

ENDOTHELIAL NITRIC OXIDE ACTIVITY & BIOAVAILABILITY DURING OBESITY CONDITIONS

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Abstract

Obesity has been recognized as a pandemic and linked with several metabolic dysfunctions. One of the hallmarks in the onset of obesity is the development of hypertension coupled with endothelial dysfunction. (ED) A fundamental feature of ED is the reduced bioavailability of the key vasodilator, nitric oxide (NO), which has important vasoactive functions. Endothelial nitric oxide is significantly produced by the isoform endothelial nitric oxide synthase (eNOS) in the vascular wall. NO production is modulated by both physiological and pathophysiological environment. Several recent studies have reported the correlation of obesity with NO bioavailability and the endogenous inhibitor of eNOS, asymmetric dimethyl arginine (ADMA). This research aims to define the mechanisms that contribute to controlling eNOS activity and NO bioavailability during obesity conditions. In this study, eight week-old male Sprague-Dawley rats were exposed to cafeteria diet for 16 weeks. Our findings show that body weight increased gradually with 44% of the CAF-fed group becoming obese (CAF-OB) and the remaining 56% becoming resistant to body weight gain (CAF-WR). Levels of plasma ADMA increased and serum nitrite concentrations were reduced in the CAF-OB group while the CAF-WR group had a decrease in plasma ADMA levels and an increase in serum nitrite concentrations. This reflects the interaction of ADMA as an endogenous inhibitor on the bioavailability of NO. In conclusion, CAF diet induces obesogenic effects leading to endothelial dysfunction in both weight gain and weight gain resistant groups.

Introduction

Obesity is defined as the abnormal or excessive accumulation of total body fat that is reported as having a BMI of more than 30kg/m².^[1] The genesis of obesity is complex that is a result due to the imbalance in the energy intake and energy expenditure.

Obesity is known to be associated with other metabolic co-morbidities and is an important risk factor for cardiovascular diseases (CVD) including endothelial dysfunction. Obesity is an important predictor of CVD, which begins when several risk factors, including lipid abnormalities, high blood pressure, impaired glucose tolerance, proinflammatory and pro-thrombotic states co-exist in obese individuals, which in turn induce endothelial dysfunction. Endothelial dysfunction (ED) is defined as the imbalance between vasoconstrictors and vasodilators produced by and acting on the endothelium leading to loss or impairment of its physiological properties.^[2] Vascular endothelium has a vital role in the maintenance of cardiovascular homeostasis in health. The endothelium, although a simple monolayer, apart from acting as a physical barrier between the vessel wall and lumen, responds to physical and chemical signals by secreting a wide range of mediators that regulate platelet aggregation, coagulation, fibrinolysis and vessel tone.

During obesity, the vascular homeostasis is disrupted due to elevated blood pressure levels and loss of vascular tone. A key signaling molecule is nitric oxide (NO) that is produced endogenously. In mammals, NO is synthesized from L-arginine by the enzyme NO Synthases (NOS) that is available in three isoforms: neuronal NOS (nNOS), inducible NOS (iNOS) and endothelial NOS (eNOS). eNOS is most abundant in the vascular endothelium which generates NO by the NADPH- and O₂-dependent oxidation of L-arginine to L-citrulline in the presence of the endothelial cofactor, tetrahydrobiopterin (BH₄).^[3] An endogenous competitive inhibitor of eNOS is asymmetric dimethyl arginine (ADMA) which inhibits NO synthesis by competing with L-arginine at the active site of eNOS. It has been hypothesized that ADMA may play a direct role in the dysregulation of L-arginine/NO pathway.^[4]

