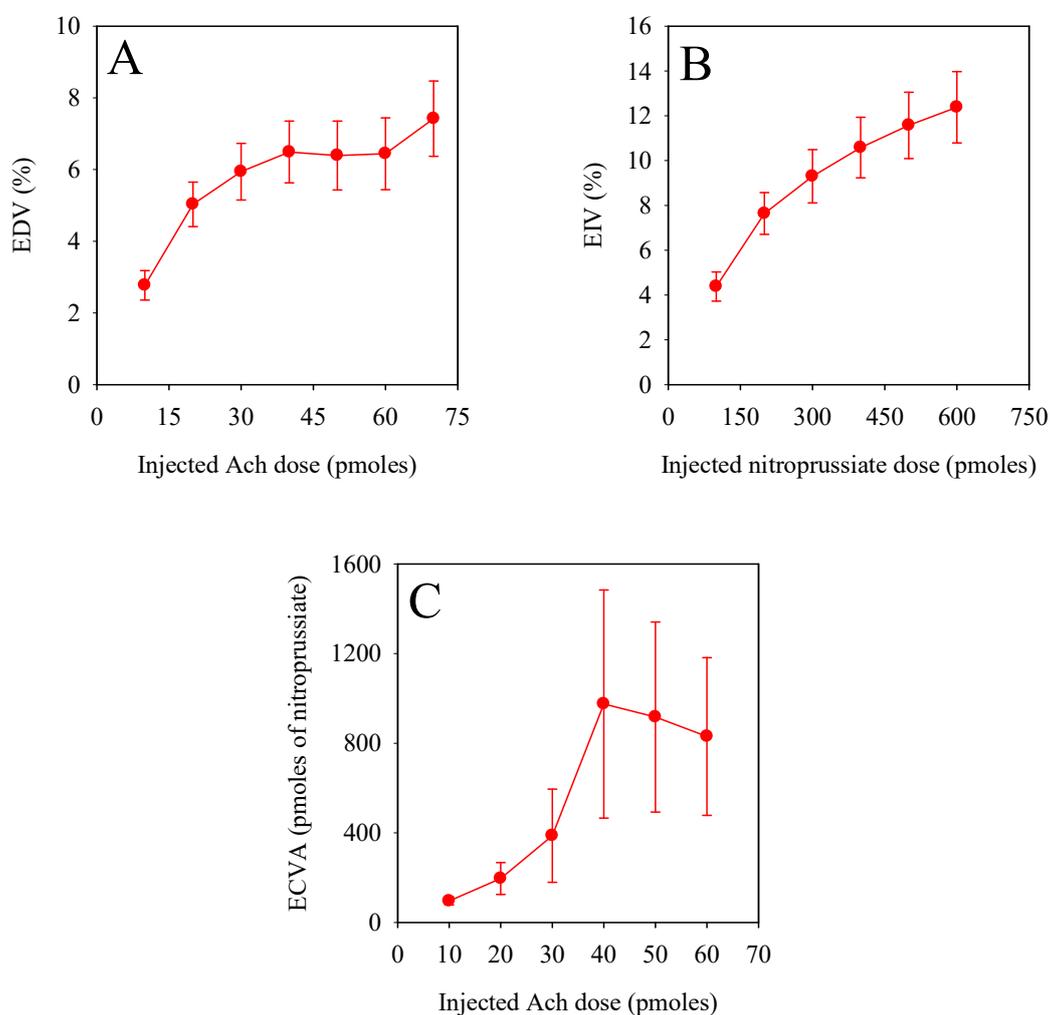
solubilized in aqueous solution, nitroprussiate degrades spontaneously and produces NO which relaxes directly the smooth muscle cells. The vasodilatation induced by nitroprussiate reflects thus the specific activity of the smooth muscle cells.

In practical terms, the EDV and EIV were determined in the isolated heart perfused according to the Langendorff's mode at constant coronary flow. In order to evaluate the effect of the vasodilatation agents, a preliminary vasoconstriction was performed using U46619, a thromboxane A2 analogue, at the concentration of 30 nM. The perfusion pressure was thus raised from 65 to values surrounding 130 mmHg. The vasodilatation agents were injected directly into the aorta at different concentrations ranging from 4 to 100 pmoles for Ach and 100 to 1000 pmoles for nitroprussiate. They entered the coronary circuit and relaxed the resistance vessels, the activity of which determines the coronary flow. Since the coronary flow was constant, the vasodilatation induced by Ach or nitroprussiate triggered a reduction of the perfusion pressure (Fig. 14): the magnitude of the vasodilatation was estimated by determining the amplitude of the decrease in the perfusion pressure and this parameter was normalized to the value of the perfusion pressure just before the injection of the vasodilatation agent. After normalisation for the input function, the dose-response curves of EDV and EIV were fitted to theoretical curves (logarithmic functions) and this allowed the estimation of the vasodilatation activity of the endothelial cells (ECVA) using the following formula:

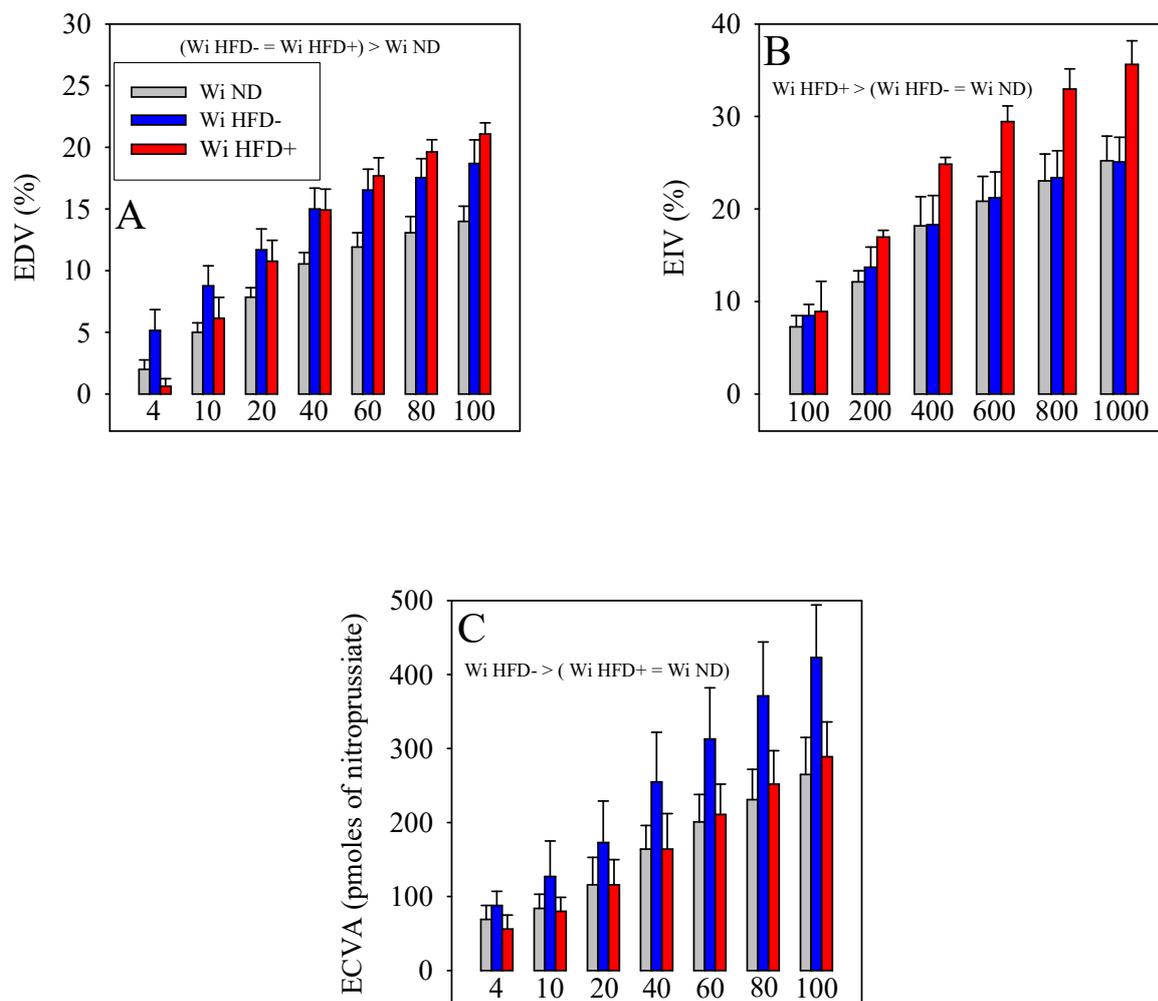
$ECVA = e^{[(\% \text{ Ach-induced vasodilatation} - b)/a]}$ , where  $a$  and  $b$  are the coefficients of the theoretical EID curve. The results were expressed in pmole equivalents of nitroprussiate. A typical representation of the obtained results are presented in Fig. 15.

