Distribution of Renin in Rat Adipose Tissue

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Introduction

Obesity and hypertension are two leading international health problems of particular concern within the United States. Obesity is often a precursor to high blood pressure which is regulated in part by the renin angiotensin system (RAS). Recent studies have also implicated the RAS as a regulator of body adiposity in human and rodent models [1,2]. Adipose (fat) tissue has been shown to display a fully functional RAS with the discovery of mRNA for renin, angiotensinogen, angiotensin converting enzyme (ACE) and angiotensin II receptor expression in adipocytes (fat cells) [1]. However, it is not yet known where renin is distributed within fat tissue.

The aim of this study is to analyze the distribution of renin in components of fat tissue.

Results

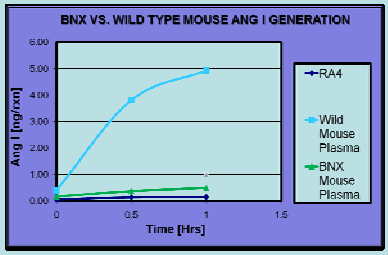

Figure 2. Proof of renin assay: Mouse plasma renin measured in a normal animal and an animal with 48 hour bilateral nephrectomy (BNX). Since plasma renin comes from the kidneys, the 48 hour BNX plasma sample should show much less renin activity, and in fact it showed a 95.5% fall.

Renin Measurement

1. **Angtiotensinogen is prepared from bilateral nephrectomized rats.**
2. **Unknown renin sample is added to angiotensinogen.**
3. **Angiotensin I is generated linearly over time in the presence of ACE inhibitor (see *Figure 2*) is directly proportional to sample renin concentration.**
4. **Angiotensin I measured by a Radioimmuno assay.**

Samples to assay:

- **Fat pad (whole)**
- **Adipocytes from collagenase digestion**
- **SV pellet after collagenase digestion**
- **Supernatant taken from fraction just above SV pellet (represents interstitial fluid (ISF)**
- **DMEM + BSA + HEPES (No collagenase) unused media (control)**
- **RA4 control**

The Renin Angiotensin System

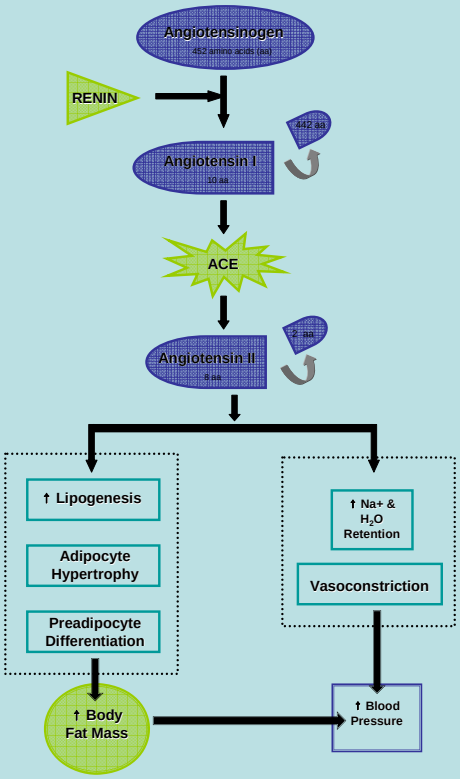

Figure 1. The Renin Angiotensin System (RAS): Renin, an enzyme released from the kidney, cleaves angiotensinogen (inactive) to form angiotensin I in the blood stream. Angiotensin I then interacts with ubiquitous angiotensin-converting enzyme (ACE) which catalyzes the formation of the major bio-active product Angiotensin II [3]. Angiotensin II acts a modulator of blood pressure and body adiposity [2].

Discussion

This project is still in its preliminary stages. Data shown above (*figure 2*) verifies successful measurement of Renin by a radioimmuno- enzymatic assay. The finer details involved in isolating the following components of fat tissue have yet to be worked out: high yield of adipocytes, stromal-vascular (SV) pellet, cell membrane and intracellular material/ cytosol. Practice assays have determined a number of obstacles. Problems encountered so far include:

- **Renin assay and collagenase digestion have learning curves. (It is not quite cook book).**
- **Dulbecco's Modified Eagle Medium (DMEM) is buffered with 20 mM HEPES. DMEM is used to store the fat pads (4°C), mince the fat pad into 20 mg sections (20°C) and to incubate the sections with collagenase (37°C). However the HEPES pK is temperature sensitive!**
- **Initial experiments did not use enough fat tissue (100mg), should use at least 300mg in order to have enough SV pellet.**
- **We are unsure how much agitation of cells is needed during collagenase digestion. More agitation leads to more cell dispersion but decreased cell number and possible renin degradation in media.**
- **Straining cells after collagenase digestion may be best with a 100um cell strainer.**
- **Fat pads need to be used quickly (can not store overnight).**
- **We are unsure of best method to separate adipocytes from supernatant (supernatant represents ISF).**

Experimental Design

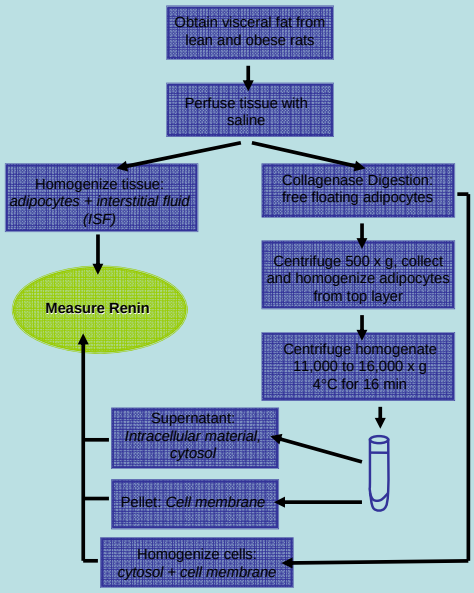

Figure 3. Experimental Design: The flow chart provides a brief description of the steps involved in separating a whole fat pad into its constitutive parts. Upon successful isolation each component will be analyzed for renin concentration with the radioimmuno-enzymatic assay described above.

Acknowledgements

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