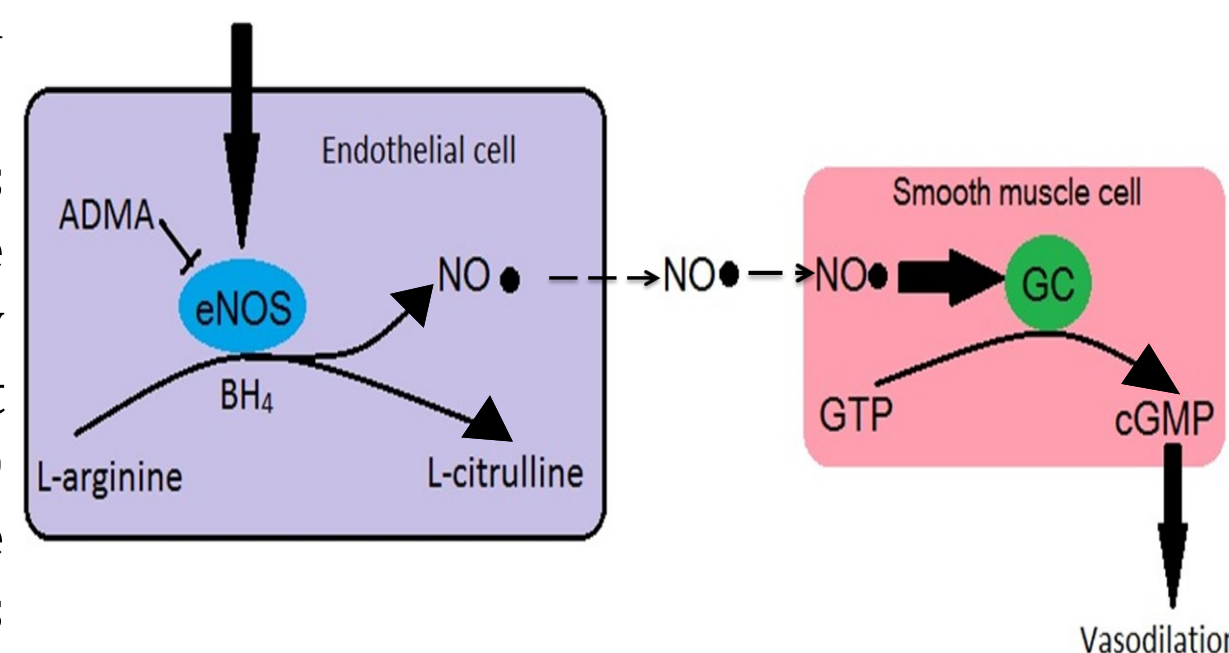