The effects of the 3-month high fat diet is presented in Fig. 16. The animals fed with the high fat diet were separated in those which gained weight (HFD+ group)

and those who did not (HFD- group). The results clearly show that abdominal obesity increased EDV irrespective of the weight gain of the animals. However, the weight gain played a role on the type of activated vascular cells: when the body weight was not increased by the high fat diet, the endothelial cells were activated and the smooth muscle cells were not. In contrast, when the high fat diet increased the body weight, the gain in EDV was only due to an activation of smooth muscle cells.



**Figure 15.** Typical behaviour of the micro-vessel cells during the measurement of the coronary reactivity of Wistar rats. The number of experiments was 10 per group. EDV: endothelial-dependent vasodilatation; EIV: endothelial-independent vasodilatation; ECVA: endothelial cell vasodilatation activity; Ach: acetylcholine. Unpublished data.



**Figure 16.** Effect of the high fat diet on the endothelial-dependent and endothelial-independent vasodilations (EDV and EIV, panel A and B, respectively) and endothelial cell vasodilation activity (ECVA, panel C). The number of experiment was 10 for the Wi ND group and 5 for the Wi HFD groups. Wi ND: Wistar rats fed the normolipidic diet; Wi HFD-: Wistar rats fed the high fat diet without body weight gain; Wi HFD+: Wistar rats fed the high fat diet with body weight gain. Absissa: panels A and C = injected acetylcholine doses expressed in picomoles; panel B: injected nitroprussiate doses expressed in picomoles.  
 Extracted from (47)

**Table 2. Effects of HF on NO function during vasodilatation**

	No inhibitor	L-NAME	Indo	L-NAME + Indo
Wi ND	11.6 ± 1.1 a	7.6 ± 1.7	9.6 ± 1.8	8.5 ± 1.7
Wi HFD-	16.1 ± 2.1 b	4.7 ± 1.3 *	12.7 ± 1.0	6.7 ± 1.8 *
Wi HFD+	17.4 ± 1.2 b	14.5 ± 4.1	9.8 ± 1.4 #	10.0 ± 2.0 #

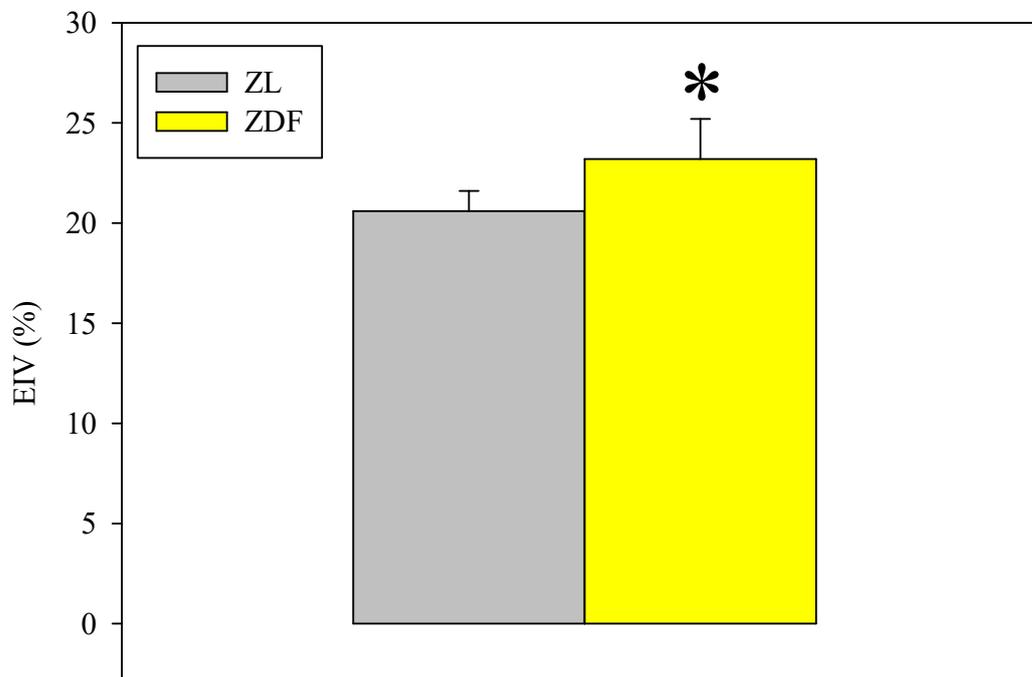
*N= 10 for Wi ND group and 5 for Wi HFD groups. The results are expressed in percentage of the perfusion value preceding the injection of the vasodilatation agent. L-NAME: L-N<sup>G</sup>-Nitroarginine methyl ester ; Indo : indomethacin ; Wi ND: Wistar rats fed the normolipidic diet; Wi HFD- : Wistar rats fed with the high fat diet without body weight gain ; Wi HFD+ : Wistar rats fed the high fat diet displaying body weight gain; a, b: a mean of the “no inhibitor” column without a common letter is significantly different; \*: significant effect of the tested inhibitor(s) in the HFD- fed group compared to the basal value of this group without inhibitor; #: significant effect of the tested inhibitor(s) in the HFD+ fed group compared to the basal value of this group without inhibitor. Extracted from (47).*

Utilization of different inhibitors (Table 2) indicates that the increase in vasodilatation induced by the HFD was related to a NO-dependent mechanism in the HFD- group, confirming the involvement of the endothelial cells. In contrast, it was related to a prostaglandin-dependent mechanism in the HFD+ group, since it was inhibited by indomethacin: this suggests that the augmented relaxation of the smooth muscle cells due to the HFD was related to the accumulation and utilization of arachidonic acid as substrate for the formation of enzymatically oxidized fatty acids.

EDV, EIV and ECVA were also determined in the ZDF rats in comparison with their lean littermates. In these animals, the EDD and ECVA were not significantly modified, but the EIV was slightly but significantly increased (Fig. 17).

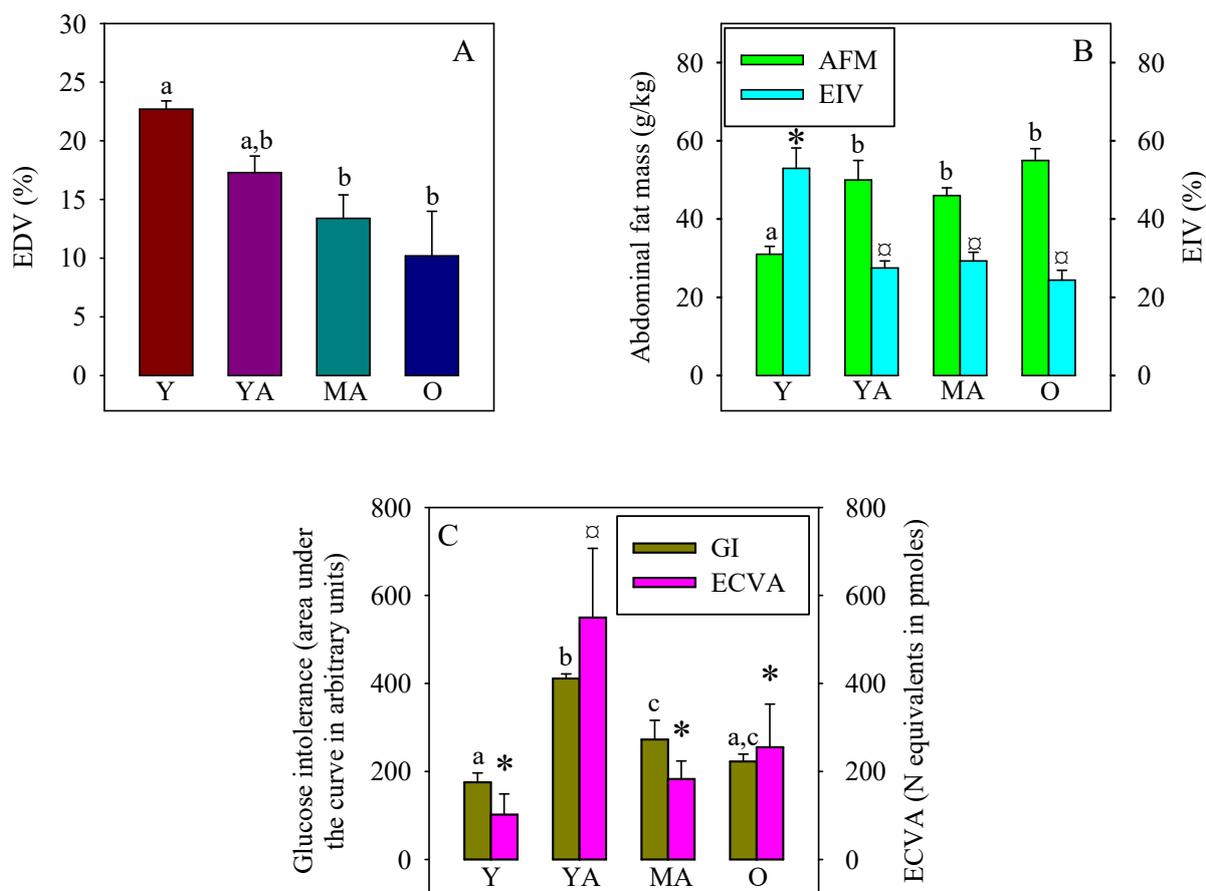
In this model of marked abdominal obesity, the ArA proportion of cardiac phospholipids was considerably increased and this probably contributed to increase the EIV. However, the high plasma glucose concentration probably attenuated the changes of vessel reactivity: indeed, we previously demonstrated

that streptozotocin-induced hyperglycaemia strongly reduces the EDV and EIV (62).