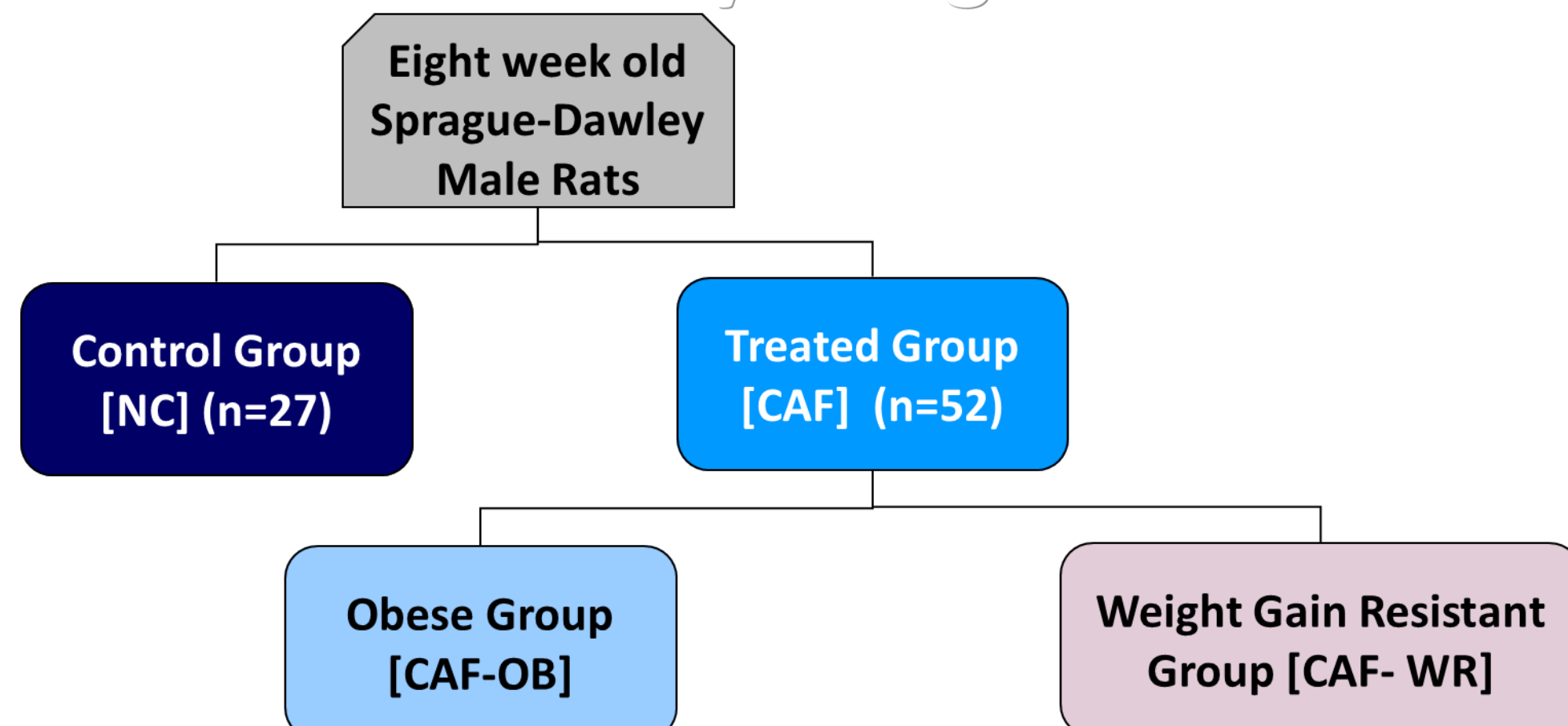