**Figure 17.** Endothelial-independent vasodilatation (EIV) in the Zucker Diabetic Fatty rats (ZDF) compared to their lean counterparts (ZL). Both strains were fed the normolipidic diet. The injected nitroprusside dose was 600 pmoles.  $N=10$  per group. \*: significantly different ( $p < 0.05$ ). Extracted from (51).

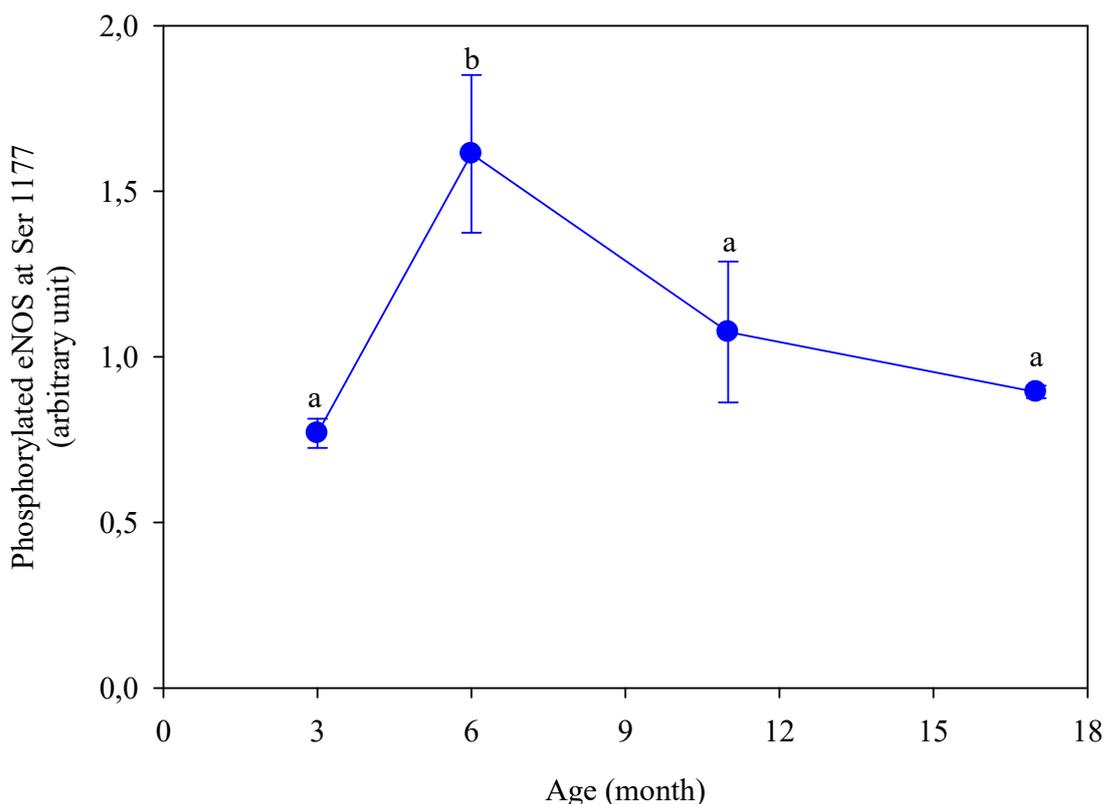
Aging also influences the effects of abdominal obesity on coronary perfusion. Coronary EDV, EIV and ECVA were determined at the ages of 3 (young), 6 (young adult), 11 (middle aged) and 17 (old) months in Wistar rats fed an equilibrated diet. These parameters of cardiac function were compared to the changes in abdominal adiposity and glucose tolerance.



**Figure 18.** Effects of aging on the endothelial-dependent vasodilatation (EDV, panel A), the endothelial-independent vasodilatation (EIV) and abdominal fat mass (AFM, panel B) as well as the endothelial cell vasodilatation activity (ECVA) and glucose intolerance (GI, panel C). All the animals were Wistar rats fed the normolipidic diet. N: nitroprussiate. a,b,c: means of EDV, AFM and GI without a common letter are significantly different. \*,□: means of EIV and ECVA without a common sign are significantly different. From (48).

As seen in Fig. 18A, the EDV gradually decreased with aging, suggesting that the heart's capacity for contractile performance was progressively compromised with time. This progression has already been described in the literature (24-26), but the influence of aging on the individual behaviour of endothelial and smooth muscle cells was not known. Our work allowed the determination of the aged-related changes in EIV (Fig. 18B), seen as high at the age of 3 months, when the smooth muscle cell relaxation capacities decreased between 3 and 6 months and thereafter plateaued at a low value. This was similar to the age-related effect on myocardial

contractility (see Fig. 10). EIV had an inverse relationship compared to the gain in abdominal fat mass: when rats were 3 month old, the abdominal fat mass was low and the EIV was high; between 3 and 6 months, when the abdominal fat mass increased, the EIV decreased and it remained low thereafter when the abdominal fat mass was maintained at a high value.



**Figure 19.** Effects of aging on the active form (phosphorylated at serine 1177) of the endothelial nitric oxide synthase (eNOS). The assay was performed in the myocardium of Wistar rats fed the normolipidic diet. Ser: serine. a,b: significantly different. From (48).

However, for endothelial cell vasodilatation capacity this was not related to the abdominal fat mass, but rather to the rate of glucose intolerance (Fig. 18C). Glucose intolerance was low at 3 months of age, but rapidly increased to a paroxysmal value at 6 months in parallel with the gain of abdominal fat mass; thereafter, when the abdominal fat mass was high, but stable, the animals adapted and glucose intolerance progressively recovered a value close to that measured in

3 month old animals. ECVA evolved similarly with a paroxysm at 6 months and a return to the basal values thereafter: the greater the glucose intolerance, the greater the ECVA. Fig. 19 shows that glucose intolerance-related increase in ECVA is related to the activity of eNOS. Phosphorylated eNOS at Ser 1177 reflects active eNOS: the activity of this enzyme paralleled the age-related modifications of glucose intolerance and ECVA indicating that glucose intolerance contributed to stimulate eNOS and to increase the ECVA when this parameter was measured according to the age.

## ***2.6 - Conclusions***

Our data show that abdominal adiposity is associated with an increased cardiac mechanical function supported by augmented cardiac perfusion capacities. Both phenomena help the organism to fight against the weight gain, since they contribute to maintain the physical capacities of the obese individual. They seem to be related to the accumulation of arachidonic acid in membrane phospholipids which stimulates the contractile function of cardiomyocytes and favour dilatation of the coronary micro-vessels. However, after this phase of gain in physical activity, cardiac function is known to steadily decline until the development of heart failure (24). The long-term phenomenon could also be related to the accumulation of arachidonic acid in cardiac phospholipids. Indeed, arachidonic acid favours the formation of the 2-series prostaglandins which are known to have pro-inflammatory effects (66). These molecules may contribute to deterioration of cardiac function in the long term and leading to cardiac failure. Improvement of cardiac function in obese individuals may thus be influenced through dietary n-3 polyunsaturated fatty acid intake which is recognized to decrease arachidonic acid in cardiac membrane (67, 68) and favour the formation of lipid mediators with anti-inflammatory properties (66).

### **3 - OBESITY PARADOX**

#### **3.1 - Introduction**

Evidence in support of obesity being associated with an increased risk of developing cardiovascular disease, has been reported by Kenchaiah *et al.* (69). They examined the incidence of heart failure in 5881 Framingham participants according to the obesity status. For women and men: compared to normal weight subjects the overweight have an increased risk of heart failure, and compared to overweight, the obese have a higher risk of developing the pathology. If the risk of developing a cardiac pathology is augmented when the body mass index (BMI) increases, the rate of survival during or after this pathology is increased in obese individuals. Horwich *et al.* (70) depicted the phenomenon known as the obesity paradox by describing the rate of survival at 5 years of patients with heart failure. They showed that the highest the BMI had the lowest the mortality rate. Lavie *et al.* (71) examined this more closely by paralleling the 3-year survival rate of patients presenting coronary heart disease with the body composition. They categorized their patients in four groups: 1) those with low body fat and low lean mass indexes which displayed the lowest survival rate; 2) those with high body fat and low lean mass indexes which had a slight but not significant augmentation of the survival rate; 3) those with a low body fat but high lean mass indexes which continued to increase their survival rate; and 4) those with high body fat and lean mass indexes which had the highest survival rate. Only the two extreme groups (low body fat and low lean mass indexes vs. high body fat and high lean mass indexes) displayed statistical significant differences in their survival rate. However, this study highlights the importance of adipose and muscular masses on the survival after a coronary heart disease. In 2013, the same group (72) published a key-study on the obesity paradox in which they evaluated the influence of cardio-respiratory fitness on the relationship between survival rate during heart failure and BMI. They showed the classical positive relationship between survival

rate and BMI in the patients with the low cardio-respiratory fitness. However, the patients with high fitness ( $\text{O}_2$  consumption  $> 14 \text{ ml O}_2 \text{ kg}^{-1} \text{ min}^{-1}$ ) displayed the highest survival rate. Furthermore, the classical relationship between survival rate and BMI almost completely disappeared, indicating that low BMI patients had an almost similar survival rate than those with a high BMI. These interesting results underline the importance of the cardio-respiratory fitness in the phenomenon of obesity paradox. The work presented in the previous chapter of this book is thus of considerable importance since it can explain the obesity paradox in patients with a low cardio-respiratory fitness. Indeed, we demonstrated that obesity preserves relatively greater mechanical performance of the heart and thus greater recovery after adverse cardiac events.

As suggested by Lavie *et al.* (71), skeletal muscle mass is of notable importance in the explanation of the obesity paradox because it can favour mobility which is a strong predicting factor in the estimation of the life expectancy (73). A high skeletal muscle mass can contribute to increase the cardio-respiratory fitness by favouring physical activity and finally protects the individual vis-à-vis premature death. However, apart from this effect on the mobility, the skeletal muscle mass can also play an important role as a substrate reserve allowing the organism to fight against the developed cardiac pathology. In this respect, adipose tissue mass should also be beneficial. Wannamethee *et al.* (74) show a positive relationship between circulating leptin and survival rate during heart failure. Yet, plasma leptin level is proportional to the amount of stored fats in the adipose tissue. This indicates that the adipose tissue mass plays also a critical role in the obesity paradox, perhaps by intervening as a substrate in order to furnish the energy necessary to fight against the pathology or by increasing the cardiac fitness. However it is unclear what is most important in the obesity paradox, the substrate effect of muscle proteins or adipose tissue fats or their capacities to promote a gain in fitness. We therefore evaluated the effect of abdominal obesity on

cardiomyocyte mitochondrial metabolism with respect to the oxidative phosphorylation and release of reactive oxygen species which can interact with the energy metabolism. The investigations were performed in male Wistar rats fed a high fat diet for 3 and 7 months. In parallel, we determined the coronary reactivity in order to know whether the increase observed after a 3-month high fat diet is maintained after 7 months of feeding.

**Table 3. Oxygen consumption of cardiac mitochondria after a 3-month high fat diet**

		Wi ND	Wi HFD
GM	State 3	123 ± 16	151 ± 8
	State 4	16 ± 1	19 ± 1
	RCI	6.6 ± 0.9	8.2 ± 0.6
Pyr	State 3	134 ± 10	124 ± 8
	State 4	22 ± 1	17 ± 1
	RCI	7.0 ± 1.0	7.4 ± 0.2
Succ	State 3	153 ± 12	161 ± 9
	State 4	45 ± 1	42 ± 1
	RCI	3.3 ± 0.1	3.8 ± 0.2
Pcarn	State 3	103 ± 14	132 ± 9
	State 4	16 ± 1	20 ± 1
	RCI	6.7 ± 0.9	6.5 ± 0.1

*N=10 per group. The results are expressed in ng atoms of oxygen/min/mg of protein. Wi ND: normolipidic diet; Wi HFD: high fat diet; GM: glutamate/malate; Pyr: pyruvate/malate; Succ: succinate/rotenone; Pcarn: palmitoylcarnitine/malate; State 3: state 3 respiration rate measured in the presence of saturating concentration of ADP; State 4: state 4 respiration rate measured in the presence of oligomycin; RCI: respiratory control ratio. Unpublished data.*

### **3.2 - Mitochondrial function after 3 months of high fat diet**

The mitochondrial oxygen consumption was determined using 4 different substrates: glutamate/malate and pyruvate/malate which are complex 1-related substrates, succinate/rotenone, a complex 2-related substrate, and palmitoylcarnine/malate which is a lipid substrate. The effects of the 3-month high fat diet is presented in Table 3. For each substrate used, mitochondrial

oxygen consumption was unaffected by the high fat diet. This was true for the rates of states 3 and 4 of the respiration, but also for the respiratory control indexes which reflect the mitochondrial integrity.

The mitochondrial release of hydrogen peroxide ( $H_2O_2$ ) measured during the state 2 respiration with Amplex red and horseradish peroxidase was evaluated with the 4 substrates previously described during the state 2 respiration rate (in the presence of the substrate, but without ADP addition). It was determined in the absence and presence of a complex 1 inhibitor (rotenone) and complex 3 inhibitor (antimycin A). When glutamate/malate or pyruvate/malate was used as a substrate (Table 4),  $H_2O_2$  release was not affected by the high fat diet in the absence or presence of the complexes 1 and 3 inhibitors. Conversely, with succinate/rotenone, the basal  $H_2O_2$  release was increased greatly by the high fat diet (+65%).

The efficiency of the complex 1 inhibitor rotenone in suppressing the difference indicated that the high fat diet-induced augmentation of the  $H_2O_2$  release was due to the reverse electron flux from complex 2 to complex 1. Finally, when the lipid substrate palmitoylcarnitine/malate was employed,  $H_2O_2$  release was unaffected by the high fat diet in the absence of inhibitor and in the presence of the complex 1 inhibitor rotenone. Conversely, it was increased noticeably when the complex 3 was inhibited by antimycin A.

Taken together, these data suggest that the mitochondrial oxidative phosphorylation was not altered by the 3-month high fat diet, but the reactive oxygen species production was increased, namely when a complex 2-related substrate was utilized. This was also true when the lipid substrate was used. This high fat diet-induced increase in reactive oxygen species production did not seem to alter the energy metabolism, but could act as a second messenger to provoke subsequent changes in cellular function.

**Table 4.** H<sub>2</sub>O<sub>2</sub> release by cardiac mitochondria after the 3-month high fat diet

		Wi ND	Wi HFD
GM	St. 2	97 ± 8	96 ± 5
	+ rot	273 ± 49	327 ± 37
	+ aa	380 ± 75	371 ± 28
Pyr	St. 2	104 ± 12	124 ± 5
	+ rot	484 ± 63	405 ± 65
	+ aa	411 ± 69	509 ± 71
Succ	St. 2	781 ± 49	1280 ± 147 *
	+ rot	202 ± 20	214 ± 14
	+ aa	429 ± 46	380 ± 37
Pcarn	St. 2	138 ± 12	162 ± 25
	+ rot	295 ± 35	337 ± 132
	+ aa	554 ± 39	930 ± 152 *

*N=10 per group. The results are expressed in pmoles of H<sub>2</sub>O<sub>2</sub>/min/mg of proteins. Wi ND: normolipidic diet; Wi HFD: high fat diet; GM: glutamate/malate; Pyr: pyruvate/malate; Succ: succinate; Pcarn: palmitoylcarnitine/malate; St. 2: state 2 respiration rate without inhibitor; + rot: with rotenone; + aa: with subsequent addition of antimycin A; \*: significantly different compared to the control value. Unpublished data.*

**Table 5.** Oxygen consumption of cardiac mitochondria after a 7-month high fat diet

		Wi ND	Wi HFD
GM	State 3	123 ± 8	180 ± 18 *
	State 4	20 ± 1	22 ± 1
	RCI	7.4 ± 0.4	8.2 ± 0.7
Pyr	State 3	149 ± 13	166 ± 19
	State 4	19 ± 1	24 ± 1 *
	RCI	9.3 ± 0.9	7.4 ± 0.5
Succ	State 3	157 ± 10	175 ± 7
	State 4	41 ± 4	48 ± 3
	RCI	4.2 ± 0.1	4.3 ± 0.2
Pcarn	State 3	121 ± 9	155 ± 22
	State 4	19 ± 1	23 ± 1 *
	RCI	7.5 ± 0.4	5.9 ± 0.9

*N=10 per group. The results are expressed in ng atoms of oxygen/min/mg of protein. Wi ND: normolipidic diet; Wi HFD: high fat diet; GM: glutamate/malate; Pyr: pyruvate/malate; Succ: succinate/rotenone; Pcarn: palmitoylcarnitine/malate; State 3: state 3 respiration rate measured in the presence of saturating concentration of ADP; State 4: state 4 respiration rate measured in the presence of oligomycin; RCI: respiratory control ratio; \*: significantly different compared to the control group. Extracted from (50).*