Fig. 2: Synthesis of nitric oxide

Objectives

- **Determine** the level of NOx bioavailability as an indicator of eNOS activity
- **Examine** the levels of ADMA as an endogenous inhibitor of eNOS
- **Evaluate** vasodilation response as measured by arterial blood pressure

Study Design



Methodology

Development of animal model	Tracking of food & water consumption, weekly body weight gain and blood pressure. Anesthetization of rats, collection & processing of blood samples on reaching targeted weight gain.
Determination of NO bioavailability	Deproteinization of serum samples using HPLC grade acetonitrile-chloroform. Quantification of nitrite in serum samples using Griess' reaction with absorbance read at 548 nm.
Determination of ADMA	Acylation of plasma samples. Enzyme immunoassay of acylated ADMA. Optical density read at 450 nm
Analysis of data	Statistical analysis of all results with one-way ANOVA using Excel.

Results and Discussion

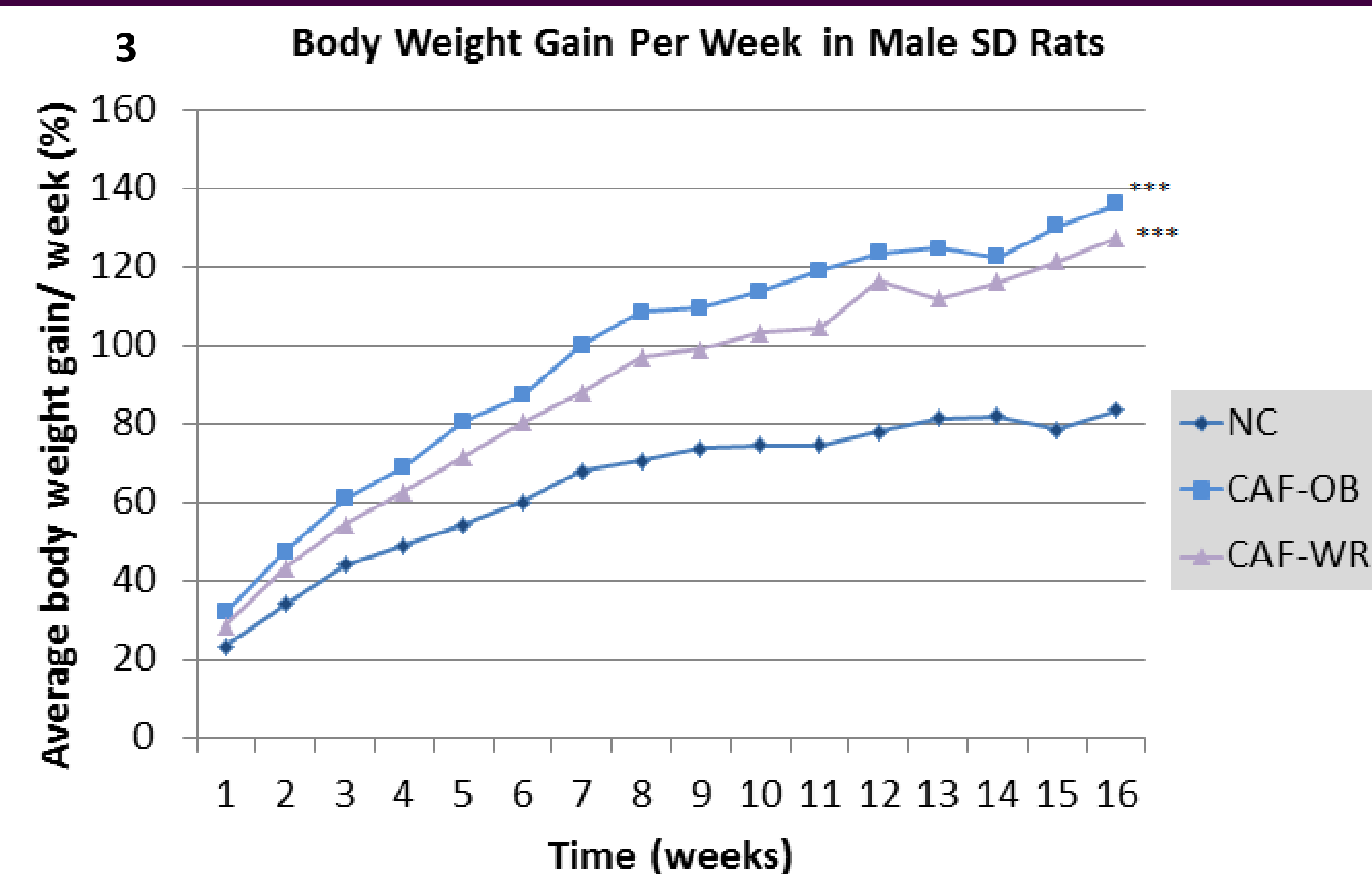


Fig. 3: Weekly body weight gain in male SD rats. CAF-fed rats exhibited significant weight gain after one week and persisted throughout the study reaching almost twice the weight compared to the control group with P-value ≤ 0.01 . Interestingly, this study also reports a finding wherein some of the CAF diet fed rats resisted weight gain.

Rats exposed to the CAF diet exhibited an initial high intake of the diet representing hyperphagia as well as reflecting the palatability and preference of the CAF diet over standard chow. Our findings are in agreement with studies showing that exposure to CAF diet induces hyperphagia^[5], leading to increased weight gain in less than half of the population^[6] while the others become resistant to weight gain^[7].