### 3.3 - Mitochondrial function after 7 months of high fat diet

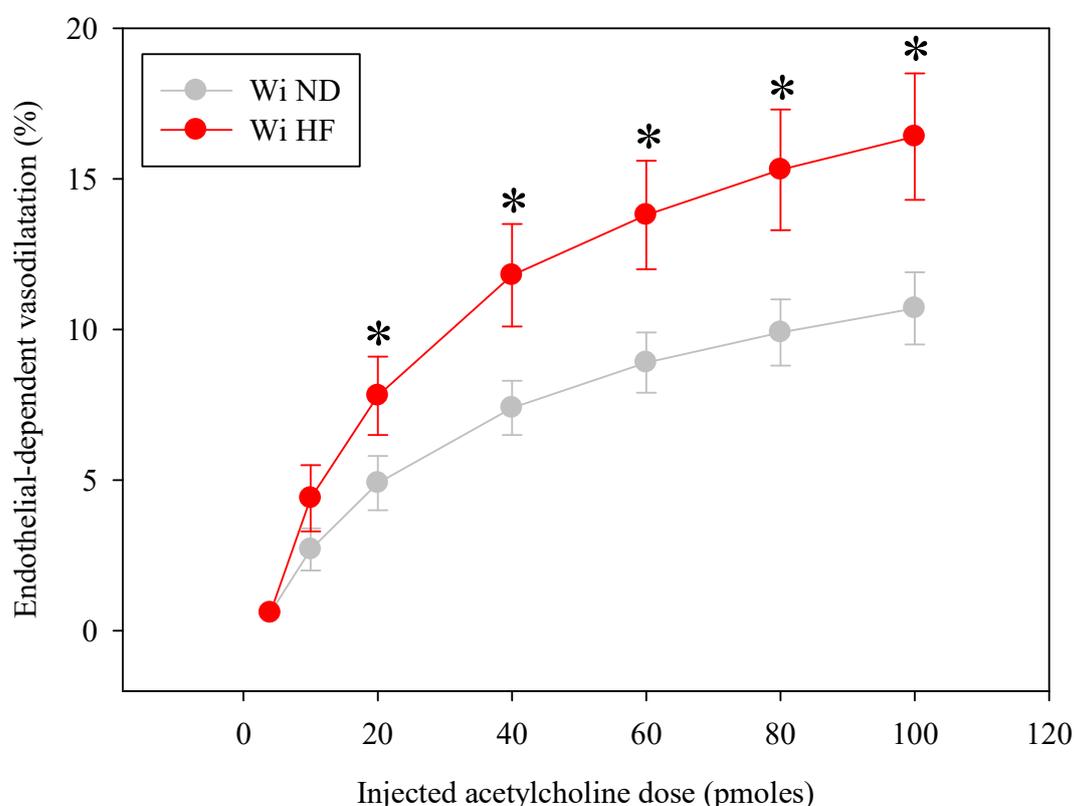
The effects of the high fat diet on the mitochondrial function was also evaluated after a 7-month supply in order to determine whether the previous changes observed after a 3-month administration had effects on energy metabolism and H<sub>2</sub>O<sub>2</sub> release. The results of the mitochondrial oxygen consumption is presented in Table 5. The most impressive result was the high fat diet-induced increase in state 3 respiration rate when glutamate/malate was used as substrate (+46%). Similar trends were noticed for the other substrates (+11, +11 and +28% for pyruvate/malate, succinate/rotenone and palmitoylcarnitine/malate), but the differences were not significant. These changes in respiration might be related to the increased production of reactive oxygen species observed after 3 months of high fat diet, which was sustained as augmented H<sub>2</sub>O<sub>2</sub> release even after 7 months when palmitoylcarnitine/malate were tested as substrates (Table 6).

**Table 6. H<sub>2</sub>O<sub>2</sub> release by cardiac mitochondria after the 7-month high fat diet**

		Wi ND	Wi HFD
GM	St. 2	77 ± 6	77 ± 5
	+ rot	245 ± 13	194 ± 17 *
	+ aa	362 ± 39	306 ± 13
Pyr	St. 2	103 ± 4	118 ± 5 *
	+ rot	271 ± 14	336 ± 39
	+ aa	399 ± 22	490 ± 81
Succ	St. 2	1680 ± 34	1978 ± 212
	+ rot	161 ± 11	183 ± 14
	+ aa	304 ± 20	424 ± 46
Pcarn	St. 2	122 ± 6	144 ± 4 *
	+ rot	183 ± 10	276 ± 26 *
	+ aa	578 ± 42	1005 ± 90 *

*N=10 per group. The results are expressed in pmoles of H<sub>2</sub>O<sub>2</sub>/min/mg of proteins. Wi ND: normolipidic diet; Wi HFD: high fat diet; GM: glutamate/malate; Pyr: pyruvate/malate; Succ: succinate; Pcarn: palmitoylcarnitine/malate; St. 2: state 2 respiration rate without inhibitor; + rot: with rotenone; + aa: with subsequent addition of antimycin A; \*: significantly different compared to the control value. Extracted from (50).*

The obesity related-increase in arachidonic acid accumulation in cardiac phospholipids or stimulation of cardiac mechanical function could also be involved in the increase in the mitochondrial oxidative phosphorylation. Our results show that abdominal obesity improves the mitochondrial energy metabolism, provided the excess abdominal fat is installed for a sufficiently extended period. Thus we next evaluated the endothelial-dependent vasodilatation of the coronary micro-vessels after 7 months of high fat diet administration.



**Figure 20.** Effect of 7-month high fat diet on the endothelial-dependent vasodilatation. *Wi ND*: Wistar rats fed the normolipidic diet; *Wi HFD*: Wistar rats fed the high fat diet for 7 months. \*: significantly different. Extracted from (50).

### 3.4 - Coronary reactivity after the 7-month high fat diet

Fig. 20 describes the coronary endothelial-dependent vasodilatation of the rats fed the high fat diet for 7 months. It appears clearly that this parameter was increased

by the high fat diet for each injected acetylcholine dose. Thus, greater coronary flow may be viable in the hearts of high fat-fed animals.

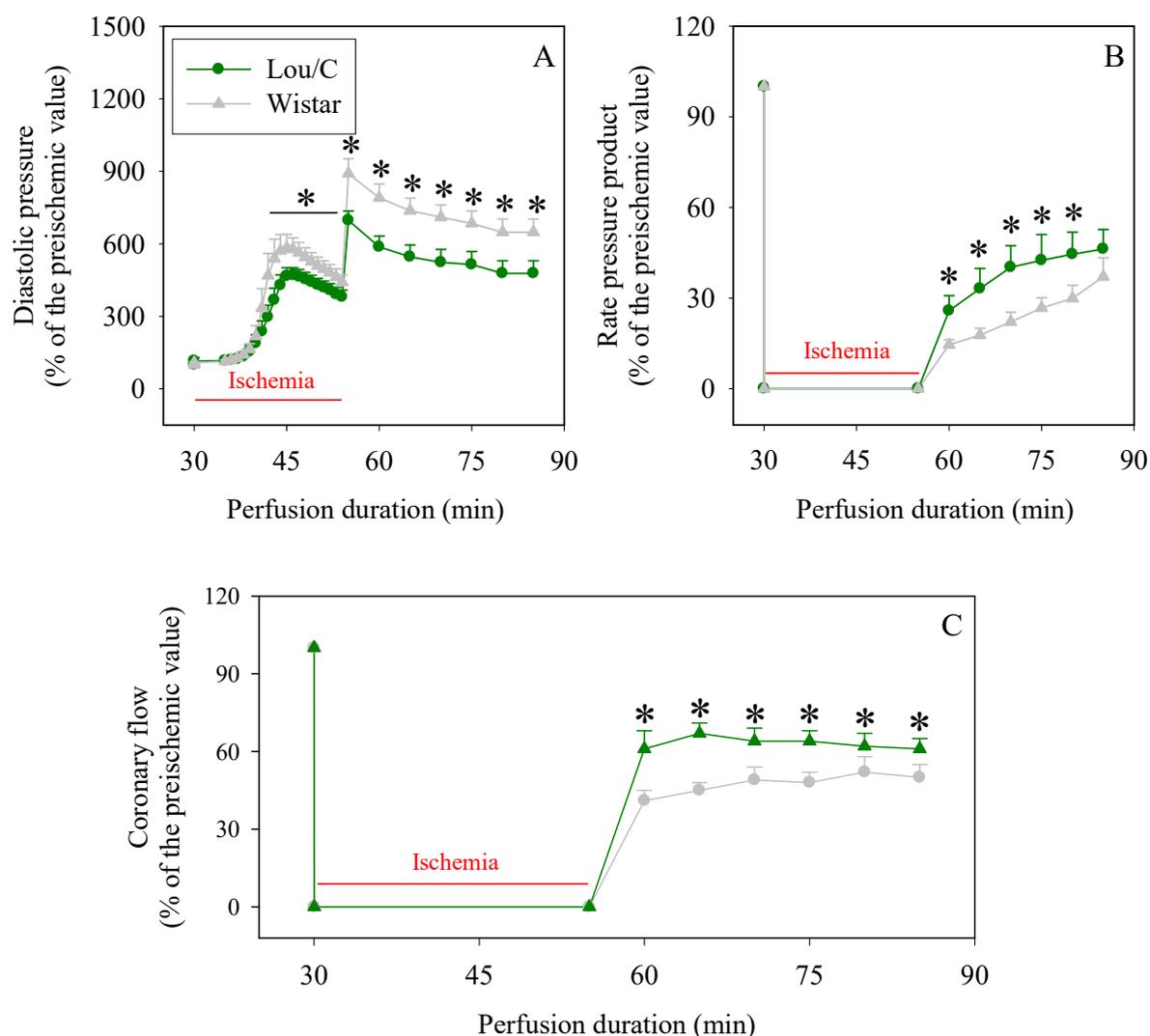
The heart relies on the amount of available substrates, with energy production under the control of several enzymatic activities which meet the Michaelis-Menten law, *i.e.*, the more abundantly available substrates, the more intense the energy production. Obese individuals have more fat and sometimes more muscle mass than lean people, and thus greater energy production capacity. Thus, the heart of obese individuals display specific qualities which may permit greater resistance and recovery from injury.