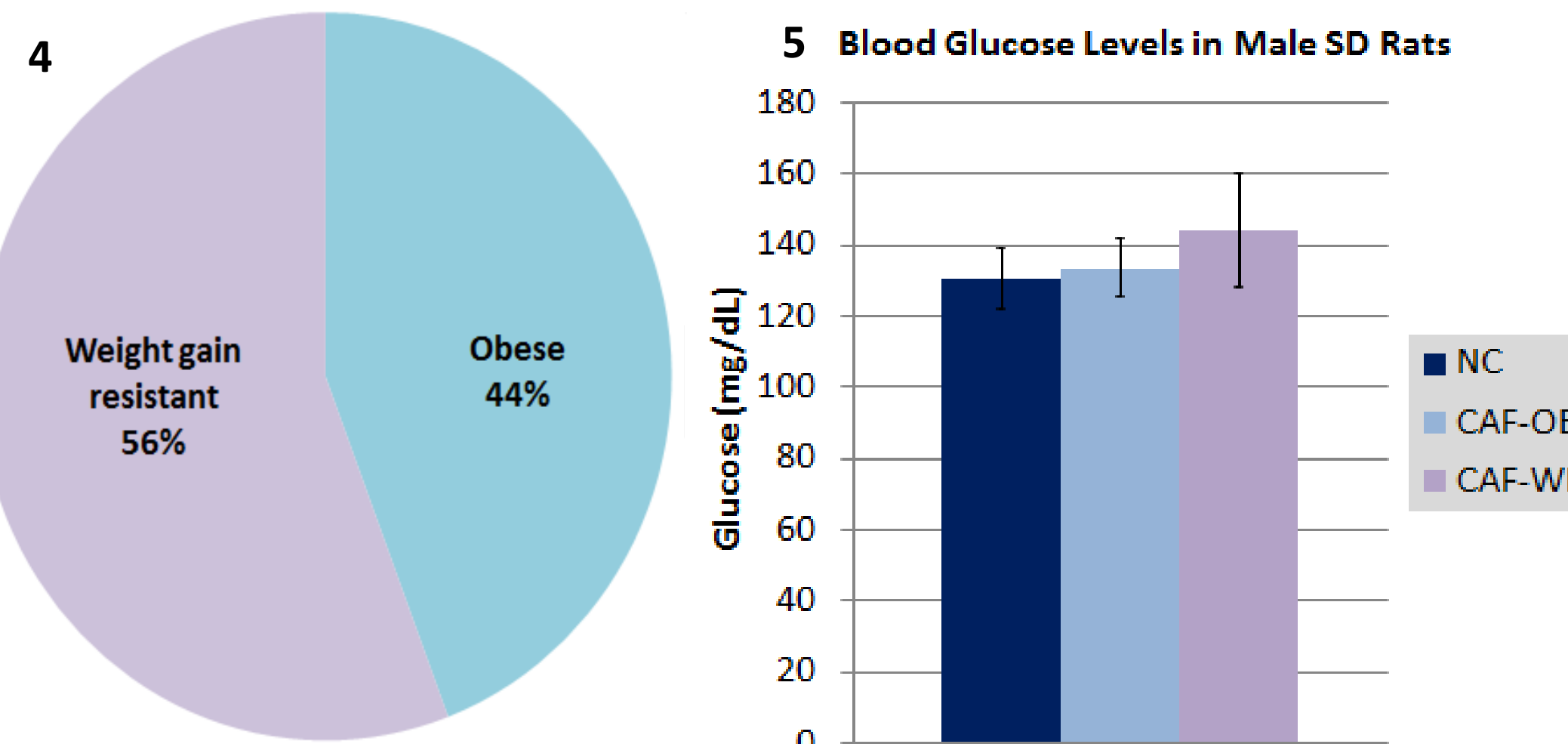


Fig. 4: Distribution of CAF-fed rats at week 16. CAF-fed rats exhibited greater total weight gain when compared to NC wherein 44% of the CAF-fed group gained 30% more from their initial weight while the remaining 56% were found to be resistant to weight gain and were similar to the NC group.

Fig. 5: The plasma glucose concentrations between the groups. There was no significant difference between the weight resistant group (CAF-WR) and obese group (CAF-OB) when compared to their control counterparts.

Regardless of the weight gain and availability of the standard chow, rats continued to consume the energy dense CAF diet in excessive amounts revealing that weight gain was not associated with high glucose levels. This indicates that hyperglycemia was not detected and the rats are in the early stages to a diabetic condition.