### ***3.5 - Effects of obesity on the severity of ischemia***

It was attractive to know whether obesity favours the obesity paradox in laboratory animals, since shifts between clinical data and experimental results obtained in animals are not systematically valid. We thus evaluated the influence of ischemia/reperfusion in the anti-obese Lou/C and Wistar rats. Briefly, the hearts were perfused in the Langendorff mode (perfusion pressure maintained with the Mariotte's flask) and subjected to 30 min of normoxia followed by 25 min of global no-flow ischemia and 30 min of reperfusion. As it was expected according to the differences in animal body weight, the rate pressure product (reflecting the cardiac mechanical activity) and coronary flow before ischemia were different in the Lou/C and Wistar rats ( $38 \pm 1$  vs  $34 \pm 1$  mHg/min for the rate pressure product and  $8.3 \pm 0.8$  vs  $6.6 \pm 0.3$  ml/min/g for the coronary flow, respectively,  $p < 0.05$  and  $0.05$ ). These parameters thus were normalized to their pre-ischemic values.

The results are presented in Figure 21. During ischemia, the contracture (increase in diastolic pressure) was rapidly ampler in the Wistar phenotype compared to the Lou/C one and it was maintained higher during reperfusion. Recovery of the rate

pressure product was reduced in the Wistar rats and this was associated with a lower recovery of coronary flow. These results contrasted with the occurrence of the obesity paradox, but they correspond to what is published in the literature when rats are fed with a HFD for ~3 months (76-78).



**Figure 21.** Effect of ischemia/reperfusion in the perfused hearts of the Lou/C and Wistar phenotypes. Panels A, B and C represent the diastolic pressure, rate pressure product and coronary flow.  $N=10$  per group. \*: significantly different. From (50).

Measurements of the mitochondrial metabolism before ischemia did not show any significant difference between Lou/C and Wistar rats (data not shown). The enhanced effects of long-duration HFD on the mitochondrial metabolism thus was not observed in this study, which contrasts with the results obtained in Wistar rats fed with the HFD diet for 7 months. Finally, occurrence of the obesity paradox was reported in rats fed with a high fat diet for 6 months (79): the recovery of the rate pressure product was improved in these high fat diet-fed rats compared to animals fed a normolipidic diet. It is noteworthy to understand that the paradox occurred only after 6 months of high fat feeding: this duration represents the time necessary for induction of the high fat diet-related increase in mitochondrial oxidative phosphorylation.

#### **4 - GENERAL CONCLUSIONS**

Obesity is associated with ectopic accumulation of fats and, sometimes, with increased skeletal muscle mass. The epidemiological studies have clearly shown that obese people display a higher risk for severe pathologies including cardiovascular diseases, cancer, etc. However, the organism adapts: when obese individuals contract one of these illnesses, they have more chance to remain alive. This is called the obesity paradox. Our works have shown that obese individuals have a heart which is more tonic than lean people probably because the accumulation of arachidonic acid in membrane phospholipids. They also have more “gas” in order to maintain an elevated cardiac function. In those respects, they are able to fight more efficiently against the lethal effect of the most severe pathologies. This explains the obesity paradox. However, it must be kept in mind that the phenomenon is not valid for all the obese people. Indeed, the obesity paradox does not occur in morbid obese, that is to say in people with a body mass index higher than 40. For these people, specific lifestyle changes or bariatric

surgery are necessary in order to bring them back to a leaner and thus more perennial phenotype.

If obesity protects against death because of greater resistance to injury, greater metabolic capacity and this potentially relatively greater 'cardiac fitness', it would be interesting to determine whether physical training may have similar beneficial effects.

## 5 - REFERENCES

1. Obesity and overweight. World Health Organization. Website: <http://www.who.int/mediacentre/factsheets/fs311/en/>
2. Overcoming obesity: An initial economic analysis. McKinsey Global Institute. Website: [https://www.mckinsey.com/~media/McKinsey/Business%20Functions/Economic%20Studies%20TEMP/Our%20Insights/How%20the%20world%20could%20better%20fight%20obesity/MGI\\_Overcoming\\_obesity\\_Full\\_report.ashx](https://www.mckinsey.com/~media/McKinsey/Business%20Functions/Economic%20Studies%20TEMP/Our%20Insights/How%20the%20world%20could%20better%20fight%20obesity/MGI_Overcoming_obesity_Full_report.ashx)
3. Czernichow S, Kengne AP, Huxley RR, Batty GD, de Galan B, Grobbee D, Pillai A, Zoungas S, Marre M, Woodward M, Neal B, Chalmers J; ADVANCE Collaborative Group. Comparison of waist-to-hip ratio and other obesity indices as predictors of cardiovascular disease risk in people with type-2 diabetes: a prospective cohort study from ADVANCE. *Eur J Cardiovasc Prev Rehabil.* 2011 Apr;18(2):312-9.
4. Waist circumference and waist-hip ratio Report of a WHO expert consultation, Geneva, 8-11 December 2008. World Health Organization. Website: [http://www.who.int/nutrition/publications/obesity/WHO\\_report\\_waistcircumference\\_and\\_waisthip\\_ratio/en/](http://www.who.int/nutrition/publications/obesity/WHO_report_waistcircumference_and_waisthip_ratio/en/)
5. Herrera BM, Lindgren CM. The genetics of obesity. *Curr Diab Rep.* 2010 Dec;10(6):498-505.
6. Sharma BR, Kim DW, Rhyu DY. Korean Chungtaejeon tea extract attenuates body weight gain in C57BL/6J-Lep ob/ob mice and regulates adipogenesis and lipolysis in 3T3-L1 adipocytes. *J Integr Med.* 2017 Jan;15(1):56-63.
7. Muenzner M, Tappenbeck N, Gembardt F, Rülke R, Furkert J, Melzig MF, Siems WE, Brockmann GA, Walther T. Green tea reduces body fat via upregulation of neprilysin. *Int J Obes (Lond).* 2016 Dec;40(12):1850-1855.
8. Kobayashi M, Kawano T, Ukawa Y, Sagesaka YM, Fukuhara I. Green tea beverages enriched with catechins with a galloyl moiety reduce body fat in moderately obese adults: a randomized double-blind placebo-controlled trial. *Food Funct.* 2016 Jan;7(1):498-507.
9. Pinel A, Pitois E, Rigaudiere JP, Jouve C, De Saint-Vincent S, Laillet B, Montaurier C, Huertas A, Morio B, Capel F. EPA prevents fat mass expansion and metabolic disturbances in mice fed with a Western diet. *J Lipid Res.* 2016 Aug;57(8):1382-97.
10. Go RE, Hwang KA, Park GT, Lee HM, Lee GA, Kim CW, Jeon SY, Seo JW, Hong WK, Choi KC. Effects of microalgal polyunsaturated fatty acid oil on body weight and lipid accumulation in the liver of C57BL/6 mice fed a high fat diet. *J Biomed Res.* 2016 May;30(3):234-42.
11. Pappalardo G, Almeida A, Ravasco P. Eicosapentaenoic acid in cancer improves body composition and modulates metabolism. *Nutrition.* 2015 Apr;31(4):549-55.