Determination of NOx bioavailability as nitrite in serum

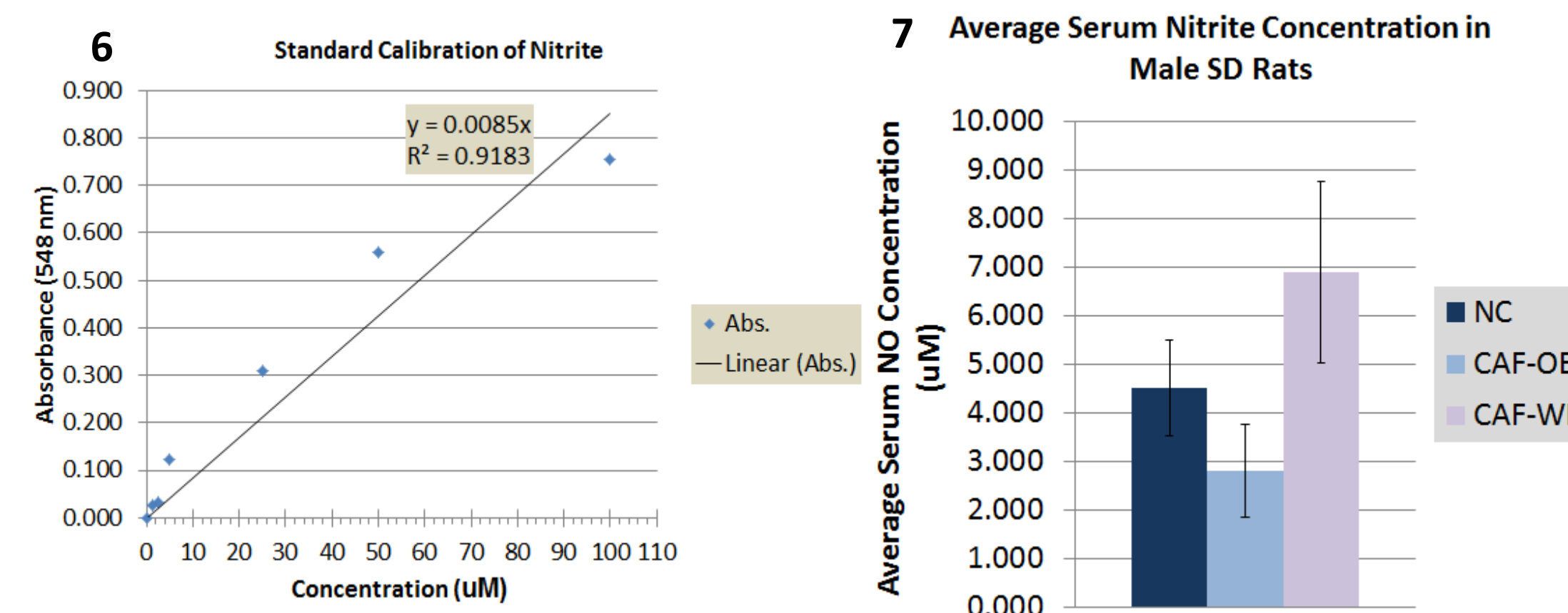


Fig. 6 & 7: Chemical reduction by Griess' reaction to determine the level of nitrite. Although the level of nitrite in CAF-OB group did not show statistical significance, the values of concentration of nitrite was diminished in CAF-OB group. Interestingly, there was an increase in the CAF-WR group yet it did not show statistical difference in comparison to NC group.

It has been hypothesized that the unaltered plasma NO levels due to CAF diet may be due to the inhibition of arginine production thus preventing the increase of NO production under CAF diet induced obesity.^[8]

Determination of ADMA in plasma

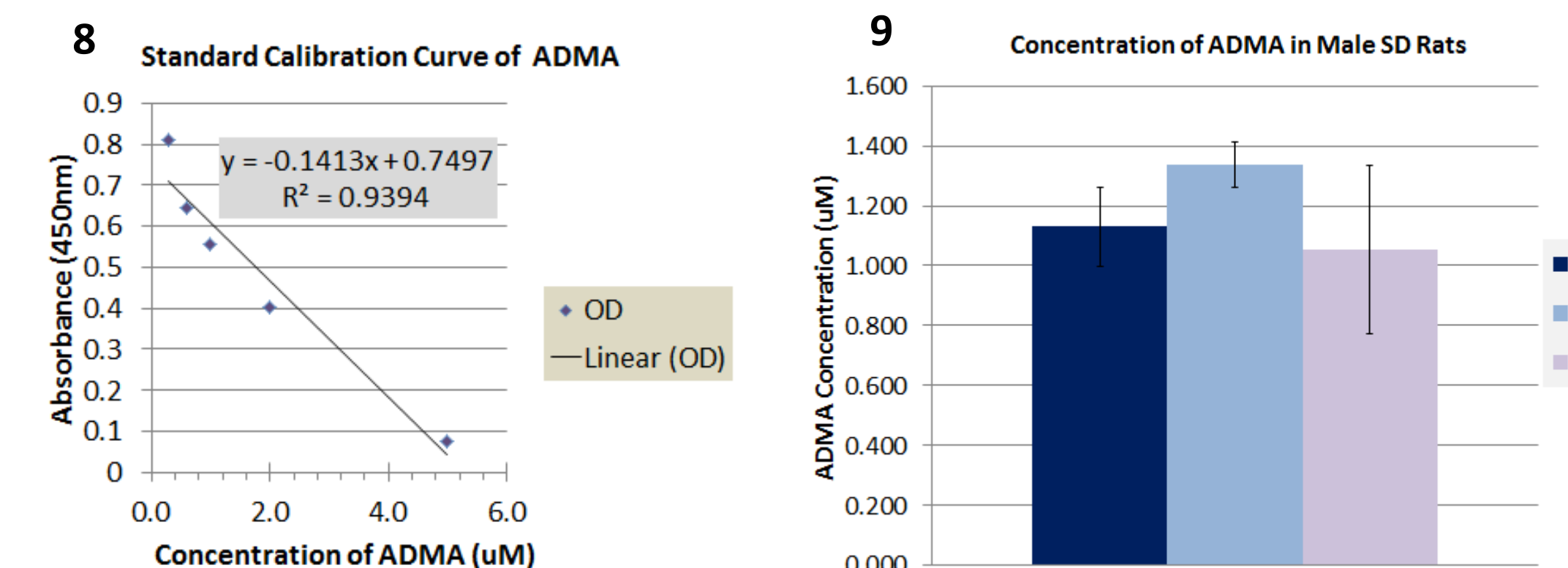


Fig. 8 & 9: Concentration of ADMA by ELISA. ADMA levels increased in obese rats when compared to their control counterparts. The CAF-WR group shows a slight decrease in the ADMA concentrations while the CAF-OB group shows an increase in ADMA concentrations relative to the NC group.

CAF diet induced obesity stimulates ADMA increase coupled with decreased NO. However, ED is detectable and manifested after the onset of hyperglycemia.^[9]

Evaluation of vasodilation response as blood pressure

Group	Initial BP (mmHg)		Final BP (mmHg)	
	Systolic	Diastolic	Systolic	Diastolic
NC	166.8±5.5	134.7±5.6	148.8±4.5	106.3±3.9
CAF-OB	161.2±6.3	123.9±6.5	166.4±4.9	119.0±4.7
CAF-WR	168.3±7.2	129.1±7.1	157.7±3.6	111.1±4.4

Table 1: Blood pressure of male SD rats. Our investigation reveals a significant drop in the blood pressure in both control and CAF-fed groups while being within range as published by other research group.

This drop in blood pressure could be due to the fact that the evaluated parameters of endothelial cell function have not deteriorated to reflect the significant changes. It has been reported that the L-arginine/ADMA ratio has a negative correlation with systolic blood pressure.^[9]

Conclusion

This study demonstrated that high consumption of CAF diet resulted in high weight gain and diet induced obesity resembling the patterns of human obesity. This pattern of obesity was not associated with significant increase in blood glucose levels yet the serum levels of endogenous inhibitor, ADMA increased above basal levels that might result in endothelial dysfunction and reduced NO production and bioavailability as explained by the serum levels of nitrite.

Interestingly, these changes were detected for the first time in weight gain resistant group that needs further investigation to understand pathophysiology of high-energy consumption and impairment of NO production and bioavailability under non-obese conditions.

References

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Acknowledgements

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