12. Munro IA, Garg ML. Dietary supplementation with long chain omega-3 polyunsaturated fatty acids and weight loss in obese adults. *Obes Res Clin Pract.* 2013 May-Jun;7(3):e173-81.
13. Munro IA, Garg ML. Prior supplementation with long chain omega-3 polyunsaturated fatty acids promotes weight loss in obese adults: a double-blinded randomised controlled trial. *Food Funct.* 2013 Apr 5;4(4):650-8.
14. DeFina LF, Marcoux LG, Devers SM, Cleaver JP, Willis BL. Effects of omega-3 supplementation in combination with diet and exercise on weight loss and body composition. *Am J Clin Nutr.* 2011 Feb;93(2):455-62.
15. Li JJ, Huang CJ, Xie D. Anti-obesity effects of conjugated linoleic acid, docosahexaenoic acid, and eicosapentaenoic acid. *Mol Nutr Food Res.* 2008 Jun;52(6):631-45.
16. Jakobsen GS, Småstuen MC, Sandbu R, Nordstrand N, Hofso D, Lindberg M, Hertel JK, Hjelmæsæth J. Association of Bariatric Surgery vs Medical Obesity Treatment With Long-term Medical Complications and Obesity-Related Comorbidities. *JAMA.* 2018 Jan 16;319(3):291-301.
17. Desai M, Beall M, Ross MG. Developmental origins of obesity: programmed adipogenesis. *Curr Diab Rep.* 2013 Feb;13(1):27-33.
18. Bassett DR, Craig BW. Influence of early nutrition on growth and adipose tissue characteristics in male and female rats. *J Appl Physiol* (1985). 1988 Mar;64(3):1249-56.
19. Erlanson-Albertsson C. How palatable food disrupts appetite regulation. *Basic Clin Pharmacol Toxicol.* 2005 Aug;97(2):61-73.
20. Patterson CM, Bouret SG, Park S, Irani BG, Dunn-Meynell AA, Levin BE. Large litter rearing enhances leptin sensitivity and protects selectively bred diet-induced obese rats from becoming obese. *Endocrinology.* 2010 Sep;151(9):4270-9.
21. Glavas MM, Kirigiti MA, Xiao XQ, Enriori PJ, Fisher SK, Evans AE, Grayson BE, Cowley MA, Smith MS, Grove KL. Early overnutrition results in early-onset arcuate leptin resistance and increased sensitivity to high-fat diet. *Endocrinology.* 2010 Apr;151(4):1598-610.
22. Gorski JN, Dunn-Meynell AA, Levin BE. Maternal obesity increases hypothalamic leptin receptor expression and sensitivity in juvenile obesity-prone rats. *Am J Physiol Regul Integr Comp Physiol.* 2007 May;292(5):R1782-91.
23. Plagemann A, Harder T, Rake A, Waas T, Melchior K, Ziska T, Rohde W, Dörner G. Observations on the orexigenic hypothalamic neuropeptide Y-system in neonatally overfed weanling rats. *J Neuroendocrinol.* 1999 Jul;11(7):541-6.
24. Habbout A, Li N, Rochette L, Vergely C. Postnatal overfeeding in rodents by litter size reduction induces major short- and long-term pathophysiological consequences. *J Nutr.* 2013 May;143(5):553-62.
25. Chen J, Huang XF, Shao R, Chen C, Deng C. Molecular Mechanisms of Antipsychotic Drug-Induced Diabetes. *Front Neurosci.* 2017 Nov 21;11:643.

26. Marketou M, Gupta Y, Jain S, Vardas P. Differential Metabolic Effects of Beta-Blockers: an Updated Systematic Review of Nebivolol. *Curr Hypertens Rep.* 2017 Mar;19(3):22.
27. Kim TN, Choi KM. The implications of sarcopenia and sarcopenic obesity on cardiometabolic disease. *J Cell Biochem.* 2015 Jul;116(7):1171-8.
28. González-Gross M, Meléndez A. Sedentarism, active lifestyle and sport: Impact on health and obesity prevention. *Nutr Hosp.* 2013 Sep;28 Suppl 5:89-98.
29. Giske L, Lauvrak V, Elvsaa IKØ, Hofmann B, Håvelsrud K, Vang V, Fure B. Long Term Effects of Bariatric Surgery [Internet]. Oslo, Norway: Knowledge Centre for the Health Services at The Norwegian Institute of Public Health (NIPH); 2014 Jan. Available from <http://www.ncbi.nlm.nih.gov/books/NBK464735/>
30. Kizy S, Jahansouz C, Wirth K, Ikramuddin S, Leslie D. Bariatric Surgery: A Perspective for Primary Care. *Diabetes Spectr.* 2017 Nov;30(4):265-276.
31. Kunnumakkara AB, Sailo BL, Banik K, Harsha C, Prasad S, Gupta SC, Bharti AC, Aggarwal BB. Chronic diseases, inflammation, and spices: how are they linked? *J Transl Med.* 2018 Jan 25;16(1):14.
32. Gadde KM, Martin CK, Berthoud HR, Heymsfield SB. Obesity: Pathophysiology and Management. *J Am Coll Cardiol.* 2018 Jan 2;71(1):69-84.
33. Gérard C, Brown KA. Obesity and breast cancer - Role of estrogens and the molecular underpinnings of aromatase regulation in breast adipose tissue. *Mol Cell Endocrinol.* 2017 Sep 15. pii: S0303-7207(17)30499-9.
34. IDF Consensus Worldwide Definition of the Metabolic Syndrome. International Diabetes Federation. Website: <https://www.idf.org/e-library/consensus-statements/60-idfconsensus-worldwide-definitionof-the-metabolic-syndrome>
35. Vague P, Raccach D. The syndrome of insulin resistance. *Horm Res.* 1992;38(1-2):28-32.
36. Smith U. Regional differences in adipocyte metabolism and possible consequences in vivo. *Int J Obes.* 1985;9 Suppl 1:145-8.
37. Engin AB. What Is Lipotoxicity? *Adv Exp Med Biol.* 2017;960:197-220.
38. Bratusch-Marrain P, Waldhäusl W. [Hepatic and peripheral insulin resistance as a cause of hyperglycemia in non-insulin-dependent (type 2) diabetes mellitus: a review]. *Wien Klin Wochenschr.* 1987 Apr 3;99(7):211-6.
39. Kraegen EW, Jenkins AB, Storlien LH, Chisholm DJ. Tracer studies of in vivo insulin action and glucose metabolism in individual peripheral tissues. *Horm Metab Res Suppl.* 1990;24:41-8.
40. Grill V. Why is insulin release from beta cells insufficient in type 2 diabetes? *Tidsskr Nor Laegeforen.* 2000 Sep 30;120(23):2796-8.

41. Daousi C, Casson IF, Gill GV, MacFarlane IA, Wilding JP, Pinkney JH. Prevalence of obesity in type 2 diabetes in secondary care: association with cardiovascular risk factors. *Postgrad Med J*. 2006 Apr;82(966):280-4.
42. Emelia J. Benjamin, Michael J. Blaha, Stephanie E. Chiuve, Mary Cushman, Sandeep R. Das, Rajat Deo, Sarah D. de Ferranti, James Floyd, Myriam Fornage, Cathleen Gillespie, Carmen R. Isasi, Monik C. Jiménez, Lori Chaffin Jordan, Suzanne E. Judd, Daniel Lackland, Judith H. Lichtman, Lynda Lisabeth, Simin Liu, Chris T. Longenecker, Rachel H. Mackey, Kunihiro Matsushita, Dariush Mozaffarian, Michael E. Mussolino, Khurram Nasir, Robert W. Neumar, Latha Palaniappan, Dilip K. Pandey, Ravi R. Thiagarajan, Mathew J. Reeves, Matthew Ritchey, Carlos J. Rodriguez, Gregory A. Roth, Wayne D. Rosamond, Comilla Sasson, Amytis Towfighi, Connie W. Tsao, Melanie B. Turner, Salim S. Virani, Jenifer H. Voeks, Joshua Z. Willey, John T. Wilkins, Jason HY. Wu, Heather M. Alger, Sally S. Wong, Paul Muntner. Heart Disease and Stroke Statistics—2017 Update: A report from the American Heart Association. *Circulation*. 2017;135:e146-e603.
43. Aune D, Sen A, Schlesinger S, Norat T, Janszky I, Romundstad P, Tonstad S, Riboli E, Vatten LJ. Body mass index, abdominal fatness, fat mass and the risk of atrial fibrillation: a systematic review and dose-response meta-analysis of prospective studies. *Eur J Epidemiol*. 2017 Mar;32(3):181-192.
44. Ammar KA, Jacobsen SJ, Mahoney DW, Kors JA, Redfield MM, Burnett JC Jr, Rodeheffer RJ. Prevalence and prognostic significance of heart failure stages: application of the American College of Cardiology/American Heart Association heart failure staging criteria in the community. *Circulation*. 2007 Mar 27;115(12):1563-70.
45. Sanjay Dandamudi, Joshua Slusser, Douglas W. Mahoney, Margaret M. Redfield, Richard J. Rodeheffer, Horng H. Chen. The Prevalence of Diabetic Cardiomyopathy: A Population Based Study in Olmsted County, MN. *J Card Fail*. 2014 May; 20(5): 304–309.
46. Habbout A, Guenancia C, Lorin J, Rigal E, Fassot C, Rochette L, Vergely C. Postnatal overfeeding causes early shifts in gene expression in the heart and long-term alterations in cardiometabolic and oxidative parameters. *PLoS One*. 2013;8(2):e56981.
47. Mourmoura E, Chaté V, Couturier K, Laillet B, Vial G, Rigaudiere JP, Morio B, Malpuech-Brugère C, Azarnoush K, Demaison L. Body adiposity dictates different mechanisms of increased coronary reactivity related to improved in vivo cardiac function. *Cardiovasc Diabetol*. 2014 Feb 27;13:54.
48. Mourmoura E, Couturier K, Hininger-Favier I, Malpuech-Brugère C, Azarnoush K, Richardson M, Demaison L. Functional changes of the coronary microvasculature with aging regarding glucose tolerance, energy metabolism, and oxidative stress. *Age (Dordr)*. 2014;36(4):9670.
49. Mourmoura E, Chaté V, Couturier K, Malpuech-Brugère C, Azarnoush K, Demaison L. Evolution from increased cardiac mechanical function towards cardiomyopathy in the obese rat due to unbalanced high fat and abundant equilibrated diet. *OCL*. 2015; 22:1-8.

50. Mourmoura E, Rigaudière JP, Couturier K, Hininger I, Laillet B, Malpuech-Brugère C, Azarnoush K, Demaison L. Long-term abdominal adiposity activates several parameters of cardiac energy function. *J Physiol Biochem*. 2016 Sep;72(3):525-37.
51. Mourmoura E, Vial G, Laillet B, Rigaudière JP, Hininger-Favier I, Dubouchaud H, Morio B, Demaison L. Preserved endothelium-dependent dilatation of the coronary microvasculature at the early phase of diabetes mellitus despite the increased oxidative stress and depressed cardiac mechanical function *ex vivo*. *Cardiovasc Diabetol*. 2013 Mar 25;12:49.
52. Niemann B, Rohrbach S, Miller MR, Newby DE, Fuster V, Kovacic JC. Oxidative Stress and Cardiovascular Risk: Obesity, Diabetes, Smoking, and Pollution: Part 3 of a 3-Part Series. *J Am Coll Cardiol*. 2017 Jul 11;70(2):230-251.
53. Reho JJ, Rahmouni K. Oxidative and inflammatory signals in obesity-associated vascular abnormalities. *Clin Sci (Lond)*. 2017 Jun 30;131(14):1689-1700.
54. Gardner PR. Superoxide-driven aconitase FE-S center cycling. *Biosci Rep*. 1997 Feb;17(1):33-42.
55. Wang B, Chandrasekera PC, Pippin JJ. Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes. *Curr Diabetes Rev*. 2014 Mar;10(2):131-45.
56. Kuryszko J, Sławuta P, Sapikowski G. Secretory function of adipose tissue. *Pol J Vet Sci*. 2016;19(2):441-6.
57. Wyatt AW, Steinert JR, Mann GE. Modulation of the L-arginine/nitric oxide signalling pathway in vascular endothelial cells. *Biochem Soc Symp*. 2004;(71):143-56.
58. Armengou A, Hurtado O, Leira R, Obón M, Pascual C, Moro MA, Lizasoain I, Castillo J, Dávalos A. L-arginine levels in blood as a marker of nitric oxide-mediated brain damage in acute stroke: a clinical and experimental study. *J Cereb Blood Flow Metab*. 2003 Aug;23(8):978-84.
59. Wijnands KA, Vink H, Briedé JJ, van Faassen EE, Lamers WH, Buurman WA, Poeze M. Citrulline a more suitable substrate than arginine to restore NO production and the microcirculation during endotoxemia. *PLoS One*. 2012;7(5):e37439.
60. Vásquez-Vivar J, Kalyanaraman B, Martásek P. The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications. *Free Radic Res*. 2003 Feb;37(2):121-7.
61. Pecker F, Amadou A, Magne S, Pavoine C. Le rôle messager de l'acide arachidonique dans le cardiomyocyte. *Med Sci (Paris)*, 1998, Vol. 14, N° 10, p.1009-16.

62. Vial G, Dubouchaud H, Couturier K, Lanson M, Leverve X, Demaison L. Na<sup>+</sup>/H<sup>+</sup> exchange inhibition with cariporide prevents alterations of coronary endothelial function in streptozotocin-induced diabetes. *Mol Cell Biochem.* 2008 Mar;310(1-2):93-102.
63. Egashira K, Inou T, Hirooka Y, Kai H, Sugimachi M, Suzuki S, Kuga T, Urabe Y, Takeshita A. Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. *Circulation.* 1993 Jul;88(1):77-81.
64. Chauhan A, More RS, Mullins PA, Taylor G, Petch C, Schofield PM. Aging-associated endothelial dysfunction in humans is reversed by L-arginine. *J Am Coll Cardiol.* 1996 Dec;28(7):1796-804.
65. Toma BS, Wangler RD, DeWitt DF, Sparks HV Jr. Effect of development on coronary vasodilator reserve in the isolated guinea pig heart. *Circ Res.* 1985 Oct;57(4):538-44.
66. Bennett M, Gilroy DW. Lipid Mediators in Inflammation. *Microbiol Spectr.* 2016 Nov;4(6).
67. Demaison L, Sergiel JP, Moreau D, Grynberg A. Influence of the phospholipid n-6/n-3 polyunsaturated fatty acid ratio on the mitochondrial oxidative metabolism before and after myocardial ischemia. *Biochim Biophys Acta.* 1994 Oct 21;1227(1-2):53-9.
68. Pepe S, McLennan PL. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. *Circulation.* 2002 May 14;105(19):2303-8.
69. Kenchaiah S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, Kannel WB, Vasan RS. Obesity and the risk of heart failure. *N Engl J Med.* 2002 Aug 1;347(5):305-13.
70. Horwich TB, Fonarow GC, Hamilton MA, MacLellan WR, Woo MA, Tillisch JH. The relationship between obesity and mortality in patients with heart failure. *J Am Coll Cardiol.* 2001 Sep;38(3):789-95.
71. Lavie CJ, De Schutter A, Patel DA, Romero-Corral A, Artham SM, Milani RV. Body composition and survival in stable coronary heart disease: impact of lean mass index and body fat in the "obesity paradox". *J Am Coll Cardiol.* 2012 Oct 9;60(15):1374-80.
72. Lavie CJ, De Schutter A, Patel DA, Milani RV. Does fitness completely explain the obesity paradox? *Am Heart J.* 2013 Jul;166(1):1-3.
73. McLeod M, Breen L, Hamilton DL, Philp A. Live strong and prosper: the importance of skeletal muscle strength for healthy ageing. *Biogerontology.* 2016 Jun;17(3):497-510.
74. Wannamethee SG, Shaper AG, Whincup PH, Lennon L, Papacosta O, Sattar N. The obesity paradox in men with coronary heart disease and heart failure: the role of muscle mass and leptin. *Int J Cardiol.* 2014 Jan 15;171(1):49-55.

75. Vitiello D, Mourmoura E, Couturier K, Faure P, Dubouchaud H, Malpuech-Brugere C, Azarnoush K, Demaison L. Increased abdominal adiposity exacerbates ex vivo cardiac reperfusion injury through augmented mitochondrial oxidative stress. *J Diabetes Metab.* 2015;6:10.
76. T. Aoyagi, J.K. Higa, H. Aoyagi, N. Yorichika, B.K. Shimada, T. Matsui, Cardiac mTOR rescues the detrimental effects of diet-induced obesity in the heart after ischemia-reperfusion, *American journal of physiology. Heart and circulatory physiology*, 308 (2015) H1530-1539.
77. E.F. du Toit, M. Nabben, A. Lochner, A potential role for angiotensin II in obesity induced cardiac hypertrophy and ischaemic/reperfusion injury, *Basic research in cardiology*, 100 (2005) 346-354.
78. G. Maarman, E. Marais, A. Lochner, E.F. du Toit, Effect of chronic CPT-1 inhibition on myocardial ischemia-reperfusion injury (I/R) in a model of diet-induced obesity, *Cardiovascular drugs and therapy*, 26 (2012) 205-216.
79. Edland F, Wergeland A, Kopperud R, Åsrud KS, Hoivik EA, Witsø SL, Æsøy R, Madsen L, Kristiansen K, Bakke M, Døskeland SO, Jonassen AK. Long-term consumption of an obesogenic high fat diet prior to ischemia-reperfusion mediates cardioprotection via Epac1-dependent signaling. *Nutr Metab (Lond)*. 2016 Nov 28;13:87.